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DEVELOPMENT OF MASS-REARING TECHNIQUES FOR *APHELINUS ASYCHIS* WALKER FOR USE IN A BIOLOGICAL CONTROL PROGRAM AGAINST RUSSIAN WHEAT APHID

by

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ABSTRACT

Mass-rearing techniques were developed for the parasitoid *Aphelinus asychis* Walker (Hymenoptera:Aphelinidae) for use in a biological control program against the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera:Aphididae). Daily ovipositional activity, host-feeding behavior, effects of population density in the rearing cages and effects of cold storage on mummies were determined.

Daily ovipositional activity peaked at day 5 after emergence of the parasitoid. Ninety-two percent of mummies resulting from ovipositional activity were oviposited the first 13 days of the parasitoid's life. Since mummification occurred 11 days after oviposition and emergence occurred 11 days after mummification, day 24 was determined to be the optimum day to harvest mummies for biological control shipments.

Host-feeding behavior was found to be steady throughout the lifetime of the parasitoid (mean longevity 31 days, ± 14.2). A mean 4.3 RWA (± 2.0) were killed per day by this behavior.

Available cage space was optimized when a parasitoid population of 100 (70% female) was used for mummy production. A host-to-parasitoid ratio of 80:1 was

determined to be optimal when host ages (instars) were random. A mean 164 mummies (± 56) per female developed at this host-to-parasitoid ratio. This high host-to-parasitoid ratio made the host density in the cage the limiting factor rather than parasitoid density. At densities of 200 and 300 parasitoids, aphid numbers were too high for the plants to sustain them, and everything died as a result.

Short-term cold storage of *A. asychis* mummies proved to be a feasible alternative to shipping mummies when conditions so dictate. Three population ages, 3, 7 and 21 days, were stored for periods of 3, 5, 7 and 14 days. Best percent emergence occurred for the 3-day old population, however, each age declined in emergence when stored for increasingly longer lengths of time. Lowest percentage emergence occurred for 21-day old mummies stored for 14 days. Twenty-one day old mummies exhibited higher percent emergence when stored for 3, 5 and 7 days than did the 7-day old mummy population. Males survived the cold storage process better than females, as most of the decrease in emergence at the longer storage times was indicated to be female mortality.

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INTRODUCTION

The Hymenoptera, as an insect order, are singularly and sharply characterized by numerous and remarkable developmental and ecological adaptations which have arisen during a long and complicated evolution. This evolution has generally been toward parasitism, for apparently the majority of the more than 200,000 species of Hymenoptera existing today are parasitic. Such speciation may have had its inception during the Permian period when the insect fauna of the world was much more diverse than it is today (Flanders 1962).

Aphelinus asychis Walker, a member of the family Aphelinidae, is a solitary endoparasitoid of aphids. The Aphelinidae number more than 500 known species, many of which play an important role in the biological control of aphids, coccids and aleyrodids (Flanders 1953).

All members of the genus *Aphelinus* are parasitoids of aphids (Homoptera:Aphididae). The genus currently contains 48 species, excluding some that have been transferred to other genera or placed in synonomy with other species (Hayat & Fatima 1990).

Aphelinus asychis is about 1 mm in length. Size varies, however, depending on the size of the aphid host, and males are generally smaller than females. This may result from females ovipositing female eggs into older, i.e., larger, aphids. How this process of sexual allocation occurs is unknown. *Aphelinus asychis* exhibits arrhenotoky, i.e., fertilized eggs give rise to females and unfertilized eggs give rise to males. Cate et al. (1977) noticed a higher percentage of females (95%) emerging from older aphids (5 days old at time of oviposition) when greenbug, *Schizaphis graminum* (Rondani), was host.

Two reproductive strategies among the parasitic Hymenoptera have been distinguished by Flanders (1962). In some species, eggs absorb host fluids and increase greatly in size during incubation (hydropic). Females in general are relatively short-lived, may carry a large number of mature eggs in their ovaries, and do not host-feed (a behavior in which females consume host hemolymph and tissues). The other type of reproductive strategy is employed by females which are generally long-lived and produce eggs that contain all the necessary nutrients for embryonic development (anhydropic). Eggs tend to be relatively large, and females exhibit synovigenesis, that is, they have only few mature eggs in their ovaries at any one time and host-feed to reach maximum egg production. In the absence of suitable hosts, anhydropic eggs may be resorbed, resulting in a slow cycle of egg maturation and egg resorption.

Aphelinus asychis employs a reproductive strategy that incorporates host-feeding at levels necessary to satisfy basic nutritional requirements. This enables the females to allocate more time and energy to oviposition (Bai & MacKauer 1990).

Aphelinus asychis is a synovigenic species with anhydropic eggs. Bai & MacKauer (1990) found host-feeding and oviposition to be mutually exclusive events. Host-feeding usually precedes oviposition, especially in newly-emerged females.

At the time of pupation, the parasitoid larva kills its host, transforming the aphid into a black, protective shell known as a "mummy." The prepupal and pupal stages develop inside the mummy until the adult emerges. It is in this protected mummy stage that the parasitoids are handled, packaged, shipped and stored for biological control purposes.

Biological control is one of the oldest methods of pest management. It has many definitions, but in the classical sense, it is the introduction of natural enemies, (predators, parasitoids and pathogens) to areas where they are not native, to control a pest, which is usually an accidental introduction to an area. Approximately 5,000 species have been tested for use in biological control programs. Of these, 270 species have led to partial (100), substantial (100) or complete (70) control. More than 80% of the successful natural enemies are parasitoids; the remainder are predators (17%) and pathogens (1%) (van Lenteren 1985).

Most problems in developing biological control programs are encountered in the selection of parasitoids to mass-produce, and in the actual mass production process. Quality control is often overlooked in insect rearing programs, and quantity produced often becomes more important than the quality of the insect being mass-produced (van Lenteren 1985). Quality here is defined as the ability of the insect to survive and reproduce in the field.

Genetic variability is usually abundant in natural, open populations. When a part of this gene pool is brought into the laboratory, the population becomes closed. The size of the founder colony directly affects how much variation will be taken from the gene pool. Laboratory selection factors differ from those in the field, causing selection of an average, or perhaps poor, genotype (van Lenteren 1985). van Lenteren suggests the following criteria to be considered to mass-rear a quality insect for use in a biological control program:

1. The effective number of parents at the start of mass breeding is much lower than the number of founder individuals; so start with a large population.
2. Compensate for density dependent phenomena (large cages).

3. Create a proper balance of competition, but do not overcrowd.
4. Set environmental conditions for the best genotype; use fluctuating abiotic conditions.
5. Maintain separate laboratory strains and cross them systematically to increase F1 variability.
6. Determine the standards that apply to the intended use of the insects, and then adapt rearing procedures to maximize those values in the domesticated strain.
7. Develop morphological and biochemical genetic markers to monitor changes between the founder population and later generations of the lab-reared strain.

The Mission Biological Control Laboratory (MBCL) in Mission, Texas, is a well-equipped facility which has the resources to effectively produce a quality parasitoid for field releases. The laboratory is a United States Department of Agriculture (USDA) Animal & Plant Health Inspection Service (APHIS) facility. Twenty-one programmable walk-in environmental growth chambers and extensive greenhouse facilities make mass-rearing of many different insect species possible.

In 1987, USDA-APHIS targeted a newly-introduced pest of wheat and barley, the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (RWA), for biological control. In 1989, *A. asychis* was chosen, mainly because of its synovigenic, host-feeding behavior, as one of the many parasitoids to mass-rear for release against RWA. The current strain being reared was collected from Chillan, Chile, by Keith Pike of Washington State University and David Reed, ARS Biological Control Laboratory, Stillwater, Oklahoma. In 1991, nine

species or geographical strains of RWA parasitoids were mass-reared at MBCL and shipped for field release.

The environmental conditions at which the RWA is reared were chosen as a result of studies done by Michels & Behle (1989) on the aphids' developmental temperature requirements for optimum growth, and general springtime climatic conditions which exist when RWA numbers rise to the level that it becomes a pest. In this way, parasitoid genotypes suitable to cooler conditions are selected for, even though this may slow down their development, and subsequently, lessen the numbers of parasitoids to release in a season.

To achieve economic feasibility, insect rearing facilities must standardize equipment and procedures whenever possible. A standard-size cage has been designed at MBCL which has been used successfully for the mass-rearing of many different insect taxa. These cages are large enough (41 x 41 x 51 cm) to allow desired searching behavior of small insects to occur, yet small enough to maintain many separate colonies of insects in one chamber. The cages are sealed to prevent escape of insects which may allow cross-contamination between the colonies.

A general mass-rearing protocol has been developed for aphidiid (family Aphidiidae) RWA parasitoids; however, a protocol for mass-rearing aphelinids is needed. The objective of this study is to develop a mass-rearing protocol for *A. asychis*. This protocol will make mass-rearing a quality insect possible using standard laboratory equipment and a minimum of manpower. The protocol can be used in a quarantine situation, where the host pest is not found in the area where the rearing facility is located. Some of the data dealing with

the biology of *A. asychis* with RWA as host may be useful in making decisions concerning their behavior in the field.

Aphelinus asychis has been recovered in the Texas Panhandle two years after the initial release. Recovery was made using sweepnets in a field of winter wheat on May 5, 6 and 19, 1992, by G. J. Michels and R. Whitaker-Deerberg, close to the original release site. Four adult *A. asychis* were recovered on May 5, one on May 6 and two on May 19. Three releases of parasitoids had been made at this site in Carson County, Texas, in April and May of 1990. Although aphidiid parasitoid species were also included in the releases, none were recovered. Final determination was made by J. B. Woolley at the Texas A&M University Biological Control Laboratory at College Station. Successful establishment of this very promising biological control organism in one area makes mass-rearing of these parasitoids even more important. Establishment of these parasitoids in many areas may firmly entrench these natural enemies of RWA into the agricultural ecosystem.

METHODS AND MATERIALS

To gain an understanding of parasitoid biology with implications for mass production, two closely related areas must be considered: the methods of culturing both the host and its host plant. Parasitoid-host-plant interactions must be treated as a unit. For this reason, the methods of host plant culturing and RWA rearing are briefly outlined in this section.

Host Plant. Host plant used was wheat, *Triticum aestivum* L., cultivar MIT. Plants were grown in a mixture of No. 2 and No. 3 vermiculite. Seed was measured volumetrically, 6-8 ml (100-125 seeds) per 10-cm pot. Pots were filled 2/3 full with vermiculite mixture, seeds were spread evenly across the top. More vermiculite mixture was placed over the seed to approximately 1 cm from the top. Vermiculite was tamped lightly. The seeds were watered immediately after planting with a mixture of 5 ml Subdue® (seed treatment for germination) and 5 ml of granular fertilizer per gallon of water. Subsequent watering was done with a dilute water soluble fertilizer mixture. The wheat seedlings were treated with a *Bacillus thuringiensis* formulation (Gnatrol®) specific for fungus gnats (Diptera:Cecidomyiidae) immediately after emergence from the soil. Temperature in the greenhouse was kept at 25-28°C. The plants were treated with a systemic fungicide to prevent powdery mildew prior to being taken into the RWA rearing chamber. Three different fungicides were used on alternate months to prevent powdery mildew resistance to the fungicides from developing. All fungicides were screened for aphid toxicity and phytoxicity before use in the mass-rearing program. Plants were sprayed with the recommended dose of fungicide. These plants were taken into the RWA rearing chamber 4-8 hours later and infested with aphids. None of the tested fungicides caused aphid

mortality, but some had a phytotoxic effect on the wheat. This phytotoxic effect was exhibited as yellowing and withering of the meristematic tissues. The plants were taken into the RWA rearing chambers and infested when they were approximately 10 cm in height.

RWA Rearing Procedures. The RWA were reared in a semi-open type rearing system. The shelves were enclosed in nylon organza cloth "shrouds" which prevented alatae (winged aphids) from escaping. The shrouds consisted of 3 shelves, which held 6 trays. Each tray held 20 pots of wheat. Twelve shrouds (in two chambers) gave a potential of 1440 pots of wheat infested with RWA. Temperatures fluctuated from 12-22°C. A 16:8 (L:D) photoperiod was supplied with a mixture of light wavelengths using incandescent and fluorescent lights. Percent relative humidity also fluctuated in a diurnal cycle with a daytime low of 55% and a nighttime high of 65%.

Fourteen trays (280 pots) of 4 cm high wheat were brought in from the greenhouse daily (Monday-Friday). Fourteen pots were used to infest 20 pots (1 tray) of fresh wheat with RWA. The pots of wheat used to infest the fresh wheat had been in the aphid chamber (infested) for one week. The ratio of 14:20 (infested to uninfested) usually ensured a population of 400-600 aphids per pot. These pots of wheat were clipped onto lightweight mesh which was placed directly on top of the fresh wheat plants. The clipped wheat was spread evenly over the entire surface of the fresh wheat so that an even distribution of aphids occurred. Two days later this mesh was removed. The newly-infested wheat was available for introduction into the parasitoid rearing cages. Since the parasitoids used 6 pots per tray, this gave a potential of 420 pots available per week. When nine species/strains

were being mass-reared, this allowed set-up of 3 cages per species each week. This staggering of the cage set-up dates provided parasitoids for shipments on a weekly basis.

For the purpose of this research, numbers of mummies which developed was used to measure oviposition. Bai & MacKauer (1990) reported that frequency distributions between number of eggs dissected 24 hours and 4 days after an attack did not differ significantly. Pea aphid, *Acyrthosiphon pisum* (Harris), was host. However, a proven positive correlation between eggs and mummies, when RWA is host, would save much time in rearing and less handling of host plant material for individual oviposition studies.

Optimum Parasitoid-to-Host Ratio. Optimum host density for ovipositional activity was studied using an individual cage method. Clear, plexiglass tubing was used to individually cage each female *A. asychis*. The 4.4 cm tubing was cut into 25 cm-long sections. These were placed over small, commercially obtained planters. Two small vents were cut into the tubing and covered with nylon organza cloth. The top of the tubing was covered with the same material.

Five to ten wheat seedlings, cultivar MIT, were grown in each cage. Just prior to infestation with the host aphids, plaster of paris was poured in a thin layer over the soil in order to facilitate observation of any aphids which mummified off the plant and to be able to observe the dead parasitoid. After the seedlings reached approximately 12 cm in height, they were infested with the desired density of aphids. Aphid densities consisted of 10, 20, 40, 60, 80 and 100 aphids per cage. Aphids were chosen randomly from the RWA colony, with no separation of instars. The aphids were allowed to settle for approximately 6 hours on the plant. One newly-emerged, mated female *A. asychis* was introduced into each cage.

Five cages containing each host density were set up. This was repeated three times, each time using parasitoids from different cage populations and different generations, for a total of 15 females studied for each density. Significant means were separated using the Student-Newman-Keuls Mean Separation Test ($P < .001$).

Each cage was observed on a daily basis. Total number of mummies per female and date of first emergence and mummification were recorded. Date of death of the parasitoid was noted if it occurred before her progeny began to emerge.

Host-Feeding Behavior and Longevity. Host-feeding behavior and longevity were determined by placing one mated, newly-emerged female *A. asychis* into a petri dish with a wheat leaf containing approximately 50 host aphids. Every 24 hours the parasitoid was removed and placed into another identical petri dish. Transferral of the parasitoid from one petri dish to another was accomplished by waiting for the wasp to walk onto the lid of the petri dish and then moving it over onto the next dish. This was done because two other attempted methods of transferral proved to be harmful to the parasitoid. When an aspirator was used, the parasitoid died within one week, and camel hair brushes often damaged the parasitoid.

The wheat leaves were maintained by covering the cut ends with a small piece of moistened paper towel (Michels et al. 1987). This proved satisfactory for the 24 hour time period needed to keep the leaves in good condition.

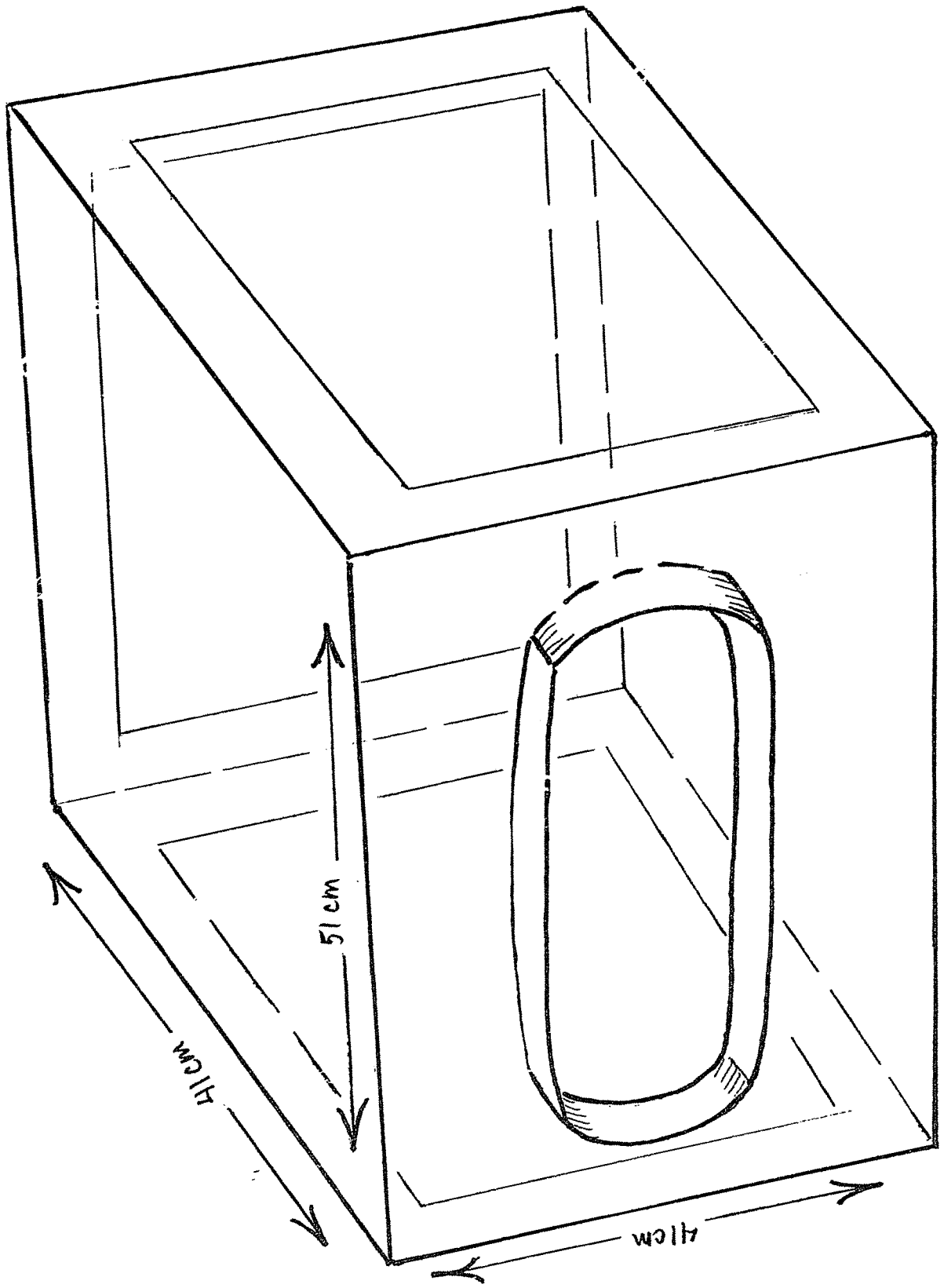
Fifty aphids were used each day to ensure that host numbers would not be a limiting factor influencing numbers of aphids parasitized or fed upon each day. Aphids which were dead from host-feeding were recorded. This was done daily until the death of the

parasitoid. Appearance of the dead aphids was used as the criterion for determination of host-feeding. Aphids which had been killed by this behavior look significantly different from those dead from other causes. The aphid has a yellowish color, a withered look, and the ovipositor wound with hemolymph exuding from it can usually be seen under low magnification. This criterion for determination of host-feeding activity was used by Bai & MacKauer (1990). This was done for a total of 15 parasitoids. Nine of the 15 were studied for daily ovipositional activity.

Length of Ovipositional Period and Daily Ovipositional Activity. To ascertain daily ovipositional activity, aphids from the host-feeding study which were still alive after the 24 hour exposure period were transferred daily to an individual tube cage containing approximately five wheat plants. Transferring aphids rather than parasitoids avoided parasitoid mortality associated with daily handling with a brush or aspirator. Minimum handling of the parasitoids is essential if a correlation is to be assumed between parasitoid behavior in petri dishes and in a rearing cage where no handling of the parasitoids occurs. Mummies were then quantified on a daily basis after enough time elapsed for pupation to occur. This was done for the entire life of nine parasitoids.

Optimum Parasitoid Density in the Rearing Cages. Optimum parasitoid density in the rearing cages was studied using different parasitoid densities in a standard MBCL rearing cage (Fig. 1). This data was also used to determine the maximum host density and plant biomass that a standard rearing cage can accommodate without causing problems associated with condensation and plant death or damage due to high aphid populations.

Fig. 1. Drawing of mass-rearing cage used at MBCL for mass production of RWA parasitoids.



Using a host to parasitoid ratio of 80:1 (determined by the previous study), five cages were set up with parasitoid densities of 10, 50, 100, 200 and 300 per cage. The *A. asychis* sex ratio at the environmental conditions prescribed for the RWA mass-rearing program at MBCL was determined to be 70% female parasitoids. This percentage is used in calculations determining numbers of mummies per female in the mass-rearing cages. This percentage was also used to determine the probable numbers of females at the 100, 200 and 300 parasitoids/cage. The females were counted individually for the lower densities. The remaining 30% (males) were then added to the cage. These were newly-emerged individuals taken from the stock colony. For the higher density infestations, total mummies were counted prior to emergence and placed into the mass-rearing cages.

Cages were maintained using the standard operating procedure for the mass production of RWA parasitoids at the MBCL. This procedure was developed as a general guideline for many different species of parasitoids, and was not specific for aphelinids. The basic procedure is outlined as follows:

1. Cages were sanitized using a 10% solution of Lundmark's sanitizing solution.
2. Water-absorbent, white cage liners were placed on the bottom of each cage.
3. Mummies were counted (200-300, depending on species) and placed on white paper toweling and placed into the cage.
4. One streak of honey was applied to the material covering the vents of the cage, and a water source was placed in the cage. Honey was used as a food source for the males.

5. Two days after the mummies began to emerge, the host was added to the cage. The RWA was reared on wheat (cultivar MIT) in 10-cm pots containing approximately 100 wheat seedlings. Each pot of wheat contained approximately 500 aphids at the time of introduction to the parasitoids, but this number could be decreased to or increased up to 1000 aphids per pot depending on the density required.
6. The period from introduction of the host to mummification took 2-3 weeks, and care of the host plant was of utmost importance. Plants were checked daily for soil moisture content, disease and general condition. If wilting or disease occurred, the aphids were transferred to new host plants. The wheat was clipped, and the aphids were allowed to move onto fresh plants placed into the cage. If the aphid had been parasitized for a sufficient length of time, however, it could not move to the new host plant, and subsequently died when the plant it was feeding from died. A certain percentage of parasitized aphids died whenever it became necessary to move them to new host plants, so care of the original plants was important.
7. After most of the parasitized aphids had mummified, and just prior to first emergence (16-21 days at the given temperatures), the plants were all clipped at the base, and placed on the bottom of the cage so that any remaining green plant material dried out.
8. Mummies were then placed into recolonizing cages or shipped to designated release sites.

For this research, a change was made in the general protocol. The number of days after emergence begins before the host was added to the cage was changed to one. The host was introduced sooner because *A. asychis* females that are deprived of the host after emergence tend to host-feed at a higher rate than do those that have immediate access to the host (Bai & MacKauer 1990).

Since ascertaining the optimum parasitoid density for mass-rearing, and ultimately for shipment of unemerged parasitoids, was the purpose of this study, a cut-off point had to be determined for quantifying total mummies in the cage. An overlap of emergence and mummification will occur if the mummies are not processed soon enough. However, if processed too soon, mummy numbers will be less than optimal as parasitized aphids die. Twenty-four days was chosen as the total mummification time allowed before the wheat was clipped and the mummies counted for each cage. The results of the daily ovipositional study done previously were used to determine the cut-off point for mummification time used in this study.

Short-Term Cold Storage Potential. The short-term cold storage potential of *A. asychis* mummies for shipment at a later date was determined by placing 30 mummies of 3 different population ages for a varying number of days into a 4.4°C (40°F) storage area. The mummies were stored in plastic petri dishes, still attached to the wheat clippings. Since removal of the mummies from the plant may harm the parasitoid pupae within, they are handled, stored and shipped still attached to the wheat leaves.

The wheat was usually clipped at soil level 1-2 days prior to storage or shipment of the mummies. This was done so that excess moisture in the wheat leaves did not promote a problem with growth of fungus.

Because the shipment of biological control organisms must also conform to the usual constraints imposed by the "business work week," the number of days the mummies were stored was determined to conform with worker convenience. The mummies were held in cold storage for 3, 5, 7 and 14 days. The age of the mummies was calculated as number of days after mummification in the cage was first observed. Mummy age in this respect is actually a population average, because new mummies were forming each day. The longer the time before collection, the wider the range of ages put into cold storage. Population ages used in this research were 3, 7, and 21 days. The 21-24 day old population was the most important regarding storage to a mass-rearing program, because at that time maximum mummy development had occurred before the mummies were harvested. Thirty mummies of each age were stored for each time period. A control population of 30 mummies was allowed to emerge in the rearing chamber under mass-rearing conditions.

After the mummies were in cold storage for the allotted time period, they were removed and placed into the rearing chamber to await emergence. Percent emergence and sex ratio were recorded.

Data were analyzed using the SAS Statistical Analysis System for Personal Computers, version 6.06 (SAS Institute, 1988). Means and standard deviations were determined for all data except the cold storage studies. Untransformed percentages were used to analyze the cold storage data. Analysis of variance was performed on the optimum

host-to-parasitoid data and the significant means separated using the Student-Neuman-Keuls Mean Separation Test ($P < .001$).

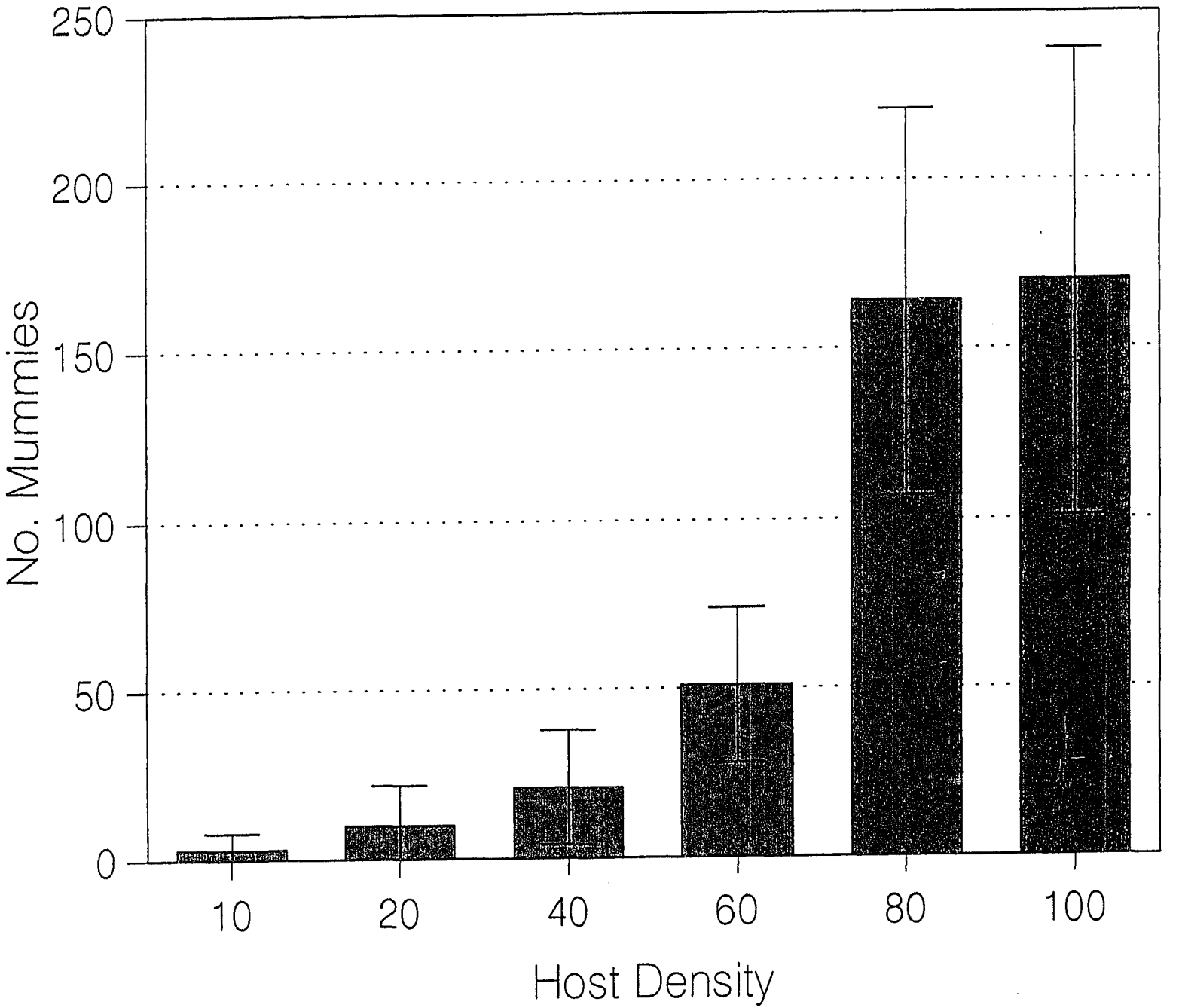
RESULTS AND DISCUSSION

Optimum Parasitoid-to-Host Ratio. In order to achieve maximum numbers of mummies per parasitoid in the rearing cages, an optimum host density per female parasitoid had to be ascertained. This was found to be 80 aphids of random ages with a mean number of 164 mummies (± 57) per female parasitoid. An analysis of variance treatment of the data, followed with a Student-Newman-Keuls test comparing the means at the different host densities, showed no significant difference in the means at the 10:1, 20:1 and 40:1 aphid-to-parasitoid ratios or at the 80:1 and 100:1 ratios. Eighty-to-one was chosen as the optimum host-to-parasitoid ratio to conserve aphids and harvest the largest percentage of mummies (Fig. 2). This density may have been lower if only the parasitoid-preferred instar of RWA had been used in the study. This was not done because it would not be feasible to separate aphid instars in a mass-rearing program.

At a host density of 10, one *A. asychis* female produced a mean number of 3 mummies (± 5). A mean number of 10 mummies (± 12) were produced at a density of 20 RWA. At a host density of 40 RWA, a mean number of 21 (± 17) were produced. At a host density of 60 RWA, a mean number of 51 mummies (± 23) were produced. And at a host density of 100, a mean number of 170 mummies (± 69) were produced.

In a population of aphids with random ages, aphid consumption by *A. asychis* should not overwhelm the reproductive capacity of the aphids. Some decline in the aphid population is acceptable as long as it does not fall below the numbers needed for optimum parasitoid ovipositional activity. In a randomly chosen host population, especially at lower densities, the number of nonreproductive nymphs compared to reproductive adults may vary

Fig. 2. Optimum host density per female parasitoid for mummy production. Graph represents the mean for 15 females.


250
200
150
100
50
0
No. Mummies
10
20
40
60
80
100
Host Density

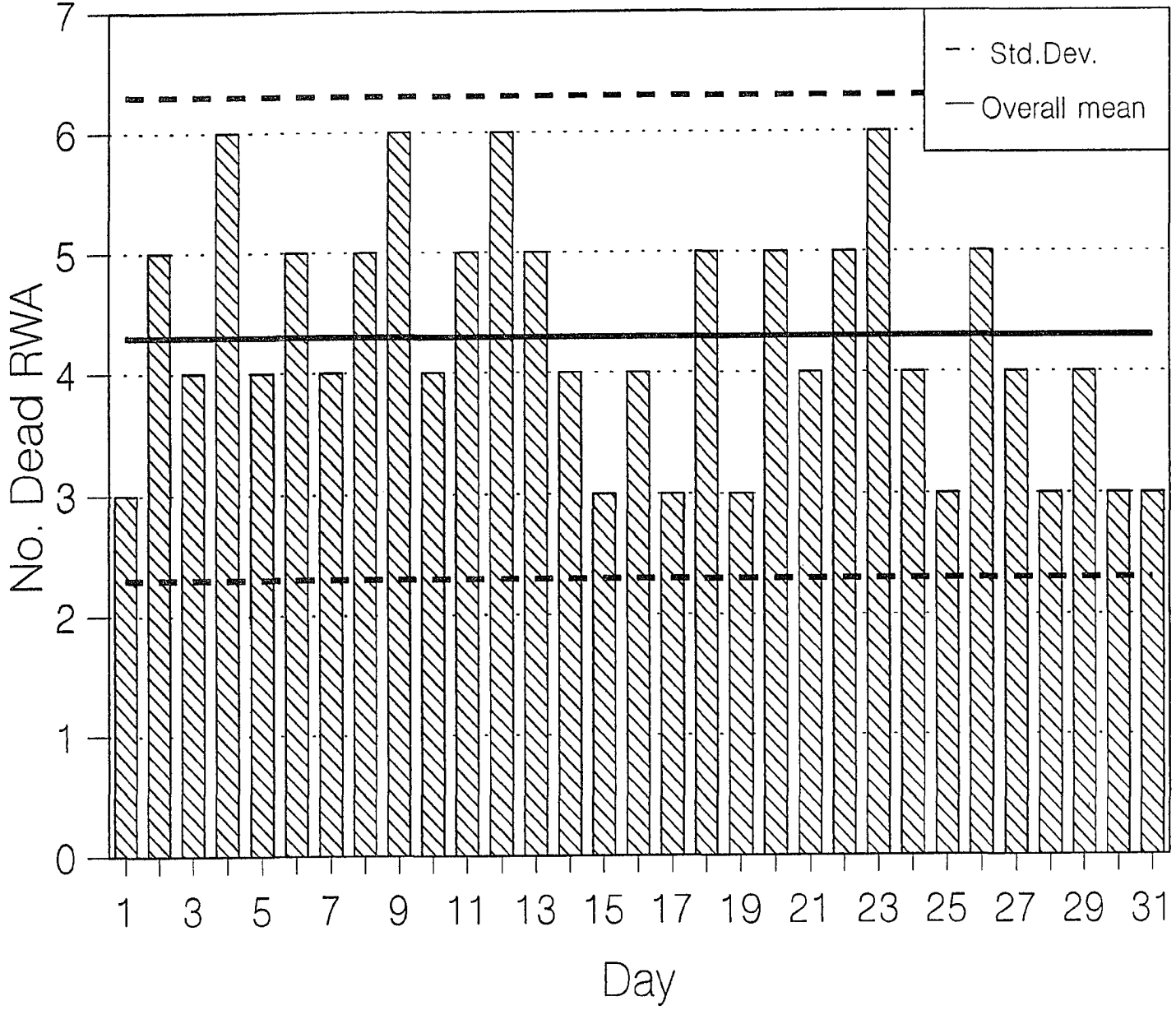
considerably. When host-feeding is density-independent, along with individual parasitoid variation and a possible aphid instar preference by the parasitoid, broad differences in mummy numbers per parasitoid at lower host densities may result. The ranges of mummies forming at the different host densities in this study help to illustrate this effect. Ranges were as follows: 0-18 (host density 10), 0-47 (host density 20), 3-61 (host density 40), 24-177 (host density 60), 78-271 (host density 80), and 86-305 (host density 100).

The parasitoid died before mummies developed (11 days) in all cases at the 10:1 aphid-to-parasitoid ratio. This indicates that once *A. asychis* has oviposited into an aphid, that aphid will no longer be used for host-feeding, even though death to the parasitoid results. Only one *A. asychis* lived until mummy formation at the 20:1 aphid-to-parasitoid ratio.

This behavior is important to a mass-rearing program. If *A. asychis* resorted to host-feeding on parasitized aphids, slight fluctuations in RWA stock colony age ratios (nonreproductive vs. reproductive) could result in drastic changes in expected mummy numbers.

Host-Feeding Behavior. Host-feeding behavior of *A. asychis* showed a remarkable lifetime stability (Fig. 3). A mean of 4.3 RWA (± 2.0) were killed each day by each female parasitoid's host-feeding activity, with a range of 1 to 10. Over an average lifespan of 31 days, *A. asychis* killed an average of 133 RWA with this behavior. Non-parasitoid aphid mortality in the control was only 6 aphids from 10 control dishes that were set up and run concurrently with the study. This was not considered significant enough to calculate into the

Fig. 3. Number of RWA killed daily by host-feeding activity of *A. asychis.* Graph represents daily mean of 15 females. *Aphelinus asychis* mean longevity of 31 days used as cut-off point.


Std.Dev.
Overall mean
No. Dead RWA
0
1
2
3
4
5
6
7
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
Day

host-feeding data. Conditions were exactly the same as the study, except no parasitoid was placed into the petri dish with the aphids.

Aphelinus asychis host-fed upon aphids every day until death. Host-feeding occurred even after oviposition ceased in most cases. Host-feeding may supply the parasitoid with necessary liquids and food for both maintenance and ovarian development. Wahab (1985) noted that females of *Aphelinus abdominalis*, which feed on aphid honeydew and water droplets, had a daily oviposition rate of only seven eggs, compared to approximately twenty eggs oviposited daily by *A. asychis* (Bai & MacKauer 1990).

Bai & MacKauer (1990) and Cate et al. (1977) also noted a density-independent steady rate of host-feeding activity. Pea aphids were the host in Bai and MacKauer's research and greenbugs were used as the host by Cate et al. (1977). When pea aphid was the host, one pea aphid per day was fed on, regardless of the host density. Only second instar pea aphids were used, as this instar was determined to be readily attacked by *A. asychis*. Pea aphids are relatively large aphids, and are much larger than RWA.

According to Cate et al. (1977), when greenbugs were used as host, an average of 1.5 aphids were fed on per day. This differs greatly with the mean number of 4.3 RWA fed on per day found in this study. Instar preference should not be a factor here because sufficient numbers of all instars were present at the host density (50) used in the study. Cate et al. (1977) determined a ratio of greenbugs killed by host-feeding compared to oviposition to be 1:7.66 for the first 15 days of the parasitoid's life (mean longevity 17.3 days at 26.7°C). When RWA is the host, this ratio becomes 1:1.23 for the lifetime of the parasitoid, since *A. asychis* kills 133 RWA over an average 31-day lifespan and parasitizes

164. This could make *A. asychis* an effective biological control agent in the field, especially if alternate hosts are available. However, it could deleteriously influence the attempted establishment of either introduced or native parasitoids and predators, a concern expressed about releases of host-feeding parasitoids by Kidd & Jervis (1989).

High levels of host-feeding are not uncommon among parasitoids which exhibit this behavior. *Tetrastichus gallerucae*, an eulophid egg parasitoid of the elm leaf beetle, destroyed 65% of its host's eggs by host-feeding behavior in laboratory studies (Hamerski & Hall 1988).

Host size does not seem to be a contributing factor to the difference in numbers of RWAs and greenbugs fed upon by *A. asychis*, since these aphids are very similar in size. Host preference may play a role here. Greenbug has been determined to be the preferred host by *A. asychis* in studies comparing greenbug, corn leaf aphid [*Rhopalosiphum maidis* (L.)] and yellow sugarcane aphid (*Sipha flava* Forbes) (Raney et al. 1971). Importations of *A. asychis* for biological control purposes in the early 1970s, targeted greenbug as the host (Jackson et al. 1971, Raney et al. 1971, Rogers et al. 1972, Esmaili & Wilde 1972). If RWA is not the preferred host, utilization of this aphid may not be as efficient as it is with greenbug, thereby "wasting" more of the host material with more unsuccessful attempts at host-feeding and ovipositing than occurs with greenbug. Before the parasitoid can host-feed, the aphid must be stung, i.e., the ovipositor is inserted to inflict a wound from which the parasitoid then feeds. Cate et al. (1977) showed that host-feeding kills the aphid, even if the aphid is not fed upon after it is stung. Aphids removed before *A. asychis* had a chance to feed never recovered and were dead in a few hours. No

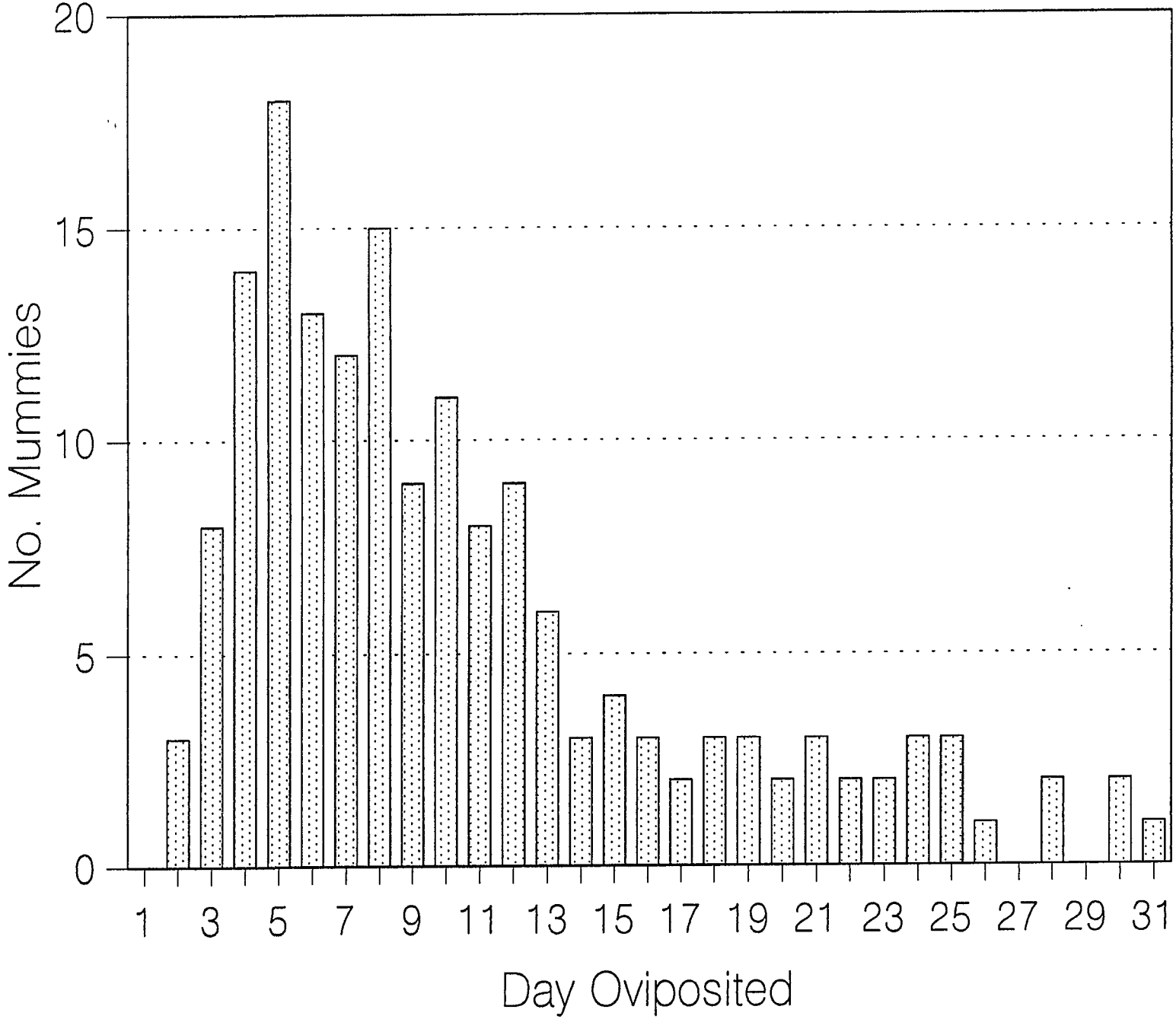
mummies developed from any of these aphids. Observational data from the MBCL indicates that this particular strain of *A. asychis,* reared from RWA, will switch to greenbug as a host with no apparent reduction in first generation numbers, while the reverse is not true. At least one generation was required to recover when *A. asychis* reared from greenbug was switched to RWA.

The *A. asychis* strain reared at the MBCL was originally collected from RWA, however, *A. asychis* utilizes several hosts, including pea aphid, greenbug, bird cherry-oat aphid and spotted alfalfa aphid (Finney et al. 1960, Rogers et al. 1972, Cate et al. 1977, Bai & MacKauer 1990).

Length of Ovipositional Period and Longevity. *Aphelinus asychis* females did not begin ovipositing until the second or third day after emergence. Of the nine females used in this study, seven began ovipositing on the second day, and the remaining two began ovipositing on the third day. Mean longevity for 15 female parasitoids was 31 days (± 14.2), with a range of 18 to 61 days. Bai (unpublished data) determined the mean longevity of *A. asychis* reared on pea aphid at 21°C to be 47 days. *Aphelinus asychis* continued ovipositional activity throughout most of its lifetime, but with relatively few eggs oviposited the last half of its life (Fig. 4A). Since *A. asychis* is synovigenic, this may be quite different, or even reversed, if host material is scarce (a condition which may often occur in the field).

Daily Ovipositional Activity. Daily ovipositional activity showed a cyclical nature, peaked at day 5 (18 mummies), gradually tapered off and increased again until another smaller peak (15 mummies) was reached at day 8 (Fig. 4A). Additional smaller peaks are also seen. These smaller peaks are followed by one or two days of less production.

Fig. 4A. Daily oviposition rate of *A. asychis* using mean for nine females. *Aphelinus asychis* mean longevity of 31 days is used as the cut-off point.


20
15
10
5
0
No. Mummies
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
Day Oviposited

Ninety-two percent of total mummy production occurred during the first 13 days of the parasitoid's life. This is significant because mummies form 11 days after oviposition and emerge 22 days after oviposition. No eggs are oviposited on day 1, and the aphids can survive on cut plants for one day before the plants wilt to the point that the aphids leave. Therefore, mummies developing from eggs oviposited between days 2 and 13 will be harvested. Day 24 is when first emergence should begin, so the mummies must be shipped or placed in cold storage at that time. There is a chance that some of these will emerge in storage, but if the mummies are harvested earlier, more are lost because of the early harvest date than are lost because of emergence in storage or in the rearing cage, i.e., more eggs are oviposited on day 13 than on day 2. Figure 4B shows the days that mummies formed, indicating the best harvest date to be day 24.

Effect of Parasitoid-to-Host Density in the Rearing Cage. The optimum number of *A. asychis* per rearing cage was 100, with 70% female and approximately 5,600 aphids added to the cage. This was achieved by using twelve pots of wheat holding approximately 450-500 aphids each. Plant biomass was kept to a minimum, and the plants were able to withstand the aphid load for the required period of time. Figure 5 illustrates the difference in mummy numbers harvested at these different parasitoid-to-host ratios. Host density, rather than parasitoid density was found to be the limiting factor in maximizing space in the rearing cages. A mean number of 11,097 mummies (± 1362) were produced.

Using a host-to-parasitoid ratio of 80:1, aphid numbers soon became too high for the plants to support. When 300 parasitoids and 16800 aphids were confined in the rearing cages, the plants died, and as a result, all the aphids died before mummification.

Fig. 4B. Days that mummification occurred. Emergence began to occur at day 25, so day 24 was designated for mummy harvest. Graph represents mean for nine females.

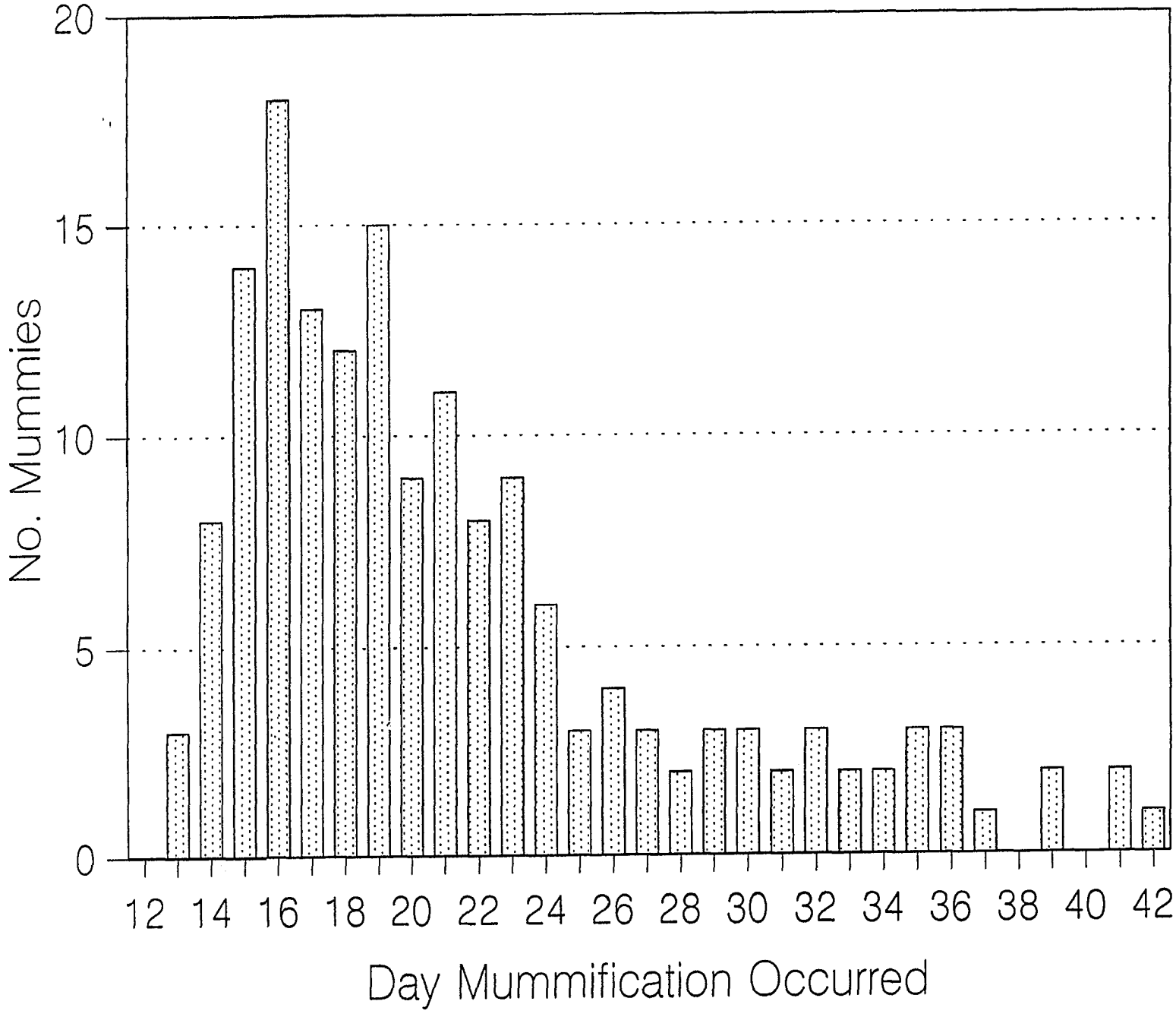
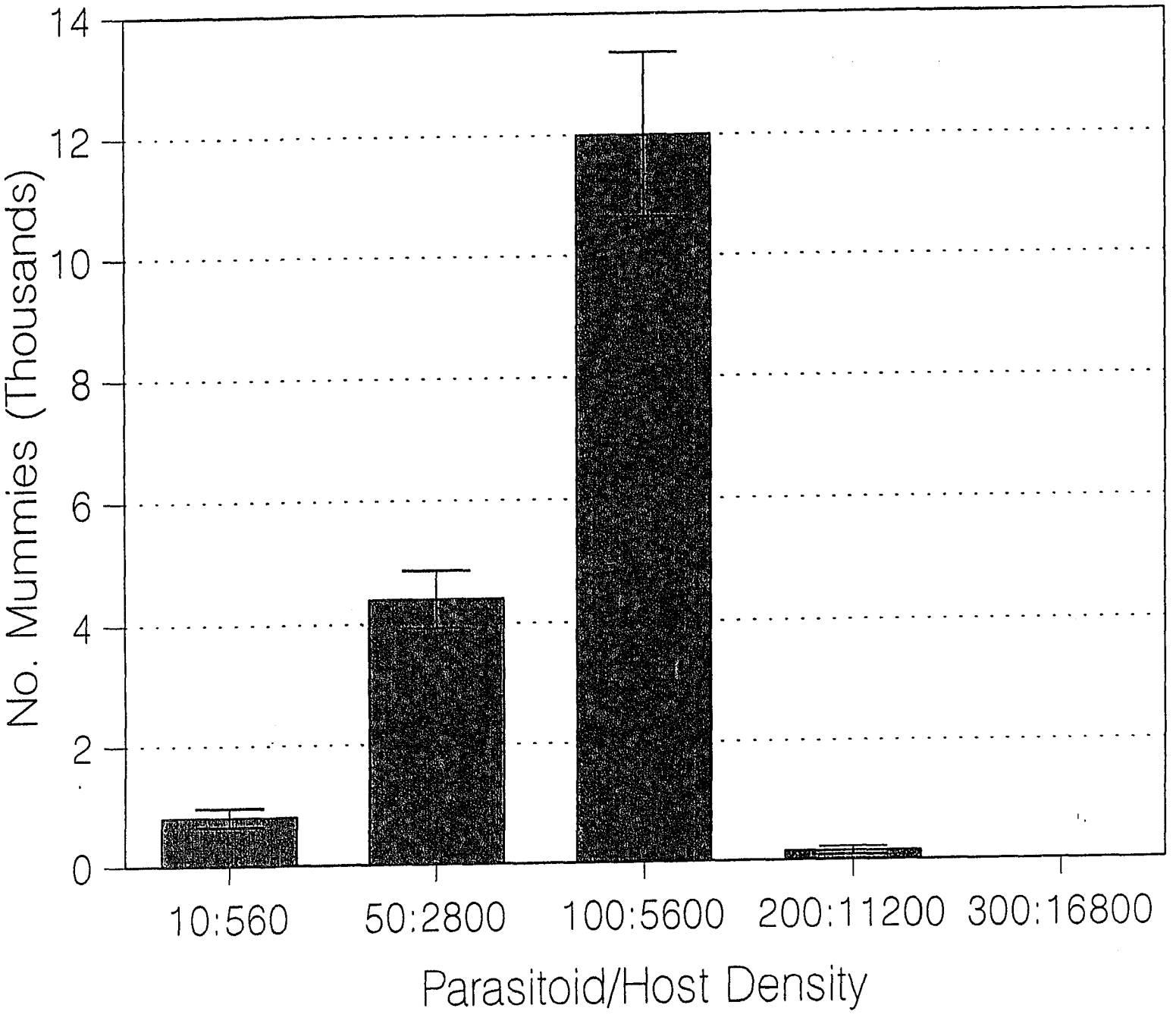

20
15
10
5
0
No. Mummies
12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42
Day Mummification Occurred

Fig. 5. Effect of different parasitoid/host densities in the rearing cages. A female parasitoid:host ratio of 1:80 was used.


14
12
10
8
6
4
2
0
No. Mummies (Thousands)
10:560
50:2800
100:5600
200:11200
300:16800
Parasitoid/Host Density

When 200 parasitoids and 11200 aphids were confined in the cages, severe plant damage resulted, and few mummies formed. A mean number of 148 mummies (± 67) were produced. Additional uninfested post of wheat were added to the cages to compensate for the heavy aphid load. This created other associated problems. Condensation from transpiration by the increased plant biomass caused high numbers of aphids and parasitoids to drown.

At a density of 10 parasitoids:560 RWA, a mean number of 797 mummies (± 159) were produced. At the 50 parasitoid:2800 RWA density, a mean number of 4400 mummies (± 467) were produced in the rearing cages.

Cold Storage Potential. Short-term cold storage of *A. asychis* mummies proved to be a feasible method for making mummies available for releases at a later date. This is sometimes necessary because of poor weather conditions at the release site, an excess of mummies from previous shipments, or an absence of the targeted host at the release site.

Esmaili & Wilde (1972) found adult *A. asychis* to be more cold tolerant than the mummies. Mummies stored at 5 °C for 15 days exhibited no emergence. Those stored for 3 days exhibited 70% emergence, and for 7 days, 10% emergence. Mummies and adult parasitoids were taken from stock colonies. The temperatures at which the stock colonies were reared may have had an effect on the cold tolerance of the mummies used in the research. This temperature was not specified. Mummies from stock colonies at MBCL were reared at temperatures fluctuating between 12 °C and 22 °C, with a mean temperature of 18 °C. This may have acclimatized the mummies used for this study, allowing a greater percentage to survive to emerge than that shown by Esmaili & Wilde (1972). A strain

difference may result in a greater percentage of emergence. The current strain was collected from an area (Chillan, Chile) where climatic conditions are temperate (Keith Pike, pers. com.). The strain studied by Esmaili & Wilde (1972) was only specified as being collected from the Mideast.

Long-term cold storage studies of the adult stage of *A. asychis* have been done by Archer & Eikenbary (1973). Best results were achieved when the parasitoids were taken out of cold storage periodically, allowed to move about and feed, and then placed back into cold storage. Only about 50% of the stored females reproduced, and those stored in this manner for 105 days reproduced at 66% of the rate for unstored females. Later studies by Archer et al. (1976) used acclimatizing and deacclimatizing procedures to make the adult parasitoids better fit for cold storage, and different feeding schedules were compared. The best survival and reproduction were by adults stored at 4.4°C.

Mummies of the aphidiid parasitoid, *Lysiphlebus testaceipes* (Cresson) were also studied for cold storage potential. Two mummy ages were used, 3 and 6 days old. They were stored for periods of 15, 30, 45, 60 and 90 days at varying temperatures. Three-day old mummies had the highest percent emergence, 65% when stored at 4.4°C for 15 days. Optimum storage temperatures were 4.4°C and 7.2°C. Fecundity between stored and unstored females was comparable. The parasitoid mummies did not store well for more than 30 days (Archer et al. 1973). For all of the above studies, the host for *A. asychis* was greenbug.

Results of this study also show that the younger the population of mummies when introduced into cold storage (4.4°C), the better percent emergence. Three-day old

mummies had the best percent emergence, 100% when stored for 3 days (Fig. 6A). Percent emergence declined as mummies, regardless of age, were kept in cold storage for increasingly longer lengths of time. The poorest percent emergence occurred for the oldest mummies (21 days) stored for the longest length of time (14 days) (Fig. 6B). Three emerged in storage. However, 21-day old mummies stored for 3, 5 and 7 days exhibited a higher percentage of emergence (92, 80 and 83%) than 7-day old mummies stored for the same length of time (83, 77 and 70%) (Fig. 6C). Emergence in the control population was 97%.

Curiously, in all cases, the percentage of males from the emergence total remained relatively stable, and the decline in percent emergence was due to female mortality.

Fig. 6A. Short-term cold storage effects on three-day old mummy population. Zero days is the control population.

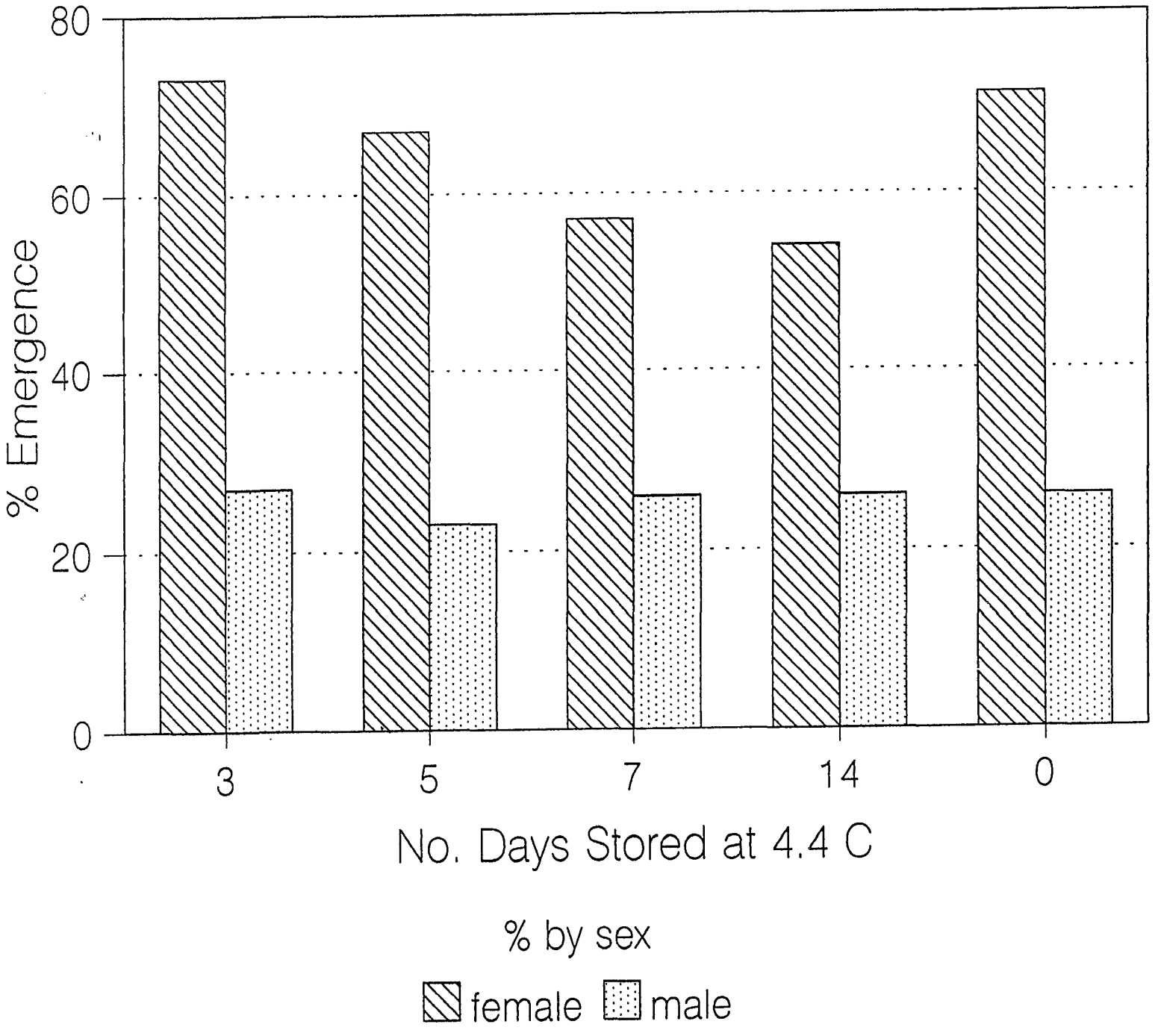

80
60
40
20
0
% Emergence
3
5
7
14
0
No. Days Stored at 4.4 C
% by sex
female
male

Fig. 6B. Short-term cold storage effect on 21-day old mummy population. Zero days is the control population.

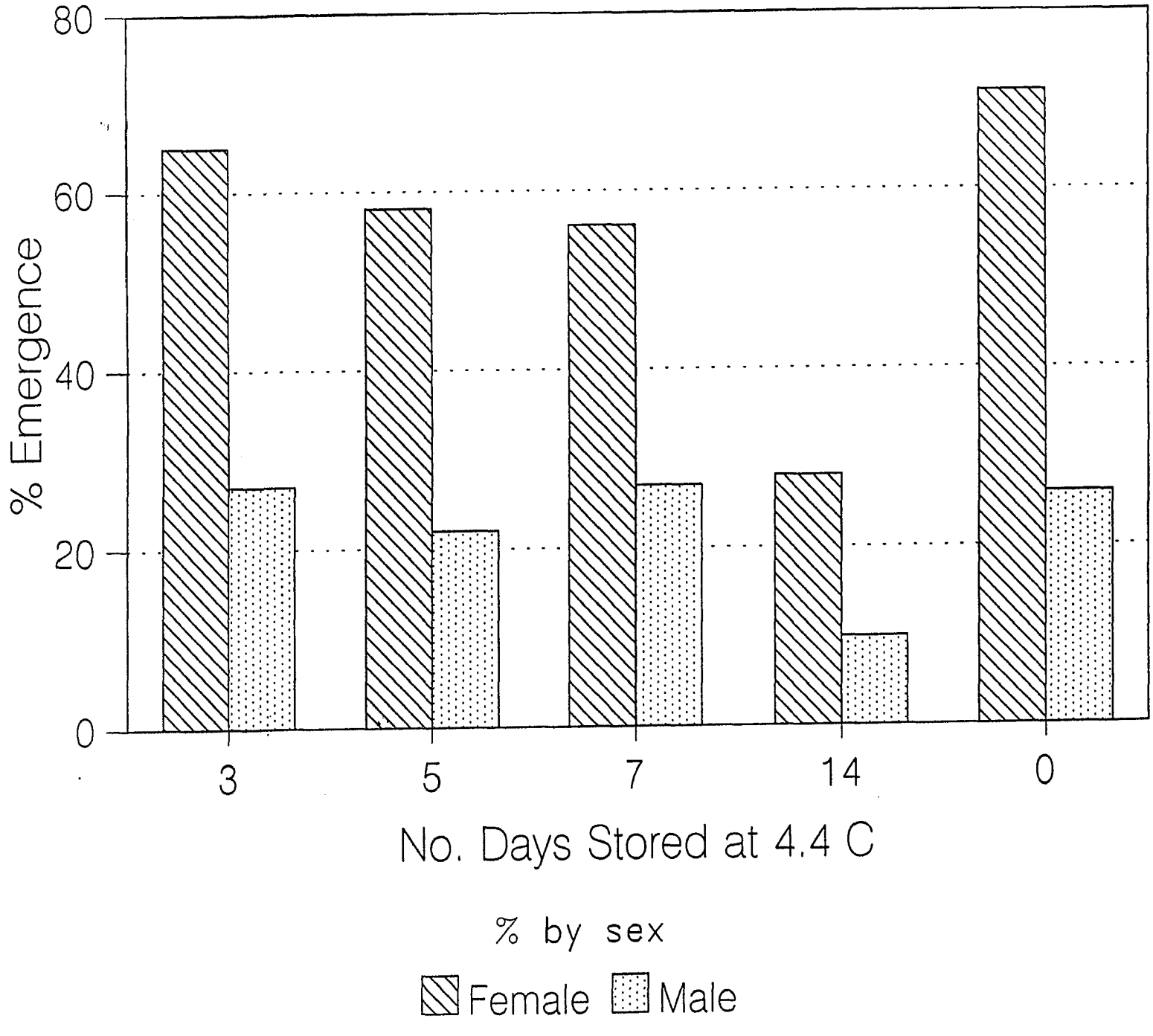
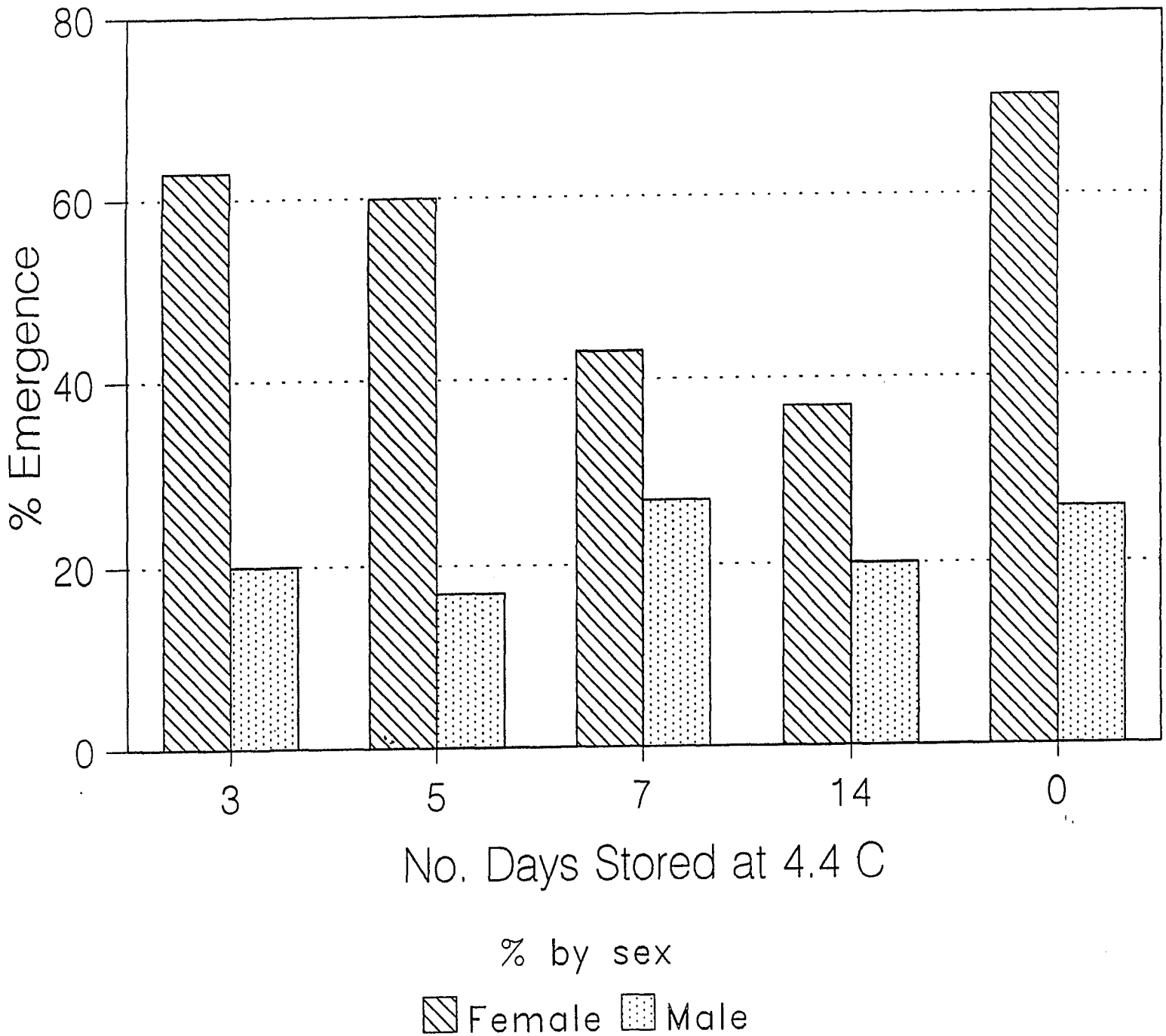

80
60
40
20
0
% Emergence
3
5
7
14
0
No. Days Stored at 4.4 C
% by sex
Female
Male

Fig. 6C. Short-term cold storage effects on seven-day old mummy population. Zero days is the control population.


80
60
40
20
0
% Emergence
3
5
7
14
0
No. Days Stored at 4.4 C
% by sex
Female
Male

CONCLUSIONS

In order to successfully mass-rear a biological control organism for field releases, many aspects of its biology must be carefully researched. Its life cycle can then be manipulated in an artificial setting so that its reproductive capacity can reach limits which make it feasible to mass-rear that particular insect. This must be done without sacrificing fitness factors genetically programmed into the insect. Size of the founder colonies, length of time these colonies are laboratory reared, and the environmental conditions at which these colonies are reared all need to be taken into account to ensure releases of an insect with a better chance of establishment in a given area.

Laboratories are generally small political arenas, with each different interest group vying for space and equipment. This makes it especially important for development of the most efficient mass-rearing protocols possible, using standard laboratory equipment and minimum manpower.

By developing a mass-rearing protocol for *A. asychis*, a general guideline for certain other members of the genus can be utilized for the mass-rearing of those organisms if the need arises. Several strains of *A. varipes* (Foerster) are currently being mass-reared at MBCL. The biology of these insects also make them good future prospects for biological control purposes.

There is a need for research on the effect of long-term cold storage on mummies. If the parasitoids could be induced to enter diapause, a hardier, overwintering mummy develops and could be stored for months. Parasitoids could be be stored during the

off-season for releases, and very large numbers would be available the following spring. This could make releases at multiple sites possible, enhancing chances of establishment.

The complete mass-rearing protocol developed for *A. asychis* reared on RWA at MBCL as a result of this research is as follows:

Environmental Conditions

Mass-rearing takes place in room-size, programmable environmental growth chambers. Lighting consists of a mixture of 60-watt incandescent and fluorescent bulbs. Fluorescent lighting consists of a 50/50 mixture of white light and standard grow-light bulbs. A bank of lights containing two of each type of fluorescent bulb is suspended 3" over the mass-rearing cages. Photoperiod is 16:8 (L:D), with incandescents lighting first in a ramping action beginning dim and increasing in intensity. Incandescents are located on front and back chamber walls. When full intensity is reached, one of each type of fluorescent bulbs in each bank turns on, 30 minutes later the second set turns on. At this time the incandescents turn off. This process takes one hour. At night the reverse occurs. This process simulates sunrise and sunset as best as possible in a laboratory.

Temperatures fluctuate in a diurnal cycle with a nighttime low of 12 and a daytime high of 22°C. This occurs in a ramping action so that the temperatures change gradually. Relative humidity is controlled in a fluctuating, diurnal cycle with a daytime low of 55%, and a nighttime high of 65%. Table 1 shows the entire 24-hour program for the environmental growth chambers, including temperatures, relative humidity and lighting.

Table 1. Actual program showing fluctuating abiotic conditions in environmental growth chambers for mass-production of RWA and its parasitoids at the MBCL.

Step	Time		Temp C	% RH	Lights
	Clock	Interval			
0	6:30	0:30	16	62	3-0
1	7:00	0:30	18	61	3-1
2	7:30	1:00	20	60	1-3
3	8:30	1:00	22	58	1-3
4	9:30	1:00	22	57	1-3
5	10:30	7:00	22	55	1-3
6	17:30	1:00	22	55	1-3
7	18:30	1:00	20	57	1-3
8	19:30	1:00	19	59	1-3
9	20:30	1:30	18	61	1-3
10	22:00	0:30	16	61	2-3
11	22:30	0:30	16	63	2-1
12	23:00	0:30	15	64	2-0
13	23.30	0:30	15	65	0
14	24:00	0:30	14	65	0
15	0:30	5:00	12	65	0
16	5:30	1:00	14	65	0

Lights: 3-0 = Incandescents Only
2-0

1-3 = Two Sets Fluorescents

0 = Total Darkness

2-3 = Incandescents Plus
2-1 One Set Fluorescents
3-1

Equipment

Sealed plexiglass cages (41 x 41 x 51 cm) are used for mass production of all RWA parasitoids at MBCL. Three sides have large ventilation openings which are covered with a very fine organza mesh or broadcloth material. Individual *A. asychis* may be very small in size, and may escape through the material, especially if it has been stretched in areas. An oblong opening in the front of the cage is covered with a sleeve made of the same material (Fig. 1).

Water is supplied to the parasitoids in 30-ml plastic condiment cups with lids. A hole is punched through the lid and a 3.5 cm-length of dental wick is placed through the hole and into the water.

A portable water wagon is used to water the host plants in the rearing chambers. A dilute water soluble fertilizer should be added to the wagon each time it is filled. The wagon should be a wand-type so that the plants can be watered in the cages with as little disturbance to the parasitoids as possible.

Release containers consist of approximately one-half liter paper containers that have modified ends. The ends are replaced with a mesh material designed to let the emerging parasitoids escape, but keep predators out. Plastic petri dish lids secured with a rubber band are placed over the ends. These are removed in the field.

Cage Preparation

Vacuum out all debris if necessary. Completely sanitize the cage by wiping entire area, including the ventilation material, with 10% Lundmark's solution or a comparable

sanitizer. Line the cage bottom with white, moisture absorbent paper, such as a commercial bench liner. Place the water source and honey into the cage just prior to cage set-up. The honey can be streaked or dotted directly on the ventilation material or on a piece of paper taped to the cage wall.

Cage Set-Up

Place approximately 100 *A. asychis* mummies (on wheat leaves) onto a white paper toweling and place them into the cage. This will ensure that at least 70 females will be available for oviposition. Gently spread out the plant material to which the mummies are attached on the toweling so that the wheat dries completely. One day after emergence of the parasitoids has begun, add the host material to the cage.

Addition of Host Material

Place twelve pots of wheat from the host RWA colony into the cage. Each pot of wheat should hold 450-500 RWA of random ages. Each person in the RWA biological control program should gain experience in estimating numbers of RWA per pot. Absolute counts should be done until estimated counts can be made with confidence. Tape the date that the host material was added on the corner of the cage. Water the plants just prior to placing them into the cage, and allow any excess water to drain. Arrange the pots so that watering can be done with ease, with the least amount of disturbance to the parasitoids as possible.

Daily Cage Maintenance and Quality Control

Each mass-rearing cage should be inspected daily. Conditions to monitor include:

1. Moisture content of the soil - Plants are under stress from high levels of aphid infestation. Water and nutrients are being removed from the plants, and drought stress will quickly kill the plant. MIT wheat, which is recommended for mass-rearing the RWA, is drought resistant, but if allowed to become too stressed, will wilt and will not recover. If the soil is dry on top, water the plants with a wand-type watering device so that pots need not be removed from the cage. When mummies begin to form, water the plants and then discontinue all subsequent watering.
2. Water condensation on cage walls - This problem can be prevented by not overwatering the plants during daily cage maintenance. If condensation does occur, wipe the cage walls with toweling, taking care not to kill parasitoids that may be on the cage walls.
3. Plant diseases - Check the plants for any disease symptoms, including fungal growth or wilt rot caused from drought stress. Roots can be attacked by the larvae of insects which can enter the greenhouse as contaminants. If these are seen in the mass-rearing cages, preventative measures can be taken. Any fungal growth on living plants is indicative of a parasitic fungal disease and should be reported immediately. Saprophytic fungi can sometimes be seen on the dead plant material or on the soil surface and may be indicative of overwatering.

4. Insect disease - Unexplained mortality or low mummy production from a cage could signal a disease problem and should be reported immediately.
5. Insect contaminants - Plants grown in a greenhouse may become contaminated with native insects prior to being taken into the insect rearing facility. Observe cage populations closely, and if an insect that does not belong there is present, carefully remove it with an aspirator for closer inspection. Identification of the insect may be very important, especially if a hyperparasitoid is suspected. Insects to look for include:

   A. Native aphids - Greenbugs (GB) and birdcherry-oat aphids (*Rhopalosiphum padi* Fitch) (BCOA) are two common contaminants of cereal grain plants. Birdcherry-oat aphids are small, round, dark colored aphids, with a red to orange posterior. Greenbugs are similar to RWA, but have a dark green stripe running down the back, and long cornicles and antennae that can be seen without magnification. In some areas, western wheat aphid [*Diuraphis tritici* (Gillete)], which is in the same genus as RWA, may be a concern. The main morphological difference is the presence of a supracaudal process (double tail) in RWA, which can be seen under magnification. Keys are available to distinguish all these aphids. If any of these aphids are found, manually kill as many of them as possible. The plants in the greenhouse should be sprayed with an insecticidal soap or short-residual insecticide until no more contamination is found. Some of these aphid species, especially BCOA, can reproduce at alarming rates since

they are not the preferred host species for *A. asychis*, and will displace RWA rather quickly.

B. Native parasitoids and hyperparasitoids - Whenever a native aphid becomes a contaminant, there exists the possibility of bringing in their own parasitoids as an additional contaminant. This can then bring about the possibility of introducing hyperparasitoids into the mass-reared colonies. These "hypers" usually look quite different from the primary parasitoids, although they are also wasps. They may have a metallic, or hump-backed appearance. Monthly quality control checks can help detect these kinds of problems.

C. Plant-attacking insects - This includes small beetles and plant sucking insects such as plant hoppers and thrips. Remove these with an aspirator and discard after they have been identified as such. Thrips can become a major problem and preventative measures may need to be taken.

D. Lady beetles and lacewings - The larvae are voracious aphid predators. Remove and destroy any stage of these insects found.

E. Cross-contamination - When many different species of parasitoids are reared in close proximity, the possibility of cross-contamination exists. Although it is impossible to separate geographical strains of the same species morphologicaly, different species can be differentiated with the use of color differences and keys. *Aphelinus asychis* has an orange abdomen which differentiates it from *A. varipes*, which has a black abdomen. Monthly checks of each cage for cross contamination are a part of quality control.

Processing of Mummies

Twenty-four days after introduction of the host material, using scissors, clip the wheat from each pot at soil level. After removing all the clipped pots, spread this plant material on the cage bottom to dry. The next day, the mummies can then be packaged for shipment or placed into cold storage for later shipments. One hundred of these mummies are to be placed into a clean mass-rearing cage that has been prepared for set-up. These 100 mummies should be divided and mixed with mummies from other cage populations of the same age to keep as much genetic diversity as possible in the cage populations. Always handle the mummies very gently so that they are not dislodged from the plants. Absolute counts of mummies should be done by personnel until estimated mummy counts can be made with confidence.

Record Keeping

Be sure to follow quarantine or semi-quarantine procedures for record keeping if they apply. A separate record book chronicling cage conditions for each generation should be maintained. This way a problem may be backtracked to its source. Include in the record book:

1. Cage I.D. number
2. Date host was added
3. Any problems encountered with the daily cage maintenance, which are outlined above
4. Non-emergence of mummies

5. Anything you consider notable may be useful in the future, e.g., host plant condition when introduced into cage

Shipment

Still attached to the wheat clippings, 250-500 mummies are carefully placed in release containers. Layer the containers in the shipping box and place a mat-type coolant between the layers of shipping containers. This ensures even cooling of the release containers. Place any required paperwork for the receiver in a plastic bag, and place on top of the contents in the shipping box.

Cold Storage

If it is necessary to delay shipment of mummies which are nearing the emergence date (24 days), package them as if for shipment in release containers and place them in a 4.4°C storage area for up to 7 days. Storing in the release containers minimizes handling of the mummies.

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