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ORGANISMS IMPACTING WATERHYACINTH IN THE PANAMA CANAL

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for the presence of arthropods and plant pathogens. Three arthropod species, *Neochetina eichhorniae* Warner, *Cornops* sp. Bruner, and *Orthogalumna terebrantis* Wallwork, were abundant on all but two sites on all sampling dates. *Orthogalumna terebrantis* was rare on two of the sites, which indicated a need for an effort to distribute this species to all areas of the Canal. One plant pathogen, *Acremonium zonatum* (Saw.) Gams, was abundant on all sites on all sampling dates. None of the species found on waterhyacinth in the Canal exerted significant pressure on the growing points of the plants. Consequently, it was recommended that additional species be introduced into the Canal for the biocontrol of waterhyacinth with top priority given to those species (e.g., *Sameodes albiguttalis* (Warren)) that feed on the newest tissues of the plant. Other species that should be considered for introduction include one plant pathogen, *Cercospora rodmanii* Conway, and two insect species, *Arzama densa* Walker and *Neochetina bruchi* Hustache.

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PREFACE

This study was sponsored by the Panama Canal Commission (PCC), Balboa Heights, Republic of Panama, and the Office, Chief of Engineers, U. S. Army, through the Corps of Engineers' Aquatic Plant Control Research Program (APCRP). The work was initiated in January 1978 at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The work was performed and the report written by Dr. Dana R. Sanders, Sr., and Messrs. Russell F. Theriot and Edwin A. Theriot of WES. Support from the PCC was provided by Messrs. C. Von Chong, F. D. Halverson, and W. P. Murdoch, Jr.

The APCRP is conducted by the Environmental Laboratory at the WES, of which Dr. John Harrison is Chief. The project reported herein was under the general supervision of Dr. Conrad J. Kirby, Jr., Chief, Environmental Resources Division, and the direct supervision of Dr. Hanley K. Smith, Wetland and Terrestrial Habitat Group. Mr. J. Lewis Decell is Manager of the APCRP.

Commanders and Directors of the WES during the conduct of this study and preparation of the report were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

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ORGANISMS IMPACTING WATERHYACINTH IN THE
PANAMA CANAL

PART I: INTRODUCTION

Background

1. Floating waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] first appeared in the Panama Canal in 1920 and has been a significant problem aquatic plant in portions of the Panama Canal for nearly 60 years.* The problem is of greatest concern in the Chagres River (Figure 1), with its many backwater areas where waterhyacinths proliferate to form large mats. During periods of high water levels, these mats flush out into the main river channel, thereby producing large rafts of plants that block the river channel to navigation. Initially, these rafts of plants moved directly into the Panama Canal, where shipping channels were temporarily blocked. To alleviate this problem, the Dredging Division of the Panama Canal Company (PCC), now the Panama Canal Commission, constructed a permanent boom across the Chagres River 0.8 km upstream from its confluence with the Panama Canal. River currents now funnel the rafts of waterhyacinths into a lagoon where they are removed by a large rake operated from a Sauerman slack-line cable (Hearne 1966). This mechanical system has the capacity to remove an average of 650 tons** of plants per day at an approximate cost of \$3.75/ton.+ Although it is very efficient at removing plants from the river and is operational only 20 percent of each year, the system must be used on an annual basis because it does not attack the source of the problem. Waterhyacinths are produced continuously in the backwater

* Personal Communication, C. Von Chong, 1980, Panama Canal Commission, Balboa Heights, Republic of Panama.

** To convert tons to kilograms multiply by 907.1847.

+ Personal Communication, W. P. Murdoch, Jr., 1980, Panama Canal Commission, Balboa Heights, Republic of Panama.

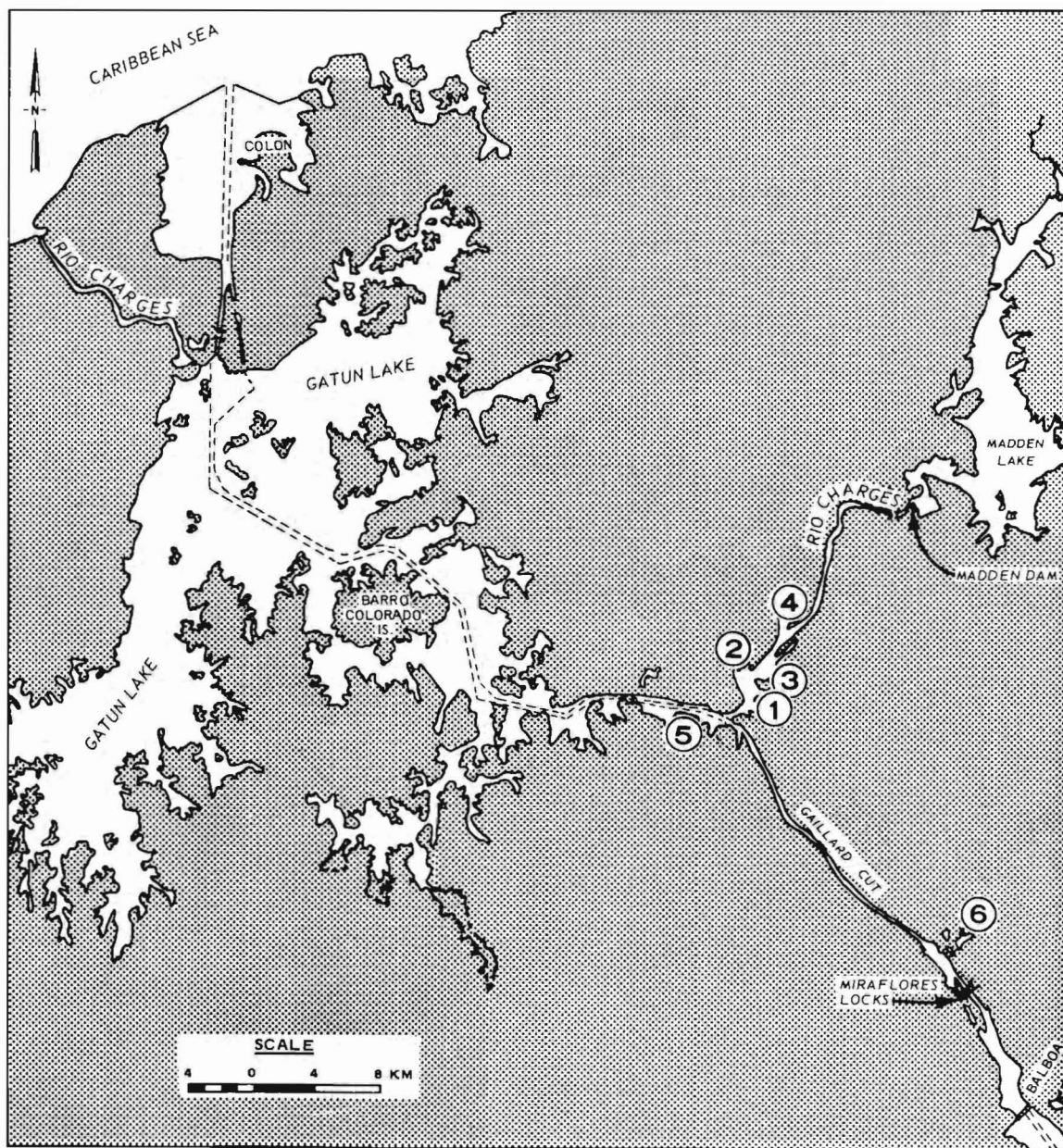


Figure 1. Map of portion of the Panama Canal in which the study was conducted. Study sites are numbered

areas and are moved by river currents to the permanent boom.

2. In 1977, the PCC asked the Aquatic Plant Control Research Program (APCRP) of the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., for assistance in finding more efficient methods for controlling problem aquatic plants in the Panama Canal. The APCRP had been actively pursuing research for the control of waterhyacinth through the use of biological agents; at that time, two insects and one plant pathogen were available or approaching readiness for use on an operational scale. After a cursory inspection of the waterhyacinth problem in the Panama Canal in 1977, WES personnel informed the PCC that the pyralid moth *Sameodes albiguttalis* (Warren), the weevil *Neochetina bruchi* Hustache, and the plant pathogen *Cercospora rodmanni* Conway should be considered for introduction onto waterhyacinth in backwater areas of the Panama Canal. The successful implementation of these biocontrol agents would provide the PCC with at least a partial solution to the waterhyacinth problem, and would provide the APCRP with much-needed data for developing efficient strategies for managing waterhyacinth with biocontrol agents. To determine the effect of any introduced organisms on the waterhyacinth population and to obtain the data needed to develop management strategies, it was deemed necessary to conduct a baseline study of organisms already present on waterhyacinth in the Panama Canal and their impacts on its population.

Purpose and Objectives

3. The purpose of this study was to determine the organisms impacting waterhyacinth in the Panama Canal prior to the introduction of biocontrol agents, and to quantify their effects on the plant population. The purpose of this report was to present the procedures used, results obtained, conclusions drawn, and recommendations resulting from this study.

4. The specific objectives of the study were:

- a. To determine the complement of organisms that impact waterhyacinth in the Panama Canal.

- b. To assess spatial and temporal variations in the abundance and impacts of these organisms on waterhyacinths in the Panama Canal.

PART II: PROCEDURE

Selection of Study Sites

5. Following an inspection of the areas of the Panama Canal in which waterhyacinth occurred, six study sites were selected in 1978 (Figure 1). Two important criteria applied in the selection of sites were: (a) the waterhyacinth population on each site must be stable and not subject to movement from the site by currents, and (b) sites were to be selected from the various areas of the Panama Canal that were representative of the waterhyacinth population. The study sites were as follows:

- a. Site 1 - Manatee Lagoon. Located on the eastern shore of the Chagres River +600 m north of the permanent boom, this 3-ha cove was heavily infested by waterhyacinths, which were retained in the cove by a temporary boom across its entrance.
- b. Site 2 - Nevada. Located in a 2-ha cove approximately 0.5 km upstream from Manatee Lagoon on the west side of the river, this site was isolated from the primary flow patterns of the river. Extensive areas of surface-matted hydrilla (*Hydrilla verticillata* Royle) also restricted the movement of waterhyacinths out of the cove.
- c. Site 3 - Las Cruces. This site, located directly across the river from the Nevada site, was in an abrupt bend in the river approximately 6 ha in size that was heavily infested by waterhyacinth. The plants were maintained in the area by interlocking with aquatic plant species that were rooted on the site.
- d. Site 4 - Stump Lake. This site was located in the backwaters of a large embayment approximately 3 km upstream from Site 3. Waterhyacinths on this site occurred primarily as dense fringes from a wetland community dominated by *Pontederia* spp. Large circular colonies of waterhyacinth also occurred on this site, held in place by surface-matted hydrilla.
- e. Site 5 - Dump 4-1/2. Site 5 was located approximately 2 km west of Gamboa in an area where sediment dredged from the ship channel had been deposited. It was a 3-ha pool, separated from the main body of Gatun Lake by a levee constructed to permit use of the pool as a nursery area for white amur (*Ctenopharyngodon idella* Val.). A combination

of the levee across the pool, flow patterns of the Chagres River, and the permanent boom across the river effectively prevented the exchange of waterhyacinths from Sites 1-4 with those found at this site.

- f. Site 6 - Red Tank Lake. This site, located approximately 12 km east of Gamboa, was a shallow lake separated from the Panama Canal by a highway. The only outlet from the lake was through a large culvert that drained water into Miraflores Lake. The major concentrations of waterhyacinth at this site occurred in shallow backwater areas away from the culvert, and were held in place by surface-matted submersed vegetation, primarily hydrilla and brittle naiad (*Najas minor* All.).

Collection of Plant Samples

6. A total of 24 waterhyacinth plants were collected from each study site on each of the following dates:

<u>Date</u>	<u>Season</u>
January 1978	Dry
April 1978	Dry
May 1979	Rainy
October 1979	Rainy
January 1980	Dry

On each site, four areas representative of the waterhyacinth population were visited and six plants were randomly selected from each area. To preclude sampling bias, the bow of the boat was pushed into the waterhyacinth mat at each location, and a small styrofoam ball was thrown into the waterhyacinth mat in front, and to either side, of the boat. The two plants nearest the ball were collected. The six plants collected from each of the four locations at each site were placed in plastic bags and transported to Gamboa, where they were maintained in an air-conditioned building until processed.

Processing of Plant Samples

Waterhyacinth

7. For each bag of plants, the total number of plants was

recorded. The number of daughter plants was determined by subtracting the number of plants collected from each area (six) from the total number of plants present in the bag. In most cases, the daughter plants were small plants attached to stolons, and were not yet floating on the water surface. After the daughter plants were separated from the parent plants, the number of leaves and flower stalks was recorded for each of the six parent plants.

Arthropods

8. Each parent plant was examined for the presence of arthropods. A cursory inspection of the plants revealed that some arthropods (e.g., *Neochetina eichhorniae* Warner) were present in abundance. Consequently, systems were developed to quantitatively assess either the abundance of these species or the extent of damage characteristically produced by the species. The following procedures were used:

- a. *Neochetina eichhorniae*. Commonly known as the waterhyacinth weevil, adults of this species feed on the pseudolamina and larvae tunnel in the petioles and stems. The number of *N. eichhorniae* adults and pupae present on each parent plant was recorded. In addition, feeding scars produced by adult *N. eichhorniae* were estimated by using the following scale:

<u>Rating Category</u>	<u>Range of Category (No. of Feeding Scars/Pseudolamina)</u>
0	0
1	1-50
2	51-100
3	101-200
4	>200

This scale was applied to each leaf of the six parent plants in each bag. Spot checks of actual feeding scar counts were made to ensure adherence to the rating scale.

- b. *Cornops* sp. This species of grasshopper was known to feed on waterhyacinth by excising sections of the pseudolamina or by rasping the epidermis of the pseudolamina. The following rating scale was applied to define the extent of *Cornops* feeding on each leaf of the plants:

<u>Rating Category</u>	<u>Range of Category (Percent of Pseudolamina Removed)</u>
0	0
1	<50
2	>50

- c. *Orthogalumna terebrantis* Wallwork. This arthropod, a galumnid mite, characteristically produced elongate tunnels in the intervacular areas of the pseudolamina of waterhyacinth. The extent of tunnel development produced by *O. terebrantis* was recorded for each leaf of each plant by using the following rating scale:

<u>Rating Category</u>	<u>Range of Category (Percent of Pseudolamina Tunneled)</u>
0	0
1	<50
2	>50

- d. Other arthropods. All petioles of one plant from each bag (four plants/site) were examined on each sampling date, and the number of arthropod larvae present was recorded for each plant. Examination of waterhyacinths collected on the first sampling date revealed numerous penetrations on the petioles of most plants. Because these penetration points represented sites for potential invasion of the plants by facultative pathogens, the number of penetration points was counted for each plant.

Pathogens

9. All plants collected on each sampling date were inspected for symptoms produced by plant pathogens. The following procedures were used to describe these microbial agents and the extent of their impacts on waterhyacinth in the Panama Canal:

- a. *Acremonium zonatum* (Sawada) Gams. Symptoms of the zonate leaf spot fungus (Form Class: Fungi Imperfecti) were found on the plants on all sites on all sampling dates. To define its impact on waterhyacinth, the following rating scale was applied to each leaf of all collected plants:

<u>Rating Category</u>	<u>Range of Category (Percent of Pseudolamina Infected)</u>
0	0
1	<50
2	>50

- b. Other pathogens. Symptoms produced by other microbial agents were described and recorded for each plant.

Beginning in May 1979, sections of leaves with these symptoms were collected and processed using standard microbiological techniques to identify the causal agents, as described in the following section.

Laboratory Analysis for Identification of Plant Pathogens

10. To isolate and identify potential bacterial and fungal pathogens of waterhyacinth, two 16-mm² sections of pseudolaminae were excised from the region bordering each disease symptom and processed as described below.

Bacterial agents

11. One of the collected sections of pseudolamina was macerated in a Petri dish, to which was added 1 ml of sterile saline solution. One loop of the supernatant was streaked onto a plate of nutrient agar (Difco) and incubated at room temperature for 2 days. Although secondary invaders and other microbial contaminants were assumed to be present, the bacterial species associated with the defined symptom was presumed to be the species producing the largest number of colonies on the nutrient agar plate. Because the isolated species could not be brought through U. S. Customs for identification in this country, and no facilities were available in the Panama Canal for performing this task, the API-20 Enterobacteriaceae System (API20E) was used for the characterization of the potential bacterial pathogens. This ready-for-use microtube system, designed for the performance of 23 standard biochemical tests from a single colony of bacteria on plating media, proved to be a simple and accurate method of obtaining the biochemical data required for the identification of Gram-negative bacterial species. A colony of the isolated suspected pathogen was touched with the tip of a sterile wooden applicator. The tip of the applicator was then inserted into a test tube containing 5 ml of sterile 0.85 percent saline solution, after which the microtubes were inoculated with the bacterial suspension and incubated at 30°C for 2 days. Results of the biochemical tests were recorded and used for identification of species by use of a differential chart (Buchanan and Gibbons 1974).

Fungal agents

12. The other collected section of pseudolamina was surface sterilized with 0.5 percent sodium hypochlorite for 2 min and rinsed twice in containers of sterile, deionized water. The section was placed on a potato dextrose agar (PDA) plate and incubated at room temperatures for 7 to 10 days. The fungal agents were identified by colony morphology on PDA and by morphology of hyphae, fruiting structures, and spores on slide cultures. Identifications of fungal agents were made from Barnett and Hunter (1972) and Bessey (1950).

Data Analysis

13. Methods of data analysis and statistical procedures used in the study were as follows:

- a. Waterhyacinth. The average numbers of daughter plants, leaves, and flowering stalks per plant for each site on each sampling date were determined by dividing the total number of respective units per site by 24, which was the total number of plants examined from each site on a given sampling date.
- b. Arthropods. Average numbers of *N. eichhorniae* adults and pupae, and arthropod penetration points per plant for each site on each sampling date, were determined by dividing the total number for each site by 24. The average number of arthropod larvae per plant for each site on each sampling date was calculated by dividing the total number of larvae by 4, which was the number of plants from each site that were examined for arthropod larvae. Average levels of feeding by adult *N. eichhorniae* and *Cornops*, and the degree of tunneling by *O. terebrantis* per leaf for each site on each sampling date, were calculated by dividing the sum of the respective rating categories by the total number of leaves examined.
- c. Pathogens. Average values for the degree of *A. zonatum* damage per leaf for each site on each sampling date were calculated by dividing the sum of the rating categories by the total number of leaves examined.

PART III: RESULTS

Characteristics of Waterhyacinth

Leaves

14. The average number of leaves per plant for each site on each sampling date ranged from 7.4 at Red Tank Lake to 10.5 at Las Cruces, both occurring in January 1978 (Table 1). There was no obvious seasonal variation in leaf number; both the high and low values for sampling dates averaged across treatment sites occurred during the dry season of 1978. However, the substantially higher average for April 1978 as compared with January 1978 indicated that the number of leaves per plant increased during the dry season. Values for sites averaged across time varied from 8.2 at Dump 4-1/2 to 9.5 at Las Cruces. Although all three growth stages of waterhyacinth* were collected, the majority of plants were the Stage III morphotype at sites 1-3. Plants at sites 4-6 were predominantly Stage I and Stage II morphotypes.

Daughter plants

15. The average number of daughter plants per plant for each site on each sampling date ranged from 0.1 at Red Tank Lake in January 1978 and at Stump Lake in October 1979 to 2.3 at Red Tank Lake in January 1980 (Table 2). The high average values at Red Tank Lake beginning in May 1979 were probably due to an intensified herbicide spray program that began in July 1978, which resulted in a reduction of the mat followed by a period of rapid vegetative reproduction from surviving plants. Except for Red Tank Lake, there were no significant differences in the average number of daughter plants between sites.

Flowering

16. The average number of inflorescences per plant for each site on each sampling date was generally low (Table 3). No definite seasonal

* Personal Communication, E. E. Addor, 1980, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. Stage I plants have only bulbous petioles. Stage II plants have both bulbous and nonbulbous petioles. Stage III plants have only nonbulbous petioles.

trend was observed, but some spatial variation was noted. Values for the Red Tank and Stump Lake sites were higher than for other sites. A greater percentage of the plants in the population at these sites was of the Stage II morphotype, the growth stage normally associated with flowering. Plants at the other sites were predominantly the Stage III morphotypes, in which flowering rarely occurs.

Arthropods

Neochetina eichhorniae

17. Adults of *N. eichhorniae* were found on all sites on all sampling dates, except at Red Tank Lake in January 1980 (Table 4). Temporal variation in the adult population was not pronounced, but the population was slightly higher during the dry season than during the rainy season. Spatial variation in the *N. eichhorniae* population was considerable; the average number of adults per plant at Manatee Lagoon was nearly twice that recorded for Las Cruces, the site with the second highest average number of adults per plant, and 15 times greater than the average recorded for Red Tank Lake. The higher values obtained at Manatee Lagoon were probably related to the concentration of waterhyacinths at the boom across the Chagres River. Plants containing the various life stages of *N. eichhorniae* floated down the river and rafted at the boom. As plants were removed and stacked on shore by the slack-line cable system, adult *N. eichhorniae* migrated from the stacked plants back to the river, where they aggregated on floating plants. Consequently, there was an accumulation of large numbers of *N. eichhorniae* adults in this area. Because of the proximity of Manatee Lagoon to the boom, there was a migration of adults from the boom area into the study site. The low values obtained at the Dump 4-1/2 and Red Tank Lake sites were probably the result of removal of plants through the chemical control of waterhyacinth. The periodic spraying with 2,4-D effectively removed the waterhyacinths, and thereby restricted the population development of *N. eichhorniae*. Any eggs, larvae, or pupae on treated plants were likely destroyed with the

plants. The waterhyacinth population then redeveloped faster than the population of *N. eichhorniae*.

18. The average number of *N. eichhorniae* pupae per plant was low at all times, but values for all sites were highest during May 1979 (Table 5). The reason for the low numbers of pupae was not apparent. Considering the higher average numbers of adult *N. eichhorniae* obtained (Table 4), it was assumed that the mortality rate for the pupal stage was very low. Due to the relatively large number of pupae in May, it was also assumed that the adult population of *N. eichhorniae* increased in late dry season and early rainy season.

19. Average index values for *N. eichhorniae* feeding scars per leaf for each site on each sampling date revealed significant spatial variation in adult feeding activity with values ranging from 1.9 at Manatee Lagoon to 0.9 at Red Tank Lake (Figure 2). A value of 1.9 indicated moderate feeding by *N. eichhorniae*, while a value of 0.9 represented light feeding. As expected, these data were closely related to the average number of adults (Table 4). The rankings of sites according to the numbers of adults (Table 4) and feeding scar indices (Figure 2) averaged over time were identical. Also, the largest average number of adults and highest average index values for feeding scars occurred on the same site and sampling date (Manatee Lagoon, April 1978). Temporal variation in the average index values of feeding scars was substantial, but was apparently not related to seasonality because both the highest and lowest average feeding scar index values occurred in January (1978 and 1980). The decrease in the recorded level of adult *N. eichhorniae* feeding beginning in May 1979 could have been related to an intensified herbicide spray program implemented by the PCC. All study sites, except Stump Lake, were subjected to periodic applications of 2,4-D. The normal resulting reduction in the waterhyacinth population would also have produced a reduction in feeding activity.

Cornops sp.

20. Average index values of *Cornops* feeding damage per leaf for each site on each sampling date ranged from a high of 1.2 (moderate feeding) at Las Cruces in January 1978 to a low of 0.1 at Stump Lake in

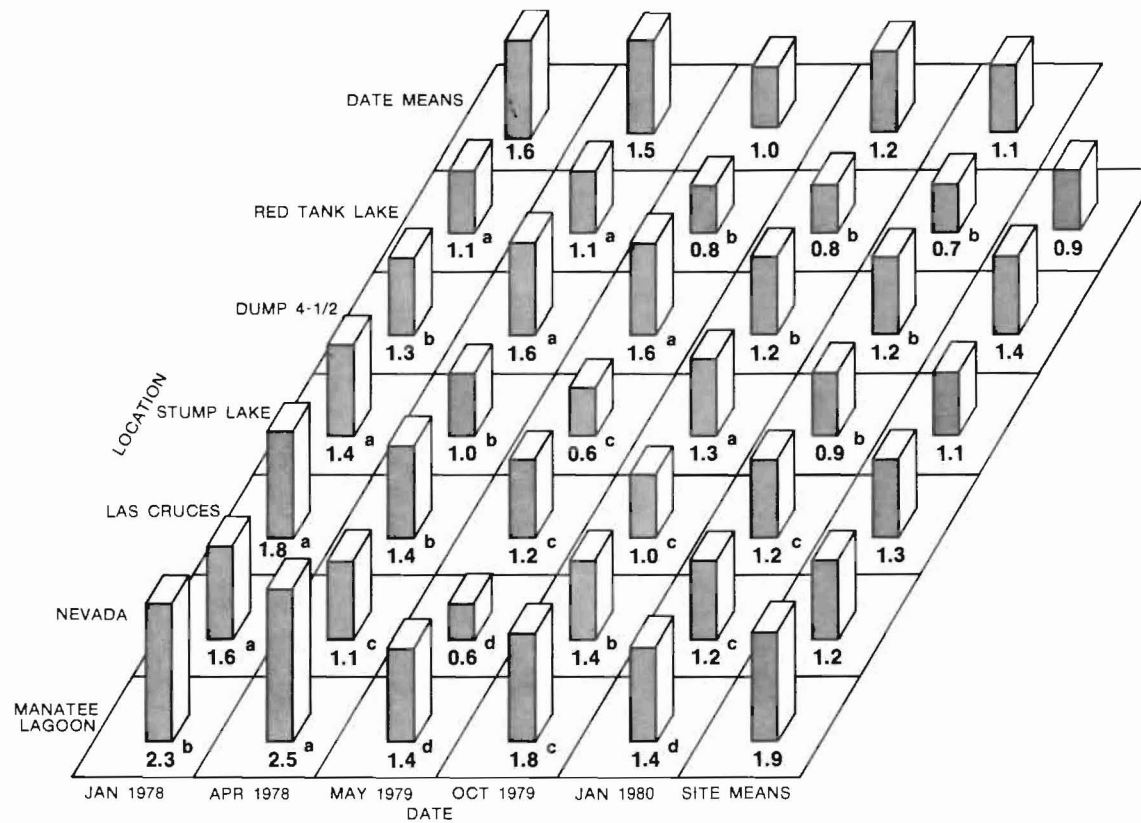


Figure 2. Average index values of *N. eichhorniae* feeding scars per pseudolamina for sites during the study. Means with the same letter within rows are not significantly different at the 0.05 probability level

April 1978 (Figure 3). The same pattern of spatial variation in feeding damage per leaf that was described for *N. eichhorniae* in the Chagres River sites (paragraph 19) occurred for *Cornops*. The highest incidence of feeding occurred in the area nearest the permanent boom. Significantly less *Cornops* feeding occurred at the Stump Lake site than at other Chagres River sites. Temporal variation in *Cornops* feeding damage was also substantial. The highest index values for its feeding damage occurred in January and April 1978, while the lowest values occurred in May 1979. The lower values in 1979 and 1980 may have been produced by a reduction in the amount of waterhyacinth as a result of the herbicide spray program. However, *Cornops* is not specific to waterhyacinth and its level of population development should not be limited significantly by the reduction in the population of waterhyacinth alone. Therefore, some other factor or factors may have been involved in the decrease in feeding damage by *Cornops* in 1979 and 1980.

Orthogalumna terebrantis

21. There was substantial spatial and temporal variation in the degree of tunneling in the pseudolaminae of waterhyacinth by *O. terebrantis* (Figure 4). Spatial variation ranged from 0.0 at Stump and Red Tank Lakes to 0.8 at Nevada. Due to the lack of exchange of waterhyacinths between Stump and Red Tank Lakes and the other sites, *O. terebrantis* may not have reached these two sites. However, it is also possible that *O. terebrantis* reached the sites, but failed to become established due to some environmental factor. Temporal variation ranged from an average of 0.0 in January 1978 to 0.7 in October 1979 and January 1980. There was a general increase in the average degree of tunneling from the earlier to later sampling dates. Reasons for the increase in activity of *O. terebrantis* with time were unclear; however, the initiation of the herbicide spray program could have enhanced the population development of *O. terebrantis*.

Arthropod larvae

22. The average number of arthropod larvae per plant for each site on each sampling date is presented in Table 6. Nearly all of these larvae were found in petioles, and they were predominantly larvae of

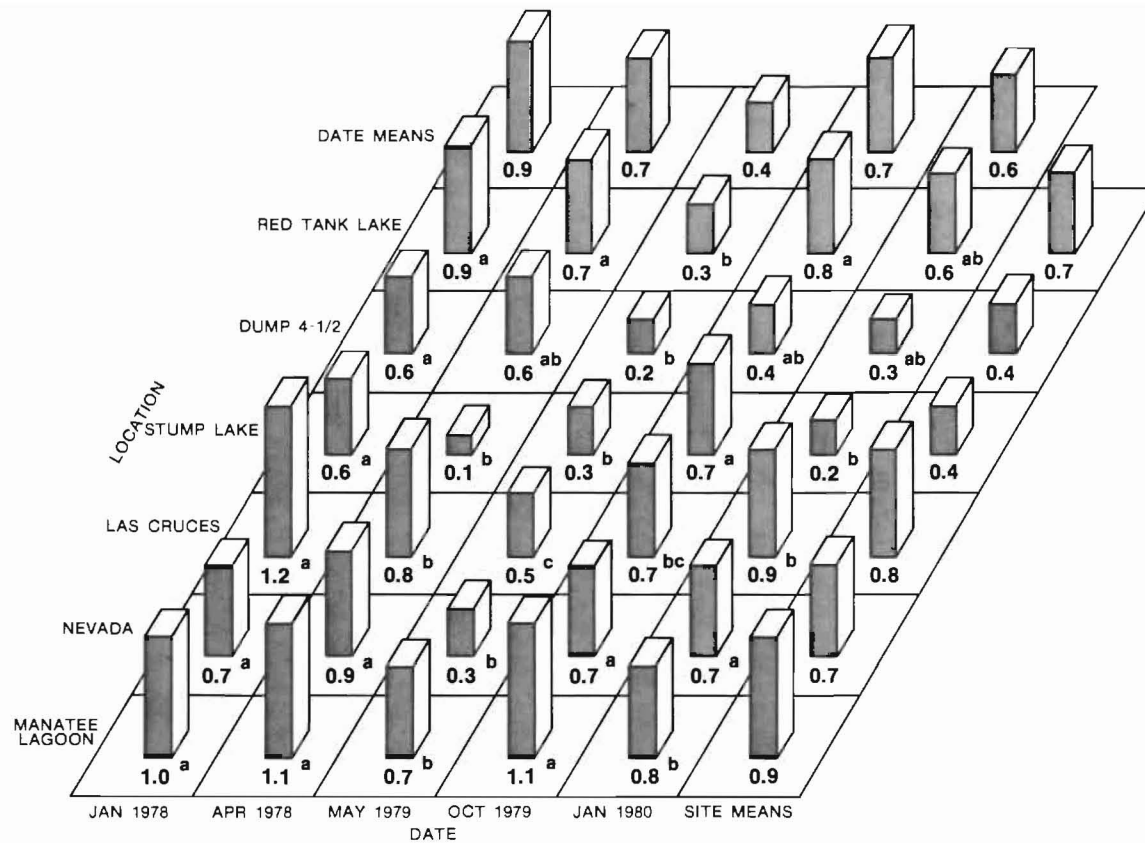


Figure 3. Average index values of *Cormops* sp. feeding damage per pseudolamina for sites during this study. Means with the same letter within rows are not significantly different at the 0.05 probability level

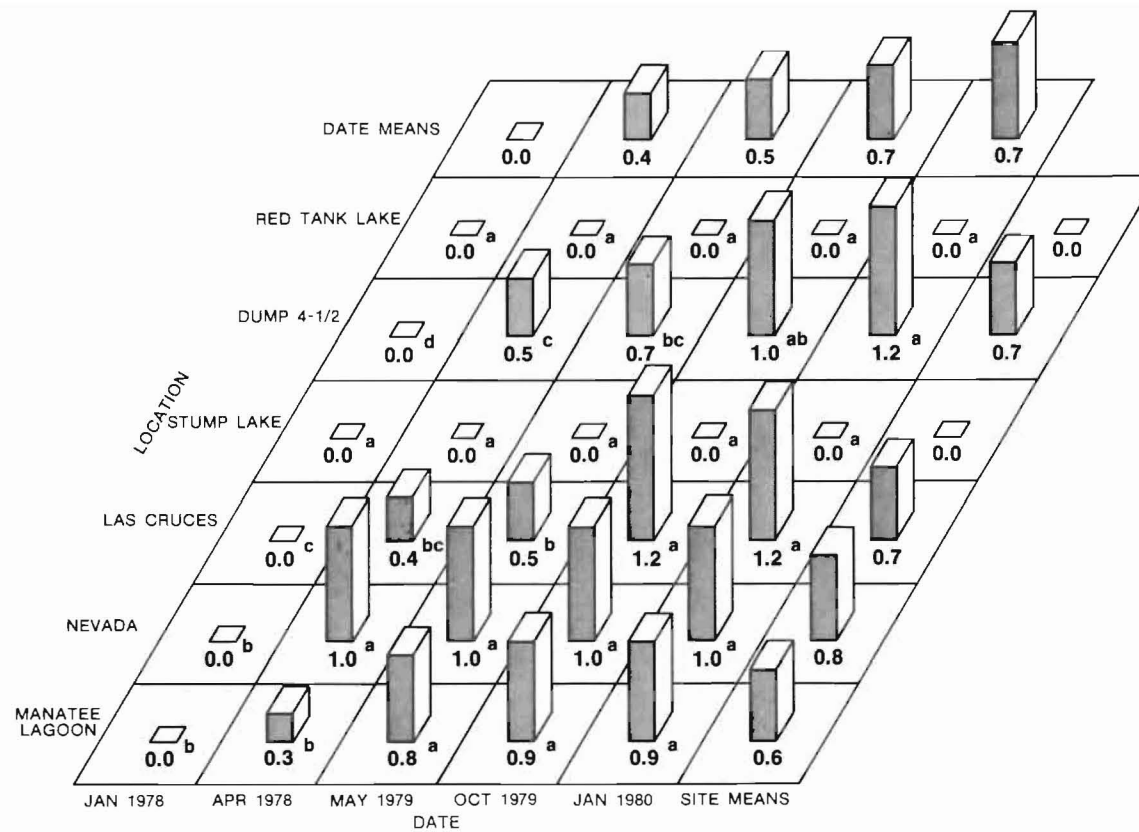


Figure 4. Average index values of *O. terebrantis* tunneling per pseudolamina for sites during the study. Means with the same letter within rows are not significantly different at the 0.05 probability level

N. eichhorniae or *Thrypticus* sp. (Dolichopodidae), a small Dipteran. Due to the inability to distinguish early instar larvae of *N. eichhorniae* from the larvae of *Thrypticus* under field conditions, and due to the large number of samples to be analyzed, all arthropod larvae were recorded as one number per plant. The average number of larvae per plant fluctuated erratically, with no definite temporal trends being evident. However, spatial variation in numbers of larvae was more pronounced; greater numbers of larvae were found in the three Chagres River sites nearest the permanent boom as compared with the other sites.

Penetration points

23. On the initial sampling date, it was apparent that a small arthropod was penetrating the epidermis of the waterhyacinth petioles. Although it was obvious that the waterhyacinth plants were not being significantly stressed by this activity, the penetration points were viewed as possible sites for entry into the plant by facultative pathogens. It was later determined that the penetration points were being made by *Thrypticus* as it oviposited beneath the epidermis. As was true for arthropod larvae, the average number of penetrations per plant was greater in the downstream Chagres River sites than in other sites (Table 7). The larger plants found on these sites had more space in the larger petioles available as oviposition sites, and this probably accounted for the larger population of *Thrypticus* in these areas. There were no evident trends in temporal variation in the number of *Thrypticus* penetration points per plant.

Pathogens

Acremonium zonatum

24. The predominant plant pathogen observed on waterhyacinth in the Panama Canal was *A. zonatum*. Average index values of *A. zonatum* damage per leaf for each site on each sampling date revealed significant spatial and temporal variation of *A. zonatum* (Figure 5). Average values for *A. zonatum* damage were significantly higher in the three downstream Chagres River sites (1-3) than in the other sites (4-6). Plants at

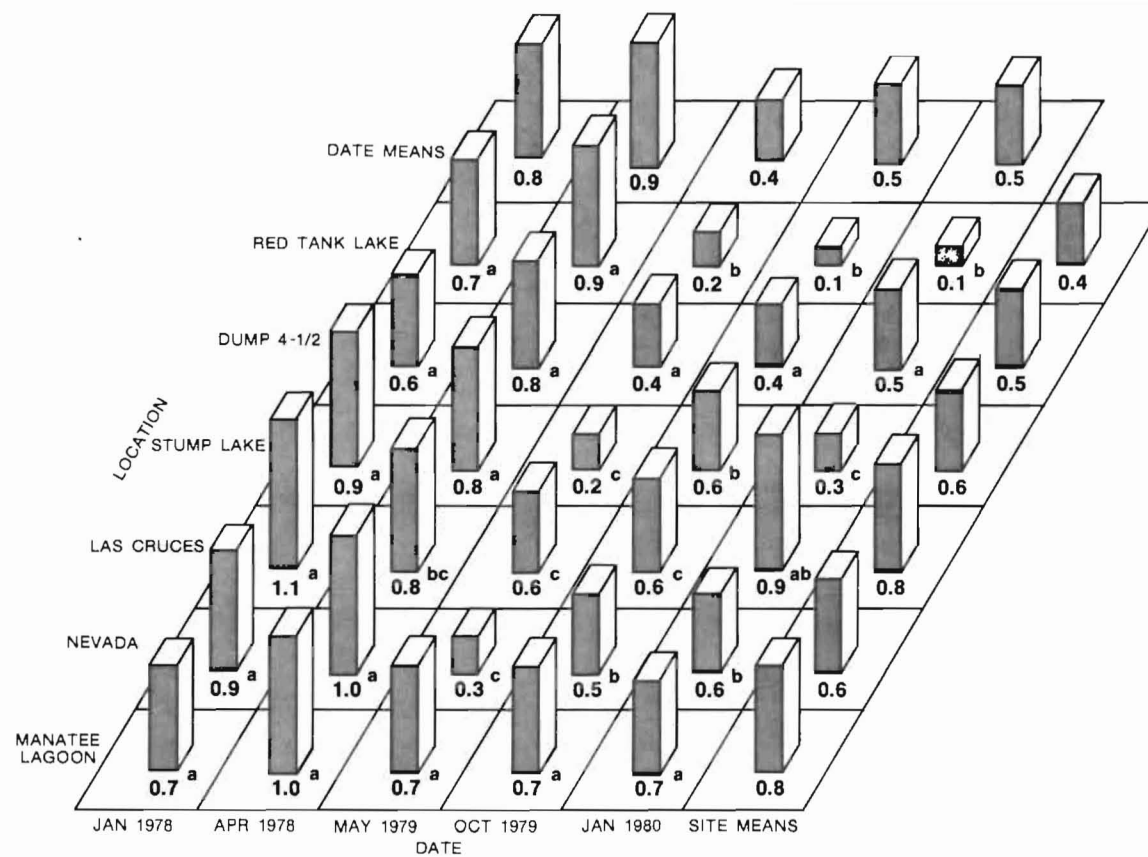


Figure 5. Average index values of *A. zonatum* damage per pseudolamina for sites during the study. Means with the same letter within rows are not significantly different at the 0.05 probability level

sites 1-3 were generally larger, more slowly growing, and had a greater percentage of mature or senescing leaves than plants in sites 4-6. Martyn (1977) found that waterhyacinths with high phenoloxidase were more resistant to infection by *A. zonatum* and that smaller, more rapidly growing plants had higher levels of phenoloxidase than the larger, more mature plants. Thus, the higher values for *A. zonatum* damage in sites 1-3 could be explained by the predominance of Stage III plants on these sites, in which phenoloxidase was low. Conversely, plants on sites 4-6 had lower values of *A. zonatum* damage because the plants were smaller and had a higher level of phenoloxidase. Temporal variation in *A. zonatum* activity also occurred, as evidenced by average values of 0.9 in April 1978, as compared to average values of 0.4 in May and 0.5 in October 1979 and January 1980. The observed variation was partially explained by seasonal changes, in which higher average values occurred during the dry season (0.8) than during the rainy season (0.5). However, average values recorded in 1979 and 1980 were significantly lower than those recorded in 1978. This could be due to the effects of a 2,4-D spray program initiated in late 1978, which resulted in most plants at sites 2, 4, 5, and 6 being destroyed by herbicides. The daughter plants produced by the surviving plants were smaller and physiologically more active. Such plants had a higher level of phenoloxidase activity, which restricted the development of *A. zonatum*.

Other microbes

25. A list of other microorganisms found in the plant samples is presented in Table 8. A total of 16 genera of microorganisms were identified from waterhyacinth, of which 10 genera were bacteria and 6 were fungi. Although 7 genera were considered to be either secondary invaders or potential pathogens, pathogenicity on waterhyacinth was not verified for any of the genera. A secondary invader is considered to be a microorganism that is normally nonpathogenic, but which may act as a pathogen if mechanical damage provides entry loci into the plant. The remaining 9 genera were considered to the contaminants or obligate saprophytes. A contaminant is a microorganism normally associated with the water environment rather than with the plant.

26. Bacteria. Four of the identified bacterial genera include species that are common plant pathogens. *Corynebacterium* sp., which was identified from leaf spots (Figure 6), was isolated from three sites in May 1979, one site in October 1979, and was not present in January 1980. These data suggest that *Corynebacterium* sp. is active on waterhyacinth only during the rainy season. *Erwinia herbicola* was isolated only once, which indicates that it is probably a secondary invader rather than a true pathogen (Figure 7). *Pseudomonas* sp. was isolated on all three sampling dates from insect and pathogen-damaged pseudolamina. Due to its widespread occurrence, *Pseudomonas* sp. produced more stress on waterhyacinth than any other bacterial agent (Figure 8), even though it appeared to be a secondary invader. *Xanthomonas* sp. was isolated from *N. eichhorniae* feeding scars on two sites in January 1980, which suggests that it is an active pathogen during the dry season.

27. Fungi. Of the six fungi isolated in this study, only *A. zonatum* occurred on all three sampling dates. It was isolated from each site on at least one sampling date. Clearly, *A. zonatum* produced greater stress on waterhyacinth than any other microorganism isolated in this study (Figure 9). Two other possibly pathogenic fungi, *Botrytis* sp. and *Fusarium* sp., were isolated in May 1979. However, neither was found in October 1979 nor January 1980, and damage produced by these organisms was minor. Prior to the study, it was anticipated that *Rhizoctonia solani*, which produces a blight on anchored waterhyacinth [*Eichhornia azurea* (Sw.) Kunth.] in the Panama Canal and has been found to be pathogenic on floating waterhyacinth in laboratory tests (Rintz 1973), would be isolated from the study sites. However, it was not isolated in this study, and does not appear to stress floating waterhyacinth in the Panama Canal.



Figure 6. Symptoms produced by *Corynebacterium* sp. on pseudolamina of waterhyacinth in the Panama Canal

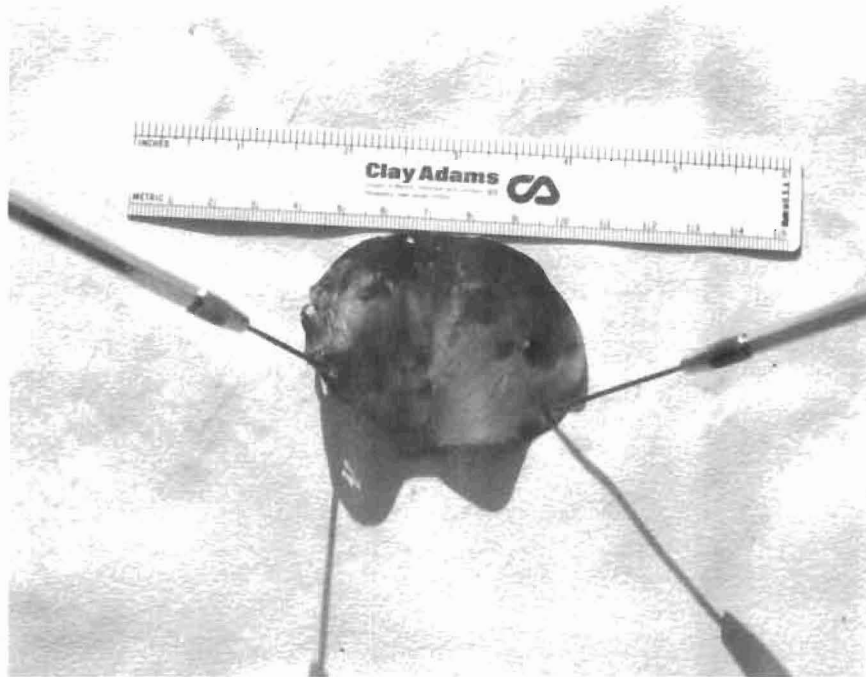


Figure 7. Symptoms produced by *Erwinia herbicola* on pseudolamina of waterhyacinth in the Panama Canal



Figure 8. Symptoms produced by *Pseudomonas* sp. on pseudolamina of waterhyacinth in the Panama Canal

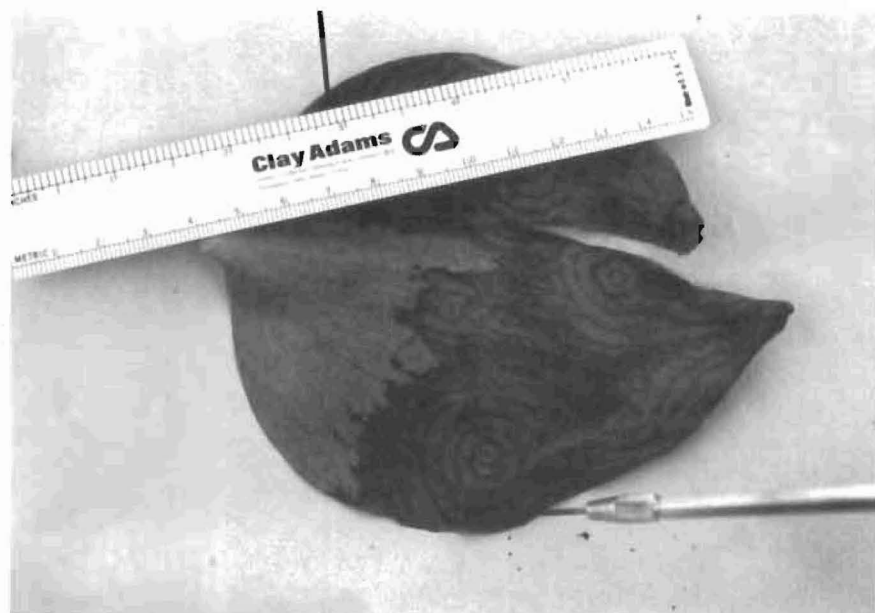


Figure 9. Symptoms produced by *A. zonatum* on pseudolamina of waterhyacinth in the Panama Canal

PART IV: DISCUSSION

Growth of Waterhyacinth

28. The uniformly high temperatures and solar radiation levels that occurred in the Panama Canal provided ideal conditions for the growth of waterhyacinth. A combination of these factors and a lack of a defined winter season would be expected to provide for a uniform mat of Stage III plants. However, several factors precluded a uniform mat of the Stage III morphotype. First, backwater sites (e.g., sites 4-6) had low rates of water exchange, which probably limited nutrient availability. Secondly, competition from hydrilla further limited water exchange and nutrient availability in backwater areas. Thirdly, the application of herbicides (2,4-D) to plants at some sites (e.g., sites 5-6) resulted in the chemical removal of significant percentages of the waterhyacinth population, which were predominantly Stage III plants before the herbicide applications. The chemical removal of the plants provided space for regrowth of the waterhyacinth populations. Plants surviving the herbicide application rapidly produced daughter plants, which were typically of the Stage I morphotype.

29. In addition to competition from hydrilla, waterhyacinth in the Panama Canal faced competition from other floating and rooting aquatic plant species. Waterlettuce (*Pistia stratiotes* L.) and salvinia (*Salvinia* spp.) typically were found interspersed with waterhyacinth, and were presumably competing with waterhyacinth for space and nutrients. In some areas, *E. azurea* was found to compete favorably with *E. crassipes* for available space, and often occurred as a monotypic stand. In the littoral zone, rooted species (e.g., grasses and sedges) were found to dominate *E. crassipes*. However, they also served as a device for anchoring the *E. crassipes* mat, and thereby afforded stability for waterhyacinth in some sites.

Arthropods

30. Feeding by *N. eichhorniae*, *Cornops* sp., and *O. terebrantis*

caused substantial damage to waterhyacinth in the Panama Canal. Evidence of *N. eichhorniae* and *Cornops* feeding was found on all sites on all sampling dates, while feeding by *O. terebrantis* was commonly observed on four of the six study sites. However, the impacts of these species on *E. crassipes* could not be determined due to the absence of data prior to their introduction in the Panama Canal. Nevertheless, it was apparent that the combined effects of these arthropod species at the population levels encountered in this study were not exerting a significant level of control of *E. crassipes*. The greatest populations for each of these species occurred at Manatee Lagoon; yet, the *E. crassipes* at Manatee Lagoon were typically of the Stage III morphotype and were taller than plants at any other site.

31. A major reason for the lack of a controlling effect by these arthropod species on *E. crassipes* is that none of them fed heavily or directly on the newest leaves or the meristematic zones of the plant. Although feeding scars produced by adults of *N. eichhorniae* were commonly found on the newest leaves, they were rarely found in quantities that were observed on older leaves. *Cornops* feeding damage was never observed on the newest leaves of *E. crassipes*, but was usually heaviest on the fourth, fifth, and sixth newest leaves. Feeding by *Cornops* on leaves with significant necrosis was rarely observed. Tunneling by *O. terebrantis* was never observed on the three newest leaves of *E. crassipes* but was greatest on leaves in the fourth through sixth positions.

32. No other arthropods were found to significantly impact *E. crassipes* in the Panama Canal. The penetration points produced by *Thrypticus* were very abundant on the petioles of *E. crassipes* at some sites; however, there was no evidence that *Thrypticus* produced any direct impact on *E. crassipes*. The larvae were very small and fed minimally on the plants. The only other arthropod species that could be inflicting some damage to the plants were an unidentified leafhopper and leafminers, but feeding damage produced by these species was rare.

Pathogens

33. Except for the presence of *A. zonatum*, *E. crassipes* in the Panama Canal was relatively free of pathogens. *Acremonium zonatum* commonly appeared on *E. crassipes* on all sites and at all seasons of the year. However, it was never observed on the pseudolamina of the three newest leaves. Typically, isolated lesions appeared first on the fourth leaf, coalesced by the sixth leaf, and all older leaves were heavily infested. Its greatest impact on *E. crassipes* appeared to be the hastening of senescence of leaves, which decreased the total photosynthesis of infected leaves. However, this impact was probably masked by the rapid production of new, uninfected leaves.

34. Other microorganisms isolated from *E. crassipes* in the Panama Canal were predominantly bacteria, none of which had the potential to significantly impact *E. crassipes*. Their association with *E. crassipes* was that of saprophytes or weak pathogens that only increased the rate of senescence.

Species Interactions

35. By applying Spearman's method for determining rank correlation coefficients to the data from all sampling dates (Siegel 1956), three positive species interactions were identified (Table 9). *N. eichhorniae* and *A. zonatum* were positively correlated on all six sites, while *N. eichhorniae* and *Cornops* sp. were correlated on all sites except Dump 4-1/2, which had a positive correlation but was not significant at the 0.05 probability level. *Cornops* sp. and *A. zonatum* were positively correlated on the three Chagres River sites nearest the permanent boom, but no correlation between these species was evident on the Stump Lake, Dump 4-1/2, and Red Tank Lake sites. These consistent positive correlations suggested that environmental conditions favorable to one of these species were also favorable to its correlated species. Conversely, environmental conditions unfavorable to one of these species were also unfavorable to the other species. Although the specific factors

responsible for these positive correlations were not identified, the greatest level of activity for all three species occurred on the Chagres River sites nearest the permanent boom. Plants on these sites were characteristically of the Stage III morphotype. On the other hand, the lowest levels of activity for these species occurred on the Stump Lake, Dump 4-1/2, and Red Tank Lake sites, in which the plants were predominantly of the Stage I or II morphotypes. Therefore, the physiological or nutritional status of the Stage III morphotype appeared to positively influence the level of activity of these three species, while the Stage I or Stage II morphotypes negatively influenced the activity levels.

36. Significant negative correlations occurred between *O. terebrantis* and *N. eichhorniae* and between *Cornops* sp. and *O. terebrantis* at the Manatee Lagoon site (Table 9). These negative correlations were probably due to the increasing level of activity by *O. terebrantis* on these sites during the study, while the greatest levels of *N. eichhorniae* and *Cornops* sp. activity occurred early in the study. Factors responsible for these shifts in activity on *E. crassipes* were not determined.

37. While processing the plant samples, it was commonly observed that the first evidence of *A. zonatum* activity occurred in portions of pseudolamina where *O. terebrantis* tunneling was in progress, and it was therefore anticipated that a positive correlation would be found between *A. zonatum* and *O. terebrantis*. However, a positive correlation was found between these species only at the Nevada site. The large number of observations that were zero for one or both species was thought to have confounded or masked any true correlation between the two species for other sites.

Combined Impact of Organisms

38. By plotting the average index values for the four major species active on *E. crassipes* in the Panama Canal, it was possible to portray the combined activity of these organisms on *E. crassipes* with respect to both time and location (Figure 10). For this purpose, it was assumed that each of the four species had the potential of producing

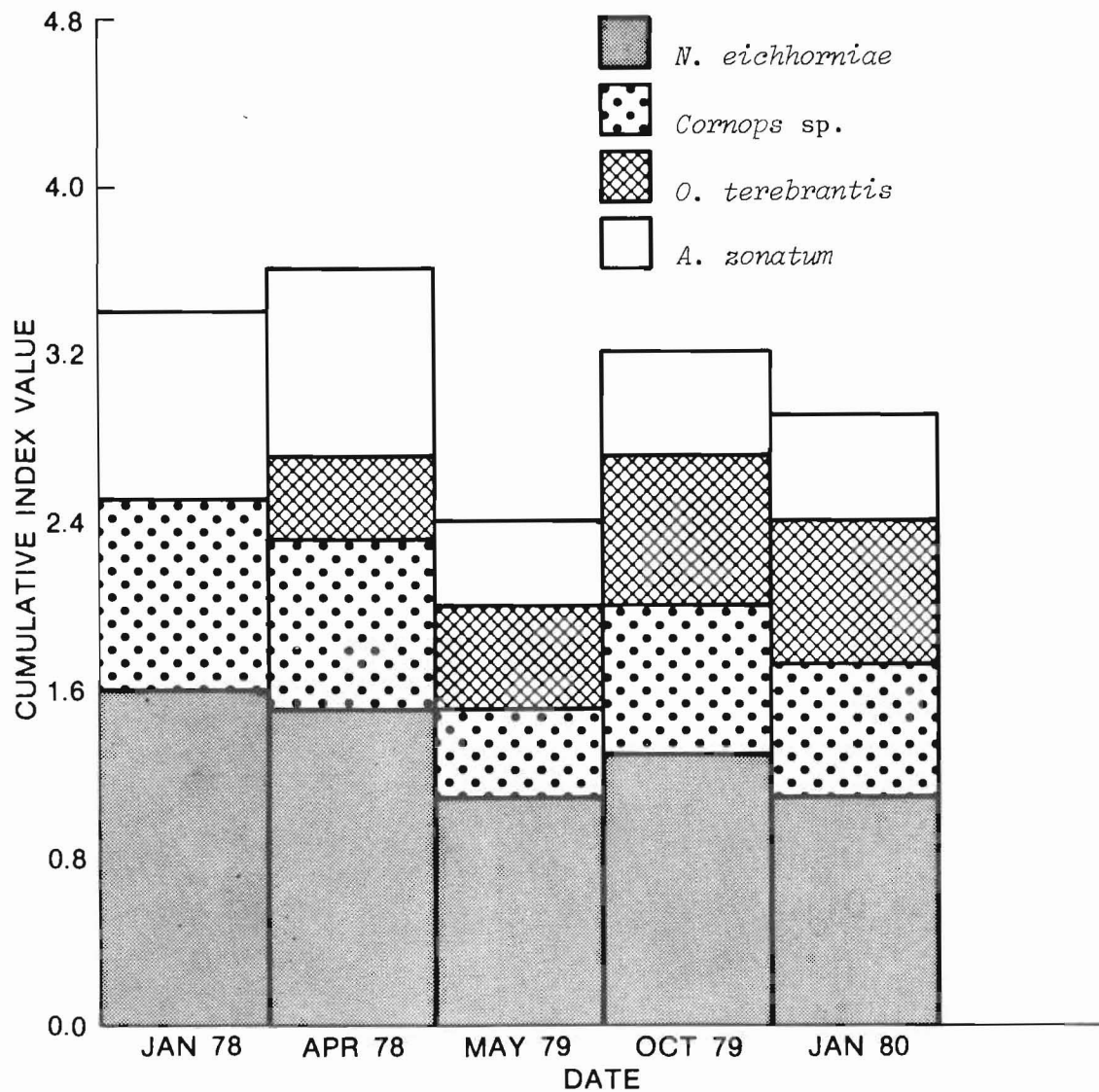


Figure 10. Temporal variation in the combined activity of *N. eichhorniae*, *O. terebrantis*, *Cornops* sp., and *A. zonatum* on *E. crassipes* in the Panama Canal

equal feeding activity on *E. crassipes*. Thus, the taller the bar on the graph, the greater the combined activity of the four species on *E. crassipes*. Temporal variation in the combined activity of these species on *E. crassipes* was substantial. The greatest overall activity occurred in April 1978, while the least activity occurred in May 1979. There was a general decline in activity of all species except

O. terebrantis in 1979 and 1980, but factors influencing the observed temporal variation were not clearly established. It is possible that the intensified herbicide spray program initiated in the spring of 1978 was a major factor influencing changes in the level of activity by these species.

39. There was also considerable spatial variation in the combined activity of the four species on *E. crassipes* (Figure 11). The greatest combined activity was observed at Manatee Lagoon, while the least activity occurred at Red Tank Lake. The level of activity was higher for the three downstream Chagres River sites than for the isolated backwater

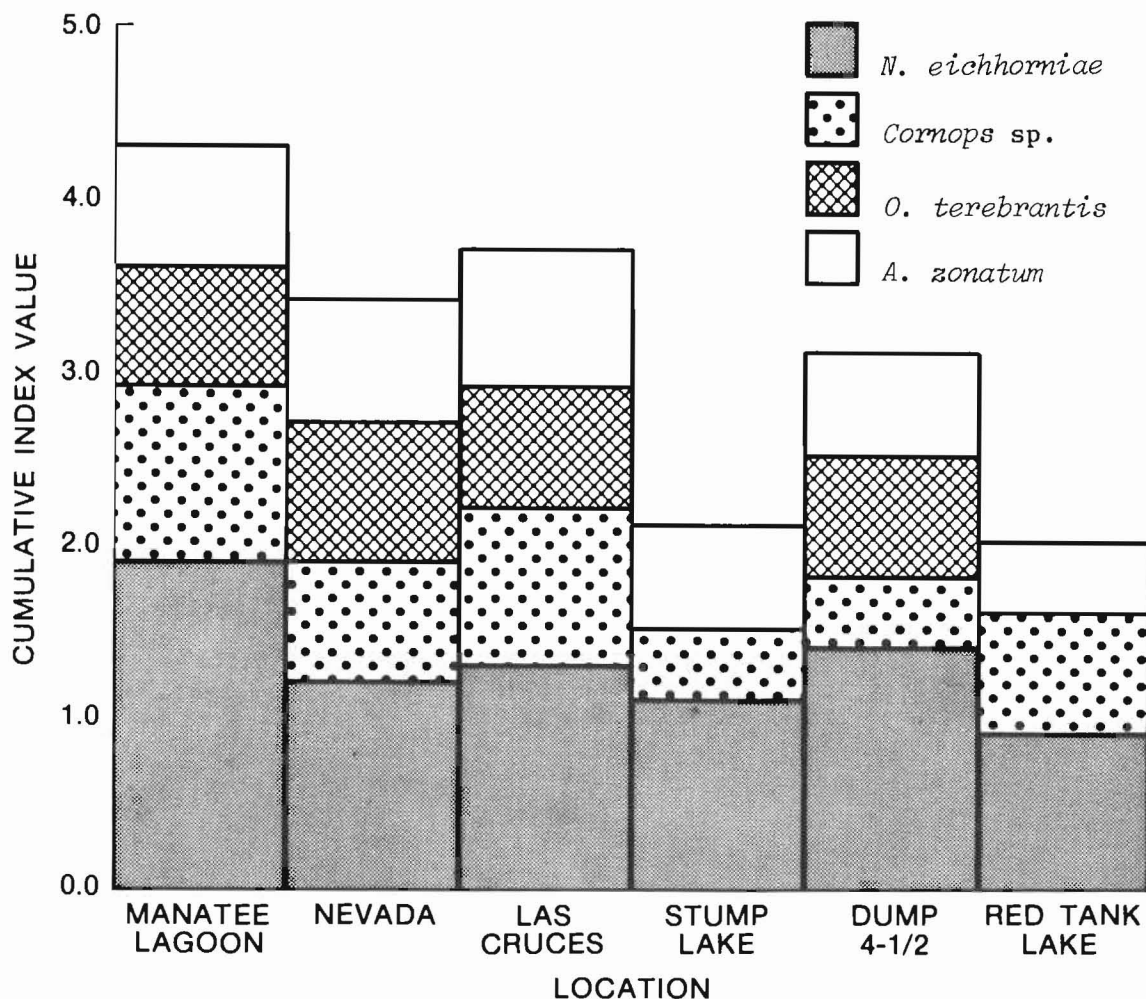


Figure 11. Spatial variation in the combined activity of *N. eichhorniae*, *O. terebrantis*, *Cornops* sp., and *A. zonatum* on *E. crassipes* in the Panama Canal

sites. Nevertheless, individuals of *E. crassipes* at the three downstream Chagres River sites were larger and more vigorous than plants in the backwater areas. Thus, it was evident that the level of overall activity by these organisms was insufficient to produce a significant level of control of *E. crassipes*.

PART V: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

40. The following conclusions were drawn from this study:
- a. Three species of arthropods, *N. eichhorniae*, *Cornops* sp., and *O. terebrantis*, fed on *E. crassipes* in the Panama Canal and produced substantial damage to the plants.
 - b. One plant pathogen, *A. zonatum*, commonly infected *E. crassipes*.
 - c. Substantial spatial and/or temporal variation in the level of activity of the four major organisms was observed.
 - d. No organisms or groups of organisms impacted the newest tissues or meristematic zones of *E. crassipes*.
 - e. *Eichhornia crassipes* was not sufficiently stressed by organisms in the Panama Canal to provide the desired level of control.

Recommendations

41. Based on the results of this study, it is recommended that:
- a. Efforts be undertaken to distribute *O. terebrantis* to all areas of the Panama Canal in which *E. crassipes* is a problem.
 - b. Other species of arthropods and plant pathogens be introduced into the Panama Canal to provide additional stress for the biocontrol of *E. crassipes*, including *Sameodes albiguttalis* (Warren), *Neochetina bruchi* Hustache, *Cercospora rodmanii* Conway, and *Arzama densa* Walker.
 - c. Highest priority be given to the introduction of species (e.g., *S. albiguttalis*) known to attack the newest tissues and meristematic zones of *E. crassipes*.

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Table 1
Average Number of *E. crassipes* Leaves Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	8.1	9.8	10.0	8.2	7.7	8.8
Las Cruces	10.5	9.5	9.1	8.5	9.8	9.5
Nevada	9.2	9.5	8.1	8.3	8.9	8.8
Stump Lake	9.1	10.2	8.5	10.0	9.0	9.4
Dump 4-1/2	6.5	8.9	8.9	8.6	8.3	8.2
Red Tank Lake	7.4	10.4	7.8	9.1	8.5	8.6
Average of all sites	8.5	9.7	8.7	8.8	8.7	8.9

Table 2
Average Number of Daughter Plants of *E. crassipes* Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	0.4	0.7	0.6	0.3	0.7	0.5
Las Cruces	0.8	0.7	0.6	1.0	1.1	0.8
Nevada	0.8	0.3	0.5	0.9	1.0	0.7
Stump Lake	0.5	0.4	1.2	0.1	1.0	0.6
Dump 4-1/2	1.1	0.3	1.2	0.4	0.6	0.7
Red Tank Lake	0.1	1.0	1.8	1.9	2.3	1.4
Average of all sites	0.6	0.6	1.0	0.8	1.1	0.8

Table 3

Average Number of *E. crassipes* Inflorescences Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	0.0*	0.0	0.0	0.0	0.0	0.0
Las Cruces	0.0	0.0	0.1	0.0*	0.3	0.1
Nevada	0.1	0.0	0.2	0.1	0.0	0.1
Stump Lake	0.1	0.1	0.4	0.0	0.3	0.2
Dump 4-1/2	0.0*	0.0	0.1	0.1	0.2	0.1
Red Tank Lake	0.3	0.2	0.2	0.3	0.6	0.3
Average of all sites	0.1	0.0	0.2	0.1	0.2	0.1

* One inflorescence was found on one plant in the 24 plants sampled on the site.

Table 4

Average Number of *N. eichhorniae* Adults Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	1.3	3.4	1.1	0.6	1.1	1.5
Las Cruces	1.5	0.2	0.4	0.2	1.6	0.8
Nevada	1.0	0.3	0.2	0.7	0.5	0.5
Stump Lake	0.6	0.2	0.0*	0.7	0.2	0.3
Dump 4-1/2	0.2	0.3	0.8	0.2	0.1	0.3
Red Tank Lake	0.1	0.0*	0.2	0.3	0.0	0.1
Average of all sites	0.8	0.7	0.5	0.5	0.6	0.6

* One adult was found on 1 of the 24 plants sampled.

Table 5
Average Number of *N. Eichhorniae* Pupae Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	0.1	0.0*	0.2	0.2	0.0*	0.1
Las Cruces	0.1	0.0	0.1	0.0	0.0	0.0
Nevada	0.0*	0.0	0.2	0.0	0.0	0.0
Stump Lake	0.0	0.0	0.1	0.0	0.0	0.0
Dump 4-1/2	0.0	0.0	0.2	0.0*	0.1	0.1
Red Tank Lake	0.0	0.0*	0.2	0.0	0.0	0.0
Average of all sites	0.0	0.0	0.2	0.0	0.0	0.0

* One pupa was found on 1 of the 24 plants sampled.

Table 6
Average Number of Arthropod Larvae Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	10.6	22.0	11.3	18.0	12.5	14.9
Las Cruces	6.2	13.8	12.8	11.3	24.0	13.6
Nevada	12.8	12.8	13.0	5.3	11.3	11.0
Stump Lake	14.8	9.3	5.5	9.5	8.3	9.5
Dump 4-1/2	3.0	7.4	15.5	9.8	10.3	9.2
Red Tank Lake	9.5	8.8	5.3	9.3	10.0	8.6
Average of all sites	9.5	12.4	10.6	10.5	12.7	11.1

Table 7

Average Number of Penetration Points Per Plant for Sites During the Study

<u>Site</u>	<u>Jan 1978</u>	<u>Apr 1978</u>	<u>May 1979</u>	<u>Oct 1979</u>	<u>Jan 1980</u>	<u>Average of all Dates</u>
Manatee Lagoon	128.3	303.0	239.1	369.6	191.0	246.2
Las Cruces	217.1	207.5	218.6	174.9	297.5	223.1
Nevada	187.5	256.8	175.5	194.1	161.5	195.1
Stump Lake	172.9	173.8	140.8	205.5	131.3	164.9
Dump 4-1/2	65.2	211.4	273.2	161.3	151.5	172.5
Red Tank Lake	90.1	131.2	83.0	162.8	127.6	118.9
Average of all sites	143.5	214.0	188.4	211.4	176.7	186.8

Table 8
Microorganisms Identified on *E. crassipes* in the Panama Canal

Taxon	Sympton	May 1979						October 1979						January 1980						Comments	
		1*	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6		
Bacteria																					
<i>Achromobacterium</i> sp.	Necrosis associated with insect damage												x				x	x	x	x	Probable contaminant
<i>Aeromonas hydrophila</i>	Brown leaf spot							x													Probable contaminant
<i>Alcaligenes</i> sp.	Chlorosis associated with <i>Neochetina</i> feeding scars																		x		Probable contaminant
<i>Corynebacterium</i> sp.	Brown lesion, leaf blight		x	x		x		x													Possible pathogen
<i>Erwinia herbicola</i>	Leaf spot					x															Probable secondary invader
<i>Enterobacter cloacae</i>	Wet leaf rot					x	x					x									Probable contaminant
<i>Proteus inconstans</i>	Necrosis associated with insect damage							x								x					Probable contaminant
<i>Pseudomonas</i> sp.	Necrosis associated with <i>Neochetina</i> feeding scars and <i>Acremonium</i> leaf spot							x	x		x				x		x			x	Possible secondary invader
<i>Serratia liquefaciens</i>	Large leaf spot					x															Probable contaminant
<i>Xanthomonas</i> sp.	Leaf spot associated with <i>Neochetina</i> feeding														x	x					Possible pathogen

(Continued)

* Numbers in this row refer to sites (see paragraph 5).

Table 8 (Concluded)

Taxon	Sympton	May 1979						October 1979						January 1980						Comments
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
<u>Fungi</u>																				
<i>Acremonium zonatum</i>	Zonate leaf spot	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x		Pathogen
<i>Ascochyta</i> sp.	Necrosis associated with <i>Neochetina</i> feeding and <i>Acremonium</i> leaf spot															x			x	Probable saprophyte
<i>Botrytis</i> sp.	Black leaf spot			x																Possible pathogen
<i>Fusarium</i> sp.	Leaf necrosis					x														Possible pathogen
<i>Paecilomyces</i> sp.	Necrosis associated with <i>Orthogalumna</i> damage												x							Probable saprophyte
<i>Zythia</i> sp.	Leaf spot associated with <i>Neochetina</i> feeding																		x	Probable saprophyte

Table 9

Correlation Coefficients for Species Interactions on *E. crassipes* in the Panama Canal

<u>Species Combination</u>	<u>Manatee Lagoon</u>	<u>Las Cruces</u>	<u>Nevada</u>	<u>Stump Lake</u>	<u>Dump 4-1/2</u>	<u>Red Tank Lake</u>
<i>N. eichhorniae</i> × <i>Cornops</i> sp.	0.4260*	0.3180	0.5350	0.3077	NS**	0.2788
<i>N. eichhorniae</i> × <i>A. zonatum</i>	0.3848	0.3628	0.5461	0.5455	0.3615	0.6685
<i>Cornops</i> sp. × <i>A. zonatum</i>	0.4978	0.5187	0.5143	NS	NS	NS
<i>N. eichhorniae</i> × <i>O. terebrantis</i>	-0.2635	NS	NS	NS	NS	NS
<i>A. zonatum</i> × <i>O. terebrantis</i>	NS	NS	0.3009	NS	NS	NS
<i>Cornops</i> sp. × <i>O. terebrantis</i>	-0.2119	NS	NS	NS	NS	NS

* All indicated values are significant at the 0.05 probability level.

** NS indicates values not significant at the 0.05 probability level.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Sanders, Dana R.

Organisms impacting waterhyacinth in the Panama Canal / by Dana R. Sanders, Sr., Russell F. Theriot, Edwin A. Theriot (Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station). -- Vicksburg, Miss. : The Station ; Springfield, Va. : available from NTIS, 1982.

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1. Aquatic weeds. 2. Insects. 3. Panama Canal. 4. Weed control--Biological control. I. Theriot, Russell F. II. Theriot, Edwin A. III. United States. Panama Canal Commission. IV. United States. Army.

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