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The University of Arizona, 1989

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EICHHORNIA CRASSIPES (MART.) SOLMS IN WASTEWATER TREATMENT: REDUCING LOW TEMPERATURE STRESS

by

Jennifer Rae Noelle Lawler

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

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WITH A MAJOR IN HORTICULTURE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1989

STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Dec. 12, 1989

Dr. David Palzkill   Date
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This thesis is dedicated to all my friends in the many corners of the world, especially Annie in Africa, my grandparents J. Wanita and Harry G. Nickle, and my parents Wanita R. Nickle and Ronald G. Lawler who planted the seed of curiosity.

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ABSTRACT

Studies were carried out from July 1988 to August 1989 to assess the growth and winter survival of water hyacinth, Eichhornia crassipes (Mart.) Solms, in treatment of secondary domestic wastewater in Tucson, Arizona. Percent of surviving overwintered plants for the following frost protection treatments from November 1988 to March 1989 was: 25 (control), 48 (plastic tarps), 70 (sprinklers), 34 (fog) and 76 (greenhouse). Both control plants and protected plants had longer roots at the effluent end of the ponds than the influent ends during winter months. Greenhouse-protected plants had greater root and entire plant lengths, and greater fresh and dry weights. Dry weight per unit area (kg m^{-2}) was higher for greenhouse plants though all protected plants showed a decline in dry weight per unit area at temperatures below 10 C.

Qualitative observations indicated that protected plants showed less chlorosis and necrosis from low temperatures than control plants, however, plants in all frost protection treatments experienced low temperature stress. Aphids were seen in some of the ponds throughout the study and contributed to severe lamina and petiole damage.

INTRODUCTION

Over the past two decades, energy limitations, overutilization of natural resources and the explosion of pollutant materials has necessitated the need for new technologies for community services in many urban and rural areas. This has held true for wastewater management, as well.

Traditional wastewater treatment systems require energy input from the treatment process to produce treated water. These conventional treatment facilities produce effluents which contain unused nutrients which represent potential energy. New natural treatment systems are designed to obtain maximum benefit from energy in wastewater as well as from the sun and wind.

Water hyacinth [Eichhornia crassipes (Mart.) Solms], an aquatic monocotyledonous macrophyte in the family Pontederiaceae, has been the focus of much research in wastewater management systems. According to Boyd (1969), water hyacinths are better suited than other aquatic plants for wastewater treatment because they are not rooted but rather float on the surface thus facilitating harvest. Water hyacinths also absorb large amounts of nutrients from water. Water hyacinths decrease biological oxygen demand (the requirement or demand of oxygen by biological components in the wastewater) as efficiently as traditional systems and also reduce total suspended solids (TSS). Algae growth is reduced more efficiently with water hyacinth than other aquatic macrophytes (McDonald and Wolverton, 1980), and water hyacinths aid in sedimentation, clarification, filtration, aeration, absorption and adsorption processes.

Low temperature stress has been identified by researchers as a critical factor in determining water hyacinth management (Dinges, 1982). Water hyacinths grow optimally in the range of 25-27.5 C but die when water temperature falls below 10 C. Because water hyacinth is of tropical origin some form of frost protection is required in climates like those found in southern Arizona.

The objectives of this study were: 1) to investigate frost protection systems which would limit the temperature stress imposed on plants during the winter and 2) to monitor the relationships of plants and insects in the water hyacinth community.

LITERATURE REVIEW

History of Eichhornia crassipes (Mart.) Solms in the U.S.

It is believed that the water hyacinth was introduced into the United States in the late nineteenth century from South America. A visitor to the world's fair in Florida is alleged to have released the plant into the state's waterways where it spread rapidly throughout the warmer areas in the Southeast and West to the point of becoming a weed (Barrett, 1989). As the plant reproduces and grows it can choke waterways disrupting river and lake traffic. In Florida, management of this plant and another, Hydrilla verticilata costs over $15 million per year (Dinges, 1982).

Morphology and anatomy

Petioles, crown, and leaves

The above water portion of water hyacinth forms a rosette near the crown and includes petioles, flower stalks, flowers, stolons and leaves made of tissue which is extremely buoyant and spongy (Figure 1). Immature petioles develop as float-like appendages and are called float leaves. Individual plants can measure from 50 cm to 120 cm from root tip to flower cluster in standing crops (O'Brien, 1981). Growth of plants is vertical and

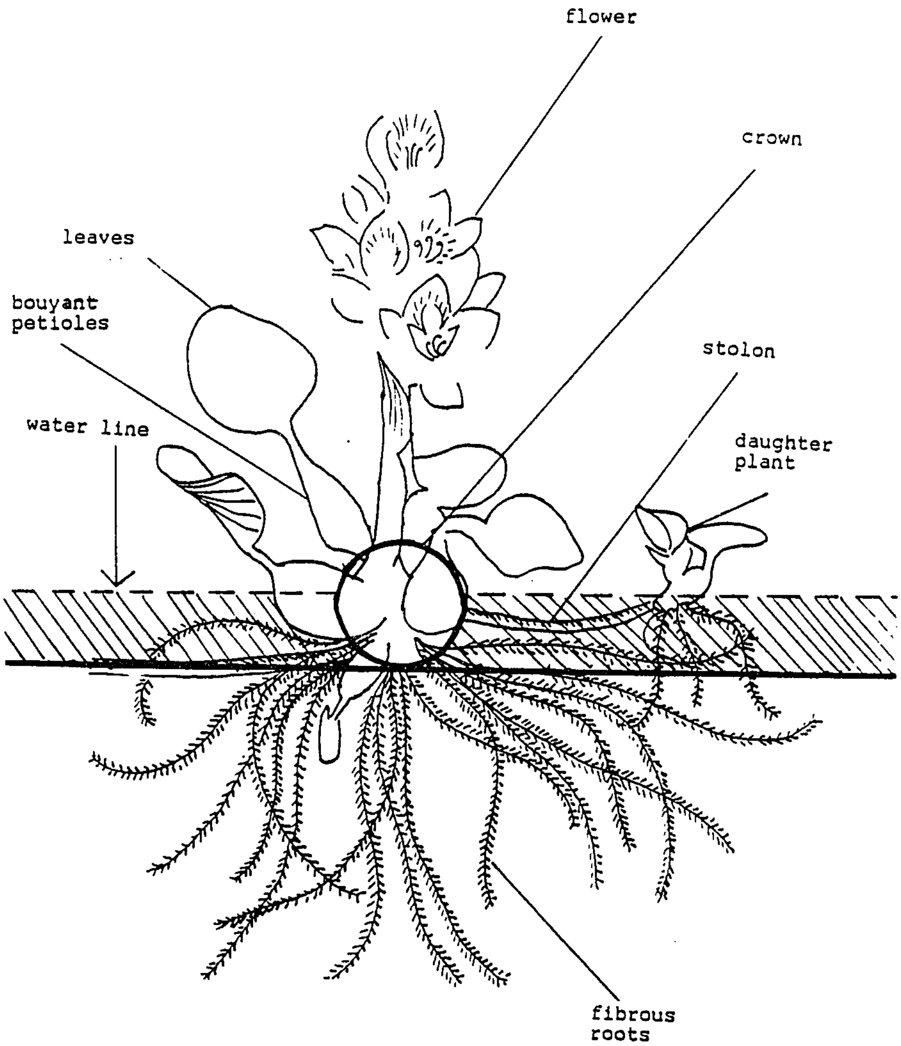

Figure 1. Crown placement and morphology of Eichhornia crassipes.

horizontal and canopy configuration varies with plant density (Debusk and Reddy, 1987).

Stolons can reach lengths of 20 to 30 cm if surface water remains open. When crowding occurs plants send out much smaller stolons, some of which may be less than 1 cm (Dinges, 1982).

Aquatic plants are classified as floating, emergent or submerged macrophytes (Smith, 1985). Water hyacinth is considered a floating macrophyte. Floating plants grow most vigorously in water deeper than 0.2 m. Cultured water hyacinth grows most effectively in 1.0 to 1.3 m of water.

Water hyacinths have stomata equally distributed on upper and lower leaf surfaces (Gopal, 1987). Calcium oxalate crystals are found throughout the palisade and mesophyll tissues (Gopal, 1987). Stem and leaf structures of water hyacinth have been identified as sensitive to salinity (Jamil, et al., 1985).

Roots

Roots of water hyacinths support microorganisms and absorb nutrients. Brown (1913) regarded water hyacinth roots as structures which anchored the plant in the river bed. Shannon (1953) identified water hyacinth roots as structures which absorbed water and nutrients. Later researchers agreed that the water hyacinth roots created a medium for the attachment of microorganisms capable of removing soluble solids. Roots also provided filtering action (Dinges, 1982; Richardson and Daigger, 1984; Reddy and Smith, 1987; Reed, Middlebrooks, and Crites, 1988).

Water hyacinths produce lateral roots which are not as regularly arranged as roots in other plants such as Cucurbita maxima (Mallory et al., 1970). Lateral roots are numerous and are initiated in the immature pericycle. They first appear as a series of closely spaced protuberances which elongate and become more distantly spaced as the primary root grows (Arnold, 1940).

Most aquatic plants produce root hairs, although some such as members of the Ceratophyllaceae, Lentibulariaceae and Podostemaceae do not (Shannon, 1953). Shannon (1953) investigated the occurrence of fibrous, feather-like root hairs of aquatic plants by visual observation. He found in a study of 209 species that most, including water hyacinths, produce abundant root hairs to increase the absorbing surface of their roots.

The structure of water hyacinth roots is affected by the nutrient status of water and cultural practices such as harvesting. Root length will vary with the amount of N and P within the water and the frequency of harvest (Reed, Middlebrooks, and Crites, 1988); higher levels of nutrients encourage shorter roots than lower levels. If no harvest occurs the roots grow until they attach to the bottom of the pond (Reed, Middlebrooks and Crites, 1988). Under these crowded conditions, plant energy is diverted from shoot growth to root growth.

Physiology

Nutrient absorbtion

Boyd (1969) found that mineral uptake rates per unit of dry matter increase when water hyacinths undergo a rapid growth phase. In water hyacinth, most of the nutrient removal occurs in the area where crown and petiole meet (Cooper and Boon, 1987) (Figure 2). In this tissue, oxygen, carbon dioxide and mineral nutrients are exchanged from the outer environment to the interior of plant tissue. Other processes involved in nutrient removal include sedimentation, plant uptake, and ammonia volitilization (Fisher and Reddy, 1987; Reed, 1988).

Nutrient composition

As with other plants, chemical composition of *Eichhornia crassipes* is a function of the environment, responding to changes in light, temperature, space, and nutrients (Debusk and Reddy, 1987). Like other herbaceous plants, such as lettuce, water hyacinths are 95 to 97 percent water (Penfound and Earle, 1948; Bates and Hentges,

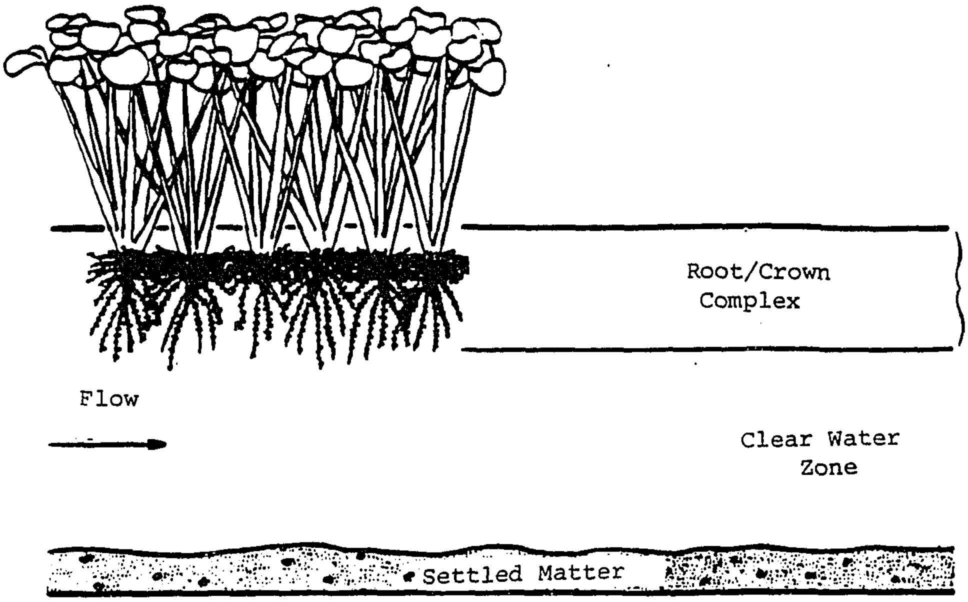

Figure 2. Cross-section of water hyacinth in wastewater treatment pond.

1976; O'Brien, 1981; Dinges, 1982). The stems and leaves of the plant contain high concentrations of K and Cl (6.5 and 6.3 percent dry weight, respectively). Other elements found in smaller concentrations are Ca, Na, and Mg. The roots contain S, Fe, Cu, Mn and Zn. Boyd (1969) found the following ion concentrations for water hyacinth tissue including lamina, petioles, and roots: 2.64 percent for N, 0.43 percent for P, 4.25 percent for K, and 1.05 percent for Mg.

Cooley, Gonzalez and Martin (1978) studied the rate of absorption by Eichhornia crassipes roots of Mn, P and Fe from a mixture of the micronutrients and concluded that the absorption rate of Mn is considerably greater than that for P and Fe. Phosphorus and Mn appeared in the leaves more rapidly than Fe. Iron began to appear in the leaves only slowly during the first 21 days of the experiment (Cooley et al., 1978).

In cultured water hyacinths, tissue N levels vary from 1.6 to 3.8 percent and P levels range from 0.31 to 1.0 percent on a dry weight basis (Boyd, 1969; Wolverton and McDonald, 1978; Wolverton and McDonald, 1979; Wolverton, 1981; Dinges, 1982).

Water hyacinths can survive in nutrient deficient water but tend to partition more of their photosynthate during times of nutrient scarcity to the root system (Dinges, 1982). Generally, root systems become more extensive as nutrient levels diminish.

Nitrogen and phosphorus removal

A number of researchers have studied N and P uptake by water hyacinths (Rodgers and Davis, 1972; Boyd, 1976; Wooten and Dodd, 1976). Additional research has been carried out comparing the rates of growth and absorption of N and P for cultivated water hyacinths and natural habitat stands (Rodgers and Davis, 1972; Boyd, 1976).

It has been estimated that a one-acre water hyacinth treatment system could remove the daily N and P found in the sewage of 800 people (McDonald and Wolverton, 1980). Water hyacinth plants absorb N five to ten times as rapidly as they do P (Boyd, 1976). Cornwell et al. (1977) estimated that water hyacinths could remove 44 percent of the P and 80 percent of the N in sewage. Scheffield (1965) studied the use of water hyacinth for nutrient removal and concluded that 99 percent of N could be removed by it compared to only 67 percent removal of N by algae covered ponds. Some researchers indicate that N removal from the solution occurs far in excess of that accounted for by the plant alone (Reed, Middlebrooks and Crites, 1988). Nitrifier organisms provide oxygen during nitrification at the root zone and microsites in benthic layers (lower portion of the pond where aerobic and anaerobic processes occur) account for denitrification (Reed, Middlebrooks, and Crites, 1988) (Figure 2).

Aquatic macrophytes prefer NH_4 over NO_3 (Reddy, 1983), and water hyacinths receive N via ammonia rather than using N from nitrification processes (McDonald and Wolverton, 1980). Microorganisms reported to be involved in these processes include <u>Azotobacter</u> <u>chroococcum</u> and <u>Azospirillium</u> <u>lipoferum</u>, both found in the roots; <u>Pseudomonas</u> spp. found in the flowers; and the purple-sulphur bacterium <u>Rhodospirillium</u> found in shoot apices (Gopal, 1987). Of all the aquatic macrophytes, floating aquatics such as pennywort and water hyacinth remove the largest amount of nitrogen at 0.19 g N m^{-2} d^{-1} and 0.20 g N m^{-2} d^{-1}, respectively. Submersed and emersed aquatics like elodea and cattail, respectively, remove 0.05 g N m^{-2} d^{-1} and 0.07 g N m^{-2} d^{-1}.

Data collected by O'Brien (1981) in Mississippi indicate that nutrient removal is greatest in shallow ponds which were harvested frequently: 3.56 g N m^{-2} d^{-1} and 0.89 g P m^{-2} d $^{-1}$. Pond depth and harvesting schedule was not specified. Longer detention times are needed for P removal than for N removal. For P removal, Reddy (1983) predicted that cattail-elodea systems would be most effective in nutrient removal. Pennywort also provided more efficient P removal than water hyacinth (Reddy, 1983).

Heavy metals removal

Heavy metals in wastewater can be removed by water hyacinths. In one experiment, plants were grown in separate solutions of various metal ions including Cu, Cd, Cr and Zn and evaluated for plant vigor, growth and morphology. When plants absorbed the Cu and Zn solution, they showed severe leaf and stem damage. In the Cd and Cr solution plants showed less leaf and stem damage (Jamil, Rao and Jamil, 1987).

In Shreveport, Louisiana the highest concentrations of metals in plants occurred during winter months. Researchers speculated that as some of the plants died metals were reintroduced into the pond, reabsorbed by the remaining plants thereby increasing the concentration of metals in the remaining plants (Black and Veatch Engineers, 1983).

Hardy and O'Keeffe (1985) in a study with cadmium (Cd) concluded that plants with greater root mass removed more Cd from solution per unit time than those with smaller root mass. Plants of different sizes and ages took up and concentrated Cd in their tissue at different rates (Hardy and O'Keeffe, 1985). Presumably the roots themselves have binding sites for metal ions required for normal growth and maintenance of the plant. Some of these sites may be utilized in the uptake of the other metal ions. However, the actual number of distinctly different metal ion binding sites on roots remains unknown.

Water hyacinth effect on pH

Jamil et al. (1985) showed that *Eichhornia crassipes* can tolerate a pH as low as 4.02, however it changes the pH of the solution toward neutrality as it grows. The mechanisms for such activity are unclear, but may be attributed to calcium oxalate precipitation when plants are grown in water treated with heavy metals (Jamil et al., 1985).

Evapotranspiration

Water loss or evapotranspiration (E) in water hyacinth lagoons varies seasonally. E or Et can be defined as actual evapotranspiration while Eo is defined as potential evapotranspiration from an open water surface. A comparative study of water hyacinth and duckweed (*Lemna*) showed the E/E_o (E actual/E potential) were generally higher in summer for water hyacinth. E/E_o for water hyacinth was not significantly correlated to mean temperature or solar radiation (Debusk and Reddy, 1987). Rodgers and Davis (1972) determined water losses to be 5.3

times greater from containers with plants than from those without. Researchers (Debusk and Ryther, 1983; Reed, Middlebrooks and Crites, 1988) reported that evapotranspiration from plants is three times greater than from open water. Debusk and Ryther (1983) reported that evapotranspiration of water hyacinth was correlated with solar radiation and mean temperature.

Debusk and Reddy (1987) studied effects of canopy structure on evapotranspiration and concluded that stocking density was also related to amount of evapotranspiration. In a comparative study of aquatic macrophytes they found that duckweed, a very low-growing floating macrophyte, did not show significant loss of water while water hyacinth showed an increase in evapotranspiration rate with increased density and amount of leaf tissue exposed.

Evapotranspiration levels off in extremely dense canopies because of self-shading from solar radiation and wind. Debusk and Ryther (1983) also showed that evapotranspiration increased with increased nutrient availability: with high nutrient availability, plants produced a small amount of root tissue, healthy long petioles and broad laminae and, therefore, higher evapotranspiration rates; with low nutrient availability plants showed thin petioles, curled laminae and extensive root systems and lower evapotranspiration rates (Debusk and Ryther, 1983).

Reproduction

Water hyacinths reproduce sexually by seed but more commonly asexually by stolon (Penfound and Earle, 1948). With regard to asexual reproduction, water hyacinths are referred to as mother or parent and daughter or ramet plants. Daughters represent the newest generation via vegetative reproduction.

The lavendar, zygomorphic flowers appear from late spring to early fall (Gopal, 1987). The plants are day neutral and tall dense stands of plants grown in high nutrient water induce flower production (Penfound and Earle, 1948). Seeds are very dense, enclosed in a capsule and minimally produced (Dinges, 1982).

Growth

Water hyacinths have some of the highest growth rates known in the plant kingdom (Wooten and Dodd, 1976; Gopal, 1987). Ten plants, for example, can produce 600,000 plants during an eight month growing season (Penfound and Earle, 1948). Wolverton (1981) has estimated a dry weight productivity of 140 metric tons ha^{-2} y^{-1}.

Effects of carbon dioxide

Water hyacinth, like *Typha latifolia* (cattail), *Juncus effusus* (soft rush), *Pistia stratiotes* (water lettuce) and *Hydrocotyle ambellata* (pennywort) have the C_3 photosynthetic pathway.

Spencer and Bowes (1986) compared plants grown at 600 microliters CO_2 per liter with plants grown at normal atmospheric CO_2 levels of 330 microliters CO_2 per liter and found that much of the assimilate formed by the mother plants caused by elevated CO_2 was allocated to the ramets or daughter plants. This temporary CO_2 rate enhancement increased the dry weight, number of leaves, leaf area per ramet and leaf area index but not number of ramets.

In a similar study, Ornes and Sutton (1974) found that 1.9 daughter plants were produced per parent plant per week with elevated greenhouse CO_2 levels.

Population effects

Efficiency of nutrient removal is linked to plant morphology and density (Debusk and Reddy, 1987). Dry matter standing crop is the decisive factor regulating the quantities of mineral nutrients absorbed per unit area by a particular species (Boyd, 1969). Plants compete for resources, particularly light, resulting in shoot length and leaf areas which are significantly increased at higher plant densities.

A plateau or ceiling exists with regard to plant density effects on nutrient removal (Debusk and Reddy, 1987). Tissue concentrations of N and P were lower at higher plant densities because plants accelerated production of fibrous, supportive tissue which have low concentrations of N and P. Plants absorbed nutrients most effectively at densities of 1000 g m^{-2} (dry weight) (Debusk and Reddy, 1987).

Characteristics associated with high yields by freshwater macrophytes are high leaf area index, minimum of structural or support tissue, large amount of foliage exposed to high irradiances, and high atmospheric carbon dioxide (Debusk and Reddy, 1987). Changes in morphology of plants were noted with respect to plant densities: at high densities the mean shoot length was greater than at lower densities (compare 37.1 cm for 150 g m^{-2} to 74.0 cm for 1000 g m^{-2}). Large lamina size and greatest total leaf area occurred at the greatest density (Debusk and Reddy, 1987).

When plants crowd and self-shading occurs due to intraplant competition, net productivity measured by dry weight decreases (Debusk and Reddy, 1987). In high standing crops under conditions of crowding new leaves replace older ones which become senescent and decompose (Debusk and Ryther, 1987). Crowding inhibits biomass production generally and causes stems to elongate in order for the leaves to acquire adequate light (Dinges, 1982). Float leaves disappear and the plants can grow up to 120 cm in height.

High quality effluent water was reported in Coral Springs, Florida in ponds with "loosely packed" hyacinths (Reed, Middlebrooks, and Crites, 1988).

The water hyacinth canopy grows in a way which minimizes evapotranspiration from supportive structures such as petioles (Center and Spencer, 1981). Canopy configuration varies with wind currents, inter-plant competition (Center and Spencer, 1981) and nutrient availability (Debusk and Ryther, 1983). Morphological changes occur as plants respond to these stresses and ultimately influence the effluent water quality. Overall morphological changes in canopy structure can be attributed to response of individual plants to maximize photosynthetic efficiency (Boyd and Scarsbrook, 1975; Center and Spencer, 1981).

Temperature

Growth effects

Temperature can impose dramatic stress on water hyacinth plants and is the limiting factor in biological wastewater treatment systems. Water hyacinths prefer water temperatures below 40 C and above 10 C (O'Brien, 1981). Air temperature must remain stable as well. Temperatures of -3 C for twelve hours will destroy leaves. Exposure to -5 C for 48 hours will kill the plants entirely (O'Brien, 1981). Water hyacinths survive freezing temperatures only if the main crown remains intact (Reed, Middlebrooks, and Crites, 1988).

In Shreveport, Louisiana, 95 percent of the plants growing in the lagoon died and sank to the bottom after the first heavy frost of 0 C (Black and Veatch Engineers, 1983). Boyd (1969) stressed that *E. crassipes* was incapable of overwintering in temperate regions.

Temperature, more than solar radiation, influences productivity of water hyacinth biomass (Debusk and Reddy, 1987). Net productivity was significantly correlated ($P<0.01$) with mean temperature. Maximum growth occurred during the months of highest temperature rather than during periods of high solar radiation.

Debusk and Ryther (1981) found that maximum growth occurred during the month of July with net productivity averages of 29.8, 38.0, 41.8, and 51.1 kg m^{-2} for densities of 150, 350, 700, 1000 kg m^{-2}. As expected, minimum plant growth occurred in November and December with averages ranging from 15.1 to 21.4 g m^{-2} at 150 and 1000 kg m^{-2} densities.

Survival and performance

In work done in 1975-76, Dinges showed that plants could help produce water of high quality (as measured by BOD and TSS levels) in winter months even though the plants lay dormant and frozen (Dinges, 1982). In Williamson Creek, Texas, during the months of November, December and January water hyacinths reduced BOD readings from 24 to 31 mg/l down to 2.4 to 10 mg/l (Dinges, 1982).

In Orange Grove, Mississippi, freezing temperatures killed the tops of plants and subsequent plant decay elevated the BOD and TSS levels during the critical three month period of January through March (Wolverton and McDonald, 1977). Cold temperatures in Roseville, California, caused die-back of stalks and leaves of plants in wastewater ponds (Hauser, 1984). The dead hyacinth plants formed a thick mat over the ponds throughout winter and early spring, but the plants grew to full density by early summer.

Insects and pathogens

Available literature on insect relationships with water hyacinth is the result of studies on natural insect enemies to control the plant. Water hyacinth stands tend to be more susceptible to insect attack during cold weather and high density (Debusk and Ryther, 1981). In natural stands root material begins to decay when plant density exceeds approximately 25 kg m^{-2} (wet weight) (Reed, Middlebrooks, and Crites, 1988). Decayed tissue provides a breeding area for insects. The most common insects reported to infest water hyacinth are caterpillars, weevils, moths, and grasshoppers (Table 1).

Perkins (1973) grouped hyacinth pests into four broad catagories: defoliators and external leaf feeders, petiole borers, leaf tunnel producers and scavengers that enhance attack by other insects. Damage from these different types of insects can take the form of direct consumption of the tissue and/or decomposition of the tissue surrounding the feeding area. Such damage, if severe, can decrease or stop reproduction entirely.

Grasshoppers, caterpillars and weevils attack the leaves creating large irregular white scars in the case of <u>Cornops</u> <u>spp.</u> and defoliation in the case of <u>Paroxya clavuliger</u>.

Table 1 . Insects attacking *Eichhornia crassipes* and how plant is affected.

Type of Insect	How plant affected
Aphids *Rhopalosiphum myphaeae* (L.)	Leaves, petioles, and crown are dessicated from aphids sucking out plant juices
Caterpillars *Acigona infusells* (Walter) *Palustra silveiraquidor* Orfila	Plant vigor is reduced in the petioles
Grasshoppers *Carnops* (Acrididae)spp. *Paroxya clavuliger* (Serville)	Large irregular white spots appear on the leaves followed by defoliation
Moths *Sameodes albiguttalis* (Warren)	Dark spots on leaf
Scarab beetles *Blattidae* spp. *Carabidae* spp. *Chalepides* spp. *Cyclocephala* spp. *Dyscinetus* spp.	Plant vigor is reduced when flowers, crown and petioles become necrotic
Weevils *Neochetina eichhornia* Warner *N. bruchi* Hustach	Leaves, petioles and crown become soft and necrotic

(Perkins, 1973; Dinges, 1982; Buckingham, 1984).

Caterpillars prefer the petioles, boring a tunnel the size of the boring larvae. Saprophytic fungi then enter the damaged tissue. This weakens the petioles to the point of breaking.

Scarab beetles (*Dyscinetus, Chalepides* and *Cyclocephala*) feed on the crown and petiole of individual plants. Brazilian and Argentinan species of *Blattidae* and *Carabidae* feed on flowers (Perkins, 1973).

Perkins (1973) reported that aphids *Rhopalosiphum myphaeae* during the adult and nymph stage damaged hyacinth plants by sucking plant juices from the petioles and leaves.

Very little has been reported on the outbreak of aphids but researchers at the Stockton, California., facility mention aphid attack as localized and insignificant (City of Stockton, 1985).

The insecticide Sevin has been used to control weevil and caterpillar infestations (Lee and McKim, 1981) and aerial spraying of malathion has been used to control spider mites (McDonald and Wolverton, 1980).

Water hyacinths are also subject to pathogen attack. In Sri Lanka five pathogenic fungi were identified as capable of producing leaf spot disease: *Myrothecium roridum*, *Cercospora piaropi*, *Curvularia tuberculata*, *Septofusidium elegantulum* and *Phaeotrichoconi crotalariae*.

Leaf spots were typically oval, yellowish brown to black and 3 to 5 mm in diameter (Hettiarachchi, Gunasedera and Balasooriya, 1983)

Other pathogenic fungi which can attack water hyacinth are *Cercospora rodmanii*, *Acremonium zonatum*, *Alternaria eichhorniae*, *Myrothecium roridum*, *Rhizoctonia solani*, *Nigrospora sphaerica*, *Cephalosporium zonatum* (Hettiarachchi, Gunasedera and Balasooriya, 1983).

Application of Eichhornia crassipes in wastewater treatment

Summary of treatment facilities using water hyacinth

Eichhornia crassipes has been the favored aquatic plant for use in wastewater treatment because it reproduces quickly and floats to promote easy harvesting (Reed, Middlebrooks, and Crites, 1988). Aquatic plants have been investigated for at least the past twenty years as part of wastewater treatment systems (Boyd, 1969; Wolverton, 1981; Stowell, 1987; Debusk and Reddy, 1987; Reed, Middlebrooks, and Crites, 1988). They reduce the chlorine requirement and other costs associated with traditional systems and generally remove nutrients from wastewater more efficiently as measured by biological oxygen demand and total suspended solids.

In the late 1960s and 1970s water hyacinths were used for research in primary and secondary treatment by the

National Space Technology Laboratory at Bay St. Louis, Mississippi; Lucedale, Mississippi, Orange Grove, Mississippi, Cedar Lake, Mississippi, and Williamson Creek, Texas. In Sri Lanka, India and Indonesia studies have focused on treatment of effluent from tanneries, rubber factories, palm oil processing plants and paper mills (Thyagarajan, 1984).

In the 1980s, temperate regions in the U.S. investigated water hyacinth use and water quality in Sugarbush, Vermont, Harwich, Massachusettes, San Diego, Stockton and Roseville, California. (Middlebrooks et al., 1982). Some of these facilities operated with domestic sewage only; other research has involved industrial sewage (Table 2).

All wastewater treatment facilities are based on several principles. Raw sewage is initially treated in primary clarifier to remove solids such as grit, grease and plastic. In traditional systems, the effluent is then pushed through activated sludge or trickling filter systems called biofilters or biotowers where bacteria and other microorganisms remove organic matter. At this second stage, water hyacinth lagoons can be used. The third step involves another clarification to remove suspended solids. The purified water is chlorinated and released (Figure 3). Many wastewater ponds experience high temperatures which

Table 2. Wastewater treatment facilities using *Eichhornia crassipes*, year, and type of wastewater effluent used (* indicates facility still in operation).

Location of facility	Year(s)	Effluent used
Bay St. Louis, Miss.	1976-77	Raw to primary
Cedar Lake, Miss.	1977	Secondary
Coral Springs, Fla.	1978-79	Secondary and tertiary
Disney World, Fla.	1978-80	Primary, secondary, and tertiary
Harwich, Ma	1984-87	Primary and secondary
Hercules, Ca.	1980-81	Primary and secondary
Lakeland, Fla.	1979	Secondary and tertiary
Lucedale, Miss	1976	Raw to primary industrial
National Space Technology Lab, Miss.	1975-77	Raw to primary
Orange Grove, Miss.	1979	Primary to secondary
Providence, RI *	1989-90	Primary industrial
Roseville, Ca.	1979-82	Secondary and tertiary
San Diego Pilot Plant, Ca.*	1983-89	Primary and secondary
Shreveport, La.	1980-81	Primary and secondary
Stockton, Ca.	1984-85	Secondary
Sugarbush, VT	1987-88	Primary and secondary
Williamson Creek (Phase I), Texas	1975	Secondary and tertiary
" " (Full scale), Texas	1976	Primary and secondary
" " (Phase II), Texas	1977-1980	Primary and secondary

(Middlebrooks, et al., 1982)

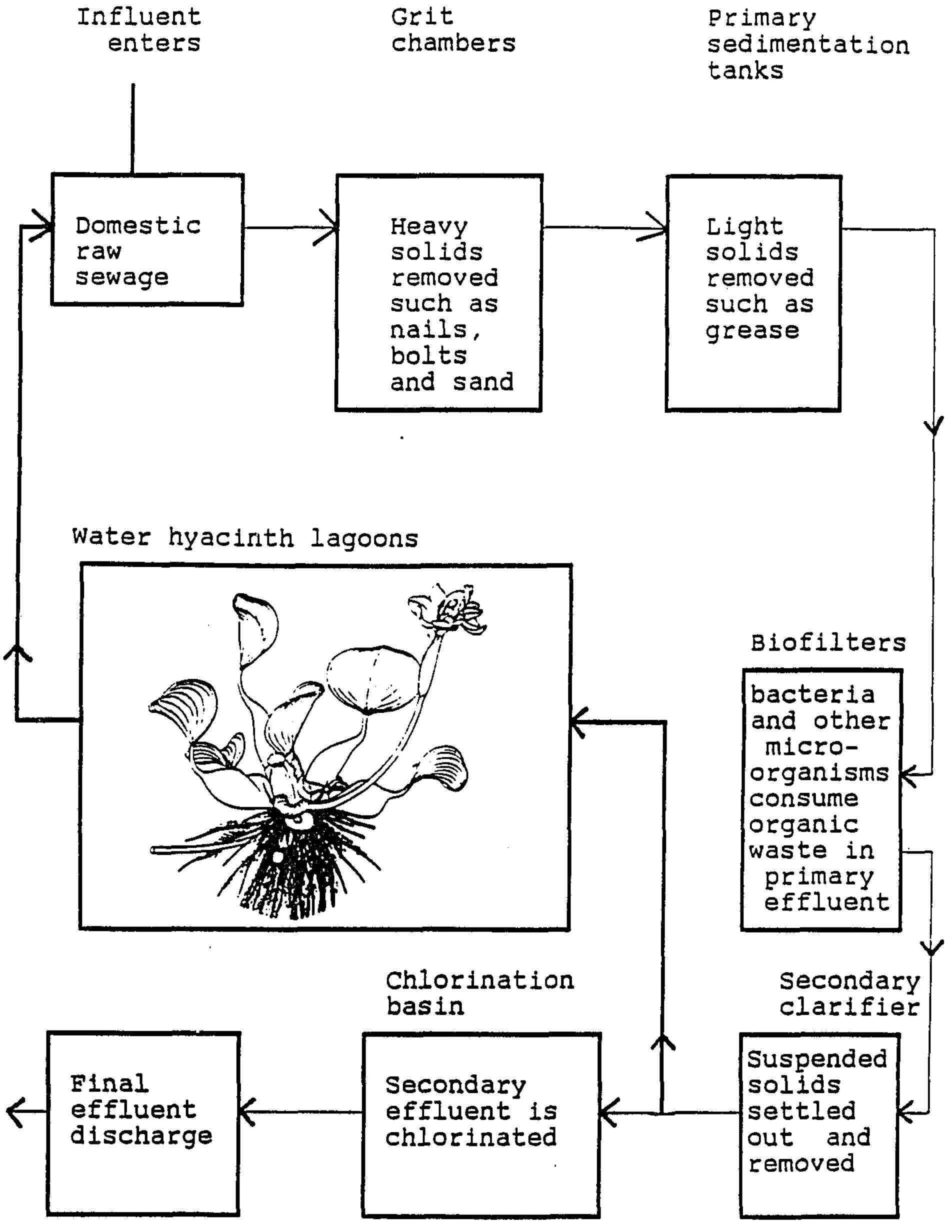

Figure 3. Flow diagram of Roger Road water hyacinth based pilot sewage treatment facility. Light lines indicate flow of wastewater in traditional systems; bold lines indicate flow of wastewater at Roger Road water hyacinth pilot research facility.

may exceed the limits of tolerance of cool water species, deplete oxygen directly or enhance the rate of microbial respiration. Two parameters used to assess the effectiveness of sewage treatment systems are biological oxygen demand and total suspended solids. For BOD a standard equilibrium concentration is 3 to 5 mg/l.

Other species of macrophytes, including Myriophyllum brasilienses (parrot feather), Paspalum fluitans (aquatic grass) and Lemna spp. (duckweed) are thought to be more likely to succeed because of their greater cold tolerance. Several submersed aquatics have also been suggested for treatment use in colder climates including Potamogeton Berentoidii and Ceratophyllum demersum and Elodea canadensis. Emergent aquatics have been put to use in temperate climates including Phragmites communis (rushes), Scripus acutus and Iris pseudacorus (Dinges, 1982). Hydrocotyle umbellata (pennywort), another floating aquatic macrophyte, is more cold tolerant that Eichhornia spp. and produces biomass at the same rate during winter and summer in tropical and subtropical environments (Reddy and Debusk, 1984).

Eichhornia crassipes contributes significantly to the "food web" of a wastewater treatment system. Through decomposition near the water surface, water hyacinth degradation enhances nitrification and denitrification processes (Wooten and Dodd, 1976). If degradation occurs too dramatically, sudd, a floating mat of partially decayed material, builds up. Sudd formation can be enhanced by frost-kill of the aerial portion of the plant (Dinges, 1982). In Georgia, Schreiner (1980) found thirty times as much detritus in an older stand compared to a younger stand. Overall, continuous leaf turnover by water hyacinth during the growing season coupled with winter dieback convert most of the leaf biomass into next season's detritus (Schreiner, 1980).

Productivity and yields

There is much speculation as to the quantity of biomass produced in an aquaculture system and very little agreement as to the amount of biomass accrued over time (Table 3). According to Smith (1985) standing crops range in density from 0.02 to 0.03 kg m^{-2} depending on water quality. Boyd (1969) places standing crop at 0.51 to 2.9 kg m^{-2} dry weight and O'Brien (1981) at 1.0 to 4.1 kg m^{-2}. In California Dinges (1976) reported 3.18 kg dry weight m^{-2}.

These variations in "standing crop density" can be accounted for by crowding and the amount of nutrient present in the water. Standing crop for water hyacinths in domestic sewage ranged from 1.0 to 3.18 kg m^{-2} dry weight.

Table 3. Summary of standing crop and productivity with location, yield reported, converted yield and source.

Location	Yield reported	Converted yield	Source
	Standing crop		
Florida	200 to 300 Mg ha^{-2}[z]	2.0 to 3.0 kg dry wt. m^{-2} [y]	Smith, 1985
Alabama	12.8 M tons dry wt. ha^{-2}	1.28 kg dry wt. m^{-2}	Boyd, 1969
Texas	44.6 to 182.9 t fresh wt. acre	1.0 to 4.1 kg dry wt. m^{-2}[y]	O'Brien,1981
Mississippi	2970 g dry wt. m^{-2}	2.97 kg dry wt. m^{-2}	Wooten and Dodd,1976
Texas	3180 g dry wt. m^{-2}	3.18 kg dry wt. m^{-2}	Dinges, 1976
	Productivity		
California	5900 kg fresh wt. ha^{-2} d^{-1}	29.5 g m^{-2} d^{-1} [x]	Hauser, 1984
Alabama	180 to 220 kg dry wt. ha^{-2} d^{-1}	18 to 22 g m^{-2} d^{-1}	Boyd, 1976
California	51 g dry wt. m^{-2} d^{-1}[w]	51 g dry wt. m^{-2} d^{-1}	Loomis and Williams, 1963

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[z] Assumed to be fresh weight.
[y] For original values reported in fresh weight plants were assumed to be 10 percent dry weight.
[x] Using the reported 5 percent dry weight.
[w] Dry weight reported for Sudangrass (S. vulgare).

Management practices

Harvesting

Harvesting is an important management practice in a water hyacinth wastewater treatment system. By appropriate harvesting, biomass production and nutrient uptake can be increased (Dinges, 1982). Harvesting hyacinths can be a potentially labor intensive and time consuming activity. One test pond in Roseville, California was harvested with a back hoe fitted with a rake 3 meters wide (Hauser, 1984). Dinges (1982) suggested periodic mowing of stems and leaves after horizontal growth to facilitate increased biomass production.

Frequent harvests are considered necessary by some to keep the plants at the optimum growth stage (Reed, Middlebrooks, and Crites, 1988). Debusk and Reddy (1987) state that harvesting affects the rate at which nutrients are removed. Frequent harvests promote biomass production and P removal but tend to inhibit N removal. Infrequent harvests promote N removal; the population of microorganisms involved in nitrification remains intact over longer periods of time and thus nitrates are removed from the water. Fisher and Reddy (1987) studied eutrophic lake water with varying amounts of water hyacinth coverage and concluded that plant uptake of N was increased with frequent biomass harvesting.

Hauser (1984) determined that routine harvesting was unnecessary to ensure effective treatment and high water quality. Harvesting did not improve overall ammonia or total nitrogen reduction in the warm season. Harvesting tended to decrease nitrification activity because removal of the plants or "bacterial support structures" meant removal of the bacteria itself.

In ponds which were not harvested, plants reached a "state of homeostasis" where biomass production ceased (Hauser, 1984). With plants no longer creating new biomass plant N uptake was reduced which increased the likelihood that ammonia removal took place via bacterial nitrification.

Dinges (1982) concluded that dry biomass production under harvesting with ideal temperatures and nutrient levels was 25 percent more than dry biomass production without harvesting. He supported the idea that harvesting at intervals was the best way to achieve maximum hyacinth biomass production.

The decision to harvest water hyacinths in a wastewater treatment system is a function of canopy height, canopy architecture and nutrient levels which affect the quality of water (O'Brien, 1981).

Aeration

Most conventional wastewater treatment ponds are mechanically aerated to maintain complete and consistent aerobic conditions. Significant amounts of energy input are required for continuous aeration. Water hyacinths and other aquatic macrophytes create aerobic environments and help reduce the need for mechanical aeration.

In their study Ogwada et al. (1984) showed that under aerobic conditions inorganic N release from decomposing aquatic macrophytes depends on the C/N ratio of the plant tissue. Forty-eight to 76 percent of the plant N and 67 to 90 percent of the plant P were released at the end of aerobic decomposition.

In Roseville, California, Hauser (1984) compared three management practices; unmanaged, aerated but not harvested, and harvested but not aerated ponds. He found that harvesting did not improve overall ammonia or total nitrogen reduction in the warm season and led to lesser reductions than that in the aerated pond in the cold season. The aerated pond produced the lowest effluent ammonia concentrations. Aerating ponds also cuts down on algae buildup and reduces odors.

Frost protection: mitigating low temperature stress

Water hyacinth survival is a function of weather and the effects of man, animal and insect. Because of low temperatures in Tucson, water hyacinth use in wastewater treatment will likely require some type of low temperature protection.

For all plants freezing stress occurs when small ice crystals form within the living cytoplasm (Geiger, 1965). Plants intolerant of frost allow for ice to grow outside of the cells.

Damaging frosts can be a combination of both radiation frosts caused by radiative cooling at night and advection frosts caused by advection of cold air and strong winds. Approaches to frost protection are twofold; heat lost by radiation can be reduced or alternatively heat can be supplied. Prevention of radiation loss is achieved by covering plants with smoke and structures such as greenhouses or shade cloth. Droplets of water in mist clouds act as good reflectors of long wavelength radiation and prevent excessive heat loss from the plant canopy (Geiger, 1965). Another method of protecting sensitive plants against frost is to supply heat by covering them with a layer of ice by spraying them with water. Every gram of water that freezes liberates

80 cal of latent heat. This released heat maintains the leaf temperature at 0 C during frost periods even though air temperature may be lower than this. Water must be continually applied as long as air temperature is below freezing. Usually the use of water as a frost protection system is restricted under clear-sky nighttime conditions (Barfield and Gerber, 1979).

In several studies, Parsons et al. (1981, 1982, 1985) used low volume microsprinkler under-tree irrigation of young citrus as a method of frost protection. Leaves at the outer edge of the canopy of treated plants were 0.6 C warmer than leaves in the control. The study concluded that this system could be used only during calm conditions and not during dry, windy nights. Evaporative cooling was found to be a major problem when water was used in windy, low dew point freezes (Parsons et al., 1985).

Most facilities using water hyacinths have avoided the need for frost protection because they are located in areas where winters are not severe. In Shreveport, Louisiana two frost protection systems were employed: an impact type sprinkler and a stream type sprinkler. Water hyacinths in the high pressure--impact-type sprinkler--irrigation zone appeared less affected by the freezes in terms of growth and development (Black and Veatch Engineers, 1983).

One of the easiest ways to protect water hyacinths is to use older plants to protect younger plants from colder temperatures (O'Brien, 1981). Older plants are no longer in the active growth phase, do not remove nutrients and are known to attract insects (O'Brien, 1981). Plants killed by low air temperature, though, will also act as a barrier to sunlight for the younger plants so a trade-off occurs with this type of frost protection. O'Brien (1981) suggested that these plants be harvested to increase nutrient removal and prevent insect infestation.

Stowell (1987) suggested that the water hyacinth mat itself reduces radiation and conductive heat loss from the wastewater during winter by blanketing the ponds.

In Japan water hyacinths without frost protection over winter successfully where the average temperature in January is 1 C (Ueki et al., 1975).

More elaborate frost protection facilities such as heated greenhouses coupled with expanded facilities for the removal of plants could be used to upgrade water hyacinth lagoon facilities (Black and Veatch Engineers, 1983). The most serious weakness with this kind of frost protection system, however, is that failure of a greenhouse heating unit could lead to death of the plants (Black and Veatch Engineers, 1983).

Other management practices

Mosquito and odor control, sludge removal and disposal of harvested plants can also pose problems for facilities using water hyacinths in the wastewater treatment system.

MATERIALS AND METHODS

General Set-up

This research was carried out from July 1988 to August 1989 at a pilot water hyacinth wastewater treatment facility east of the Roger Road Wastewater Treatment Facility in Pima County, Tucson, Arizona (lat. 32, 15 ' N and long. 110, 57 'W, alt. 813 m).

The facility includes five ponds lined with Hypalon chlorosulfonated polyethylene manufactured by Palco Linings, Inc., Stanton, California, with a thickness of 3.1 mm (Figure 4). The ponds are 9 m by 66.6 m. For this study all ponds received secondary effluent from Roger Road Wastewater Treatment Facility. Flow rates were approximately 5.2 liters per minute into each pond and entered the south end through a manifold with six openings. Influent and effluent flows were recorded daily using totalizing flow meters. The water depth was 1 m and the ponds were 1.3 m deep. The five ponds were fitted with the following frost protection systems:

The first pond has no frost protection system and serves as the control.

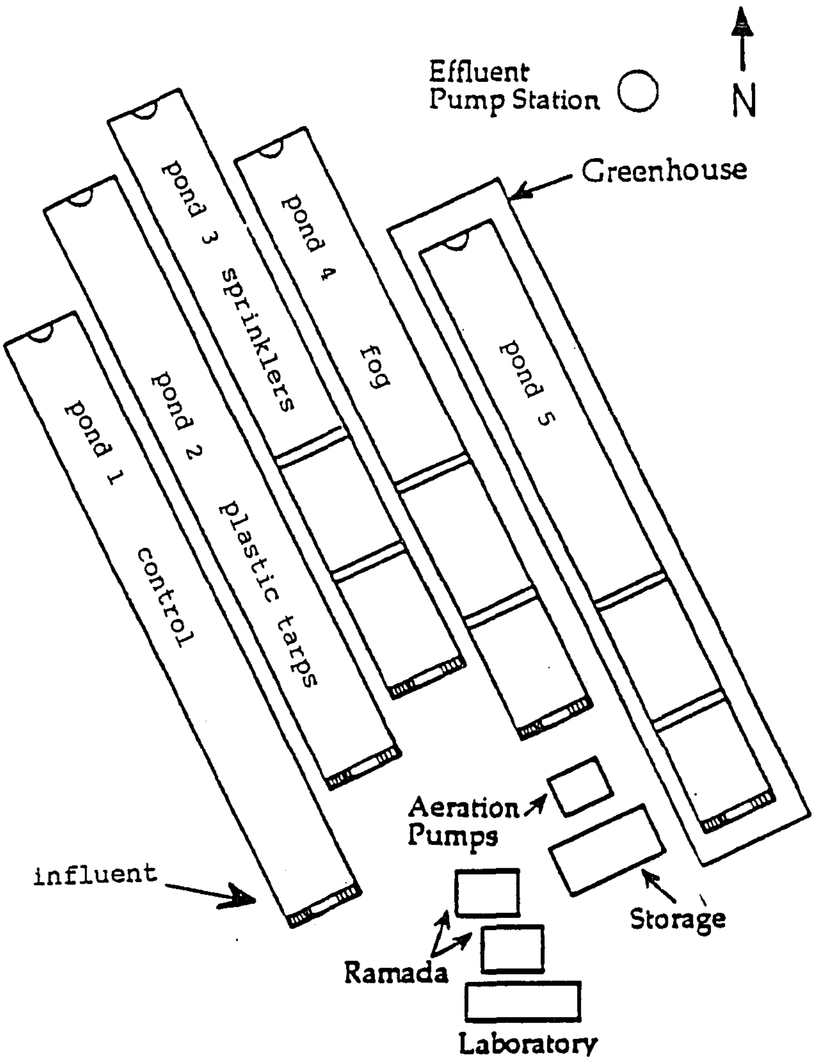


Figure 4. Schematic of Roger Road water hyacinth pilot research facility.

Pond 2 has five plastic tarpaulins, four 20 x 13.3 m and one 6.6 x 40 m. which are manually pulled over the pond.

Pond 3 uses five Rainbird sprinklers with Nelson Alpha II nozzles spaced every 10 m that spray recycled effluent from the ponds over the plants at approximately 9 liters per minute.

Pond 4 has a Mee fog system (Mee Industries Inc., El Monte, California, model 3000) equipped with 128 nozzles that collectively deliver 1.040 liters per minute of municipal water. A 1.6 m frame covered with polyethlene film is constructed around the pond to contain the fog.

The last pond is contained within a Stuppy greenhouse which was erected in October 1988 with automatic side curtains (UV polyfilm) which are thermostatically controlled and made by A.T. Newell Co., Inc., in Burmingham, Alabama.

For data collection purposes, each pond was divided into influent (0 to 22 m), center (23 to 44 m), and effluent (45 to 67 m) areas. Ponds 3 (sprinkler), 4 (fog) and 5 (greenhouse) have bridges approximately 9 m by 2 m across both the influent and effluent areas.

On July 19, 1988, 5,000 water hyacinth plants were obtained from various private individuals in the Tucson area and introduced into pond 2 where they were allowed to grow and multiply. Approximately equal numbers of these plants were divided and then placed into each of the five ponds on August 9,1988. By September 8, 1988 the five ponds were completely covered with plants.

From September 1988 to August 1989 data was collected on individual plants, biomass production and winter survival of plants in the five ponds.

Individual plant analysis

The analysis of individual plants included measurements of root length, entire length of the plant, and fresh and dry weight. Simultaneously, qualitative assessment of plant growth and development, health and ecology was taken and included weekly photographic documentation of plants within the entire pond with observations made as to color, chlorosis, necrosis, presence of insects and turgidity of plants. These data were recorded for each section (influent, center, effluent) of each pond. Twice a month, three plants were sampled randomly from these points for a total of 18 plants for each pond per harvest. Since access to plants was limited, sampling was restricted to approximately one meter from the edge of the pond for ponds 1 and 2. Bridges facilitated sampling at the influent and center of the fog, sprinkler and greenhouse ponds (Figure 4).

In all cases, the effluent point was sampled from the shore. After these plants were harvested the following data was collected:

Root length was measured from the top of the crown to the tip of the longest root.

Entire plant length was measured from the tip of the longest leaf to the tip of the longest root.

Fresh weight was determined by placing plants (after blotting) into a 4000 ml container and weighing them with an O'Haus Dial-a-gram balance.

After fresh weights were determined plants were placed in labelled paper bags and dried in a drying shed for five days at 60 C. Dry weights were then determined.

Weekly observations of plants in each pond were made as to leaf turgidity, color, number of flowers, necrosis, chlorosis, biomass coverage, presence of insects and symptoms of insects observed. Observations were made by walking down each pond, pulling plants for closer observation of insect infestation, and noting the presence of additional fauna (or flora in some cases) in or around the pond(s).

Weekly photographs were taken of plants in each pond from a point on the south and north ends. Close-up photographs were taken at designated positions along the pond to record the condition of the plants at the influent, center and effluent sections.

Biomass

Fresh and dry weights were collected to measure biomass production starting in January 1989 and continuing to August 1989. Canopy heights were determined from September 1988-August 1989. In order to sample the plants at random locations within the ponds, a "Genie-boom"--an hydraulic lift either gas or electric operated which is activated from the ground or the platform by the operator--was used. Plants were sampled from 0.25 m^2 areas using a metal or plastic guide placed over the plants. Plants were then sliced out of this area using an electric carving knife or hand saw. Three plots of 0.25 m^2 from the influent, center and effluent ends of each pond were randomly located and then removed. Once freed from surrounding biomass, all the plants in the 0.25 m^2 area were weighed, and a sub-sample was bagged and labelled to be placed in a drying shed for seven days at approximately 60 C. After approximately seven days depending on the time of year, the samples were removed from the drying shed and dry weights were determined and the total biomass kg m^{-2} was calculated for each point in each pond.

Canopy height was measured bi-weekly from September 1988 to August 1989 using a meterstick. Because the plants were difficult to access in all five ponds, measurements had to be taken from the shore. Three samples were made by extending the meterstick at arms length to the water surface as determined by the point at which the stick reached the water. The distance from the water surface to the top of the leaf nearest to the meterstick represented the canopy height. Three samples were taken at three areas in each pond for a total of nine samples per pond.

Survival study

Data on plant survival after over-wintering was collected. On November 11, 1988 fifty plants in each pond were tagged for visual monitoring and evaluation. Because of limited access to the plants themselves, tagging was restricted to those plants within "arms-reach " from the shore. Using a random numbers table to locate the plant to be sampled, plants were tagged every few feet with flagging tape and numbered one through fifty. The tape was knotted as securely as possible over the largest petiole close to the crown.

On March 29, 1989 tagged plants were harvested. Plants were removed and categorized as: 1) plants and/or tags not found, 2) tags found but not attached to any plant 3) tags attached to a dead petiole which was attached to a dead plant 4) tags attached to a dead petiole which was attached to a live plant, and 5) tags attached to a live petiole on a live plant.

The pond number, plant number and location of the plant (left or right of pond, south or north) was recorded as well.

Percent living plants was calculated using the total number of plants originally tagged (N=50) and the number of plants actually recovered. Categories 4 and 5 above were defined as living plants.

Additional data collected

Average rainfall, minimum and maximum ambient air temperatures, air temperature inside the greenhouse, air temperature at and in the plant canopy, water temperature at the crown, ambient water temperature, and wind direction and speed were recorded daily.

RESULTS AND DISCUSSION

Weather

Air temperature

Many nights of freezing temperatures were experienced during this study and unprotected plants were subjected to severe leaf and petiole damage. Sixty nights of 0 C or lower temperatures occurred (Figure 5). Nights of <10 C occurred from November 16, 1988 to March 4,1989; the lowest temperature recorded was -10 C on December 28, 1988. Temperatures in the greenhouse never dropped below 0 C.

Water temperature

Water temperature in all ponds ranged from 12 C to 25 C throughout the winter and never reached 10 C, a temperature known to be lethal to water hyacinths. The influent end tended to be approximately 5 C warmer than the effluent (Figure 6). Ponds 1-4 were slightly cooler than the greenhouse.

Frost survival

Fifty plants in this study were tagged on November 15, 1988 and recovered on March 15, 1989. Survival percent living and dead was used to assess the effectiveness of frost protection systems during winter.

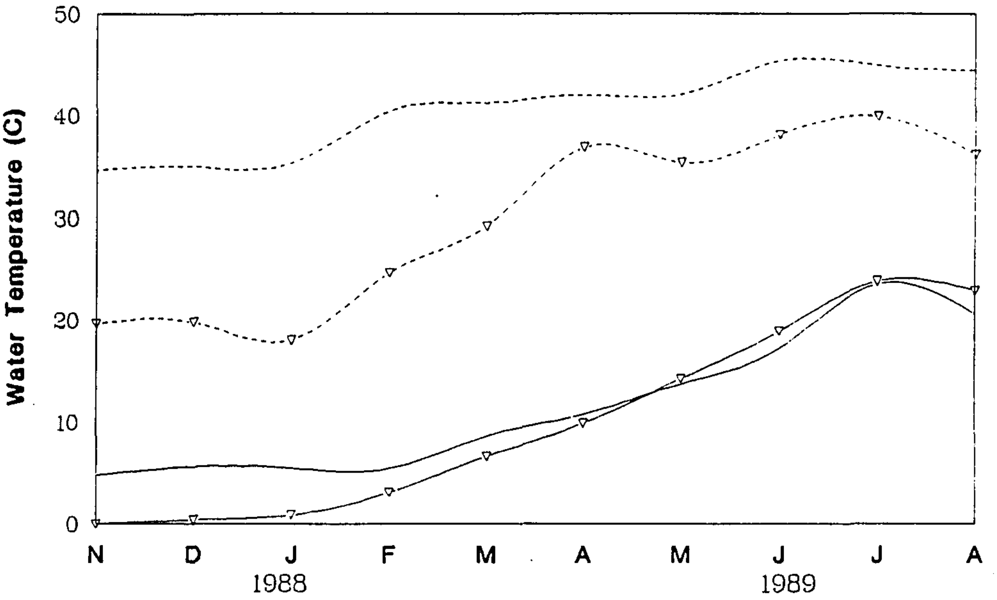


Figure 5. Average monthly air temperatures for the period November 1988 through August 1989 at the Roger Road water hyacinth pilot research facility (ambient maximum [-▽-], ambient minimum [—▽—]; greenhouse maximum [- - -], greenhouse minimum [——]).

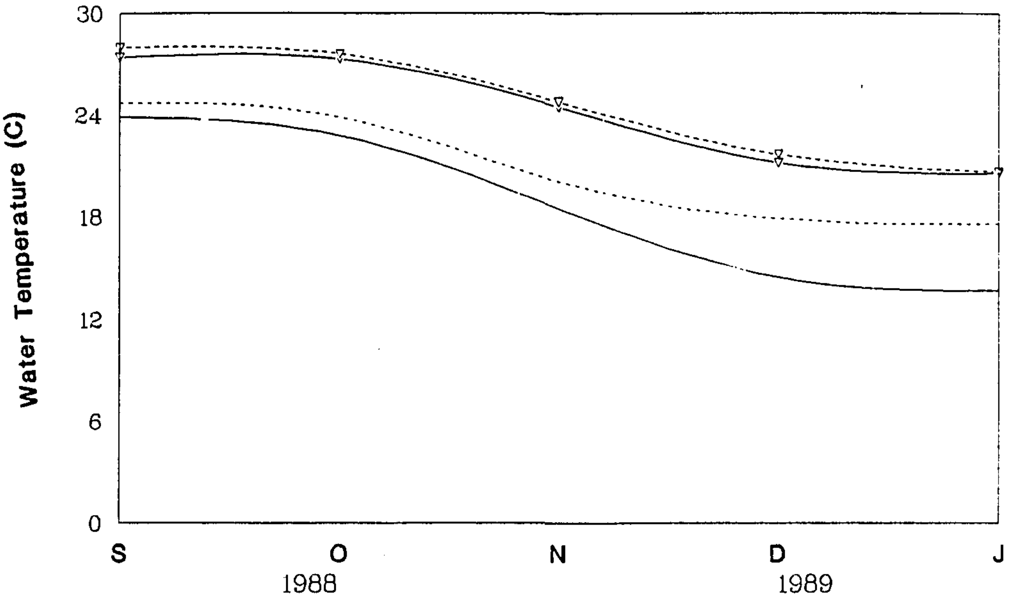


Figure 6. Pond water temperature for the period September 1988 through January 1989 at the Roger Road water hyacinth pilot research facility (ponds 1-4, influent [—▽—] effluent [——]; greenhouse influent [-▽-], effluent [- - -]).

The percent of tagged plants living on March 15, 1989 was 48, 70, 34, and 76 for tarps, sprinklers, fog and greenhouse treatments (Table 4). The highest percentage recovered came from ponds with the sprinkler system and the greenhouse. Seventy percent of the plants protected with sprinklers and 76 percent of the plants grown in the greenhouse survived winter. Sprinkler protected plants showed the highest survival percentage though a smaller number of plants (27 out of 50) were actually recovered (Table 4). In the sprinkler protected pond, ice insulated the plants from low temperatures whereas only 25 percent of tagged plants in the control lagoon survived. All forms of frost protection treatments resulted in higher survival than the control pond.

Overall, plants in all five ponds, including the control, survived winter well enough to recover and repopulate all ponds by May 1989. As indicated by Reed, Middlebrooks and Crites (1988), the crowns of the plants survive because of warm water temperatures. Warm water temperatures throughout this study likely helped keep water hyacinths alive through the winter.

Temperature stress

With the first frost on November 15, 1988 (-1.5 C) some visual damage was observed in plants in ponds 1 (control)

Table 4. Survival of water hyacinth plants coming out of winter.

Raceway Description	Number Recovered (NR)	Percent Living		Percent Dead	
		N=50	N=NR	N=50	N=NR
Control	32	8 (4)	25	56(28)	75
Plastic tarps	31	30(15)	48	32(16)	52
Sprinklers	27	38(19)	70	16(8)	30
Fog	35	24(12)	34	46(23)	66
Greenhouse	21	32(16)	76	10(5)	24

Note: Numbers in parentheses indicate actual number of plants recovered.

and 2 (plastic tarp) with lamina and petioles becoming brown, curled and necrotic. Many upper canopy leaves died and collapsed exposing younger leaves and unprotected plants to colder temperatures. Plants with sprinkler, fog and greenhouse protection during this first freeze appeared to sustain less injury than the unprotected control plants and plants protected by plastic tarps. In December plants in all five ponds suffered temperature stress as an average minimum temperature of 0.5 C for ambient air temperature was recorded for the month. Unprotected plants and those protected by plastic tarps suffered extensive necrosis and deformity during the months of December and January. This damage was apparent in older plants until March 1989. Water has been used effectively in protecting citrus (Parsons et al., 1981, 1982, and 1985) and appeared to protect water hyacinth above-water tissue in much the same way. Water hyacinths protected by sprinklers and fog showed less lamina and petiole damage, perhaps because of water-driven frost protection measures. Heavy fraying of leaves occurred in the plants exposed to water from the sprinklers throughout the frost season. Plants protected by fog showed more severe upper canopy damage (necrosis and chlorosis) than plants protected by sprinklers and experienced some frayed leaves as well.

General plant morphology

From July/August 1988 to September 1988 observations showed the water hyacinths were green, turgid, healthy and reproduced rapidly. Upper canopy debris was observed in ponds 1-4 from November to April and appeared to result from plant die-off caused by frost and aphid attack. In the greenhouse from October 1988 to May 1989 biomass die-off occurred mainly as a result of insect stress as temperature stress was not a factor (Appendix A).

Plant morphology reflected temperature and insect stress throughout the frost period. Few daughter plants were produced and root length decreased with lower air temperatures (Figure 7). Petiole turgidity as well as general plant turgidity decreased as ambient temperatures decreased. Sampling of plants became increasingly more difficult as the upper canopy deteriorated during the winter.

Flowering occurred in control, tarp and greenhouse ponds in May, June, July and August of 1989. Penfound and Earle (1948) indicated that flowering was induced by tall dense stands of water hyacinths. This was not the case in this study as biomass density was high in all ponds but flowering occurred only in three of the five ponds studied.

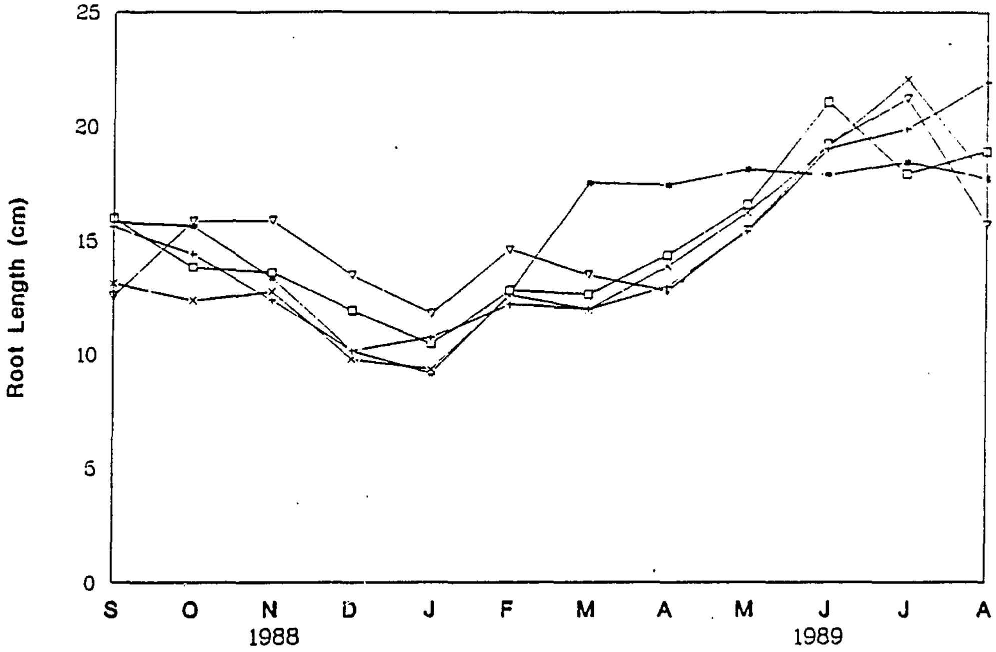


Figure 7. Root length of water hyacinth plants for the period September 1988 through August 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [—□—] sprinklers [—×—], fog [—▽—], and greenhouse [—‡—]).

Individual plant measurements and temperature stress

Not only was survival increased with frost protection treatments, but data on growth of individual plants also suggested that use of sprinklers or a greenhouse provided more frost protection than other systems investigated. Plants in all ponds showed reduced root length with lower temperatures. Means reported showed distinct trends with regard to low temperatures and standard deviations indicate high variability among plants sampled within each frost protection treatment pond (Appendix B). Root lengths in the greenhouse were greater than those in ponds 1-4 from September 1988 to November and from March to May 1989. Root lengths for plants in the greenhouse in February and June through August 1989 were lower than those for ponds 1-4 (Figure 7). In February, plants in all ponds showed similar average root length but in March the root lengths in the greenhouse increased dramatically then stabilized in May, June and July. Roots from plants in other ponds grew rapidly in April through June becoming longer than those in the greenhouse in June (Figure 7). During winter (November 1988 through February 1989) root lengths in the fog protected pond were greater than control plants and other treatment plants. In March the plants in the greenhouse showed the longest roots. Root length is a function of nutrient status of the water (Reddy and Debusk, 1987) and longer roots in the greenhouse may have indicated a shift toward more nutrient poor water. During the winter short roots in the greenhouse indicated more nutrient rich water whereas longer roots in the fog protected pond indicated nutrient poor water. Plants also responded to lower temperatures by decreases in root/shoot ratios.

Unprotected plants and those protected with plastic tarps had shorter canopy heights (Figure 8) from November 1988 through August 1989 and less fresh and dry weight (Figures 9 and 10) in April 1989 compared to their counterparts protected by sprinkler, mist and greenhouse systems. Despite dry weights in January and February being lower in the greenhouse than in other ponds, greenhouse plants outperformed those in other ponds for all seasons. Fresh and dry weights in the greenhouse were greater in October, November, December 1988 and August 1989 and canopy heights of greenhouse plants remained greater as well by fifty percent in January, February, March, April, May and June (Figure 8).

Regardless of the season, plants were longer at the effluent end of all ponds than at the influent end. Exceptions to this occurred in April when plants were consistently longer at the influent end than at the effluent end and roots of plants in the greenhouse for May were longer at the influent end.

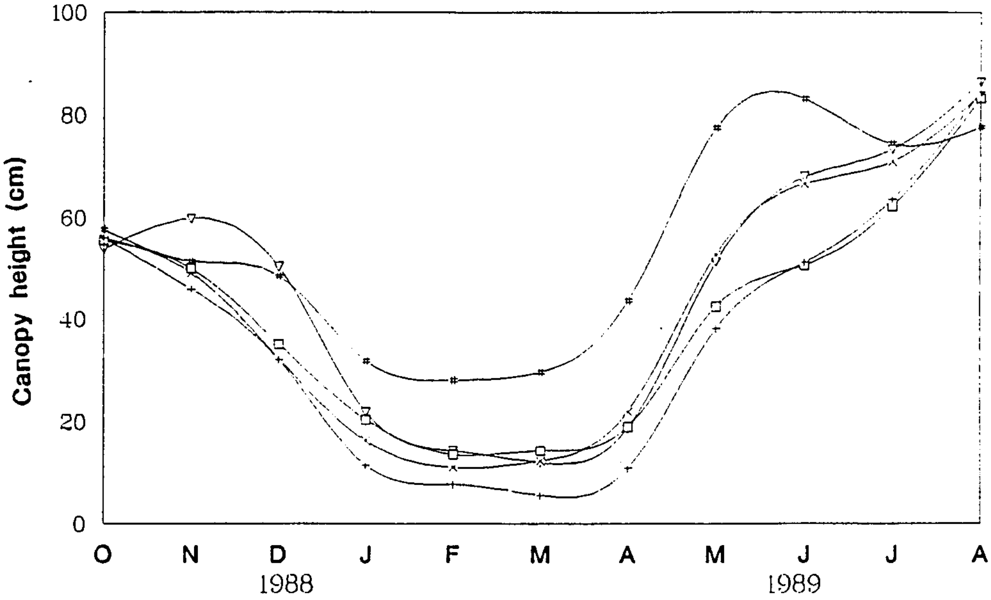


Figure 8. Canopy depth of water hyacinth plants for the period September 1989 through August 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [—□—], sprinklers [—×—], fog [—▽—], greenhouse [—#—]).

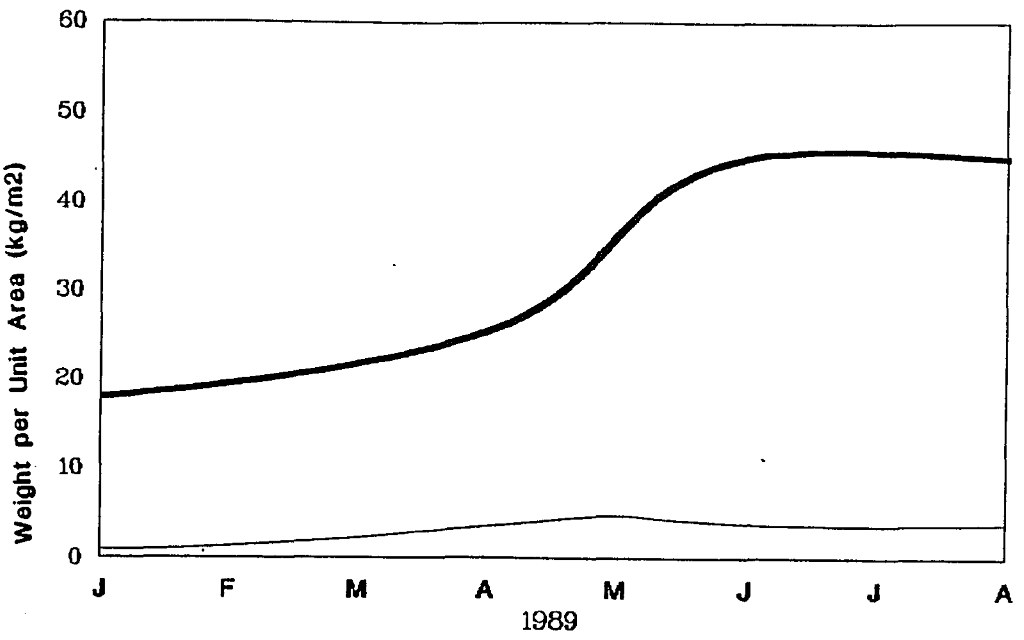


Figure 9. Fresh and dry weights per unit area (kg m^{-2}) of water hyacinth plants for the period January through August 1989 at the Roger Road water hyacinth pilot research facility (averaged for all ponds, fresh weight [▬▬], dry weight [——]).

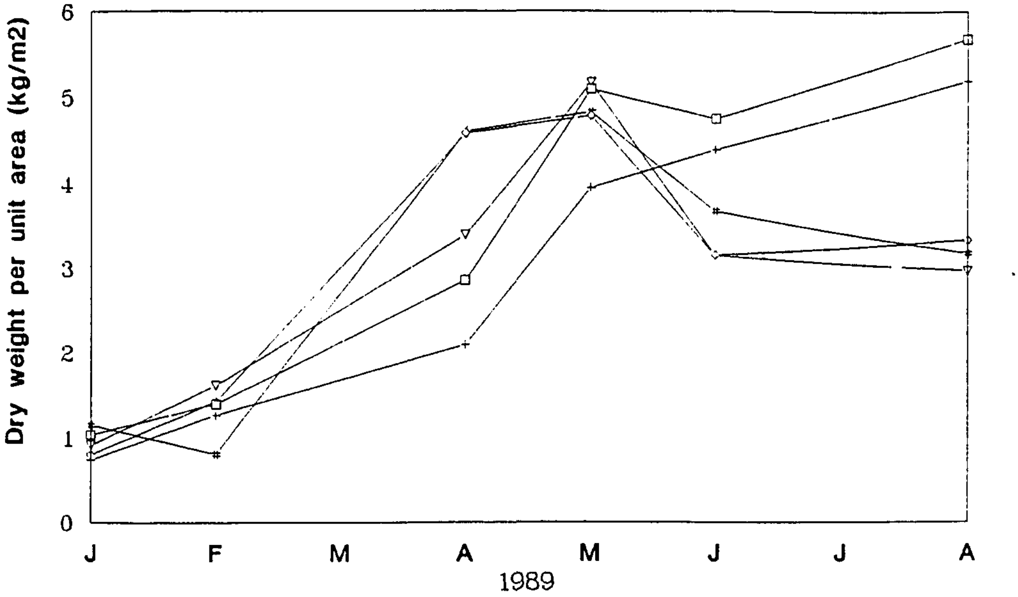


Figure 10. Dry weight per unit area (kg m^{-2}) of water hyacinth plants for the period January through August 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [—□—], sprinklers [—×—], fog [—▽—], and greenhouse [—#—]).

Effectiveness of frost protection measures

In terms of effectiveness of frost protection measures on plant growth as measured by root length (cm) and entire plant length (cm) the following observations were made: Plants in the fog system had longest roots but shortest plants. Plants in the greenhouse grew largest (Figure 11) but showed similar size roots as sprinkler, plastic tarp protected and control plants (Figure 7). Plants protected by fog and plastic tarps had similar values for entire plant length, root length and fresh and dry weights while plants protected by the sprinklers and the greenhouse had somewhat higher values. Plants with no frost protection showed the lowest values for these measurements confirming water hyacinth sensitivity to cold temperatures (O'Brien, 1981; Warshall, Jennings, and Cunningham, 1984).

Biomass analysis

Plants in the greenhouse showed greater canopy heights than their outdoor but frost protected counterparts. In February canopy heights for the greenhouse plants were 35 cm and those for plants in ponds 1-4 were 14 cm, 17 cm, 16 cm, and 18 cm respectively (Figure 8). Heights of the upper canopy from January to May 1989 for ponds 1-4 were very close to one another. In contrast, canopy heights measured for November and December 1988 plants in the fog system were at least 10 cm taller than control and sprinkler protected plants (Figure 11).

From February through April plants in all five ponds showed a steady increase in dry weight per unit area, a range of 0.90 to 1.85 kg m^{-2} for all ponds for February. The values peaked in May with a range of 3.9 to 5.2 kg m^{-2} for ponds 1-5 (Figure 10). Percent dry weight for individual plants increased from 4 percent in January to 10-14 percent in May for plants in all ponds (Figure 12). These values are greater than those of 5 percent found in other wastewater treatment facilities reported by Dinges (1982) and Reed, Middlebrooks, and Crites (1988). In April percent dry weight was highest for plants protected by fog (19 percent) and lowest for control plants (6 percent) (Figure 12) indicating that more biomass was preserved in the fog protected pond than in the other ponds. Perhaps water prevented frost damage from occurring as intensively, although this was not the case in pond 3 (sprinklers).

Plants grown in the greenhouse at the effluent end consistently showed a higher fresh and dry weight per unit area than plants at the influent end in all seasons. For plants protected by tarps, sprinklers and fog higher fresh and dry weights per unit area were found at the effluent end in the warmer months (May through August) than at the influent end. Entire plant length at the influent end was

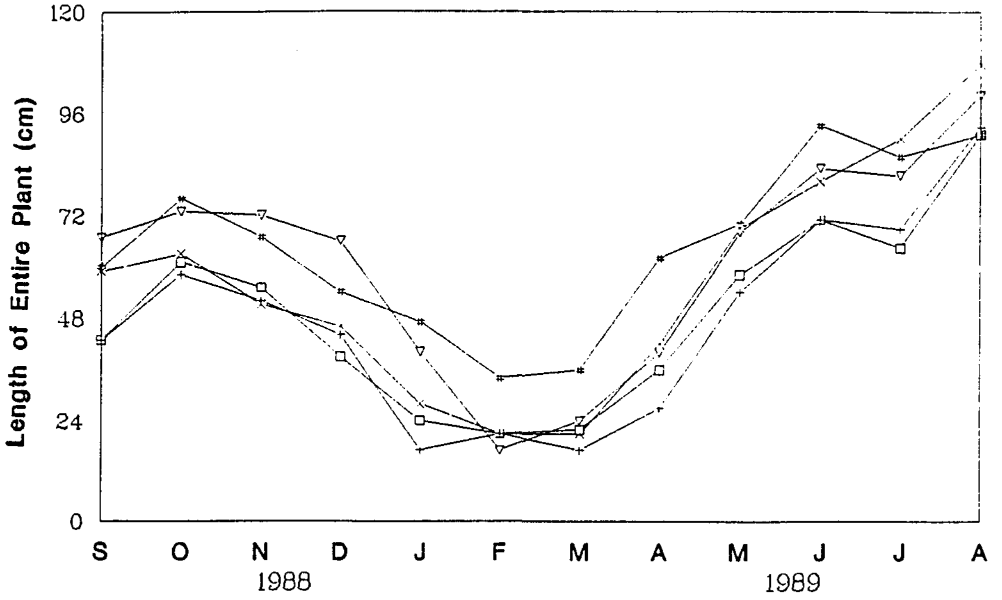


Figure 11. Length of entire water hyacinth plant for the period September 1988 through August 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [—□—], sprinklers [—×—], fog [—▽—] and greenhouse [—#—]).

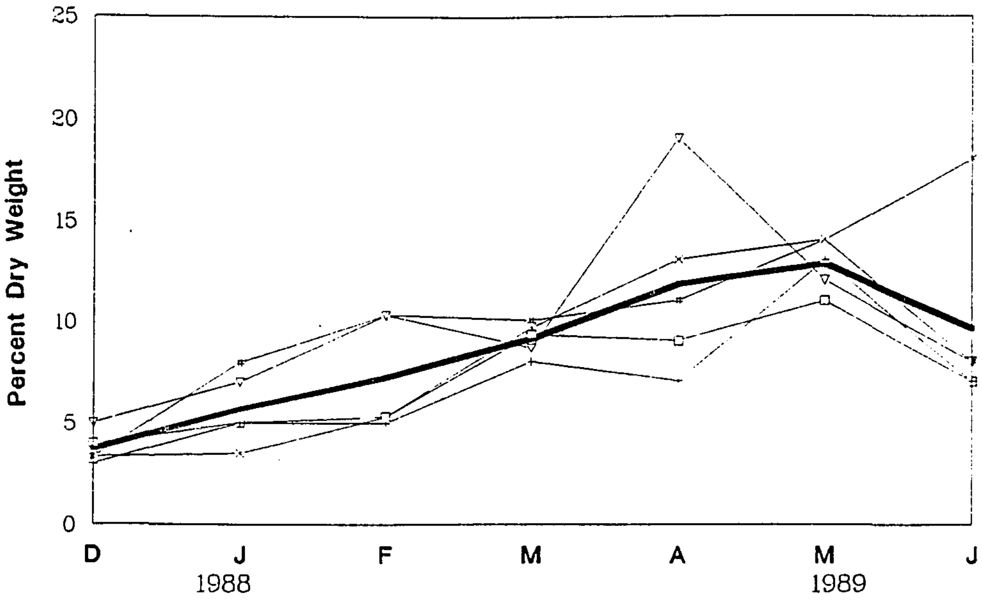


Figure 12. Percent dry weight of water hyacinth plants for the period December 1988 through August 1989 at the Roger Road water hyacinth pilot research facility (control [—+—] plastic tarps [—□—], sprinklers [—×—], fog [—▽—], greenhouse [—#—], and average of all ponds [——]).

consistently greater in the greenhouse than in other treatment ponds and the control. Plants at the effluent end of all ponds from December 1988 to April 1989 were slightly larger than their influent counterparts (Figures 13 and 14) but greenhouse plants at the effluent end were larger only in November, January, February, March and April 1989.

Recovery from winter

Although extensive lamina and petiole damage occurred in the greenhouse from aphid attack, plants remained healthy throughout the winter. Plants in the greenhouse tended to show leggy growth with older plants persisting at the effluent end of the raceway for several months after new plants had emerged. The rate of turnover for plants in the greenhouse appeared slower than that of plants in ponds 1-4 (Appendix C).

In March, temperatures increased and plants regained their characteristic green color and turgidity as plants produced multiple daughter plants. From March to June plants were healthy except for small areas which were chlorotic from insect stress. In July and August 1989, all ponds experienced overcrowding and dry weight per unit area decreased as well.

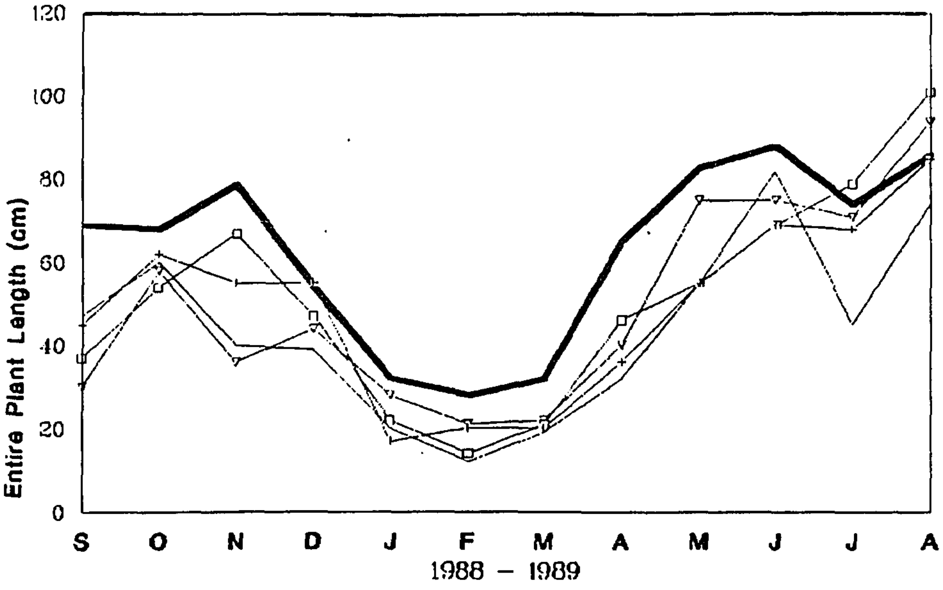


Figure 13. Entire water hyacinth plant length at the influent end for the period September 1988 through June 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [-□-], sprinklers [—×—], fog [—▽—], and greenhouse [——]).

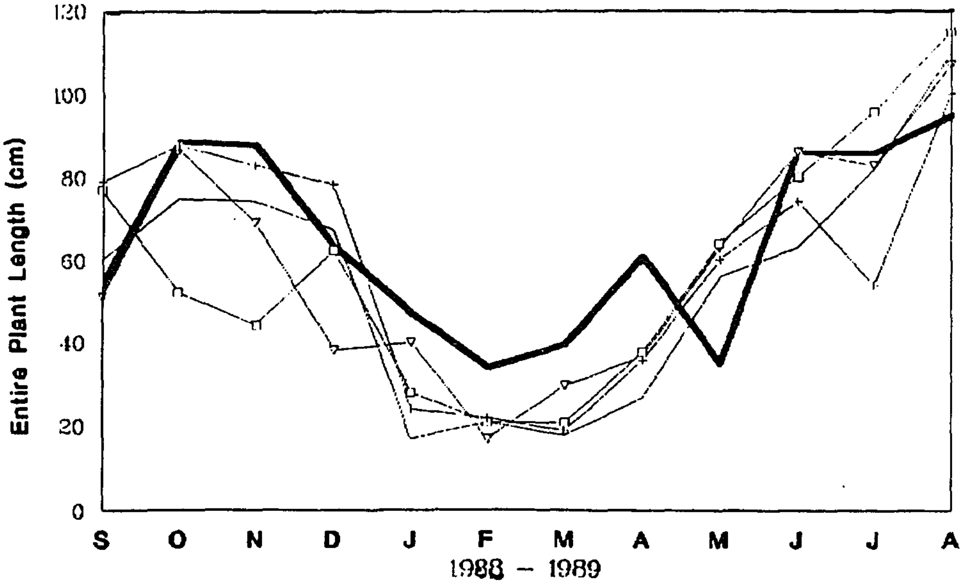


Figure 14. Entire water hyacinth plant length at the effluent end for the period September 1988 through June 1989 at the Roger Road water hyacinth pilot research facility (control [—+—], plastic tarps [-□—], sprinklers [—✕—], fog [—▽—], and greenhouse [▬▬]).

Insect stress

While aphid problems have been rarely reported in the literature (City of Stockton, 1985) several aphid infestations occurred throughout the year at the pilot facility, during the fall, winter and spring (October 16, 1988; January 11-23, 1989, February 1, 1989; April 21, 1989; May 5, 10, 17, 1989). In October 1988, January, February, April, and May 1989 plants were attacked by aphids only in certain ponds. Plants at the influent end of the greenhouse were attacked in January, February and May; plants without frost protection were attacked in April and May. Plants protected by fog were attacked in October 1988 and January 1989 (Appendix D).

Symptoms included chlorosis and necrosis of the lamina and petioles, and severe streaking of the lamina which was the result of aphid attack and Safer soap application on April 25 and May 10. The insects did not appear to attack the crown system or the root system as these remained intact on older as well as younger plants.

Upper canopy plant die-off from aphid attack seemed to occur consistently throughout the year from the influent end to the effluent end in affected ponds.

SUMMARY AND CONCLUSIONS

Low temperatures from November 1988 to March 1989 affected the growth and development of Eichhornia crassipes in wastewater treatment ponds in Tucson, Arizona. Plants protected from frost survived low ambient temperatures more than the unprotected plants in the control pond. Crown tips of many plants in all treatments and control survived freezing temperatures because water temperatures remained above 10 C. These plants recovered and reproduced when the weather became warmer. The highest percent of living plants were found in ponds fitted with some type of frost protection system. Growth slowed in response to low temperature stress on individual plants but varied with the frost protection system used.

Plants protected by sprinklers and the greenhouse survived low temperatures better than those in other treatments. Plastic tarps provided some protection but less than that provided by the fog system, sprinklers or the greenhouse. Highest biomass yields in winter months occurred in the greenhouse; lowest yields occurred in the unprotected control pond.

Water hyacinths responded to freezing temperatures as shown by changes in plant morphology for frost protected and unprotected plants. Plants in the greenhouse were 10 cm larger and showed approximately 10 cm higher canopy heights than plants protected by other frost protection measures. The average percent dry weight for plants in all ponds ranged from 4 to 13 from January to June.

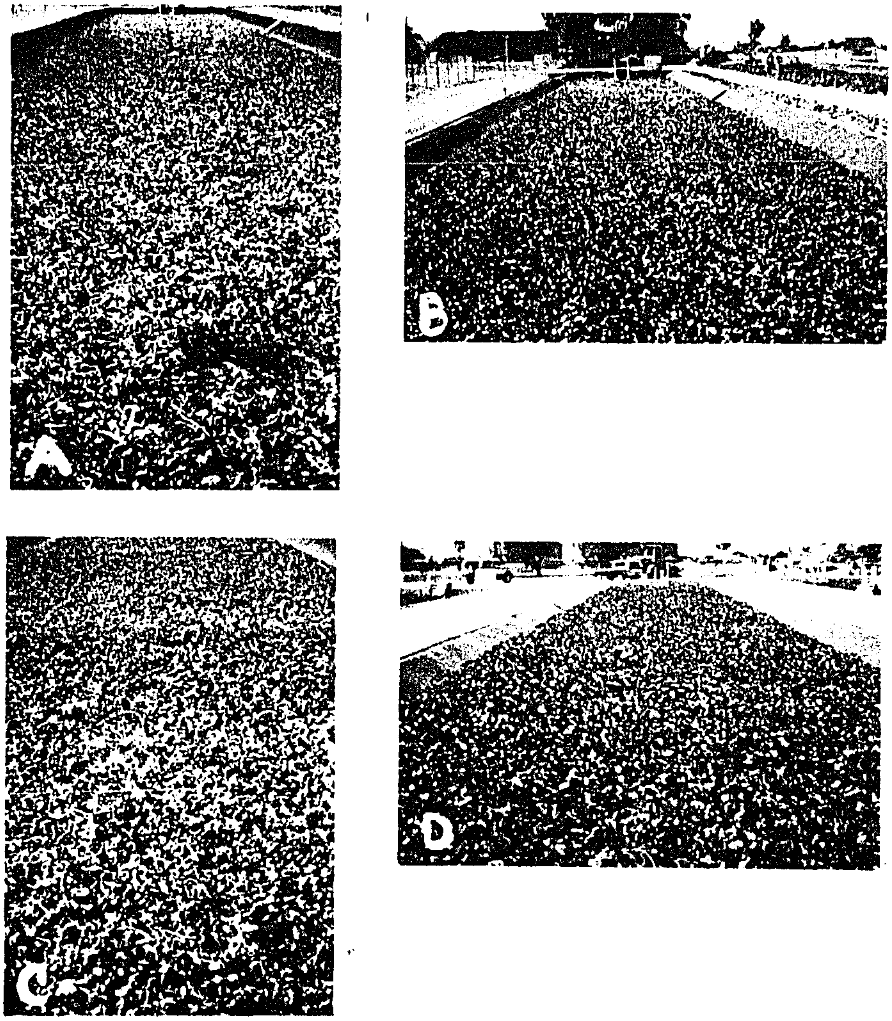
Root structure of plants was affected by freezing ambient temperatures. Water temperature remained above the critical 10 C minimum level for water hyacinth survival, therefore the crown of protected and unprotected plants remained intact. Plants protected by frost were larger, had longer root lengths and had greater dry weight per unit area than unprotected plants.

Water hyacinths in all five ponds showed differing degrees of chlorosis and necrosis during the winter. Unprotected plants showed more damage than protected plants and plants in the greenhouse showed the least damage following winter.

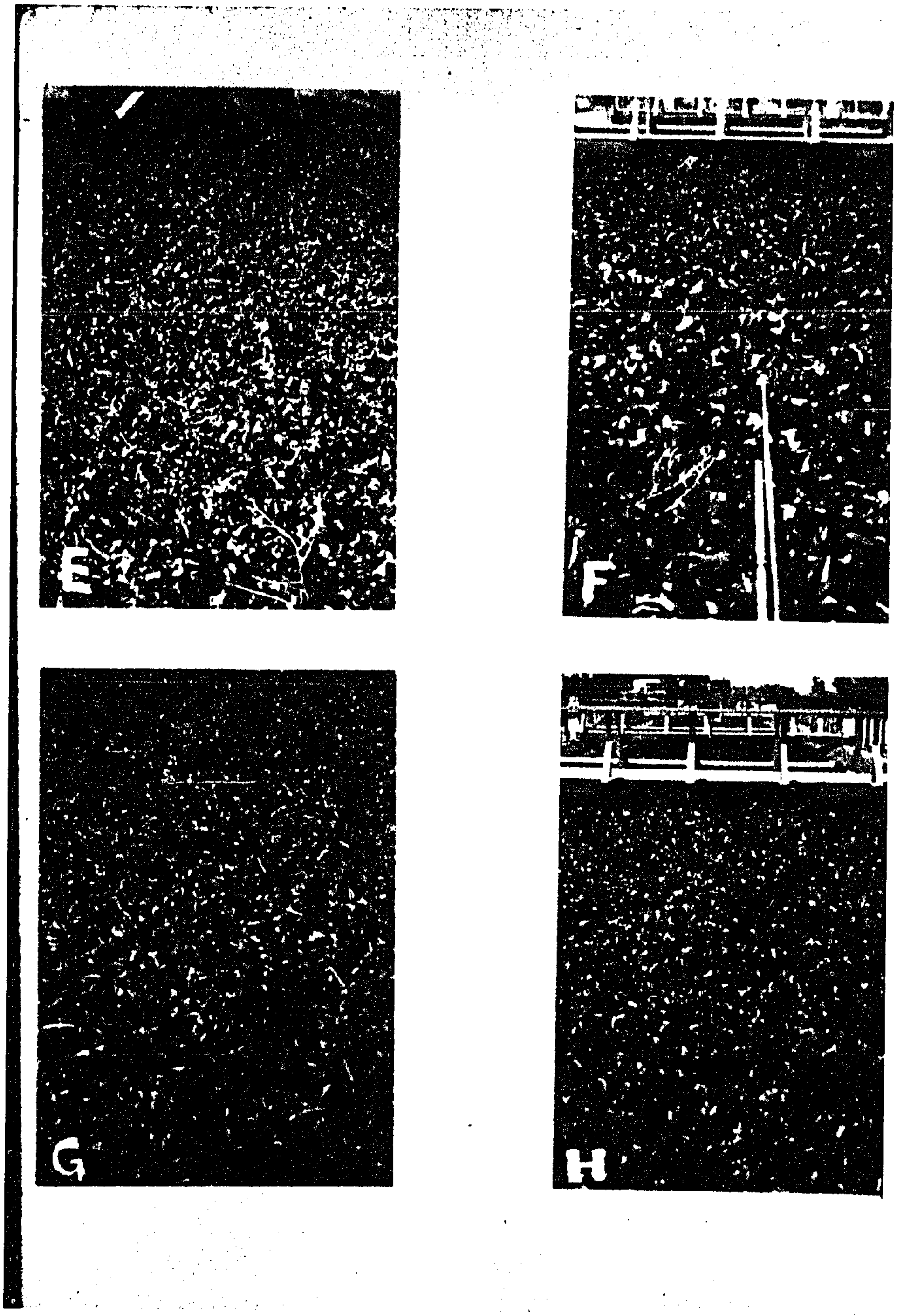
Plants in all five ponds rapidly re-established a complete closed canopy without introduction of new plant material suggesting that below water tissue (crown and roots) may be subject to low temperature stress and remain viable if water temperatures remain above 10 C.

Water hyacinths in all ponds were affected by aphid attacks during the twelve month study and all ponds experienced leaf and petiole aphid attack two weeks before the first frost with greenhouse and fog ponds being severely effected. Plants therefore entered the colder temperatures already stressed. It appeared that regeneration and reproduction of new hyacinth plants adjusted for plants killed by insect infestation and temperature stress. By the end of April all five ponds were totally covered with water hyacinths.

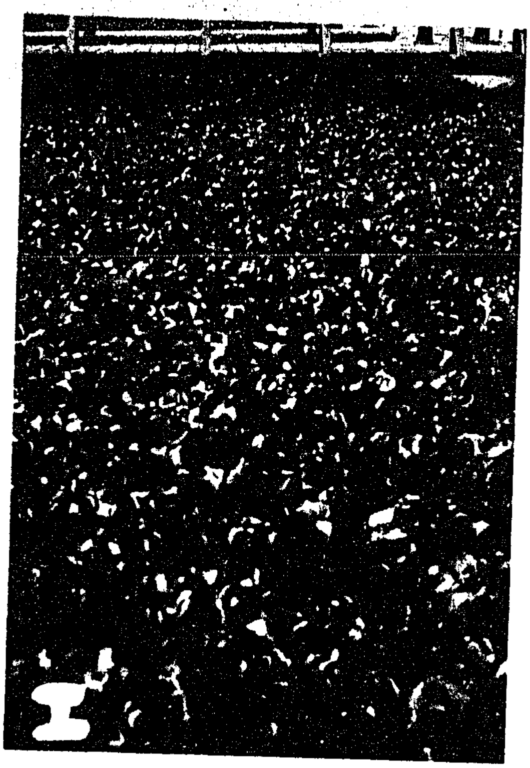
This study shows that Eichhornia crassipes (Mart.) Solms can be grown successfully in temperate regions such as southern Arizona where air temperatures can drop as low as 0 C during a period of four months. Recovery of plants is possible without frost protection, but to maintain a minimum of tissue damage some type of frost protection measures must be used. Sprinklers and fog provided relatively similar protection from low temperatures and the greenhouse plants appeared healthiest throughout winter. Future study of relationships between water quality and plant health will help identify whether frost protected plants provide cleaner water than unprotected plants.

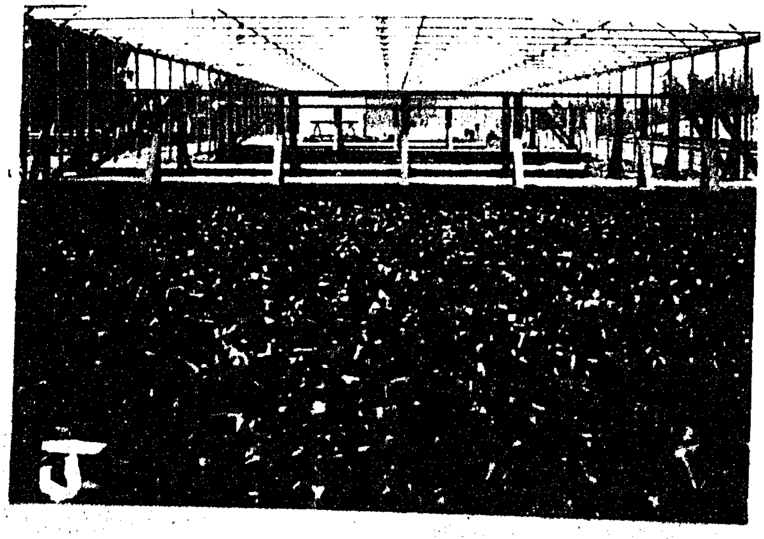


APPENDIX A. Tissue damage (January 1989) and recovery (April 1989) for plants at the Roger Road water hyacinth pilot research facility for control (A and B), plastic tarps (C and D), sprinklers (E and F), fog (G and H), and Greenhouse (I and J).



APPENDIX A--(continued)





APPENDIX A--(continued)

Appendix B. Mean and standard deviations for measurements of canopy height, entire plant length, root length, and dry weight per unit area (kg m^{-2}) for control, plastic tarps, sprinklers, fog and greenhouse ponds from September 1988 through August 1989.

Month/ Year	Canopy height (cm)	Entire plant Length (cm)	Root Length (cm)	Dry weight per unit area (kg m^{-2})
		Control		
Sept	--	43+/- 8	16+/-6	--
Oct	56+/- 8	58+/- 8	14+/-3	--
Nov	46+/-13	52+/-14	12+/-4	--
Dec	32+/-10	44+/-14	10+/-7	--
Jan	11+/- 5	21+/-10	11+/-6	0.8+/-0.4
Feb	8+/- 2	21+/- 7	11+/-6	1.2+/-0.9
Mar	6+/- 3	17+/- 3	12+/-3	--
Apr	11+/- 3	28+/- 8	13+/-3	2.0+/-0.5
May	38+/-12	54+/-10	15+/-1	3.9+/-1.1
Jun	63+/- 8	73+/- 4	19+/-3	4.4+/-1.8
Jul	64+/-19	69+/-19	20+/-5	--
Aug	84+/-14	93+/-18	22+/-6	5.1+/-2.7
		Plastic tarps		
Sep	--	44+/-24	13+/- 7	--
Oct	56+/-10	61+/-20	13+/- 3	--
Nov	66+/-11	55+/-30	13+/- 5	--
Dec	35+/-10	39+/-20	12+/- 3	--
Jan	20+/- 5	21+/- 6	11+/- 4	1.9+/-0.4
Feb	14+/- 4	21+/- 3	13+/- 4	1.4+/-0.9
Mar	14+/- 3	22+/- 5	13+/- 2	--
Apr	19+/- 3	36+/- 7	14+/- 3	2.8+/-0.6
May	42+/-10	56+/-11	16+/- 3	5.0+/-0.7
Jun	56+/- 5	72+/- 5	21+/- 4	4.7+/-1.9
Jul	62+/-18	64+/-12	18+/- 3	--
Aug	83+/- 7	91+/-39	19+/- 2	5.6+/-3.5
		Sprinklers		
Sep	--	59+/-14	13+/-3	--
Oct	56+/- 8	63+/-14	12+/-3	--
Nov	49+/-14	51+/-30	8+/-6	--
Dec	32+/-10	46+/-15	10+/-4	--
Jan	16+/-10	29+/-11	11+/-4	0.8+/-0.3
Feb	11+/- 5	21+/- 4	12+/-3	1.4+/-0.6
Mar	12+/- 3	21+/- 6	12+/-3	--

APPENDIX B--continued

Month/ Year	Canopy height (cm)	Entire plant Length (cm)	Root Length (cm)	Dry weight per unit area (kg m^{-2})
Apr	22+/- 5	41+/-10	14+/-3	4.5+/-0.8
May	53+/- 9	69+/-11	16+/-3	4.7+/-0.7
Jun	71+/-12	81+/- 9	19+/-2	3.1+/-0.8
Jul	71+/-12	85+/-10	22+/-4	--
Aug	84+/- 9	108+/-12	18+/-3	3.3+/-1
		Fog		
Sep	--	67+/-10	13+/-3	--
Oct	54+/-11	73+/-11	8+/-8	--
Nov	60+/-11	73+/-36	11+/-8	--
Dec	50+/-12	66+/-15	13+/-5	--
Jan	22+/-13	35+/-10	12+/-5	0.9+/-0.3
Feb	14+/- 5	20+/-14	14+/-5	1.6+/-0.7
Mar	12+/- 3	24+/- 6	12+/-6	--
Apr	19+/- 4	40+/- 9	13+/-3	3.2+/-0.3
May	51+/-11	68+/-14	15+/-2	5.1+/-1.3
Jun	67+/- 9	83+/- 6	19+/-3	3.1+/-1.5
Jul	73+/-11	82+/-10	21+/-5	--
Aug	86+/- 7	100+/-13	16+/-5	2.9+/-1.3
		Greenhouse		
Sep	--	60+/-12	14+/-2	--
Oct	58+/- 9	76+/-21	8+/-8	--
Nov	51+/-10	67+/-37	9+/-7	--
Dec	48+/-16	54+/-18	10+/-3	--
Jan	22+/-10	38+/-16	8+/-3	1.1+/-0.2
Feb	28+/- 5	34+/-15	13+/-4	1.5+/-0.5
Mar	30+/- 5	36+/-10	17+/-4	--
Apr	44+/- 6	62+/-11	18+/-4	4.5+/-0.9
May	77+/-13	70+/-38	18+/-2	6.3+/-4.8
Jun	86+/-10	93+/-17	18+/-2	3.6+/-1.4
Jul	74+/-11	87+/-14	18+/-3	--
Aug	78+/- 9	91+/-10	18+/-3	3.1+/-1.3

APPENDIX C. Ratings of plant health given frost protection systems, ambient temperature, and range of days. Percent of biomass necrotic/chlorotic is classified as 1=75-100, 2=50-75, 3=25-50, 4=5-25, 5=0-5; ctl=no frost protection (control), Trp=plastic tarpaulin, Spr=sprinklers, Fog=fog, and GRh=greenhouse.

Dates of minimum temperatures	Range of minimum ambient temperatures C	Frost Protection Systems Ctl	Trp	Spr	Fog	Grh
		Description of Plant Health				
17 NOV.- 28 NOV.	-4.0 to 6.0	1	2	2	2	1
		Control and plastic tarps total deformity and necrosis from a combination of aphid attack and low temperatures. Sprinklers, fog and greenhouse showed less response to frost though plants in the greenhouse suffered dramatically from aphid attack.				
29 NOV.- 8 DEC.	-1.0 to 7.0	1	2	3	3	1
		Plants at the influent of all five ponds returned as young, green plants from aphid attack and temperature stress.				
9 DEC.- 19 DEC.	-1.0 to 8.5	2	3	3	3	1
		Plants in control and plastic tarps lost turgidity. In sprinkler and fog plants were chlorotic and necrotic but overall appeared healthier. Plants in the greenhouse started to come back at the influent				

APPENDIX C--continued

		Ctl	Trp	Spr	Fog	Grh	
20 DEC.- 5 JAN.	-10.0 to 12.0	2	3	3	3	2	Plants in control and tarps were brown and dead in upper canopy;lower canopy green. Plants in sprinkler and fog were leggy/frayed at influent and effluent. Greenhouse plants less chlorotic and more turgid.
6 JAN.- 17 JAN.	-8.0 to -1.0	1	1	2	2	2	In control plants showed severe damage with no green understory. Necrotic plants in the plastic tarp pond showed chlorotic understory. Plants in sprinkler pond are frayed and leggy with high necrosis at effluent. Loss of entire plant turgidity in control, tarp, sprinkler and fog. Greenhouse plants show small percent brown leaves compared to center and effluent upperstory with much chlorosis and necrosis. High canopy, large leaves, good turgidity, leggy petioles in the greenhouse.
18 JAN- 22 JAN	-1.0 to 4.0	1	1	1	2	3	Control, plastic tarp,and sprinkler plants all showed necrosis. Fog plants some understory green. Plants in the greenhouse experienced aphid infestation.

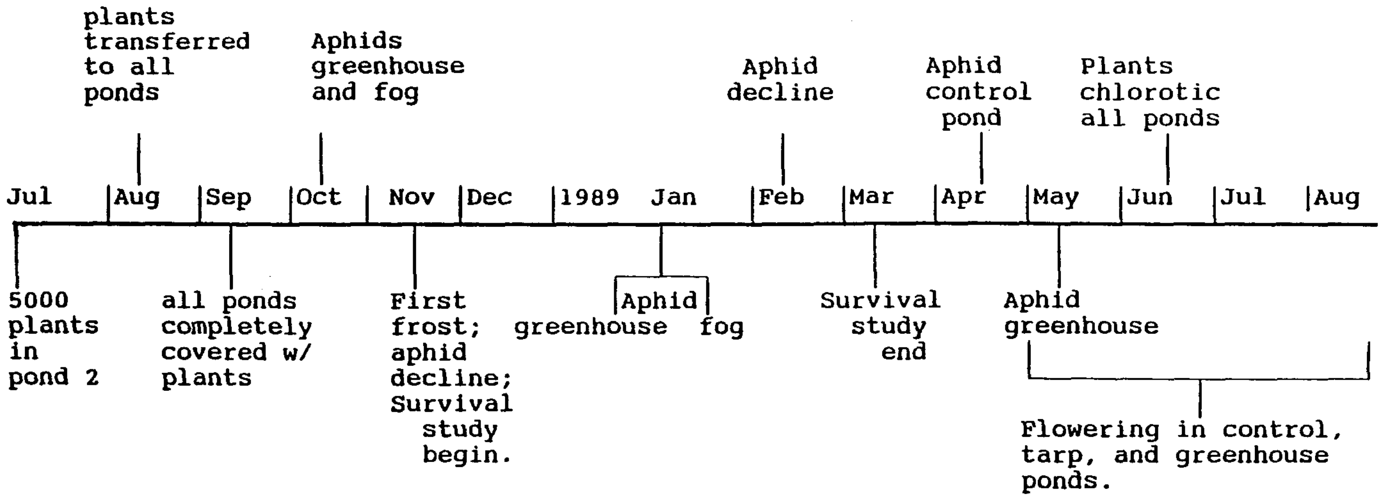
APPENDIX C--continued

		Ctl	Trp	Spr	Fog	Grh
23 JAN- 31 JAN.	-1.0 to 8.0	1	2	2	2	3
		All plants in contorl were deformed and necrotic. Plants in plastic tarp, sprinkler and fog ponds gained turgidity; most older plants are dead Plants in R5 at effluent are brown with high canopy (older plants) and those at influent and center are green.				
1 FEB- 12 FEB.	-2.0 to 7.0	2	2	2	2	2
		Control, tarp, sprinkler and fog plants green yellow understory. Older plants dead and visible. Plant in greenhouse were streaked, chlorotic, and necrotic from aphids.				
13 FEB- 19 FEB.	-1.0 to 5.0	3	3	3	3	2
		More green plants at influent of tarp, sprinkler and fog ponds. Plants in greenhouse remain yellow-green-brown from aphid damage and low temps.				
20 FEB- 26 FEB.	-1.0 to 10.5	3	3	3	3	2
		Plants in ponds 1-4 green w/older plants at effluent end w/brown leaves. Plants in greenhouse chlorotic and necrotic from application of Safer soap in early February.				

APPENDIX C--continued

		Ctl	Trp	Spr	Fog	Grh
27 FEB- 19 MAR.	-3.0 to 9.0	3	3	3	3	3
		Highest % of green young plants occurred at the influent and center of all five ponds. Plants at effluent greenhouse were chlorotic and streaked from soap application and aphid damage.				
20 MAR- 26 MAR.	3.0 to 15.0	4	4	4	4	4
		Plants in all points of all ponds green and healthy.				
27 MAR- 31 MAR.	4.0 to 9.5	4	4	4	4	4
		same as above				
1 APR- 16 APR.	8.0 to 13.0	4	4	4	4	4
		same as above				
17 APR- 23 APR.	9.0 to 15.0	4	3	3	3	4
		Plants at the effluent tarp, sprinkler and fog pond have spot chlorosis. Control and greenhouse plants are uniformally green.				
24 APR- 4 MAY.	3.5 to 12.0	1	3	3	3	4
		Aphids attacked on 4/21 inR1. the control pond. Plants chlorotic and necrotic; treat w/Safer soap.				

Appendix D. Timeline of plant-related activites at Roger Road water hyacinth pilot facility from July 1988 through August 1989.



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