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REARING, STORING, AND EFFICACY STUDIES ON *ARZAMA DENSA* FOR RELEASE PROGRAMS AGAINST WATERHYACINTH

by

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This research was conducted to develop mass rearing and dispersal techniques for large-scale releases of *Arzama densa* as a biological agent for the management of waterhyacinth.

Life history information on *A. densa* was obtained from field observations of natural populations and from laboratory studies. Various artificial diets and insect storage techniques were tested for rearing and synchronizing life

(Continued)

20. ABSTRACT (Continued).

stages of *A. densa* in release programs. Methods were tested that might be used to release *A. densa* on an operational scale. Various densities of *A. densa* larvae were evaluated for efficacy against waterhyacinth in outdoor pools.

The life history studies showed that natural enemies greatly reduce field populations of *A. densa*. Augmentation would likely be more effective in July/August or spring when parasite/predator populations are low. Nine consecutive laboratory generations of *A. densa* were successfully reared on an artificial diet that included a water extract of waterhyacinth. Storage of larvae at 8° C permitted synchronization of releases with little mortality. It was found that larvae can be successfully dispersed in segments of waterhyacinth petioles, but this method is labor-intensive. *Arzama densa* at a rate of one third instar larva/two plants successfully controlled waterhyacinth in artificial pools.

PREFACE

The study reported herein was performed under Interagency Agreement WESFR-79-83 with the U. S. Department of Agriculture (USDA), Science and Education Administration (SEA), Southern Weed Science Laboratory, Stoneville, Miss. Funds for this work were provided by the U. S. Army Engineer District, New Orleans, through the Aquatic Plant Control Research Program (APCRP), assigned to the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The study was conducted and the report was prepared by Drs. R. G. Baer and P. C. Quimby, Jr., of the Southern Weed Science Laboratory.

The appointment of Dr. Baer as a research associate was facilitated by a cooperative agreement with the Entomology Department, Mississippi State University, headed by Dr. T. J. Helms.

The authors wish to thank Dr. C. G. McWhorter, Laboratory Chief, Southern Weed Science Laboratory, for his support throughout the study. The authors also thank Mr. J. M. McWilliams of the Southern Field Crop Insect Management Laboratory, Stoneville, for building and lending them oviposition cages and for providing an environmental chamber with humidity control.

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The authors express their gratitude to the following scientists for their identifications of natural enemies: Drs. C. W. Sabrosky (Tachinidae), R. W. Carlson (Ichneumonidae), P. J. Spangler (Hydrophilidae), and O. S. Flint (Corydalidae), Beltsville Agricultural Research Center, USDA-SEA, Beltsville, Md.; Dr. N. I. Platnick (Erigonidae), The American Museum of Natural History, New York, N. Y.; and Dr. P. P. Sikorowski (Virus), Department of Entomology, Mississippi State University, Mississippi State, Miss.

Specimens acquired during this study were deposited in the Mississippi Entomological Museum, Mississippi State, Miss.

The research was monitored at WES by Dr. D. R. Sanders, Sr., Dr. A. F. Cofrancesco, Mr. E. A. Theriot, and Mr. R. F. Theriot of the Wetland and Terrestrial Habitat Group (WTHG). The study was conducted under the general supervision of Dr. John Harrison, Chief, Environmental Laboratory; Dr. C. J. Kirby, Jr., Chief, Environmental Resources Division; and the direct

supervision of Dr. H. K. Smith, Acting Group Chief, WTHG. Mr. J. L. Decell was Manager of the APCRP at WES.

Directors of WES during the study and preparation of this report were COL J. L. Cannon, CE; COL N. P. Conover, CE; and COL T. C. Creel, CE. Technical Director was Mr. F. R. Brown.

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PART I: INTRODUCTION

Background

1. Waterhyacinth, *Eichhornia crassipes* (Mart.) Solms, is a perennial, herbaceous, floating, freshwater weed. It presently infests about 400,000 ha of water bodies which include rivers, canals, streams, reservoirs, and coastline areas in the southeastern United States and along the coast of central California (Figure 1). Waterhyacinth has been ranked eighth among the world's worst weeds and, of the eight, is the most important aquatic weed problem (Holm 1969; Holm et al. 1977).

2. Waterhyacinth mainly reproduces asexually by stolons (Batanouny and

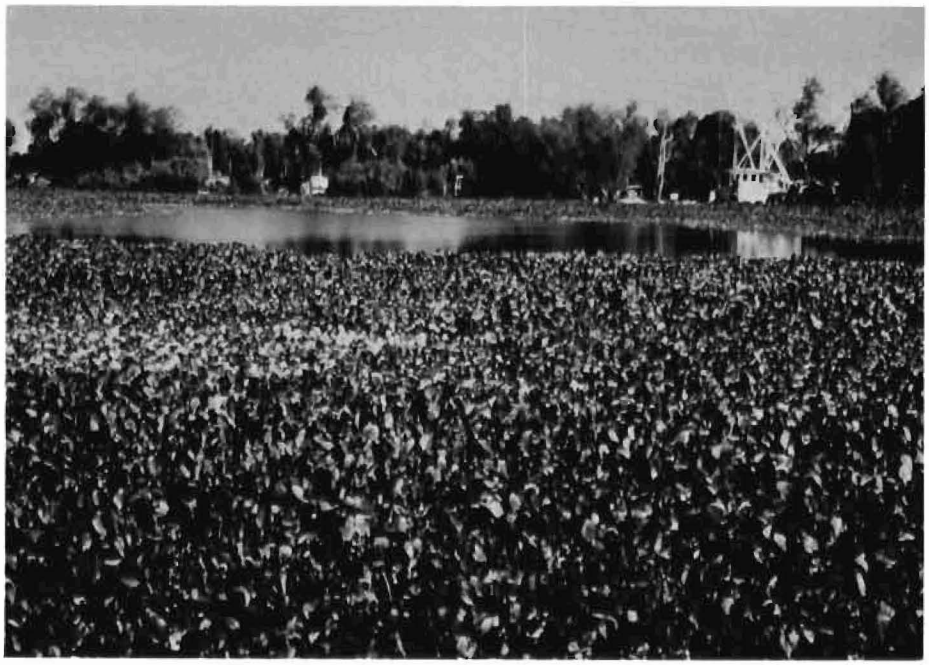

Figure 1. Waterhyacinth infestation at Venice, La.

* The common name for ***Arzama densa*** Walker (Lepidoptera: Noctuidae) approved by the Entomological Society of America is pickerelweed borer.

El-Fiky 1975). As these "runners" grow, new plants form at their tips. Penfound and Earle (1948) reported that waterhyacinth plants could double in number in 11 to 15 days, depending upon weather conditions. Such growth potential allows waterhyacinth to invade areas very quickly and form dense, floating mats of plants. The mats break apart and move to other locations. Major agricultural, navigational, and health related problems arise from these floating rafts (Zeiger 1962; Bock 1966; Sculthorpe 1967). Waterhyacinth reduces the oxygen concentration of the water, impedes water flow, restricts commercial and recreational water traffic, and serves as refuges for insect vectors of human and animal diseases.

3. Although it is difficult to arrive at a sound monetary estimate of damage caused by waterhyacinth, studies indicate figures in the millions of dollars (Spencer 1973, 1974). Waterhyacinth is the most troublesome and costly aquatic weed problem in Louisiana.

4. Many methods of control--chemical, biological, ecological, mechanical--have been attempted in an effort to reduce the spread of waterhyacinth (Sculthorpe 1967; Robson 1974). Cost, feasibility, and environmental effects have played important roles in the success and/or failure of these control methods.

Purpose and Objectives

5. The purpose of this 3-year study was to contribute to the biological control of waterhyacinth by developing mass rearing and dispersal techniques for large-scale releases of the native moth *Arzama densa* Walker (1864) (Lepidoptera: Noctuidae) (Figure 2). Although the native host plant is pickerelweed, *Pontederia cordata* L., previous studies on the life history of *A. densa* indicate that the larvae severely damage waterhyacinth (Vogel and Oliver 1969a, b; Center 1975, 1976) (Figures 3 and 4). These authors state that if satisfactory methods of rearing were developed, field populations could be supplemented with laboratory-reared larvae.

6. The main objectives of this study were:

   a. Provide biological information from field observations of natural populations and from laboratory investigations of *A. densa* collected in the field.

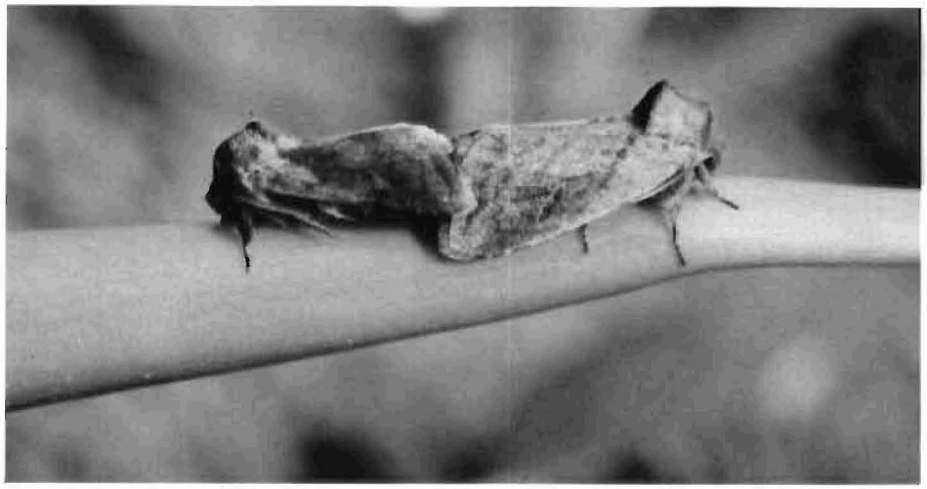

Figure 2. A mating pair of *Arzama densa* Walker (♂ on left)

Figure 3. Seventh instar *Arzama densa* larva feeding on meristematic tissue of waterhyacinth above the water surface

Figure 4. Seventh instar *Arzama densa* larva feeding in crown region of waterhyacinth below the water surface

b. Develop techniques of rearing *A. densa* for release programs and concomitantly provide biological data on at least nine consecutive laboratory-reared generations.

c. Develop techniques for storing various life stages of *A. densa* so that releases might be synchronized on an operational scale.

d. Develop methods of release on an operational scale.

e. Test the efficacy of *A. densa* as a biological control agent against waterhyacinth in outdoor pools.

PART II: MATERIALS AND METHODS

Collecting Trips

7. Seven trips were taken at different times of the year to the same locality in Venice, La., to collect *A. densa* and waterhyacinth needed for the establishment of a laboratory colony (Figure 1). At the collecting sites plants were thoroughly searched for *A. densa* and associated organisms. *Arzama densa* larvae were transferred to an artificial diet in petri dishes for transport to the laboratory. Egg masses and pupae were placed directly on the prepared diet which provided food for the newly hatched larvae and moisture for the pupae. Larvae infested with parasites, predators, and pathogens of *A. densa* were kept in separate petri dishes. Petri dishes were placed in a cooler containing a small amount of ice for the journey back to the laboratory.

8. In the laboratory, the field-collected larvae, pupae, and eggs were placed in an incubator under a 24-hr-dark period at 26° to 30° C and 70 percent relative humidity (RH). Larvae were supplied daily with freshly cut plant material or with a fresh diet every week or as needed until pupation or death. Egg masses were also placed directly on the diet. Individual pupae were weighed and placed in 30-ml clear plastic cups. Each cup was wrapped with wet Kimwipes® which were moistened daily with distilled water. A plastic petri dish top served as the cover of the container. Records were kept on pupation and emergence of *A. densa*.

9. Three methods were used on the sixth trip in an attempt to collect adult moths in the field. A crude pheromone extract was prepared from 1- to 2-day-old virgin female moths. Pressure was applied to the abdomen of the moth with blunt forceps, thus extending the last two abdominal segments. The segments were clipped with scissors and placed in a solution of n-hexane:anhydrous ether (1:1 v/v). Traps (Ganyard and Brady 1971) were baited with this extract and placed among the waterhyacinths at leaf height. Another collecting method employed a black light hung against a white sheet adjacent to the waterhyacinth stand. The black light and the pheromone studies were conducted between 9-11 p.m. CST. Also, waterhyacinth leaves were cut in half with scissors to release plant volatiles (kairomones), perhaps needed as a stimulant for oviposition. The plants were examined the next day for egg masses.

Preparation of Artificial Diet

10. Waterhyacinths were collected in the field and were reared in the greenhouse at the Delta States Research Center at Stoneville, Miss. Plants were grown in pools of water containing a 10 percent modified Hoagland's solution. The crown, leaves, and peduncles were rinsed in distilled water and freeze dried in a Virtis® freeze dryer. Excess plant material was stored at -14° ± 2° C. The freeze-dried plant material was ground in a ball mill into a fine powder. This waterhyacinth powder was substituted for the alfalfa powder used in a modified cabbage looper diet (Henneberry and Kishaba 1966; Ignoffo 1963) which was made with commercially available Vanderzant-Adkisson diet mix. Other changes in the looper diet included the addition of more water and the Vanderzant vitamin mix/modified (Table 1).

11. If freeze-dried waterhyacinth was not available, an extract of waterhyacinth was prepared by blending 50 g of fresh leaf and stem tissues in 500 ml distilled water for 2 min. The extract was filtered through organdy cloth and was added during the blending of the diet. In addition to this extract, 55 g of cellulose was added during diet preparation.

12. The agar was dissolved in 4000 ml boiling hot water in a blender for 30 sec. The waterhyacinth powder and the Vanderzant-Adkisson commercial diet mix were added. This solution was blended for 1 min. The sorbic acid, choline chloride, formaldehyde, methylparaben, and potassium hydroxide solutions (Table 1) were added and blended for 1 min. The 300-ml suspension (ascorbic acid, aureomycin, vitamin A, and the commercial Vanderzant vitamin mix--Table 1) was added and blended for 2 min to obtain a homogeneous mixture. About 20 ml of the diet was then poured into 30-ml clear plastic cups. Alternatively, diet was poured into stainless steel trays, cooled, and shredded in a meat shredder (Figure 5). All diets were refrigerated in plastic bags at 3° ± 2° C and warmed to room temperature as needed for developing larvae.

Rearing Procedures

13. Larvae and pupae, collected from the trips to Venice, La., developed to adult moths which provided the initial stock colony. Oviposition cages measured 45 cm long by 55 cm high by 11 cm wide. The cages, equipped with removable aluminum-framed plastic window screens, were placed in an

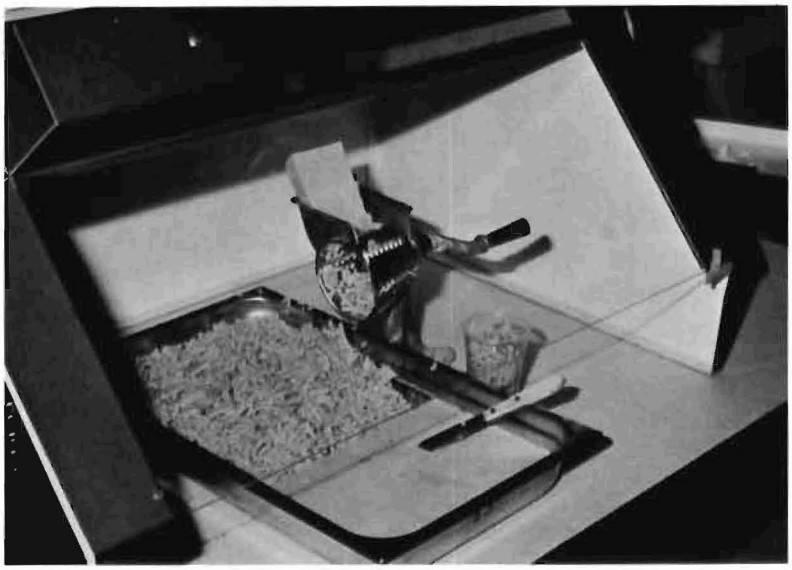

Figure 5. Cooled diet being shredded and placed in plastic cups with the developing larvae

environmentally controlled room (27°/21° C day/night, 12 hr light:12 hr dark (12:12 L:D) cycle, 80 to 90 percent RH) (Figure 6). A constant circulation of air around the cages was supplied by vents in the room. Because adults have vestigial mouthparts, they were not supplied with a food source.

14. A mating ratio of 1:1 (male: female) was used for each of nine consecutive generations. Ratios of 2:1 and 3:1 (male:female) were tried to check for an increase in oviposition and eclosion rates.

15. Moths laid their eggs singly or in masses on the removable plastic window screens. Eggs were removed either by slight pressure exerted from the blunt end of a small brush or by gently dislodging them between the thumb and forefinger. Eggs were sieved, weighed (Figure 7), and then sterilized in a 20 percent formaldehyde solution

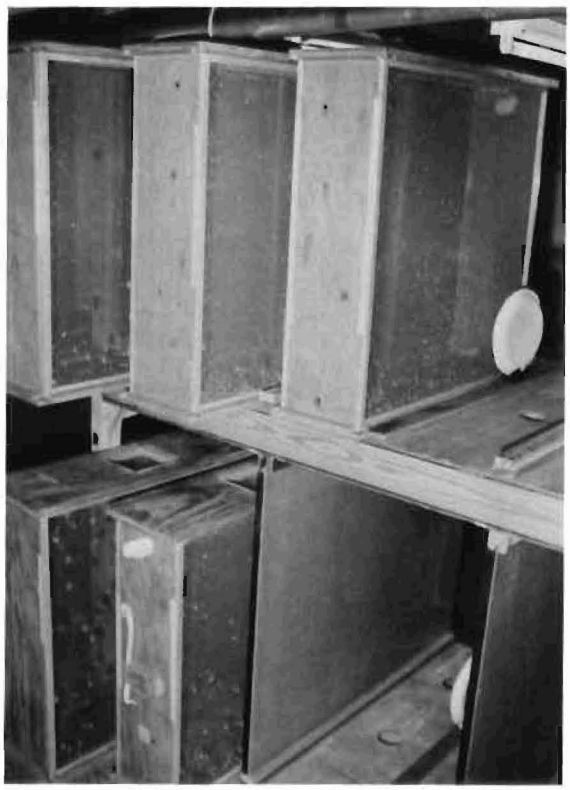

Figure 6. Oviposition cages in environmentally controlled room

Figure 7. After eggs were removed from screens, they were sieved and weighed (2 g = 10,000 eggs)

for 1 min and rinsed in distilled water for 5 min. Fifty eggs were transferred directly to each diet cup under a microbiological hood and capped with a wax-coated paper lid. The lids prevented the escape of larvae that hatched in 5 to 6 days. After the larvae developed to the third instar, the entire contents of the small cups were transferred to 296-ml clear plastic cups. Shredded diet was added until the cups were filled. An inverted petri dish bottom was provided as a top for the container. Laboratory generations one through five completed their development on solid poured diet while generations six through nine were reared on shredded diet.

16. After the larvae molted to the seventh instar, the cups were emptied onto 1-m^2 wood frame screens (Figure 8). The screens were placed on top of empty square plastic pools in the greenhouse (27°/21° C day/night, natural L:D cycle, 30 percent RH). The larvae pupated and were then picked out of the drying diet (Figure 9).

Pupal Weight

17. The weighing of pupae represented a method to determine if diet-fed individuals differed from field-collected individuals. Thirty pupae, fifteen of each sex, collected from the field were compared with thirty individuals from each of the nine laboratory-reared generations. They were placed in an

Figure 8. Seventh instar *Arzama densa* larvae in drying diet

Figure 9. *Arzama densa* pupae collected from drying diet (N = 5000)

incubator for 24 hr at 25° ± 1° C and 70 percent RH and then weighed. Duncan's multiple range test was used to compare mean weight differences.

Storage Capabilities

Eggs

18. Eggs were held at several different temperatures to determine whether eclosion could be delayed. Groups of 20 1-day-old *A. densa* eggs were placed on moist paper toweling, lowered into 30-ml plastic cups, and covered with a cardboard lid. One hundred cups containing 20 eggs each were transferred to an incubator 16:8 L:D for each of four different temperatures (8°, 14°, 20°, 25° C). Ten cups were removed daily from each temperature regime for 10 days and allowed to remain at room temperature (21° C). Newly eclosed first instar larvae were counted daily.

Larval growth rate

19. Larvae were maintained on diet at different temperatures (8°, 14°, 20° C) to determine if their growth rate could be retarded. Twenty 296-ml cups each containing ten third instar stage larvae were used at each temperature. Cups were held at each temperature regime for 6 weeks after which the number of live larvae and their stage of development were recorded.

Pupae

20. To determine the effects of temperature on eclosion, groups of four petri dishes containing ten pupae each were lined with moist filter paper and held at temperatures of 8°, 14°, or 20° C. One petri dish was removed weekly from each temperature regime over a period of 4 weeks and placed in a cage at room temperature (21° C) for emergence. The number of adults emerging from each group was recorded.

Applying Eggs and Larvae to Waterhyacinth

Larvae

21. In the laboratory, 50,000 diet-reared third instar larvae were distributed among cut leaf petioles. These petioles measured 5 to 7 cm in length. This procedure was accomplished 1 day before field application to allow the larvae to burrow into the plant tissue.

Eggs

22. The application of *A. densa* eggs to plants required the modification of a 3-ℓ stainless steel sprayer. The spray nozzle and hose were removed

and replaced with a kitchen sink sprayer hose and nozzle. The nozzle was modified with only three 1.0-mm-diam holes. These holes allowed for *A. densa* eggs to pass through freely because the eggs measured 0.8 mm in diameter. The sprayer also was fitted with a pressure gauge and the eggs were sprayed out at 15 psi (103 kPa). A xanthane gum:water solution (5 g/ℓ) was prepared as a modification of the method described by McWilliams (1979). This solution permitted the eggs to become suspended and sticky enough to adhere to the leaves. The material also dried and eclosing larvae were not "glued-in" the egg.

Outdoor Efficacy Tests

23. At Stoneville, eight pools (2.5 × 1.5 m) were set up in an outdoor location. Each pool contained a 10 percent modified Hoagland's solution and 400 rosette waterhyacinths (Figure 10). Plants were allowed 1 week to adjust to this new environment. Two pools were controls (Figures 11 and 12) and six pools were treated (three different treatments) on 14 May 1981 with 50, 100, or 200 *A. densa* third instar larvae (Figure 13). Larvae were released from a petri dish in the center of the pool onto the vegetation. Numbers of live plants (daughter plants and lateral shoots) and total dry weight of ten randomly selected live plants (leaves, stems, roots) per pool were recorded at the end of each week for four consecutive weeks.

Figure 10. Outdoor pools at Stoneville, Miss.

Figure 11. Control pool at
start of experiment

Figure 12. Control pool after 4 weeks

Figure 13. Treated pool (400 plants initially) with 200 *Arzama densa* larvae after 4 weeks

PART III: RESULTS

Field Data

24. The numbers of *A. densa* and other associated organisms collected on seven trips are presented in Table 2. *Arzama densa* populations were highest 1 to 2 m from the shoreline, especially near overhanging vegetation. These data represent the results of parasites and diseases recovered from individuals collected in the field.

25. The number of *A. densa* larvae found on the field trips ranged from 2 to 177 (Table 2). Ratios of various instars were similar for each trip. Larvae apparently overwinter in all larval stages.

26. No parasites, predators, or diseases were found associated with field-collected pupae or egg masses. Because of this, natural enemies of pupae or eggs were not included in the data in Table 2. Numbers of pupae and egg masses encountered were highest at the beginning of August. The one egg mass collected on trip five resulted in 47 larvae. Six egg masses were encountered on trip six. The number of eggs per mass averaged 35 and ranged from 20 to 45. Only five individuals failed to hatch from the total collection of 210 eggs.

27. Most of the *A. densa* larval mortality due to insects in the field was a result of parasitism by *Campoletis oxylus* (Cresson) (Hymenoptera: Ichneumonidae) (Figure 14) in the fourth instar *A. densa* and *Lydella radicis* (Townsend) (Diptera: Tachinidae) (Figure 15) in the seventh instar *A. densa*. In one case the insect predator, *Chauloides* sp. (Neuroptera: Sialidae), was found feeding on a late instar larva. A granulosis virus (Figure 16) was the only pathogen noted. This virus was encountered in the field in December 1978 and 1979. Although this virus accounted for the loss of 110 and 6 larvae in 1978 and 1979, respectively, in the laboratory, it was believed that favorable laboratory conditions greatly enhanced the incidence of this pathogen.

28. None of the field-collected larvae that were placed on freshly cut plant material completed their development. However, 30.7 to 85.6 percent of the larvae completed their development on the artificial diet.

29. Moths were not attracted by blacklight or pheromone tests. Excised plant tissue experiments in the field resulted in no egg masses.

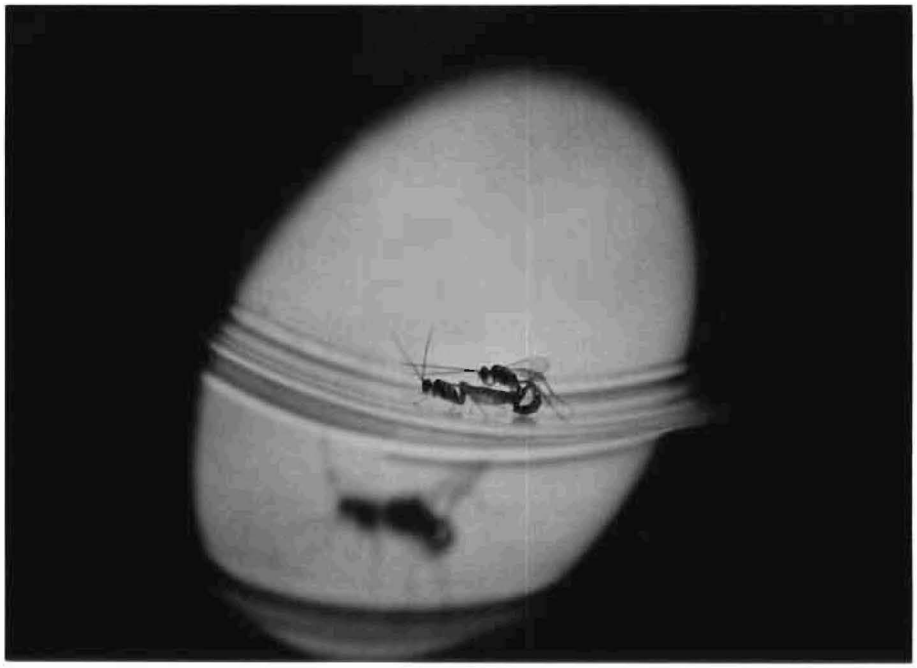

Figure 14. *Campoletis oxylus* (Cresson) in copula

Figure 15. *Lydella radicis* (Townsend) adult and puparium with parasitized seventh instar *Arzama densa*

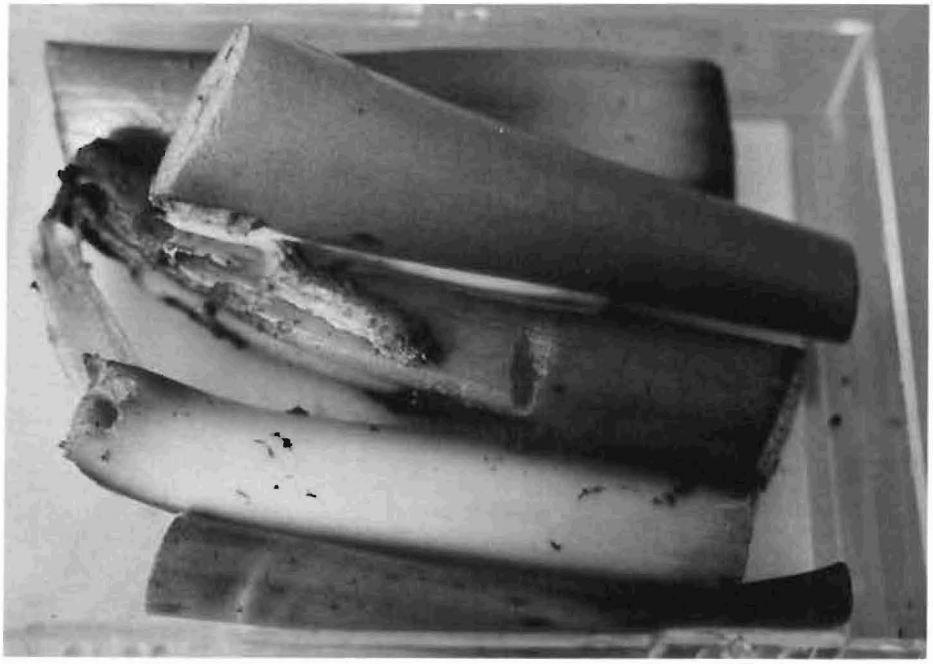

Figure 16. Granulosis virus exuding from seventh instar *Arzama densa*

Diet

30. Mold and bacteria were problems on the diet. However, these problems were reduced if the eggs were properly sterilized and handled under a microbiological hood. Microbial contamination was further reduced by autoclaving the Vanderzant-Adkisson commercial insect diet mix component of the diet. The waterhyacinth extract provided several new concepts in diet preparation. Fresh waterhyacinth was readily available from the greenhouse. This permitted immediate diet preparation. The alphacel or cellulose provided bulk necessary for digestion. The waterhyacinth extract also could be frozen and stored in the refrigerator. Preliminary studies using extract diet resulted in a similar mean developmental period as the freeze-dried waterhyacinth diet.

31. According to Center (1976), the mean developmental time of *A. densa* reared on fresh waterhyacinth was 54 days. In our studies, nine consecutive laboratory generations were reared over a 2-1/2-year period (Table 3). The data showed a decrease in the mean developmental time from egg to adult with succeeding generations. This decrease was especially noticeable when shredded diet was used starting with the F_6 through the F_9 generation. Generations F_8

and F_9 on shredded diet showed a decrease in total developmental time as compared with Center's report of those receiving only the natural food source. The sex ration was variable throughout the generations and ranged from 1.1 to 1.6:1 (males:females). The generation time averaged 66 days throughout the nine generations even though generations F_6 through F_9 were reared on shredded diet. The average survival rate over the nine generations was 63.1 percent.

32. Table 4 shows fecundity data comparisons between field-collected and laboratory-reared *A. densa*. The 28 females collected in the field averaged 297 eggs. This number was never attained with any of the laboratory-reared females. An average output per female of 204 eggs/generation was attained throughout nine generations. The percent of eggs eclosed remained similar throughout the nine generations and only slight differences were noted when compared to the field-collected individuals.

Rearing Studies

33. Mating studies involving two or more males per female indicated no significant increase in the oviposition or eclosion rates as compared with the 1:1 (male:female) ratio. Dissections of mated adult females revealed only one spermatophore per female. Moths emerged 11 to 13 days after pupation. Timing of placing emerging adults together in crepuscular light for mating purposes was critical since they only live for 4 to 6 days. Males lose their vigor 3 days after emergence. Females become "egg bound" or are unable to oviposit 2 days after emergence. Adult longevity could be extended another day by exposing them to a continuous light source in a moist environment. About 70 percent of the total oviposition occurred on the first and second nights after placing the moths in the mating cages. Sieving the eggs provided a method to separate egg clusters and remove insect parts.

Pupation

34. By spreading and drying the diet, about 70 percent of the seventh instar larvae pupated. Field-collected and F_1 through F_9 laboratory-reared pupae were compared (Table 5). There was a significant increase in weight of both sexes combined in the F_1 and F_7 generations due to the heavier females of those generations. No significant decrease in pupal weights was noted among

any of the comparisons. These results indicated that died-fed individuals were as large or larger than those collected in the field.

Storage Capabilities

Eggs

35. Moderate temperatures over a narrow range and time in storage affected viability and hatchability of the eggs. The eggs kept at 25° C hatched in 5 to 7 days whether taken out or left in the incubator (Table 6). A 91 percent eclosion rate was recorded. A nearly similar situation occurred with the eggs held at 21° C. Eighty-five percent of the eggs eclosed in 7 to 10 days. The hatch time was delayed a few days and the eclosion rate was slightly lower in comparison with those eggs kept at 25° C. Center (1976) noted an 80.7 percent eclosion rate at 25° C. The temperature range of 21° to 25° C closely resembles the natural field conditions during the summer months in Venice, La.

36. Cool temperatures extended storability time and reduced viability. Eggs held at temperatures of 8° and 14° C withstood storing for the entire 10-day period (Table 6). No eggs eclosed in the incubator. Eggs removed daily from the incubator eclosed 5 to 8 days after each of the ten removal dates. At 14° C the percent eclosion rate declined in relation to time. Eggs removed after the first day showed a 91 percent eclosion rate; those after the fifth day showed an 84 percent rate, while those held for 10 days had a 71 percent eclosion rate. A more pronounced decrease in rate of eclosion occurred with eggs held at 8° C. After the first day, 84 percent eclosed; after the fifth day, 69 percent eclosed; and after the tenth day, 48 percent eclosed. Thus, it is apparent that there is a relationship between temperature and time. Moreover, this study indicates that the eggs of *A. densa* can be stored and can be considered as having a "shelf-life."

Larvae

37. In a manner similar to the tests on eggs, the potential of storing larvae was investigated. After 6 weeks, 67.5 percent of the 200 larvae held at 20° C pupated and one individual emerged as an adult in the diet cup (Table 6). A total of 135 larvae pupated and 61 were either prepupal or seventh instar (4 larvae died). Those larvae at 14° C were slightly retarded in respect to their development. The furthest development was to the sixth

instar; however, only 6 were counted. A total of 131 larvae were recorded in the fifth instar, 47 in the fourth, and 3 in the third instar. After 6 weeks at 8° C, 81 larvae were counted in the fifth instar, 79 in the fourth instar, and 16 in the third instar. Larvae could be held for 6 weeks at 8° and 14° C with very little mortality, but their size and weight were very much reduced.

Pupae

38. Additional studies were conducted on the storability of pupae at various temperatures. From the pupae held at 20° C (Table 6), adults started emerging on day 9 and continued until day 14. This temperature was probably close to autumn field temperatures. A total of 92.5 percent eclosed. The 10 pupae held at 14° C for 1 week started emerging as adults 16 to 19 days from the start of the pupal period. Only 9 of the 10 emerged as adults in the cage. At 14° C, 9 adults emerged from pupae held 2 weeks, 7 emerged from those held for 3 weeks, and only 6 emerged from the pupae after 4 weeks. At 14° C, after 2, 3, or 4 weeks, all adults emerged 9 to 12 days after being removed from the incubator. At 8° C, eclosion decreased drastically with time; after 4 weeks, all pupae were dead. It is evident that pupae can be stored at various temperatures, but, after continuous exposure to these temperatures, mortality increases in relation to time.

Applying Larvae and Eggs to Waterhyacinth

Larvae

39. At a selected site at Norco, La., in early May 1980, the petiole segments with third instar larvae were distributed by hand into the waterhyacinth mat. The cut petioles floated on the water surface and the larvae were observed transferring onto intact plants in the mat. Personnel from the U. S. Army Engineer Waterways Experiment Station (WES) set up the plots, oversaw the application, and conducted later evaluations (Confrancesco 1982).

Eggs

40. At a selected site in Lake Salvador, La., on 15 April 1981, 35,000 eggs were suspended in 1 ℓ of solution and sprayed onto each of three plots (105,000 total eggs). Personnel from WES set up the plots and were responsible for the application and subsequent evaluations.

Outdoor Efficacy Tests

41. Outdoor pools of waterhyacinth were used to conduct efficacy tests (Table 7). Four weeks was sufficient time for the larvae to migrate from the central point of the pool to the outer edges. The larvae migrated by swimming from plant to plant on the water surface and also by tunneling through the stolons. Many individuals were in the prepupal stage at the end of the fourth week.

42. Waterhyacinth in the two control pools doubled in number and tripled in total dry weight biomass by the end of the fourth week (Table 7). Perhaps the plants would have increased more than twofold, but space limitations within the pools restricted this increase.

43. In the two pools with 50 larvae each (one larva/eight plants), the number of waterhyacinth averaged 446 after the first 3 weeks (Table 7). By the end of the fourth week, most *A. densa* had completed their larval development, and the number of plants were starting to increase. An increase was also recorded for the total dry weight biomass.

44. The plants in the two pools with 100 larvae each (one larva/four plants) showed a more pronounced response. The average number of plants after 4 weeks was reduced to less than half (from 396 to 186). A similar reduction was noted in the dry weights.

45. In the two pools with 200 larvae each, or one larva/two plants, the average number of plants drastically decreased from 389 to 71. The larvae were noted to migrate to the far edges of each pool, then move around the pool in search of plants. The biomass decreased by about fivefold during the 4 weeks.

46. Even though larvae were completing their development at the end of the third week in the four pools with 100 and 200 larvae each, daughter plants from injured plants did not increase in number in the fourth week.

PART IV: DISCUSSION

Field Data

47. All preadult life stages of *A. densa* were collected in the field during 1978 and 1979 (Table 2). Eggs were collected in late summer only. Larvae were by far the most abundant life stages, but were scarce in early spring. The active larvae can escape some predation and parasitization but stationary eggs and pupae are vulnerable.

48. The field studies indicate that two to three generations of *A. densa* occur annually (Table 2). Because of essentially two peaks/year in the number of larvae, these data agree with the statement by Vogel and Oliver (1969b) that *A. densa* is bivoltine or possibly trivoltine. However, the developmental time of each given generation probably depends on the local weather cycles encountered by that generation.

49. It is perhaps misleading that no parasites, predators, or diseases were found associated with field-collected pupae or egg masses. The extreme vulnerability of these stationary life stages to rapid predation may simply mean that, at the times of collection, predators had already "cleaned up" most of the exposed eggs and the defenseless pupae.

50. From laboratory studies of mating and oviposition, researchers can predict how the parasitic wasp, *Campoletis oxylus*, affects early instar *A. densa* larvae. However, very little is yet known about the biology of the tachinid fly, *Lydella radicis*. It is speculated that the female tachinid oviposits on plant tissue where larvae are actively moving. Then the egg could possibly be injested by a larva or adhere to its cuticle. This study found that the granulosis virus could be controlled by proper sanitation in the laboratory. However, the effect of the virus or other pathogens on larvae after manipulative releases is unknown.

51. Why the first collection of *A. densa* larvae did not complete development on excised waterhyacinth in the laboratory (Table 2) is open to speculation. Actually, only 20 larvae remained that were not parasitized. Perhaps some essential nutrient(s) from the crown of waterhyacinth were missing from their diet. The second collection of larvae was also fed on excised petioles, but those which were not parasitized died of viral disease before additional steps of sanitation were introduced into the laboratory. A satisfactory

artificial diet precluded further attempts to feed larvae on excised waterhyacinth, so the problem of incomplete development was not pursued.

52. The blacklight, pheromone, and kairomone attraction trials for adults were not successful, but these tests involved only a two-night period on one field trip. Although pupae were present, it is speculated that the timing may have been premature for that generation so that no adults or only a few were present in the area.

Diet

53. The presence of waterhyacinth, either as a freeze-dried powder or as a water extract in the artificial diet, apparently provided a feeding stimulant that was essential to successful completion of the life cycle. This stimulant was not identified; however, identification should be possible as the stimulant appeared to be rather stable. Evidence for stability was its persistence through water extraction.

54. The gradual decrease in developmental time of *A. densa* on diet may just be due to increasing adaptation of the laboratory colony to artificial conditions. The decrease in developmental time caused by shredding the diet was probably a manifestation of increased feeding (more edges for the larvae to bite into). Shredding the diet appeared to improve its physical condition with better aeration and less mold. Also, the diet may have contained more essential growth nutrients and vitamins than the plant itself.

55. Although egg production by laboratory-reared females remained quite consistent at around 200 eggs/female, this was only about two thirds of the egg production rate of females collected as prepupae or pupae in the field. The reason for this reduction in fecundity, apparently due to laboratory procedures, is unknown. However, it is speculated that the laboratory conditions for the adults were not as satisfactory as those in the field. Further studies are needed on temperature, humidity, and photoperiod effects on adult moths through emergence, mating, and oviposition.

Rearing Studies

56. Considering the critical timing of placing adults together for mating purposes, any large-scale program for rearing *A. densa* requires adequate

mating cages, lighting, and available labor. Labor is required for at least part of each day, 7 days/week. The male and female moths are easily distinguished, and this facilitates setting up mating pairs. Labor is also involved in removing eggs from the mating cages. Since the eggs have a tough exterior and are not easily damaged by sieving, this operation can be done rapidly.

Pupation

57. Pupation rate was affected by environmental conditions, but pupal weights were quite consistent. Spreading and drying the diet on a screen with prepupae enhanced pupation; thus, one might speculate that this diet, being dryer than that in a closed cup, provides conditions close to those found in the petiole of waterhyacinth, i.e., the natural pupation site. This spreading operation, however, increases the labor involved in the rearing. The consistent weights of the pupae and a comparison with individuals collected in the field provide additional evidence of the adequacy of nutrition in the artificial diet.

Storage Capabilities

Eggs

58. Acceptable eclosion rates (>85 percent) were obtained when eggs were stored at 21° to 25° C, although some did not eclose until day 10. These data are limited but suggest that egg storage at 25° C would speed up the life cycle in a mass production effort.

59. Eggs can be stored for at least 10 days at a cooler temperature (8° or 14° C), but viability is reduced with time. In a mass rearing program aimed at producing larvae on a specific date, storage of eggs at 14° C would provide a 10-day delay at a cost of about 20 percent in viability. Storage at 8° C could not be recommended because of an unacceptably large loss of more than 40 percent in viability as compared with eggs stored at 25° C.

Larvae

60. The possibility of delaying the life cycle of *A. densa* to meet a specific release date appears very promising through the refrigerated storage of larvae. The fact that larvae could be held for 6 weeks at 8° or 14° C with little mortality indicates the possibility of considerable flexibility in the

timing of a mass-production program. Obviously, the limitation in this instance would be refrigerated storage space because the larvae are stored in diet cups. Also, reliability of the power supply would be important and a back-up generator should be considered.

Pupae

61. Much less flexibility exists for the refrigerated storage of pupae than for larvae. Pupae could be stored for 2 weeks at 14° C with a predicted mortality of 10 percent, but storage at a colder temperature or for a longer period greatly increased mortality. Also, tests were not conducted on reproductive rates of prechilled pupae, so this would be an unknown factor in a mass rearing program.

Applying Larvae and Eggs to Waterhyacinth

62. Although applying larvae in petiole segments was apparently a successful dispersal method, this technique is labor-intensive and alternate methods should be sought. Preferably, an alternate method would not require live plant material.

63. Spraying eggs appeared to be a very efficient method for initial dispersion of a large number of insects. However, in retrospect, this technique may never be successful because eggs are so rarely found in nature. Eggs are very vulnerable to predation. The artificially sprayed eggs have no protection and would be defenseless against spiders or other general predators. Also, natural morning dew may loosen the adhered eggs on the plant. If this technique is considered further, eggs should be selected that will hatch 1 to 2 hr after application.

Outdoor Efficacy Tests

64. The outdoor pool efficacy tests showed that *A. densa* larvae migrate from fed-upon plants to "clean" plants. Although the pools provided a limit to the out-migration, in nature such barriers would not usually exist. Thus, the movement of larvae to areas outside of artificially designated plots is a real possibility in the field and could complicate evaluation of manipulative releases. On the other hand, such movement could be an asset in a classical

release program as movement should enhance survival of insects after inoculative releases.

65. The doubling in number and tripling in biomass by the waterhyacinth plants in the control pools is indicative of the legendary growth potential of this aquatic weed. Such rapid growth imposes a major constraint on a manipulative biocontrol program: the waterways manager would have to inundate the biomass with the biocontrol agent(s) to prevent the weeds from overcoming the agent's suppressive power.

66. The results of placing one larva/eight plants showed that the growth of waterhyacinth overcame the suppressive power of the insects.

67. The treatment of one larva/four plants was sufficient to cut the numbers and biomass of waterhyacinth in the pools to less than half. This would still be far less than the number of larvae needed to accomplish acceptable control.

68. Acceptable control of waterhyacinth was obtained in the pools with one larva/two plants. The few remaining plants were sufficiently injured so that new daughter plants or ramets were not produced in the fourth week.

69. In contrast to the above, note that the plants treated with one larva/eight plants (paragraphs 43 and 66) were increasing in number and biomass. Although control was achieved by one larva/two plants, the larvae were limited in their movement by the pool edges, and the actual number needed for control of waterhyacinth in a field situation is still unknown. It is predicted that a number greater than one larva/two plants would be required for control in situations where outward migration could occur.

PART V: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

70. The purpose of this 3-year study was to contribute to the biological control of waterhyacinth by developing techniques to mass rear and disperse the native moth *Arzama densa* through large-scale releases. This purpose was met through accomplishment of the main objectives. The following conclusions relate to these objectives:

a. Field studies on *A. densa* indicate that two to three generations occur annually. Natural enemies play an important role in checking the populations of *A. densa* during certain times of the year. Natural populations at Venice, La., could be augmented with laboratory-reared insects and would likely be more effective in July/August or possibly in spring when parasites and predator populations are low. Larvae would probably be the best life stage to release in an augmentive sense because they can escape some predation through their mobility; eggs and pupae are stationary and vulnerable. Manipulative releases of larvae past the fourth instar should provide additional escape from parasitism by the wasp, *Campoletis oxylus*.

b. *Arzama densa* can be mass reared on an artificial diet provided that a water extract of waterhyacinth is included. Nine consecutive laboratory generations were reared with an average survival rate of 63 percent from egg to adult.

c. *Arzama densa* in the egg, larval, or pupal stages can be stored at cool temperatures to prolong the life stage. However, the larval stage would be most easily prolonged with the least mortality. Refrigeration of larvae at 8° C (or less) should permit synchronization of releases.

d. More research is needed to develop a better method of release of *A. densa* on an operational scale. Eggs could be sprayed out but would likely be subject to heavy, rapid predation and adverse weather. Larvae could be dispersed in segments of waterhyacinth petioles, but this method is labor-intensive.

e. *Arzama densa* at a rate of one larva/two plants controlled waterhyacinth in an artificial pool situation; however, the number required to control waterhyacinth in the field remains unknown. It is estimated that two larva/one plant would be a good starting place for a field test. Thus, some larvae could be lost to predation and control of waterhyacinth could still occur.

Recommendations

71. The following recommendations are made:

a. Various mold inhibitors should be tested in the diet to attempt to increase the pupation rate of *A. densa* without having to remove the larvae from the diet containers.

b. Improved techniques should be developed for storing larvae for longer periods. Temperatures below 8° C should be tried.

c. The larvae of *A. densa* should be subjected to various herbicides in the diet and by actual dipping in solutions. Various combinations of this insect and herbicides should be tested in an integrated approach to control the waterhyacinth.

d. Other methods of applying the larvae should be developed.

e. The manipulative integrated approach should be tested in wooded areas where spraying waterhyacinth is impossible.

f. The host range of *A. densa* should be investigated further. This insect would have potential as a classical biocontrol agent in other countries if the host range proved to be sufficiently narrow.

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Table 1

An Artificial Diet for Rearing *Arzama densa*

Constituent	Amount
Wheat germ	96 g
Casein (vitamin free)	112 g
Sucrose	112 g
Wesson salt mix modified*	32 g
Cellulose	47 g
Linseed oil	0.8 ml
Cholesterol	0.2 g
Vanderzant-Adkisson Insect Diet Mix*	400 g
Waterhyacinth powder**	55 g
Agar	90 g
Sorbic acid	6 g
Choline chloride (10%)	36 ml
Formaldehyde (10%) (37% actual)	15 ml
Methylparaben (38% in 95% ETOH)	18 ml
Potassium hydroxide (4.0 M)	18 ml
The following suspended in 300 ml distilled water:	
Ascorbic acid	15 g
Aureomycin	0.5 g
Vanderzant vitamin mix/modified*	9 g
Vitamin A acetate	0.5 g
Distilled water	4000 ml

* Obtained from Bio-Serv, Inc., P. O. Box 100-B, Frenchtown, N. J. 08825.

** An extract of waterhyacinth and cellulose can be substituted.

Table 2

Life Stages of *Arzama densa* and Associated Organisms Collected in the Field

Trip No.	Date	*Arzama densa*			Parasites and Diseases of *A. densa* Larvae			Percent Mortality	Percent of Larvae Completing Development on Artificial Diet
		Larvae	Pupae	Egg Masses	Diptera	Hymenoptera	Virus		
1	11/17-19/78	46	1	0	11	15	0	56.5	*
2	12/4-7/78	169	0	0	45	14	110	100	*
3	1/22-24/79	29	1	0	12	1	2	53.5	30.7
4	3/20-22/79	2	0	0	2	0	0	100	0
5	7/9-10/79	36	4	1	2	2	0	11.1	81.2
6	7/30,31-8/1/79	136	10	6	3	10	0	9.5	78.0
7	12/4-7/79	177	0	0	29	28	6	35.6	85.6

* No individuals tested on artificial diet; the larvae were fed excised petioles of waterhyacinth.

Table 3

Life History Data for *Arzama densa* Reared on Artificial Diet

Generation	Eggs Tested No.	Sex Ratio (♂:♀)	$\bar{X}$ (and Range) Developmental Time (Egg to Adult) days	Percent Survival (Egg to Adult)
Field collected	72*	1.2:1	--	--
F_1	150	1.3:1	83 (67-112)	69.4
F_2	150	1.3:1	79 (64-101)	62.1
F_3	150	1.5:1	71 (39-99)	63.2
F_4	150	1.5:1	79 (43-120)	59.8
F_5	150	1.6:1	65 (35-104)	60.1
F_6	150	1.6:1	62 (39-102)	58.2
F_7	150	1.2:1	58 (36-92)	61.2
F_8	150	1.4:1	51 (37-89)	65.8
F_9	150	1.1:1	44 (28-71)	68.5
Total F_1-F_9				
$\bar{X}$	150	1.38:1	66 (43-88)	63.1

* Individuals acquired as larvae.

Table 4

Fecundity Data from Field-Collected and Laboratory-Reared *Arzama densa*

Generation	♀♀ No.	Total Number of Eggs	$\bar{X}$ Eggs/♀ No.	Percent of Eggs Eclosed
Field collected (30 July-1 Aug 1979)	28	8,314	297	95.2
F_1	21	4,736	225	97.3
F_2	181	42,061	232	93.5
F_3	200	53,643	268	94.8
F_4	78	25,248	224	96.1
F_5	290	60,348	208	88.3
F_6	248	49,731	201	90.6
F_7	1203	163,330	136	96.1
F_8	1213	181,848	150	93.8
F_9	1545	280,830	182	91.6
Total F_1-F_9	4979	861,775	1836	840.9
$\bar{X}$/generation	553	95,752	204	93.4

Table 5

Mean Pupal Weights of 15 Individuals of Each Sex from Field-Collected and F_1 through F_9 Laboratory-Reared Generations*

	Field Collected mg (±SD)	F_1 mg (±SD)	F_2 mg (±SD)	F_3 mg (±SD)	F_4 mg (±SD)
Male	291[b] (±56)	331[b] (±74)	312[b] (±56)	310[b] (±68)	320[b] (±43)
Female	397[b] (±59)	458[a] (±98)	374[b] (±76)	382[b] (±84)	374[b] (±62)
Both sexes	344[b] (±80)	395[a] (±170)	343[b] (±73)	346[b] (±84)	348[b] (±59)

	F_5 mg (±SD)	F_6 mg (±SD)	F_7 mg (±SD)	F_8 mg (±SD)	F_9 mg (±SD)
Male	322[b] (±49)	311[b] (±51)	324[b] (±61)	327[b] (±49)	323[b] (±54)
Female	374[b] (±66)	370[b] (±65)	442[a] (±58)	383[b] (±67)	376[b] (±74)
Both sexes	348[b] (±63)	353[b] (±57)	398[a] (±88)	345[b] (±61)	350[b] (±64)

NOTE: Same letter indicates no significant difference ($p \leq 0.05$).

* Pupae held in incubator for 24 hr at 25° ± 1° C and 70 percent RH before weighing.

Table 6

The Effect of Cool Temperature Storage on Various Life Stages of *Arzama densa*

Life Stage	Temperature °C	Days in Storage	Days to Hatch	Percent Eclosed	Temperature °C	Days in Storage	Days to Hatch	Percent Eclosed
Eggs*	25	--	5-7	91	14	10	5-8	71
	21	--	7-10	85	8	1	5-8	84
	14	1	5-8	91	8	5	5-8	69
	14	5	5-8	84	8	10	5-8	48

Life Stage	Temperature °C	Weeks in Storage	Percent Pupated	Percent Prepupal-7th Stage	Percent 6th Stage	Percent 5th Stage	Percent 4th Stage	Percent 3rd Stage	Percent Dead	Percent Adults
Larvae**	20	6	67.5	30.5					1.5	0.5
	14	6	--	--	3	65.5	23.5	1.5	6.5	--
	8	6	--	--	--	40.5	39.5	8	12	--

Life Stage	Temperature °C	Weeks to Removal	Days from Removal to Eclosion	Percent Eclosed (Adults)	Percent Dead	Temperature °C	Weeks to Removal	Days from Removal to Eclosion	Percent Eclosed (Adults)	Percent Dead
Pupae†	14	1	16-19	90	10	8	1	9-12	70	30
	14	2	9-12	90	10	8	2	9-12	20	80
	14	3	9-12	70	30	8	3	12	10	90
	14	4	9-12	60	40	8	4	--	0	100

* Ten cups of 20 eggs each were exposed to each temperature/storage time regimen.

** Two hundred third stage larvae were exposed to each temperature.

† Lots of 10 pupae each were exposed to each temperature/time of removal regimen; at 20° C, 92.5 percent of 40 pupae eclosed to adults after 9-14 days and no further emergence occurred.

Table 7

Mean Numbers of Plants and Mean Dry Weights in an Outdoor Efficacy Study of *Arzama densa* Against Waterhyacinth*

Treatment	Week 1		Week 2		Week 3		Week 4	
	Plants No./Pool	Dry Weight g	Plants No./Pool	Dry Weight g	Plants No./Pool	Dry Weight g	Plants No./Pool	Dry Weight g
Controls	422[a]	1406[a]	501[a]	1969[a]	648[a]	2850[a]	826[a]	4151[a]
50 Larvae/pool**	397[a]	1368[a]	402[b]	1332[b]	446[b]	1468[b]	523[b]	2030[b]
100 Larvae/pool	396[a]	1266[a]	301[c]	998[c]	249[c]	831[c]	186[c]	599[b]
200 Larvae/pool	389[a]	1157[a]	288[c]	932[c]	174[c]	565[c]	71[c]	230[c]

NOTE: Means (within a column) followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

* Started with 400 plants/pool, 2 pools/treatment.

** Third instar larvae at initiation of study.