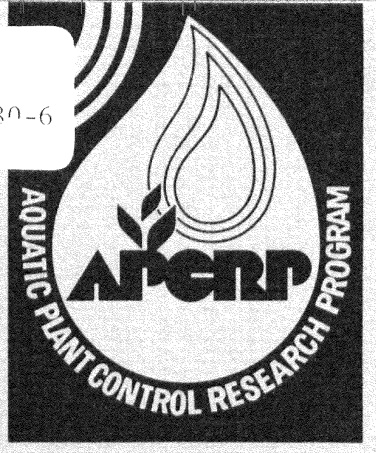

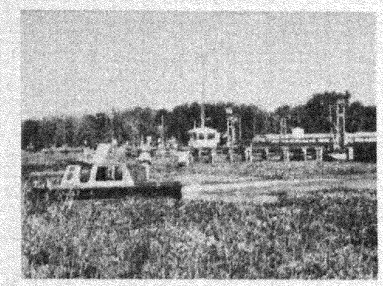

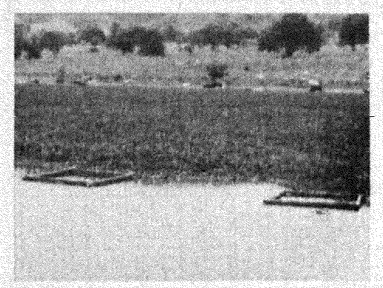

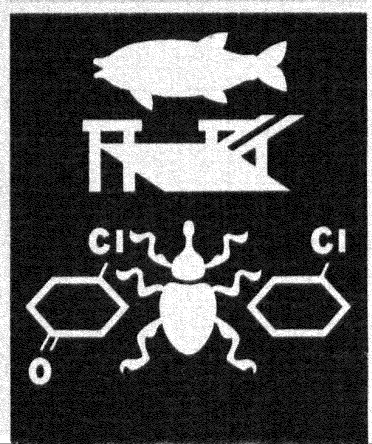

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FIELD STUDIES AND LABORATORY REARING OF *ARZAMA DENSA* WLK., A BIOLOGICAL CONTROL AGENT AGAINST WATERHYACINTH

By R. G. Baer and P. C. Quimby, Jr.

U. S. Department of Agriculture
Science and Education Administration
Southern Weed Science Laboratory
Stoneville, Miss. 38776

December 1980

Final Report

Prepared for U. S. Army Engineer District, New Orleans
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A study of biological control of waterhyacinth using a native moth, *Arzama densa* Walker, is presented herein.

Previous studies have shown that the larvae, in sufficient numbers, can severely damage populations of the target aquatic plant. Workers in biocontrol generally feel that if sufficiently large populations of the insect could be produced to supplement field populations, this agent could be an effective

(Continued)

20. ABSTRACT (Continued).

tool in limiting infestations of waterhyacinth.

The study reported herein includes the biology of the insect and effects of various diets on the insect's growth. This report also describes the most successful rearing methods found thus far for *Arzama densa*.

PREFACE

The study reported herein was performed under Interagency Agreement WESRF-79-83 with the U. S. Department of Agriculture, Science and Education Administration (USDA-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), 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. D. L. Shankland.

The authors wish to thank Dr. C. G. McWhorter, Laboratory Chief, Southern Weed Science Laboratory at Stoneville, for the use of the facilities. We are grateful to two Insect Pathologists, Dr. J. V. Bell, USDA, Stoneville, and Dr. P. Sikorowsky, Department of Entomology, Mississippi State University, for identifying the pathogens encountered during this study. We also extend our appreciation to Mr. J. McWilliams for the use of the oviposition cages.

Sincere thanks are extended to Mr. D. G. McMinn and Ms. M. C. Crittenden, technicians at Stoneville, for technical assistance and valuable suggestions in diet preparations, mass-rearing procedures, field collecting, and raising sufficient quantities of waterhyacinth under greenhouse conditions.

The research was monitored at WES by Dr. D. R. Sanders, Sr., and Mr. R. F. Theriot of the Environmental Laboratory (EL) Wetland and Terrestrial Habitat Group (WTHG). The study was conducted under the general supervision of Dr. John Harrison, Chief, EL, 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 is Manager of the APCRP at WES.

Directors of the WES during the study and preparation of this

report were COL J. L. Cannon, CE, and COL N. P. Conover, CE. Technical Director was Mr. F. R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

U. S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

Multiply	By	To Obtain
acres	4046.856	square metres

FIELD STUDIES AND LABORATORY REARING OF *ARZAMA DENSA* WLK., A BIOLOGICAL CONTROL AGENT AGAINST WATERHYACINTH

PART I: INTRODUCTION

1. Waterhyacinth, *Eichhornia crassipes* (Mart.) Solms, is a perennial, herbaceous, floating, freshwater weed that presently infests about 1 million acres* of water including rivers, canals, streams, reservoirs, and coastline areas in the southeastern United States. Waterhyacinth has been ranked as eighth among the world's worst weeds and as the single most important aquatic weed (Holm et al. 1977; Holm 1969).

2. Waterhyacinth mainly reproduces asexually by stolons (Batanouny and El-Fiky 1975). As these runners grow, a new plant forms at its tip. Penfound and Earle (1948) reported that doubling time in numbers of individuals was from 11 to 15 days, depending upon weather conditions. This growth potential allows waterhyacinths to invade areas very quickly and form dense floating mats of plants (Figure 1). Major agricultural, navigational, and health-related problems arise from these floating rafts (Bock 1966; Zeiger 1962; Sculthorpe 1967). Waterhyacinths reduce the oxygen concentration of the water, impede water flow, restrict commercial and recreational water traffic, and serve as refuges for insects that vector human and animal diseases.

3. Although it is difficult to arrive at a sound monetary estimate of expenditures caused by waterhyacinth and its control, studies indicate figures well into the million of dollars (Spencer 1973, 1974). The Southern Weed Science Society lists waterhyacinth as second among the most troublesome and costly weeds in Florida.

4. Many methods of control--chemical, biological, ecological, mechanical--have been attempted in an effort to reduce the spreading and the losses caused by the plant (Sculthorpe 1967; Robson 1974). Cost,

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* A table of factors for converting U. S. customary units of measurement to metric (SI) is presented on page 5.

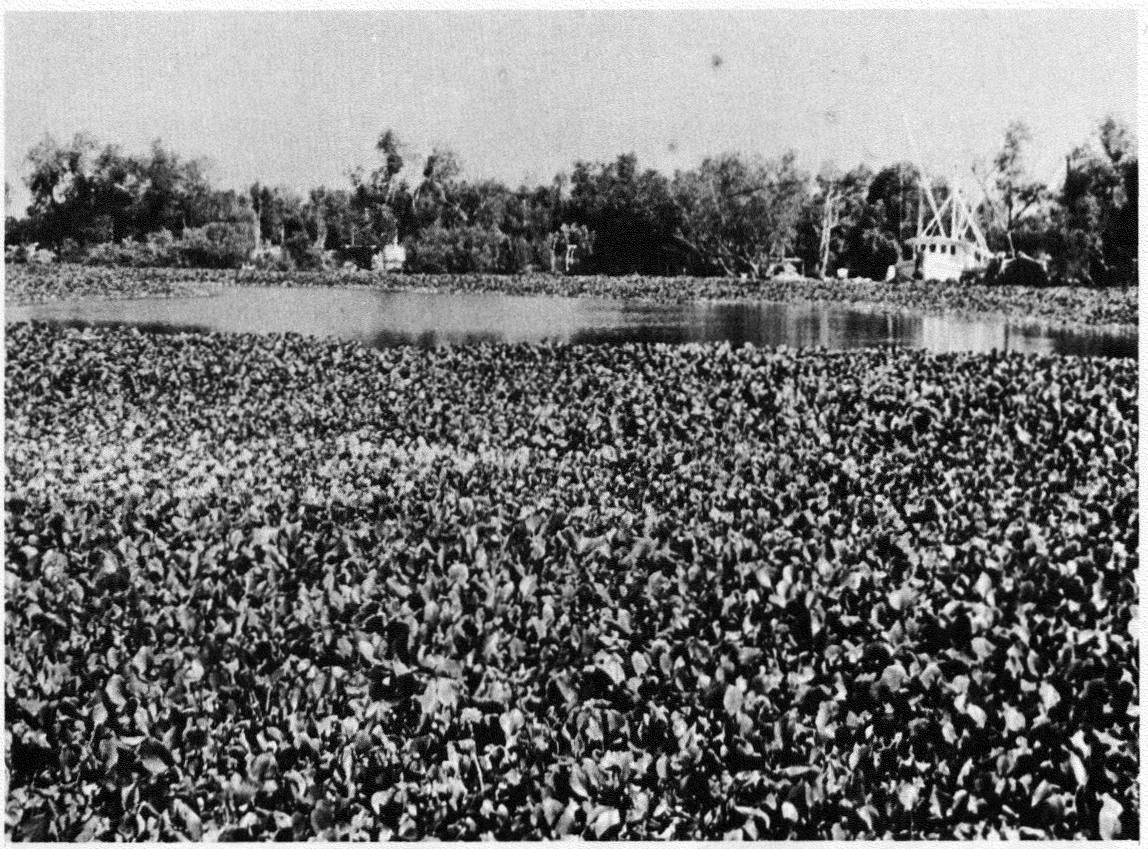

Figure 1. An infestation of waterhyacinth in Venice, La.

feasibility, and environmental effects have played important roles in the success and/or failure of these control methods.

5. This study involves the biological control of waterhyacinth using the native moth, *Arzama densa* Walker (1864) (Lepidoptera: Noctuidae) (Figure 2). Previous studies on the life history of *Arzama densa* indicate that the larvae severely damage waterhyacinth although the native host plant is pickerelweed, *Pontederia cordata* L. (Vogel and Oliver 1969 a,b; Center 1975, 1976) (Figures 3 and 4). These authors state that if satisfactory methods of mass rearing were developed, field populations could be supplemented with laboratory-reared larvae.

6. The main objective of this study was to develop a rearing method for *A. densa*. This study provides information on field observations of natural populations and the life history of *Arzama densa* under laboratory conditions.

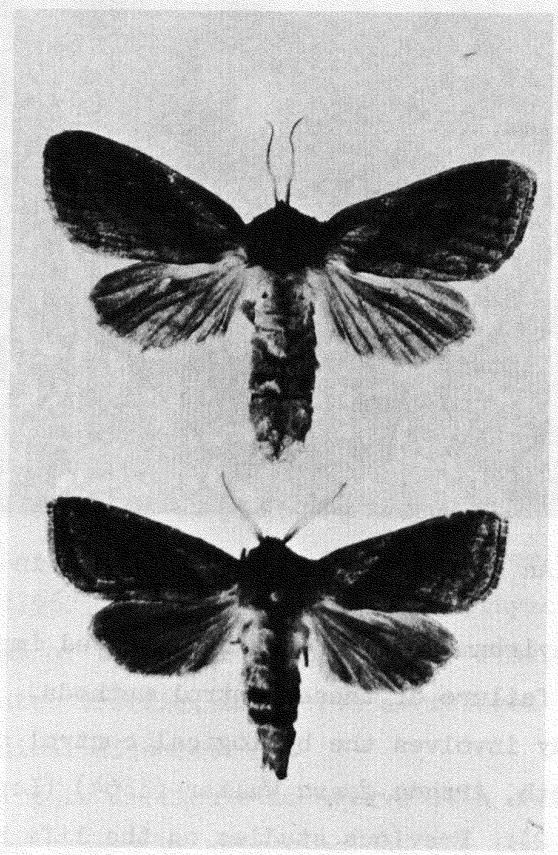

Figure 2. *Arzama densa* Walker,
♀ on top, ♂ on bottom

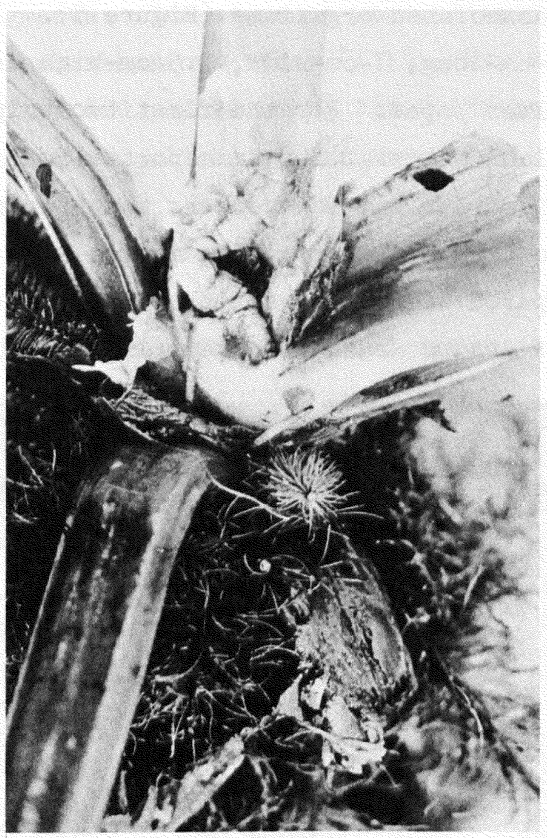

Figure 3. *Arzama densa* larva feeding on a terminal bud of waterhyacinth

Figure 4. *Arzama densa* larva feeding on the crown portion of waterhyacinth

PART II: MATERIALS AND METHODS

Collecting Trips

7. Six trips were taken to the same locality in Venice, La., to collect *Arzama densa* and waterhyacinths needed for the initial establishment of a laboratory colony. At the collecting sites, plants were thoroughly searched for *Arzama densa* and associated organisms (Figure 5). *Arzama densa* larvae were placed in 10-cm-long, 7-cm-wide, 3.5-cm-high clear plastic boxes lined with wet filter paper. For the first two trips, larvae were provided with cut fresh plant material for transportation back to the laboratory. For the last four trips, larvae were transferred to an artificial diet for transport to the laboratory. Egg masses and pupae were placed directly on the prepared diet. Larvae infested with parasites, predators, and pathogens of *Arzama densa* were kept in separate

Figure 5. Mr. D. G. McMinn searching plants for *Arzama densa* in Venice, La.

plastic boxes. All boxes and collected waterhyacinths were placed in an ice chest containing a small amount of ice for the journey back to the laboratory.

8. Three methods were used on the last trip 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, thereby extending the last two abdominal segments. The segments were clipped and placed in a solution of n-hexane:anhydrous ether (1:1). Traps (Ganyard and Brady 1971) were baited and placed among the waterhyacinths at leaf height. The second method of collecting adults employed a black light hung against a white sheet. The black light and the pheromone studies were conducted between 9 and 11 p.m. central standard time (CST). In the third method, waterhyacinth leaves were cut in half with scissors to release plant volatiles perhaps needed as a stimulant for oviposition. The plants were examined the next day for egg masses.

9. In the laboratory, all individuals 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 fresh 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 tissues and placed in a 1-litre wax-lined ice cream container. Tissues were moistened daily with distilled water. A plastic petri dish top provided the cover of the container. Records were kept on pupation and emergence of *Arzama densa*.

10. The number of larval instars of *Arzama densa* were determined by molted head capsules. Diseased instars were isolated from healthy individuals.

Diet for Rearing *Arzama densa*

Constituents

11. Waterhyacinths were collected in the field and reared in the

greenhouse at the Delta States Research Center at Stoneville, Miss. Greenhouse plants were grown in pools of water containing a 10 percent modified Hoagland's solution. Roots and flowers were pruned, leaving the crown, leaves, and leaf and flower stems. These parts were rinsed in distilled water and freeze-dried. Excess plant material was stored at -14° ± 2°C. The plant material was ground in a ball mill into a fine powder. The waterhyacinth powder was substituted for the alfalfa used in a cabbage looper diet (Henneberry and Kishaba 1966; Ignoffo 1963). Other changes included the addition of more water and a different vitamin mixture (Table 1).

Preparation

12. The agar was dissolved in 4000 ml boiled water in a blender for 30 sec. The waterhyacinth powder and the Vanderzant-Adkisson diet were added, and the solution was blended for 1 min. The sorbic acid, choline chloride, formaldehyde, methylparaben, and potassium hydroxide solutions were then added and blended for 1 min. The 300-ml suspension (ascorbic acid, aureomycin, vitamin A, and vitamin mix) was added, and the entire mixture was blended for 2 min to obtain a homogeneous mixture. About 20 ml of the liquified diet was then poured into 30-ml clear plastic cups or 35 ml into 100- by 15-mm plastic petri dishes under a microbiological hood. The diet was stored in a refrigerator at 3° ± 2°C and warmed at room temperature as needed for the developing larvae.

Rearing Procedures

13. Adult moths acquired from individuals collected from the trips to Venice, La., provided the stock colony. Oviposition cages measured 45 cm long, 55 cm high, and 11 cm deep. These cages had removable, aluminum-framed plastic window screens. The cage was placed on top of a 134.5-cm-long, 104.5-cm-wide, 29-cm-deep plastic pool containing waterhyacinth and was enclosed in a 1-m^3 screen cage (Figure 6). The entire apparatus was placed either in an air-conditioned greenhouse (27°/21°C day/night) or outdoors. The timing of emerging adults for mating purposes was critical since they only lived for 4 to 6 days.

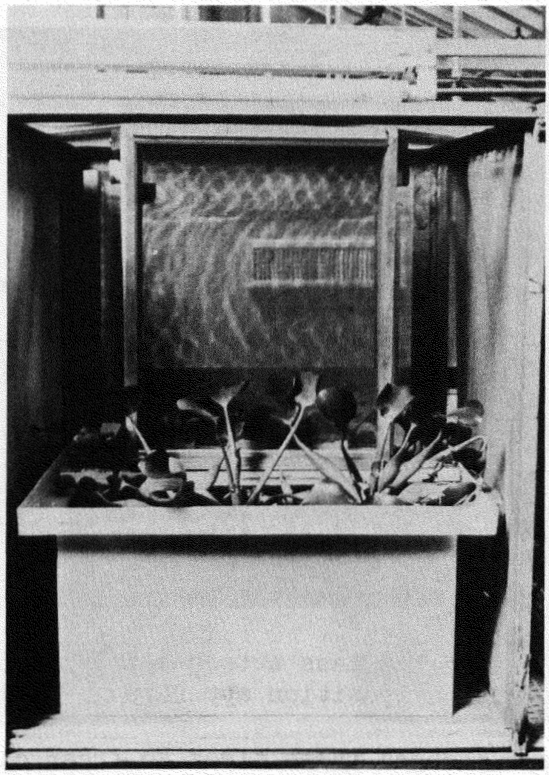

Figure 6. Oviposition apparatus in air-conditioned greenhouse

Males seemed to lose their vigor 3 days after emergence. Females became egg bound about 2 days after emergence. Adult longevity could be extended an extra day by exposing them to continuous light. Adults were not supplied with a food source because they have vestigial mouthparts. Various matings included wild or field-collected, wild × laboratory, and laboratory × laboratory individuals.

14. Moths laid their eggs singly or in masses on the removable plastic screens (Figure 7). 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 counted, sterilized in a 20 percent formaldehyde solution (37 percent actual) for 1 min, and rinsed in distilled water for 5 min. Twenty eggs were transferred directly to each diet cup under the microbiological hood and capped with a wax-coated paper lid. The lids prevented the escape of first instar larvae that hatched in 5 to 6 days.

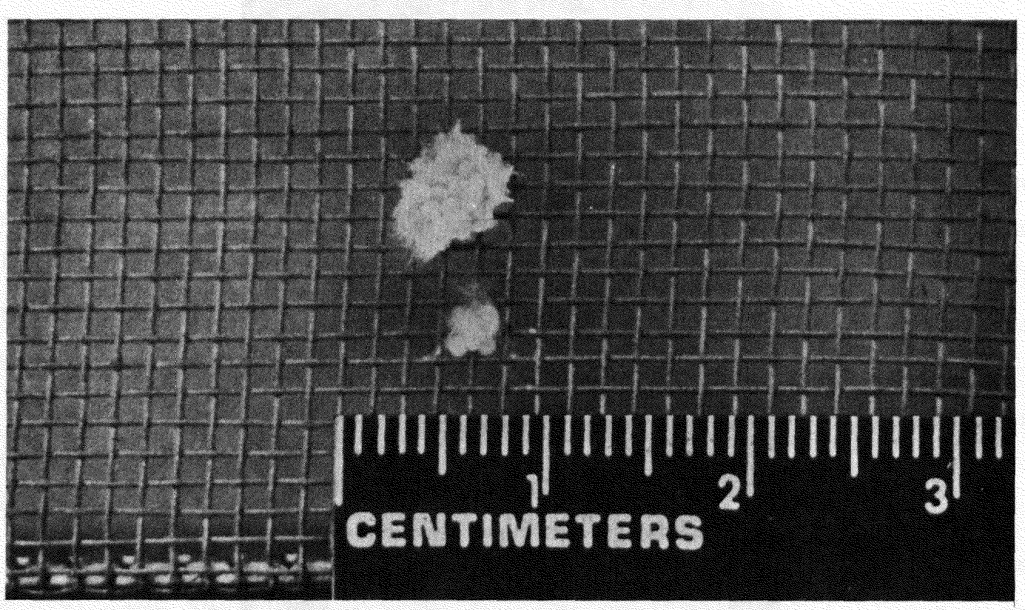

Figure 7. Egg mass attached to screen of oviposition apparatus

15. Larvae were changed three times to fresh diet in petri dishes after the 3rd, 5th, and 6th molt. Figures 8 and 9 show the 3rd and 7th instar, respectively, and Figure 10 shows the larvae in the incubator. Molting was determined by counting the number of head capsules. At the 5th instar change, five larvae were placed per petri dish until development to pupae. Pupae from laboratory rearings (Figure 11) were placed in an incubator for 24 hr at 24° to 26°C and 70 percent RH and then weighed.

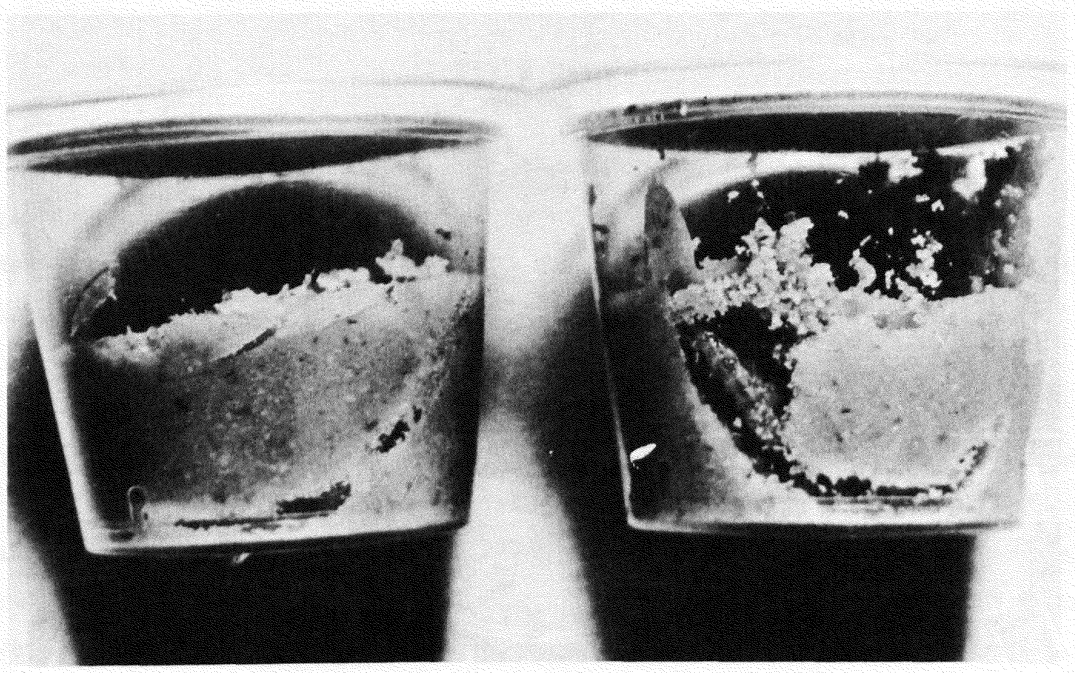

Figure 8. Third instars in diet cups

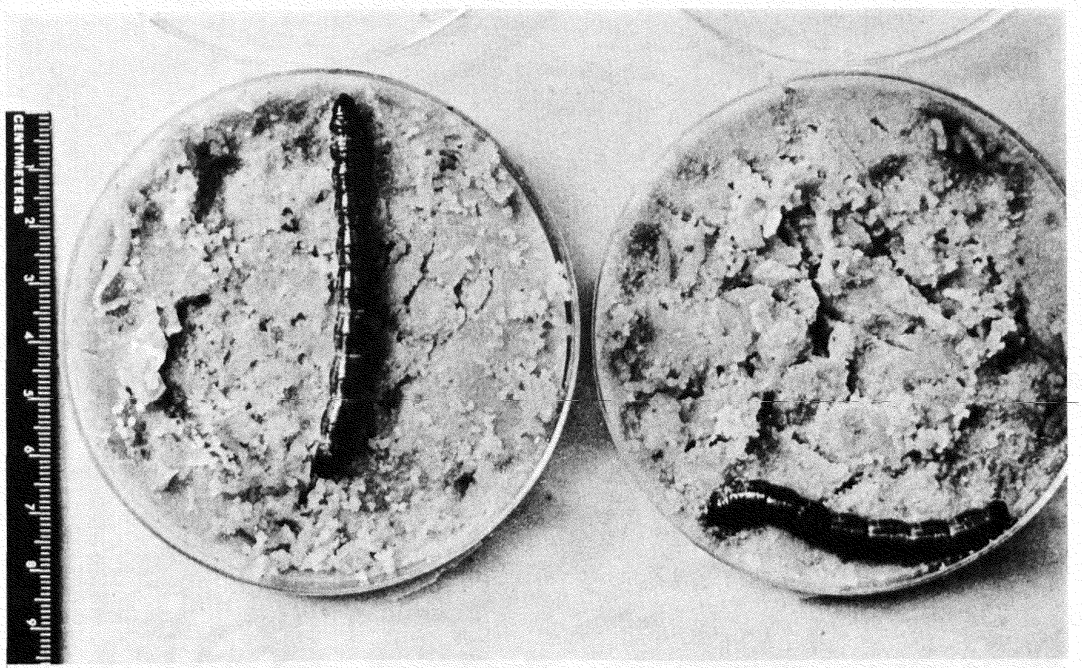

Figure 9. Seventh instars in petri dishes

Figure 10. Stacks of petri dishes containing larvae in incubator

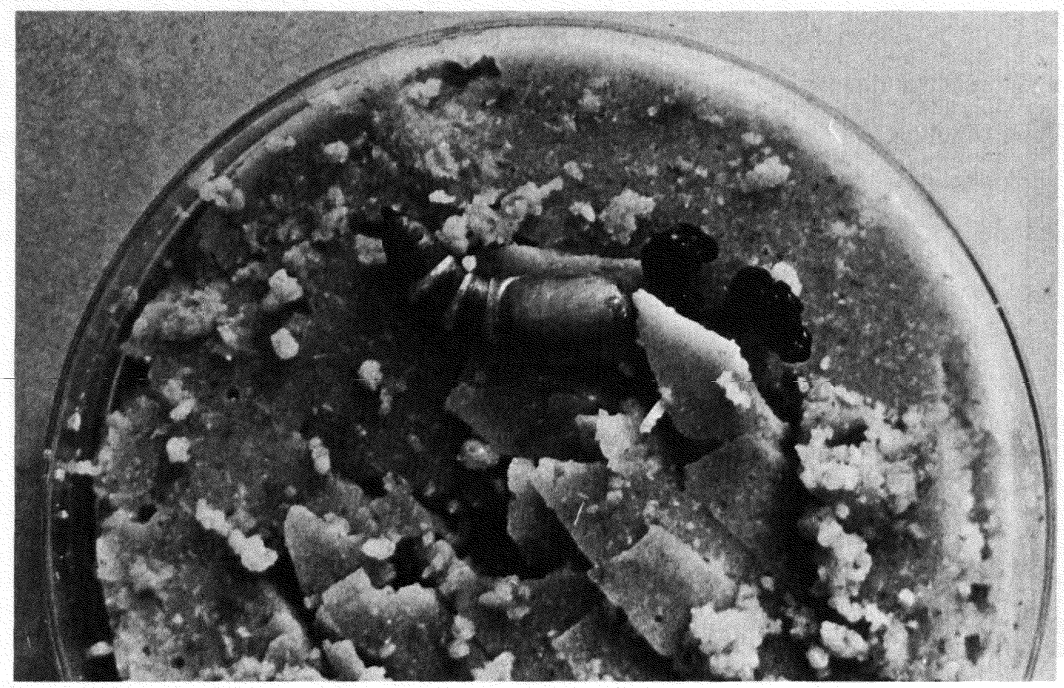

Figure 11. Newly formed pupa on diet

PART III: RESULTS

Field Data

16. The relative numbers of *Arzama densa* and other associated organisms collected on six trips are represented in Table 2. *Arzama densa* populations were highest 1 to 2 m from the shoreline and especially near overhanging vegetation. These data represent the total field and laboratory results of collected individuals.

17. The number of *Arzama densa* larvae found on the field trips ranged from 2 to 169 (Table 2). Ratios of various instars were similar for each trip. The beginning of December and August represented the peak collecting periods. The authors agree with the statement by Vogel and Oliver (1969b) that *Arzama densa* is bivoltine or possibly trivoltine; however, the developmental time of each generation may vary with climatic differences and variation within each generation. Various instars were found during the winter months; thus, larvae apparently overwinter in all larval stages.

18. No parasites, predators, or diseases were found associated with pupae or egg masses. Because of this, they 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 5 resulted in 47 larvae. Six egg masses were encountered on trip 6. The number of eggs per egg mass averaged 35 and ranged from 20 to 45. Only 5 individuals failed to eclose from the 210 eggs.

19. *Arzama densa* larvae were plagued by parasites, predators, and pathogens except for July and the beginning of August. Most of the larval mortality in the field was a result of parasitism by *Campoletis oxylus* (Cresson) (Hymenoptera: Ichneumonidae) (Figures 12 and 13) in the 4th instar and *Lydella radicis* (Townsend) (Diptera: Tachinidae) (Figures 14 and 15) in the 7th instar. In one case, the insect predator, *Chauloides* sp. (Neuroptera: Sialidae), was found feeding on a late instar larva. *Arzama densa* populations were very low in March, apparently because of bird predation. A nuclear granulosis virus was the only

Figure 12. Larva of the parasite *Campoletis oxylus* (Cresson) (Hymenoptera: Ichneumonidae) spinning pupal cocoon. Note larval skin of *Arzama densa*

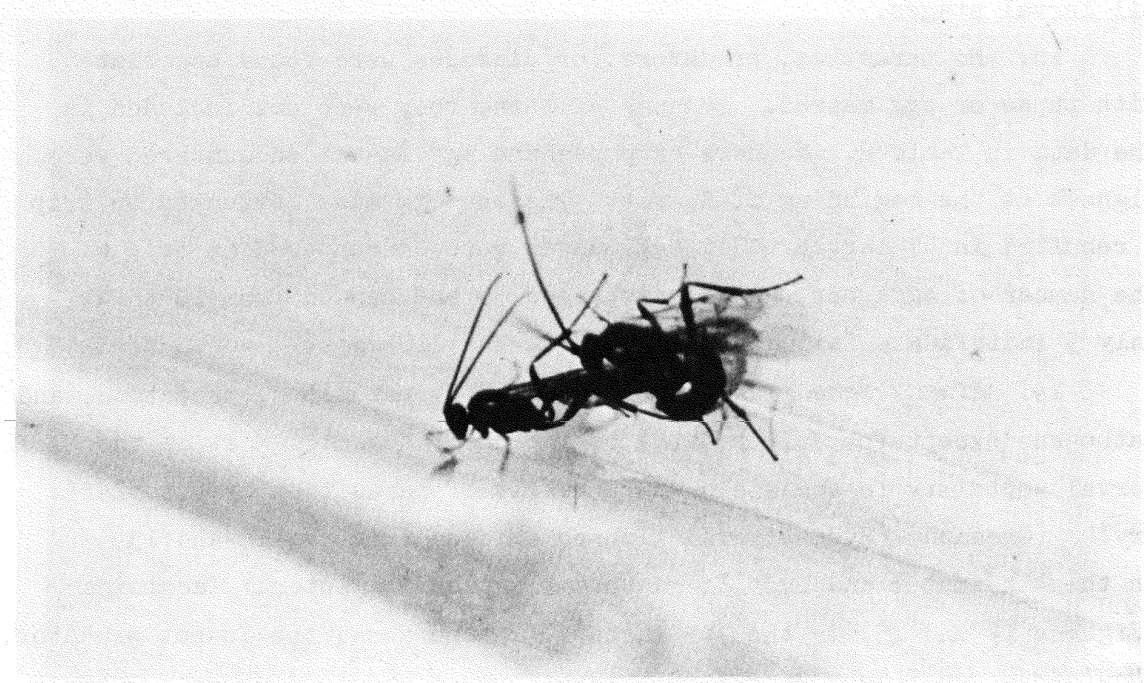

Figure 13. A mating pair of *Campoletis oxylus*

Figure 14. Three larvae and three puparia of the parasite *Lydella radicis* (Townsend) (Diptera: Tachinidae) on a 7th instar of *Arzama densa*

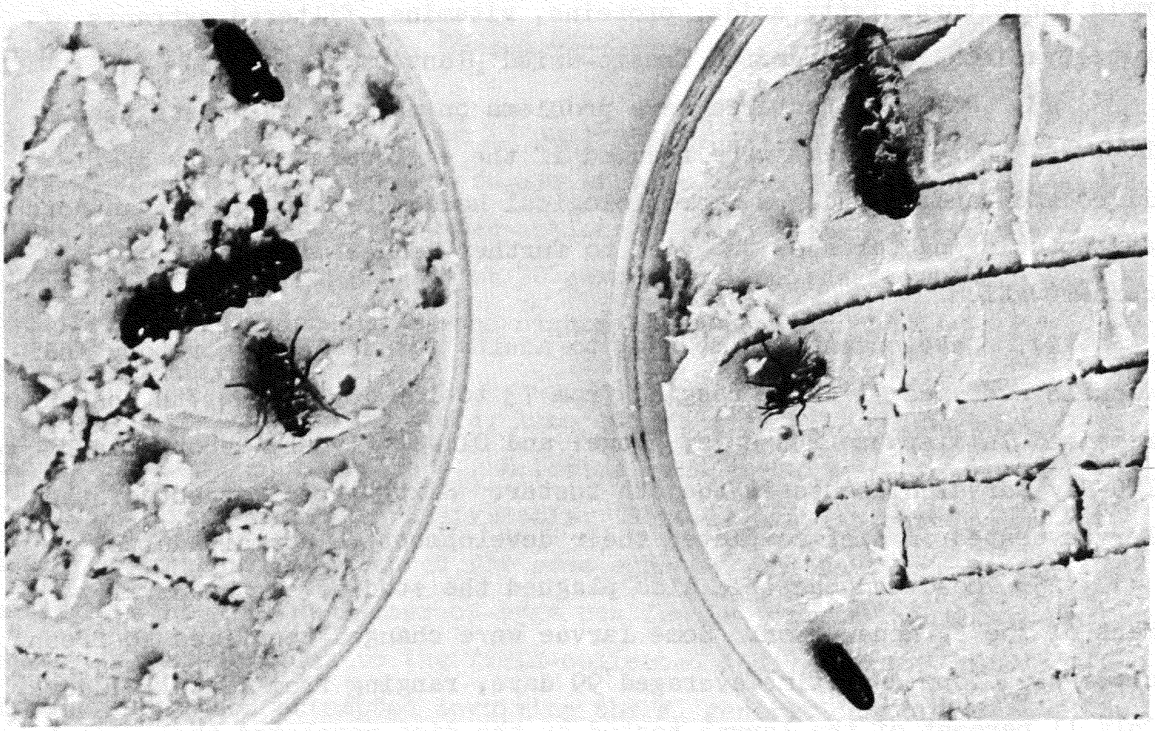

Figure 15. Adults of *Lydella radicis*

pathogen noted. The pathogen was encountered in the field in December. Although this virus accounted for the loss of 110 larvae in the laboratory, it is felt that favorable laboratory conditions greatly enhanced the incidence of this pathogen.

20. Field-collected larvae were placed either on freshly cut plant material or diet. None of the larvae on freshly cut plant material completed their development. However, 30.7 to 81.2 percent of the larvae completed their development on the diet.

21. Moths were not attracted by blacklight, pheromone, or excised plant tissue experiments in the field.

Diet

22. The meridic diet represents the most successful of more than 20 different diets tested on field-collected, F_1, and F_2 generation individuals. The other diets contained different and varying amounts of mold inhibitors, fatty acids, proteins, vitamins, filtered extracts of waterhyacinth, and separated freeze-dried plant parts.

23. Mold and bacteria were problems on many of the diets tested. However, these problems were reduced if the eggs were properly sterilized and handled under a microbiological hood. Presently, the authors are autoclaving parts of the diet to further reduce microbial contamination.

24. Developmental time (egg to adult) for the F_1 laboratory individuals averaged 91 days, ranging from 73 to 163 days. One 7th instar remained in diapause 233 days. Vogel and Oliver (1969b) reported a 25- to 120-day larval diapause in the 6th instar. Sixty-three percent of the larvae tested on diet completed their development to normal adults.

25. Mold and bacteria also plagued the studies on the development of the F_2 generation. Some larvae were changed ten times to fresh diet. Developmental time averaged 99 days, ranging from 78 to 127 days. Only 11 percent of the larvae tested on the diet completed their development because of severe pathogen problems.

26. The developmental time of the F_2 generation was longer than

that of the F_1 generation. Males in both the F_1 and F_2 generation had a slightly shorter total developmental period than the females.

Weight of pupae

27. Pupal weights were compared among field-collected (wild), F_1, and F_2 laboratory-reared generations (Table 3). Field-collected individuals also included prepupae or larvae that pupated in the laboratory without having fed on the diet. Weighing of the pupae represented a method to determine if diet-fed individuals differed from field-collected individuals.

28. The Duncan's multiple range test was used to compare mean differences. There was a significant increase in weight of both sexes from the field and F_1 generations to the F_2 generation. Although some single sex comparisons were not significantly different, no significant decrease with time was noted between any of the comparisons. These results indicated that diet-fed individuals were as large or larger than those collected in the field.

Mating Studies

29. Moths emerged 11 to 13 days after pupation. Studies involving two or more males per female or vice versa indicated that males and females only mate once. Dissections of mated females revealed one spermatophore per female. About 70 percent of the total oviposition occurred on the first and second nights after placing the moths in cages for mating.

30. Comparisons of field, laboratory, and field × laboratory matings are listed in Table 4. Eclosion rate was highest in matings involving field-collected individuals. However, variations of egg handling procedures accounted for some eggs failing to hatch in all matings. The average number of eggs per female for the F_1 laboratory colony was about equal to the field-collected individuals. Only two matings have been attempted involving the F_2 generation adults and these indicate a low fecundity rate with a large percentage of infertile eggs. The responses of wild males and laboratory-reared

females were tested in two matings. Fertile eggs were produced, indicating that laboratory-reared individuals can mate with wild individuals.

PART IV: DISCUSSION

31. Field studies on *Arzama densa* suggest that two to three generations occur annually. Natural enemies play an important role in checking the populations of *Arzama* during certain times of the year. Possibly, natural populations could be augmented with laboratory individuals but only during July and the beginning of August when parasites and predator populations are low. The use of *Arzama densa* to control waterhyacinth may have potential in countries where it is free of its natural enemies (Center 1976).

32. A diet was needed to rear sufficient quantities of *Arzama densa* for a possible rearing program. However, the question of reduced fecundity arises with the F_2 laboratory colony. Personal communication with Dr. Pritham Singh, author of the book, Artificial Diets for Insects, Mites and Spiders (1977, Plenun Data Company, N. Y.), brought this possibility to the authors' attention. Perhaps the pupal weight comparisons provide clues to the fecundity problem. The comparisons indicate a trend towards heavier individuals as they progress to the next laboratory generation. The diet may contain an excess of nutritional constituents as compared to the normal plant food for the insect.

33. Preliminary tests, started after this report, indicate a pheromone to be the agent causing the reduced fecundity. Females in a crowded situation refused to mate due to an inhibition response. Isolated females with males mated normally and resulting eggs were 95 percent fertile.

PART V: RESEARCH PLANS

34. Several more trips are planned to Venice, La., to collect more information about *Arzama densa* and associated parasites and predators. Further modifications of the diet will include reductions of certain constituents.

35. Studies are under way on the possibility of combining *Arzama densa*'s effectiveness with a herbicide. Immersing the larvae in herbicide (suggested by Dr. P. C. Quimby, Jr.) or combining a herbicide or pathogen in the diet will be evaluated.

36. While on a trip to Venice, La., the authors collected the weevil, *Sphenophorus* sp. (Coleoptera: Curculionidae). Larvae and adults were present in the basal portions of dry, shoreline plants, mining the roots and growing meristem areas of waterhyacinth. Larvae placed on the waterhyacinth diet completed their development. The authors are presently trying to establish a colony of these weevils to evaluate their potential for biological control.

37. The introduced moth, *Sameodes albiguttalis* (Warren) (Lepidoptera: Pyralidae), will be evaluated on various diets. A successful diet would have value in a rearing program. Possibly, this moth in combination with a herbicide or a pathogen will have a synergistic effect against waterhyacinth.

38. Obviously, an integrated method is needed to control waterhyacinth, possibly involving several biological organisms combined with other types of control methods.

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Table 1

A Meridic* Diet for

Rearing *Arzama densa*

Constituents	Amount of Vanderzant-Adkisson Insect Diet/ Modified** (400 g)
White 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
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% ethanol (ETOH))	18 ml
Potassium hydroxide (4.0 M)	18 ml
The following suspended in 300 ml distilled water:	
Ascorbid acid	15 g
Aureomycin	0.5 g
Vanderzant vitamin mix/modified**	9 g
Vitamin A acetate	0.5 g
Distilled water	4000 ml

* A diet chemically defined except for one substance (waterhyacinth powder).

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

Table 2

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

Trip No.	Date	*Arzama densa*			Parasites and Diseases of *Arzama densa* Larvae			Total % Mortality	Total % of Larvae Completing Development	
		Larvae	Pupae	Egg Masses	Diptera	Hymenoptera	Virus		Fresh Cut Plants	Specified Diet
1	11/17 to 19, 1978	46	1	0	11	15	0	56.5	0	--*
2	12/4 to 7, 1978	169	0	0	45	14	110	100	0	--
3	1/22 to 24, 1979	29	1	0	12	1	2	53.5	0	30.7
4	3/20 to 22, 1979	2	0	0	2	0	0	100	0	0
5	7/9 to 10, 1979	36	4	1	2	2	0	11.1	--	81.2
6	7/30, 31 to 8/1, 1979	136	10	6	3	10	0	9.5	--	78.0

* Dash means no individuals tested.

Table 3

Mean Pupal Weights (in milligrams) of Each Sex from Field-Collected, F_1, and F_2 Laboratory-Reared Individuals

Sex	Field Collected	F_1	F_2
Male	301^b (SD ± 24)	307^b (SD ± 54)	387^a (SD ± 71)
Female	381^b (SD ± 46)	441^a (SD ± 73)	486^a (SD ± 71)
Both sexes	341^b (SD ± 54)	374^b (SD ± 93)	436^a (SD ± 85)

Note: Same letter indicates no significant difference ($\alpha \leq 0.05$).
Standard deviations are in parentheses.
Pupae were placed in an incubator for 24 hr at 24° to 26°C and 70 percent RH.

Table 4

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

	No. Matings	Percent of Eggs			Total Eggs	$\bar{X}$ Eggs/♀
		Eclosed	Not Eclosed	Infertile		
Field (wild)	13	82.7	15.1	2.0	3980	306
F_1 laboratory	6	65.8	30.0	4.1	1924	321
F_2 laboratory	2	15.2	0	84.8	138	69
F_1 ♀ × wild ♂	1	3.4	1.7	94.9	179	179
F_2 ♀ × wild ♂	1	56.6	40.8	2.6	309	309