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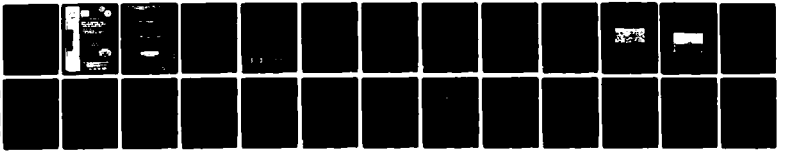

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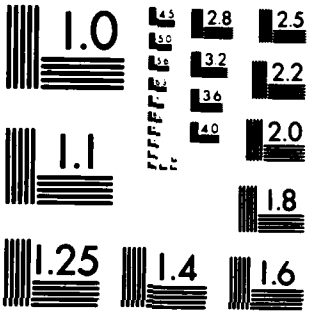

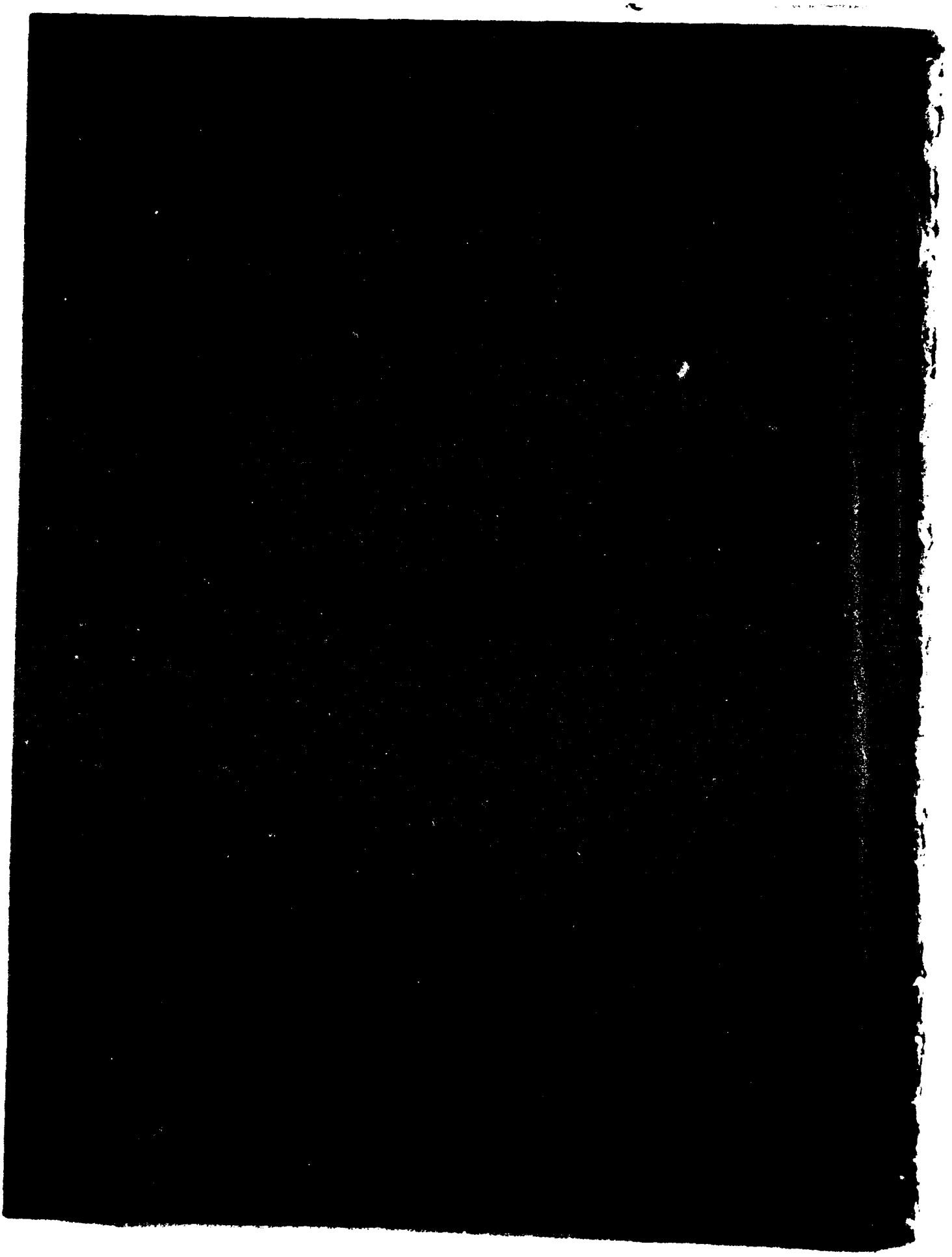

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This research was conducted to evaluate the use of Arzama densa as a biological agent for the management of waterhyacinth. Third instar larvae were released at different rates, and their impact was monitored over a 4-month period. A statistically significant reduction in biomass was noted in the experimental plots along with an increase in the production of daughter plants. The treatment did not reduce surface coverage.

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Preface

Funds for this study were provided to the Aquatic Plant Control Research Program (APCRP) through the Department of the Army Appropriation No. 96X3123, "Operations and Maintenance General," by the U. S. Army Engineer District, New Orleans.

The work was performed by Dr. Alfred F. Cofrancesco, Jr., Dr. Dana R. Sanders, Sr., Mr. Russell F. Theriot, and Mr. Edwin A. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), U. S. Army Engineer Waterways Experiment Station (WES). This report was prepared by Dr. Cofrancesco. Additional assistance was provided by Drs. Ronald G. Baer and Paul C. Quimby, Jr., of the U. S. Department of Agriculture, Science and Education Administration (USDA-SEA), Southern Weed Science Laboratory, Stoneville, Miss.

This study was conducted under the direct supervision of Dr. Hanley K. Smith, Acting Chief, Wetlands and Terrestrial Habitat Group, and the general supervision of Dr. Conrad J. Kirby, Jr., Chief, ERD, and Dr. John Harrison, Chief, EL. Manager of the APCRP at WES was Mr. J. Lewis Decell.

Commander and Director of the WES during this study was COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

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Contents

List of Figures

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	4046.873	square metres
inches	25.4	millimetres

IMPACT OF AUGMENTED FIELD POPULATIONS OF *ARZAMA DENSA* LARVAE ON WATERHYACINTH

Introduction

Background

1. Waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) has caused significant problems since its introduction into the United States in the 1880's. Since 1970, the waterhyacinth population in Louisiana has averaged more than one million acres.*,** These floating aquatic plants continue to pose a severe threat to navigation, fisheries, and recreational use of the waterways.

2. Traditionally, many chemical and mechanical methods have been employed for the management of aquatic plants. More recently, biological methods have been developed and implemented for management of selected aquatic plants (Coulson 1977). Two insect species, *Agasicles hygrophila* (Selman and Vogt) and *Vogtia malloi* (Pastrana), have been used to severely impact and provide the desired levels of management of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb) in the southeastern United States.

3. At present, several exotic and native species are being evaluated as potential agents for the biological control of waterhyacinth. *Neochetina eichhorniae* (Warner), the mottled waterhyacinth weevil; *Neochetina bruchi* (Hustache), the chevroned waterhyacinth weevil; and *Sameodes albiguttalis* (Warren), the Argentine waterhyacinth moth, are exotic insect species that have undergone extensive host-specificity studies prior to their release on waterhyacinth in the United States. Two native species have also been found to significantly impact waterhyacinth: a moth, *Arzama densa* (Walker), and a leafspot fungus, *Cercospora rodmanii* (Conway).

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* Personal Communication, Donald Lee, 1981, Louisiana Department of Wildlife and Fisheries, Baton Rouge, La.

** A table of factors for converting U. S. customary units of measurement to metric (SI) is presented on page 4.

4. This study focuses on *A. densa*, a native, North American noctuid moth. Prior to the introduction of waterhyacinth into the United States, the larvae fed on pickerelweed (*Pontederia cordata* L.), but now also utilize waterhyacinth as a food source. Larvae tunnel into the petioles and crown of the waterhyacinth plant and produce extensive feeding damage (Center 1976). However, the impacts of *A. densa* on the waterhyacinth populations have been limited and unpredictable because naturally occurring populations of *A. densa* are so highly parasitized in the fourth and seventh instar that large populations of *A. denza* seldom occur (Vogel and Oliver 1969).

5. Both laboratory (Baer and Quimbly 1980) and small-scale field studies (Center 1976) indicated that *A. densa* larvae will impact waterhyacinth. These studies led to the formulation of a plan for employing the larvae on a large scale as a biological control agent of waterhyacinth. Large numbers of larvae of the same instar would be released on a selected waterhyacinth mat in early spring. It was hypothesized that such a release would allow the larvae to develop without a corresponding increase in the parasite population. Under natural conditions, a parasite population will increase slowly in response to an increase in the host population. Since the *A. densa* larvae are more susceptible to parasitism during the fourth and seventh instars, mass releasing a synchronous population would enable a greater percentage of *A. densa* larvae to survive to the pupal stage.

Purpose and objectives

6. The purpose of this research was to evaluate the use of *A. densa* as a biological agent for the management of waterhyacinth. The objectives were:

a. To evaluate the impacts of *A. densa* on waterhyacinth through the augmentation of field populations by the mass release of laboratory-reared larvae.

b. To determine the population levels of *A. densa*.

Materials and Methods

Larvae

7. *Arzama densa* larvae used in this study were obtained from the

U. S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Southern Weed Science Laboratory, Stoneville, Miss. Larvae were collected from the field and taken through several generations on an artificial diet developed at the laboratory. Eggs were collected from the laboratory population in early April and larvae were reared to the third instar (Baer and Quimby 1980). On the day prior to release, the third instar larvae were allowed to tunnel into freshly cut petioles for transportation to the site (Figure 1).

Figure 1. Waterhyacinth petioles into which *A. densa* larvae have tunneled

Site selection

8. Three 0.1-ha plots were established in a canal that paralleled U. S. Highway 61 at Norco, La. (Figure 2). Forty thousand larvae were released at the site by dispersing petioles containing the larvae throughout the site by hand (Figure 3). Ten thousand larvae were released on the low rate plot and thirty thousand larvae were released on the high rate plot. The remaining plot was used as a control. To ensure that the same plants were maintained within the plots throughout the study, a series of 4-in. polyvinyl chloride (PVC) booms were placed across the canal.

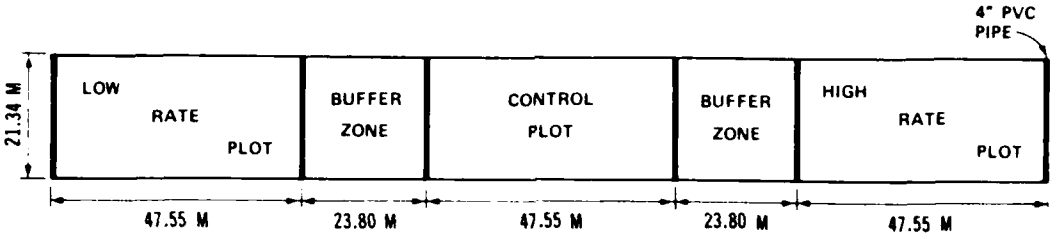

Figure 2. Arrangement of *Arzama* test plots

Figure 3. Dispersing of petioles containing *Arzama* larvae throughout a test plot

Sampling procedure

9. On each sampling date (Table 1) the following procedures were used for sampling the plots. Ten 0.25-m^2 frames were randomly selected from each of the three plots. The locations of the frames within each plot were determined by using an ordinate system and a random numbers table. There were 156 possible sampling points on the x-axis, while there were 70 possible sampling points on the y-axis for each plot. A combination of two values falling within the ranges of the x-axis and the y-axis were selected for each of the 10 frames. If the values of two frames in the same plot overlapped, the set of coordinates was dropped and another set was selected. All plants with crowns inside each frame were collected for examination. To prevent excessive disturbance to the waterhyacinth mat, watershoes were employed to collect the plant samples.

10. Visual estimates of the total surface area covered by waterhyacinth were also conducted for each plot on each sampling trip. Estimates were made independently by three individuals, based on the amount of open water in relation to the length (47.55 m) and width (21.34 m) of each plot. The three estimates were averaged to give a mean estimate of the surface area covered by waterhyacinth.

Processing of Plant Samples

Waterhyacinth data

11. The height of the center plant from the waterline was recorded in each frame prior to the removal of any plants. The total number of plants and daughter plants inside each frame was recorded. A daughter plant was considered to be any plant that did not possess functional roots and which was attached to the parent plant by a stolon. Biomass (wet weight) was determined by weighing the plants from each frame after allowing 1 min for excess water to drain. The number of petioles of two randomly selected plants from each frame was recorded.

Arthropod data

12. All plants within each frame were examined for arthropods or

Results

Waterhyacinth

15. Biomass. Means for waterhyacinth biomass for all posttreatment sampling dates in the treatment plots are presented in Table 2. Application of Duncan's multiple range test revealed that the waterhyacinth biomass in each plot was significantly different from the other, with the control having the highest biomass, the low rate plot having the next, and the high rate plot having the least biomass when comparing all posttreatment sampling periods.

16. Waterhyacinth biomass was also compared between plots for each sampling period using ANOVA and Duncan's multiple range test. One month after the release of *A. densa*, the two treated plots had significantly lower biomass values than the control plot (Figure 4). A significant difference in biomass continued between the high rate plot and the control for the duration of the study. During the August and September sampling periods, the biomass of the low rate plot increased and was not significantly different from the control plot.

17. Density. Waterhyacinth densities in the three plots during the study are presented in Figure 5. Prior to treatment, the density in the low rate plot was significantly lower than in the other two plots. An ANOVA indicated a significant reduction in the density in the high rate plot as compared to the control plot for the sampling periods following the application of the larvae (Table 3).

18. Daughter plants. In the first sampling period after the release of the larvae, there was a significantly greater number of daughter plants present in the treated plots as compared to the control plot (Figure 6). This relationship continued between the high rate plot and the control plot throughout the study. The number of daughter plants in the low rate plot was lower than in the control plot only for the July and August sampling periods.

19. Height. Plant heights between plots were very similar throughout the study. Height generally increased through August and then dropped drastically in September for all three plots. However,

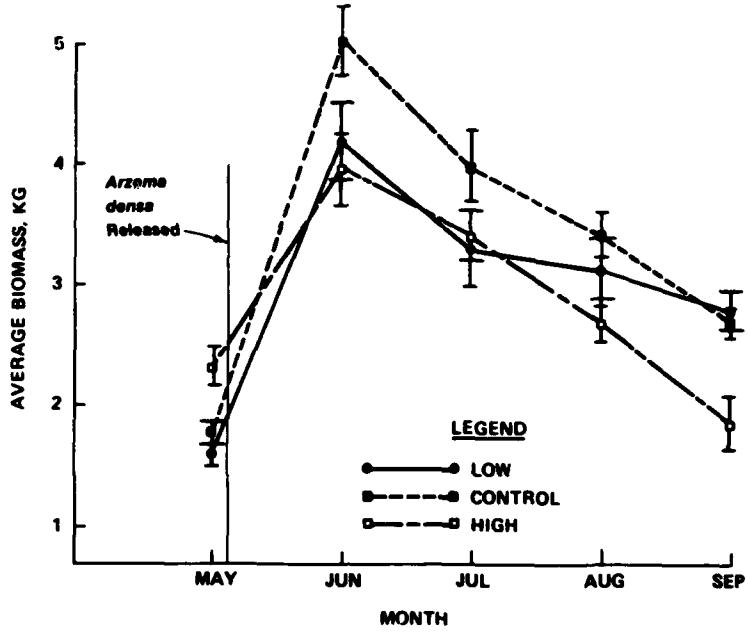

Figure 4. Average biomass of waterhyacinth for all test plots

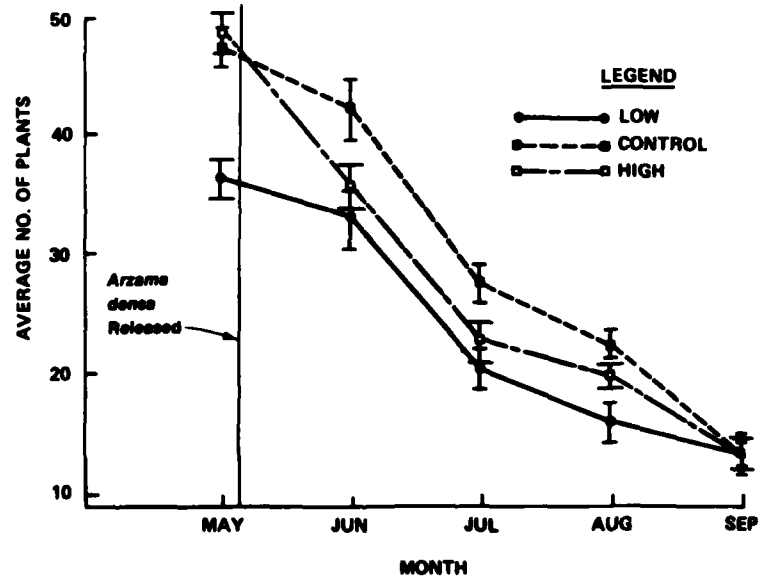

Figure 5. Average number of waterhyacinth plants for all test plots

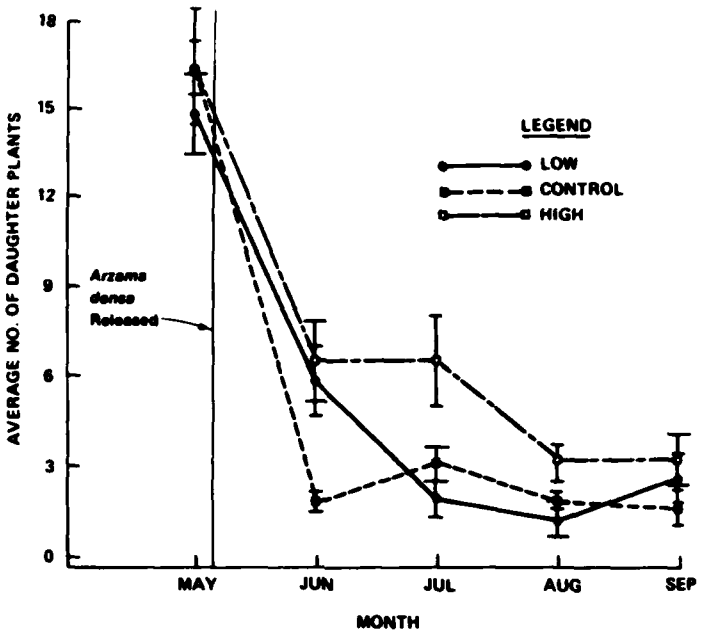

Figure 6. Average number of daughter plants for all test plots

significantly lower values were noted for the high rate plot during the July and August sampling periods when compared to the other two plots (Figure 7).

20. Petioles. In May, the average number of petioles on plants varied considerably between plots (Figure 8). Initially, the low rate plot had the largest number of petioles per plant. An ANOVA through time conducted on the low rate plot indicated no significant change in the number of petioles per plant (Table 4). The same analyses conducted on the control plot and the high rate plot indicated a significant increase through time in the number of petioles per plant. The increase noted in these two plots appeared to follow a similar pattern.

Surface area

21. The percentage of surface water covered by waterhyacinth did not change throughout the entire study. On each sampling trip, the percentage of surface water covered was 100 percent for all plots. No

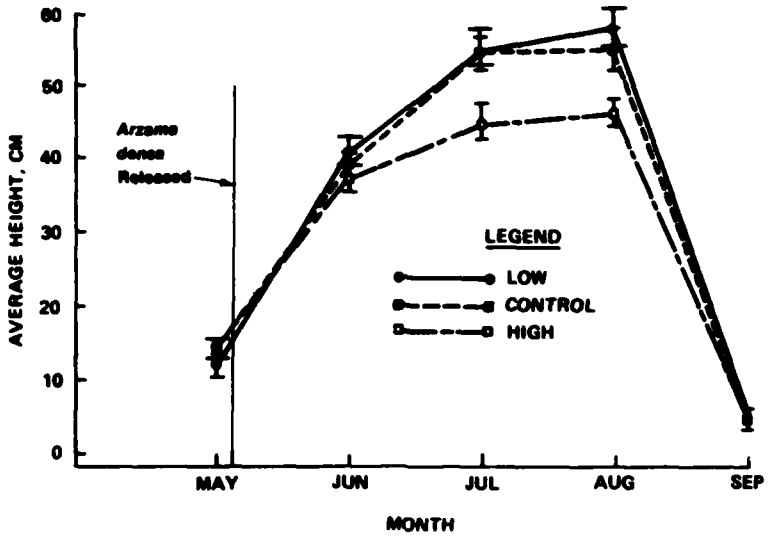

Figure 7. Average height of waterhyacinth for all test plots

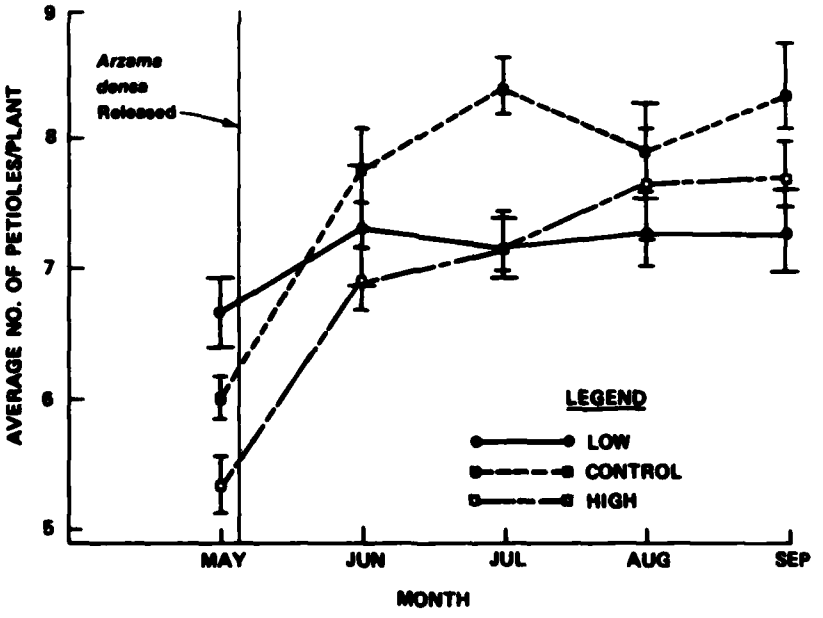

Figure 8. Average number of petioles/waterhyacinth plant for all test plots

open water areas were ever noted for any of the plots, even during periods of low density.

Arzama densa

22. The proportion of plants damaged by *A. densa* on each plot is presented in Figure 9. After the release of the *Arzama* larvae, the two treatment plots had a significantly higher number of plants with larval damage than the control plot. Increased levels of damage were apparent in the high rate plot through the August collection. The low rate plot maintained a low level of *Arzama* damage throughout most of the study, increasing only slightly in September. Except for July, the proportion of damage in the control plot remained low. Collections in the field during July for Center (1976) and Baer and Quimby (1980) also indicated natural increases in the population of *Arzama* larvae.

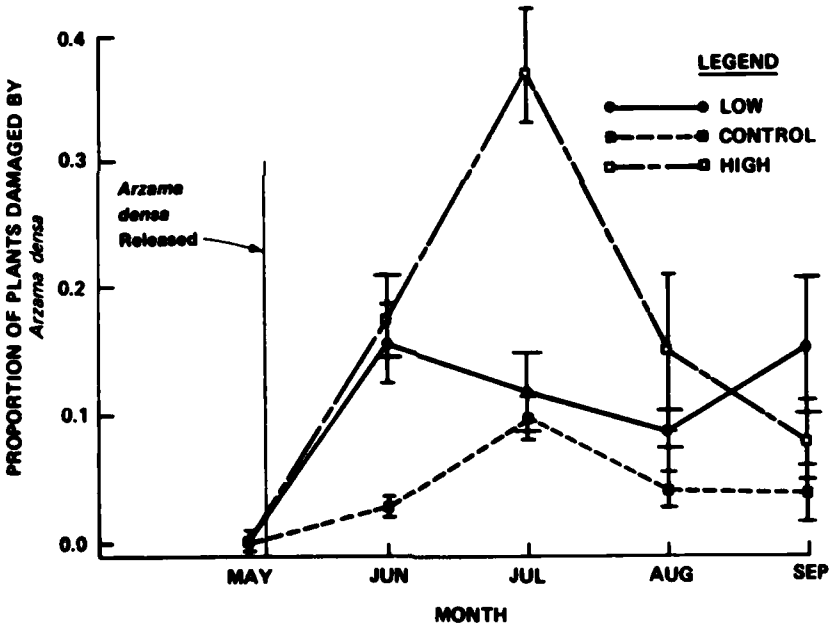

Figure 9. Proportion of waterhyacinth plants damaged by *A. densa* larvae for all test plots

23. The actual *A. densa* larvae collected on the site did not compare very closely to the changes in the proportion of damaged plants. No apparent buildup in larval population was noted during the time that the second generation should have been developing on the plots (Table 5),

even though there was a slight increase in the proportion of plants damaged on the low rate plot.

Neochetina

24. Larval damage. Since the early instars of *Neochetina* larvae are often difficult to find, it was determined that calculating the proportions of plants having larval damage would be a more useful indication of impact. The high rate plot and the control plot exhibited similar trends in the proportion of plants damaged by *Neochetina* larvae (Figure 10). The low rate plot also exhibited an increase in the proportion of plants damaged, but the increase was not as rapid as in the other two plots. A significant difference was noted in the low rate plot when compared to the other two plots for the June and July collections.

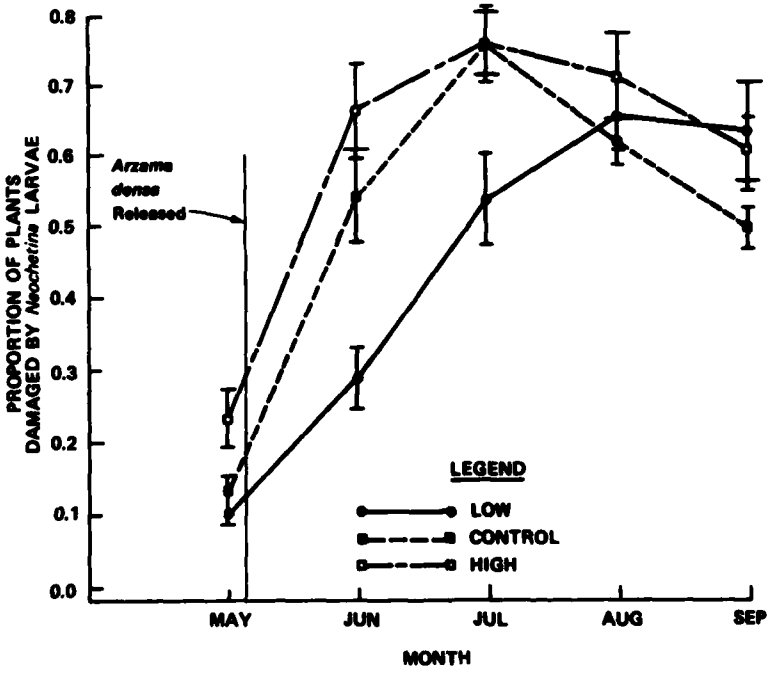

Figure 10. Proportion of waterhyacinth plants damaged by *Neochetina* larvae for all test plots

25. Adult feeding. The feeding scars on the pseudolamina produced by adult *Neochetina* increased through time in all three plots (Figure 11). An ANOVA performed for each sampling date indicated that

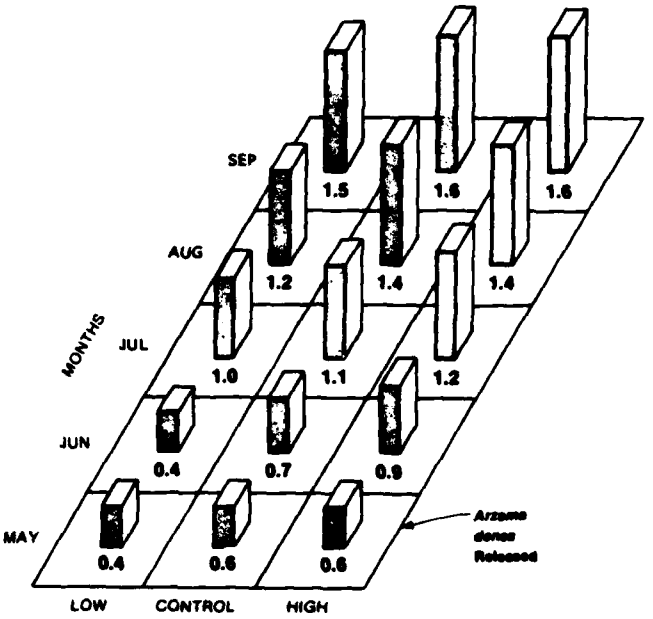

Figure 11. Average index of *Neochetina* feeding scars for all test plots

significant differences in the adult *Neochetina* feeding activity of the three test plots occurred only in June, when the low rate plot had significantly lower levels of adult feeding than the other plots (Figure 11).

Pathogen damage

26. Pathogen damage increased through time in all three plots but no major differences were noted between plots for any sampling period (Figure 12). No pathogen that was known to cause significant damage to waterhyacinth was isolated. The only damage the plants received was from normally saprophytic organisms whose impact increased uniformly across plots during the senescent period (August and September).

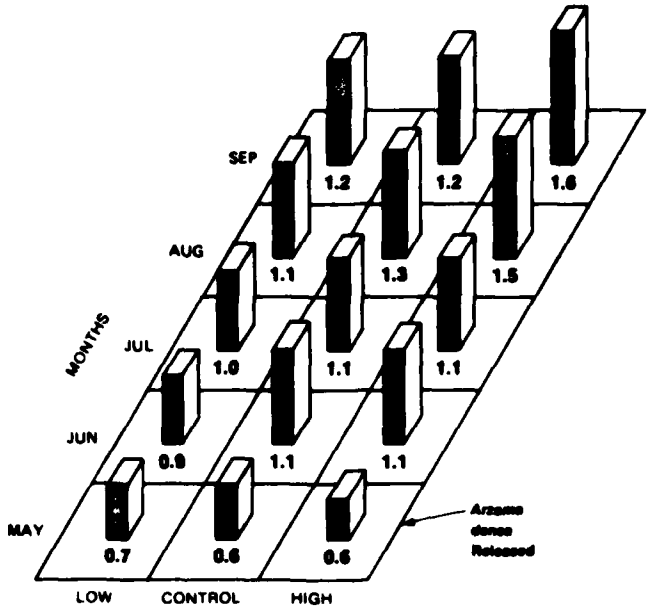

Figure 12. Average disease index values for all test plots

Discussion

27. This study revealed that, initially, *A. densa* larvae impacted waterhyacinth plants when mass released. A significant reduction was found in biomass between the test plots and the control plot 1 month after release of the larvae. Additionally, the proportion of plants damaged by larvae increased significantly in the high rate plot through July.

28. Plant density changed through time in all plots, with a significant difference being noted between the high rate plot and the control plot. The larval impact on plant densities was not apparent in September. There were no differences in plant densities of the plots; however, morphological differences such as number of petioles were noted. Plants in the control plot had more petioles than plants in high rate plots, which contributed to the differences in biomass noted between plots in September.

29. The production of daughter plants closely correlated with the

proportion of plants damaged by *A. densa* larvae. The larger number of daughter plants in the test plots in June was attributed to the presence of the *A. densa* larvae, which tunneled into and destroyed the crown of the plants. The resultant destruction of the crown of the plants stimulated the production of daughter plants. The decline in daughter plant production after July suggested that sufficient numbers of larvae were not present to stimulate daughter plant production.

30. Estimates of the total surface area covered by waterhyacinth indicated that, even at the lowest plant densities, no visual impact was noted. A smaller plant-to-insect ratio is probably needed to achieve visual damage.

31. The released larvae progressed through their life cycle on the test plots. When the organisms reached the adult stage (July), a reduction in the proportion of damaged plants was observed. No second-generation increase of the *A. densa* population was noted. The large acreages of waterhyacinth surrounding the test plots afforded a good location for the dispersal of the adult population emerging from the test plots, which could account for the lack of a second-generation buildup of *A. densa* on the test plots.

32. The increase in the proportion of plants having *Neochetina* larval damage was similar between the high rate plot and the control plot. An increase was also noted in the low rate plot, but this was a more gradual increase. Although there were some differences in the *Neochetina* larval damage between plots, it did not alter the uniformity of the plots, and thus had no effect on the results of this study.

33. In monitoring the adult feeding scars of *Neochetina*, only minor differences were noted between plots on individual sampling trips. In general, the feeding scars increased on all plots through time. This would indicate that the *Neochetina* population was building during the sampling period. This buildup in the *Neochetina* population was also observed in the proportion of larval damage, which reflected the general seasonal trend noted in other studies dealing with waterhyacinth.

34. The extent of pathogen damage was generally low throughout the study. The levels of pathogen damage did not significantly impact

any particular plot more severely than another; thus, any differences noted between plots were not attributable to pathogen damage.

Conclusions

35. Based on results obtained in this study, the following conclusions have been drawn:

a. Although *A. densa* larvae applied at a ratio of one larvae per six plants produced measurable reductions in plant biomass, this treatment rate did not produce a significant change in the surface coverage of waterhyacinth on treated sites.

b. The release of a large number of *A. densa* larvae on waterhyacinth populations does not ensure that an increase in the population of *A. densa* will occur in subsequent generations. Without an increase in the population of larvae, the surface coverage of waterhyacinth will continue to cause significant problems.

c. Modifications of the application procedure of *A. densa* will be necessary to achieve the desired level of waterhyacinth control. It will be necessary to increase the insect-to-plant ratio in order to obtain a significant reduction in the surface coverage of waterhyacinth.

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Table 1

Sampling Schedule

6 May 1980	Pretreatment
2 June 1980	Posttreatment
14 July 1980	Posttreatment
5 August 1980	Posttreatment
8 September 1980	Posttreatment

Table 2

Mean Waterhyacinth Biomass of Treatment Plots During the Study

Mean*	N	Plot
3.79[a]	40	Control
3.35[b]	40	Low rate
2.98[c]	40	High rate

* Means followed by the same letter are not significantly different at P >0.05 according to Duncan's multiple range test.

Table 3

ANOVA for Densities of the High Rate and Control Plots

Source	df	SS	F-Value
Months	3	6782.45	80.76*
Plots	1	245.00	8.75*
Interactions	3	112.50	1.34 ns
Error	72	2015.60	
Total	79	9155.5[illegible]	

Note: ns = nonsignificant.
* Significant at 0.05 level.

Table 4

ANOVA of Petioles

Source	df	SS	F-Value
Months	3	6.21	0.95 ns
Plots	2	35.17	8.06*
Interactions	6	9.42	0.72 ns
Error	108	235.75	
Total	119	286.55	

Note: ns = nonsignificant.
* Significant at 0.05 level.

Table 5

Number of *Arzama densa* Collected in Ten 0.24-m^2 Frames

Trip	Control	Low Rate	High Rate
May	0	0	0
June	2	9	10
July	1	0	1
August	0	1	2
September	0	0	1

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Final report.
"Prepared for U.S. Army Engineer District, New Orleans and Office, Chief of Engineers, U.S. Army."
At head of title: Aquatic Plant Control Research Program.
Bibliography: p. 20.

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Cofrancesco, Alfred F.
Impact of augmented field populations of Arzama : ... 1982. (Card 2)

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