Water Nutrients, Plant Nutrients, and Indicators of Biological Control on Waterhyacinth at Texas Field Sites

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ABSTRACT

The impact of biological control agents on waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) may depend on water nutrient levels, via their effects on plant nutrients and biomass. This study examined associations between water and plant nutrients, and between nutrients, plant biomass and damage, and placed these associations in the context of variable field site disturbance related to chemical and mechanical control and natural factors. Fifteen sites in coastal Texas were sampled, some repeatedly. Water samples were analyzed for dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP). Plant biomass, damage to leaf laminae by adult waterhyacinth weevils (*Neochetina* spp.), necrosis caused by a fungal plant pathogen (*Cercospora piaropi*), and leaf nitrogen (N) and phosphorous (P) content were determined. DIN was positively correlated with leaf N and P content. Water and plant nutrients were not linked to plant biomass. Plant N content was negatively associated with leaf damage by waterhyacinth weevils. In December 2003, DIN and SRP values were 2.6- and 2.2-fold higher, respectively, at five sites on the Rio Grande that had been subjected to mechanical and chemical control than at four sites off of the river. In 2004, sites at which natural disturbance and/or plant removal were frequent did not vary from low-disturbance sites in water or plant nutrient levels or in plant biomass. Damage by weevils and coverage by *C. piaropi* were 3.1-fold and 1.4-fold higher, respectively, at sites with low disturbance. The role of biological control agents in limiting waterhyacinth growth and invasion depends in part on interactions between water and plant nutrients, plant nutrients and weevil damage, and disturbance factors acting on weed populations.

Key words: *Eichhornia crassipes*, nutrients, biological control, aquatic weed, disturbance.

INTRODUCTION

Waterhyacinth colonizes rivers, canals, and reservoirs in the southeastern U.S. and California. This floating aquatic weed can survive and grow under a wide range of water nutrient concentrations, to as low as 0.05 mg/L (= ppm) nitrogen, supplied as either nitrate (Shiralapour et al. 1981) or ammonia (Tucker 1981), and 0.01 ppm phosphate (Boyd 1976). Waterhyacinth is occasionally used in sewage treatment systems because the plants absorb nutrients in excess of growth needs directly from the water column (Gossett and Norris 1971, Haller and Sutton 1973, Ornes and Sutton 1975, Wooten and Dodd 1976, Gopal and Sharma 1981, Wilson et al. 2005). Studies in which water nutrient levels were controlled have shown positive relationships between water and plant nitrogen levels (Rogers and Davis 1972, Wilson et al. 2005). Increases in water nitrogen may also lead to enhanced phosphorous uptake (Reddy et al. 1989) or have no effect (Sato and Kondo 1981), as can supplementation of water phosphorous (Ornes and Sutton 1975, Reddy et al. 1990). Few studies have examined water-plant nutrient associations in waterhyacinth growing at field sites, which are likely to vary in nutrient inputs and in levels of disturbance caused by natural factors and plant control.

Two weevils introduced to control waterhyacinth (*Neochetina eichhorniae* Warner and *N. bruchi* Hustache) are widespread in Texas (Grodowitz et al. 1991, Moran 2004a), and beyond (Center et al. 1999, 2002). However, waterhyacinth persists at many sites, and other methods of control are often required. Interactions between plant damage, insect growth and reproduction, and plant nutrients are important in determining the efficacy of biological control agents feeding on aquatic weeds (Room et al. 1989, Spencer and Ksander 1999), and on terrestrial plants (Waring and Cobb 1992). Waterhyacinth weevil reproduction is reduced at sites with crowded plants containing low nitrogen and phosphorous levels (Center et al. 1999). Weevil feeding itself reduces plant nutrients when plant densities (Center and Van 1989) or water nutrients (Heard and Winterton 2000) are high. Infection of leaves by the native fungus, *Cercospora piaropi* Tharp, is associated with pre-existing weevil damage (Moran 2004b) and slow plant growth (Charudattan et al. 1985).

Mechanical and chemical control of waterhyacinth and natural environmental disturbance could influence biocontrol damage by removing host plants, and also by altering relationships between water nutrient availability, plant nutrient uptake, and the size, biomass and density of surviving plants (Reddy and D'Angelo 1990, Mangas-Ramirez and Elias-Gutierrez 2004). This study evaluated associations between water and plant nutrients in the context of biocontrol damage to waterhyacinth at 15 field sites in Texas. The importance of variation in disturbance in determining water and plant nutrient levels, biomass, and biocontrol damage were also examined.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

MATERIALS AND METHODS

Plant and Water Sampling

Fifteen sites were sampled once or repeatedly between May 2003 and June 2004. In May 2003, plants and water at nine sites in the Lower Rio Grande Valley of Texas (latitude 25 to 26°N, longitude 97° 25 to 57 min W) were sampled. Five of these sites were located on the Rio Grande, between river-miles 40 and 100. The other four sites were canals and small reservoirs located 1-20 km north of the river. In July 2003, sites were sampled for water nutrients only, as plants covering approximately 60 ha on the Lower Rio Grande in South Texas, including all five of the on-river sites, were removed by state authorities using mechanical shredding, and 111 ha were treated with glyphosate (480 g/L N-(phosphonomethyl) glycine, applied at a rate of 6.9 L per ha) (Monsanto, St. Louis, MO). In December 2003, plants were sampled at the four off-river sites, and water samples taken at all nine sites. In June 2004, plants were sampled at the four off-river sites and one site on the river, and water samples collected at all nine sites. Plant and water samples were also collected at Lake Texana State Park, located on a 4450-ha reservoir located near Edna, Texas (28°57.63N, 96°32.45W), and at four sites in the Houston area (29° 08 to 10 min N, 95° 30 to 50 min W): Peyton Creek, a 25-km stream draining into East Matagorda Bay, and three ponds (< 1 ha) located in satellite units of San Bernard National Wildlife Refuge.

Plants were sampled by throwing a 0.25 m² pipe square into infestations and determining shoot density. Either one (May 2003) or two (all other times) squares were sampled per site. Live root and shoot fresh weight were determined for 10 washed, blotted plants collected from each square (15 plants in May 2003). On five plants per square, scars caused by adult waterhyacinth weevils were counted on the lamina (blade) of the two youngest unfurled leaves, which are preferred by adult weevils for feeding (Center 1985). Scar counts were expressed as scars per cm² leaf area by measuring areas on a LI-3100 meter (Li-Cor, Lincoln, NE), or, for sites outside the Lower Rio Grande Valley, by measurement of laminar leaf length and use of a regression (area = (9.92*laminar length)-22.11; R^2 = 0.89). In June 2004 only, the percentage of adaxial leaf surface covered with necrosis indicative of infection by *C. piaropi* was visually estimated on the oldest unfurled leaf, on which symptoms are most widespread (Charudattan et al. 1985, Moran 2004b). To sample water nutrients, four 150-ml water samples were collected at 0 to 0.5 m water depth from a boat or using buckets thrown from the shore. Samples were stored in acid-washed plastic bottles and transported on ice to the laboratory. For plant nutrient analysis, all live leaf laminae from two plants per square were pooled, rinsed in deionized water, frozen and ground on dry ice, freeze-dried, and stored in acid-washed tubes at 25°C.

Water and Plant Nutrient Determination

Water samples were pre-filtered under vacuum through #4 Whatman filter paper (Whatman, Gardiner, NY) syringe-filtered (0.7 µm glass fiber) (Millipore, Milford, MA), and frozen at -20°C on the day of collection, and were stored for no more than one month prior to analysis. Plastic storage bottles and all glassware used in sample preparation and analysis were soaked overnight in 0.1M hydrochloric acid and rinsed in deionized water. All water analyses methods were modified from APHA (1998). Samples were not hydrolyzed or distilled before analysis. The phenate method was used to determine NH_3-N concentrations using 5 ml of sample (approximate detection limit = 0.05 mg NH_3-N L⁻¹). The reaction solution contained 0.24% (mass/volume, m/v) phenol, 0.066 M sodium citrate, 0.039 M sodium hydroxide, 0.0023% (v/v) sodium hypochlorite, and 0.0028% (m/v) sodium nitroprusside. Reactions were incubated in the dark at 25°C overnight and read at 625 nm in a GeneSys-2 spectrophotometer (Spectronic, Rochester, NY). NO_3NO_2-N was determined via cadmium reduction of 10 ml samples using a nitrate detection kit (approximate detection limit = 0.01 mg NO_3NO_2-N L⁻¹) (Hach, Loveland, CO). Reactions were incubated at 25°C for 10 minutes and read at 500 nm. Values for NH_3-N and NO_3NO_2-N were summed to yield dissolved inorganic nitrogen (DIN). Soluble reactive PO_4-P (SRP) was determined in 5 ml samples with the ascorbic acid method (approximate detection limit = 0.02 mg PO_4-P L⁻¹). The reaction solution contained 0.8 mM ammonium molybdate, 0.24 M sulfuric acid, 10.3 mM ascorbic acid, and 0.07 mM potassium antimony tartrate, and was incubated for 1 h at 25 C and read at 885 nm. NH_3-N and NO_3NO_2-N analyses were run in duplicate. A dilution series of mixed wastewater effluent (Hach) (stock concentrations: 2 mg NH_3-N L⁻¹, 4 mg NO_3-N L⁻¹, and 2 mg PO_4-P L⁻¹; also contains 50 mg $SO_4$2-L⁻¹, 25 mg COD L⁻¹, and 8 mg TOC L⁻¹) was used to construct linear standard curves.

Plant nitrogen (N) and phosphorous (P) (% dry mass) in lyophilized (-40°C, 72 h) leaf samples were determined commercially (Texas Plant and Soil Laboratory, Edinburg, TX) using methods modified from Allen (1989). To obtain plant N content, samples (0.2 g) were pretreated with 3 ml Rankers' solution (6.4% salicylic acid in sulfuric acid) and digested with a modified semi-Kjeldahl method using sodium thiosulfate, selenium oxychloride and perchloric acid. Filtered samples were treated with sodium hydroxide, mixed with Nessler's reagent and read immediately at 420 nm. To determine plant P content, samples (0.2 g) were digested with mixed perchloric-nitric-sulfuric acids (Allen 1989) and %P measured as for water samples.

Statistical Analyses

All data for plant biomass, biological control damage, water DIN and SRP and plant N and P were averaged across plants and squares to yield one data point per site, per time, except for 2004 *C. piaropi* necrosis scores, which were summed across five plants per sampling square to yield two observations per site. Water DIN and SRP and weevil scar density data were log-transformed to meet normality requirements. Plant N and P data (percent dry weight) were arcsine-square root transformed. To examine water-plant nutrient associations across all sampling sites and times at which both water and plant data were available (n = 22 sampling events), canonical correlations were calculated between pairs of vari-

able groups using PROC CANCORR in SAS (Version 9.1, SAS Institute, Cary NC) (SAS 1999). The variable groups were: water DIN and SRP; plant N and P; root and shoot biomass; and laminar damage by waterhyacinth weevils on the two youngest unfurled leaves. When the first canonical correlation (based on Wilk's lambda) was significant, Pearson correlations among individual variables were calculated. To obtain a trend line for significant correlations, untransformed DIN, plant N and P and weevil scar density data were graphed and various regressions were tested to maximize R^2 (Sigmaplot Version 9.0, Systat, Point Richmond, CA). ANOVAs were performed to compare water DIN and SRP in samples from five sites on the Lower Rio Grande subjected to prior chemical and mechanical control versus four sites off of the river that were not subjected to this control event, (SAS PROC GLM). The 10 sites sampled for plants in South Texas and Houston in June 2004 were categorized as having either a relatively high level of disturbance, based on observations of both water flow and occasional, partial mechanical removal (four irrigation canals in the Lower Rio Grande Valley and Peyton Creek in Houston) or a relatively low disturbance level (one reservoir in the Lower Rio Grande Valley, Lake Texana near Edna, TX, and three ponds in the Houston area). ANOVA was used to compare these two sets of sites, except for summed *C. piaropi* necrosis scores, which were compared using the Poisson distribution and maximum-likelihood estimation in SAS PROC GENMOD.

RESULTS AND DISCUSSION

Associations Involving Water and Plant Nutrients

DIN concentration in summer 2004 samples ranged from 0.08 to 0.50 mg L^{-1} in the nine sites that were sampled repeatedly in 2003-2004, within ranges reported in studies of waterhyacinth in the field (Center and Spencer 1981) and in plant cultures (Gossett and Norris 1971, Haller and Sutton 1973, Boyd 1976, Reddy et al. 1989). NO_3NO_2-N constituted an average of 72% (range 51% to 88%) of DIN. SRP concentrations (0.10 to 0.83 mg L^{-1}) were higher than levels typically found in Texas surface waters (Rizzo et al. 2000). Based on their ranges, it is likely that neither DIN nor SRP levels were limiting to plant growth (Wilson et al. 2005). DIN and SRP concentrations were positively correlated (r = 0.58, n = 38, P < 0.001). Plant N content ranged from 2.0 to 4.0% dry weight (DW), and plant P content from 0.10 to 0.32% DW, levels consistent with those in cultivated plants (Boyd 1976, Gopal and Sharma 1981, Tucker and Debusk 1983, Reddy et al. 1989, 1990) and field plants (Center et al. 1999). Plant N and P contents were positively correlated (r = 0.67, df = 22, P < 0.001).

Water nutrients were canonically correlated to plant nutrients ($F = 5.75$, df = 4, 36, P = 0.001). In pairwise correlations, DIN concentration was positively correlated to plant N (r = 0.65, P = 0.001) and P (r = 0.65, P = 0.001) (Figure 1A, B). An exponential model with a plateau ($y = y_0 + a(1-b^x)$) appeared to fit the DIN-plant N data (Figure 1A) ($R^2 = 0.42$) and the regression coefficients (except for b) were significant (P < 0.01). A sigmoidal relationship similar to those shown in Wilson et al. (2005) had a slightly higher R^2 (0.43), but the coefficients were not significant. A positive linear trend ($y = y_0 +$
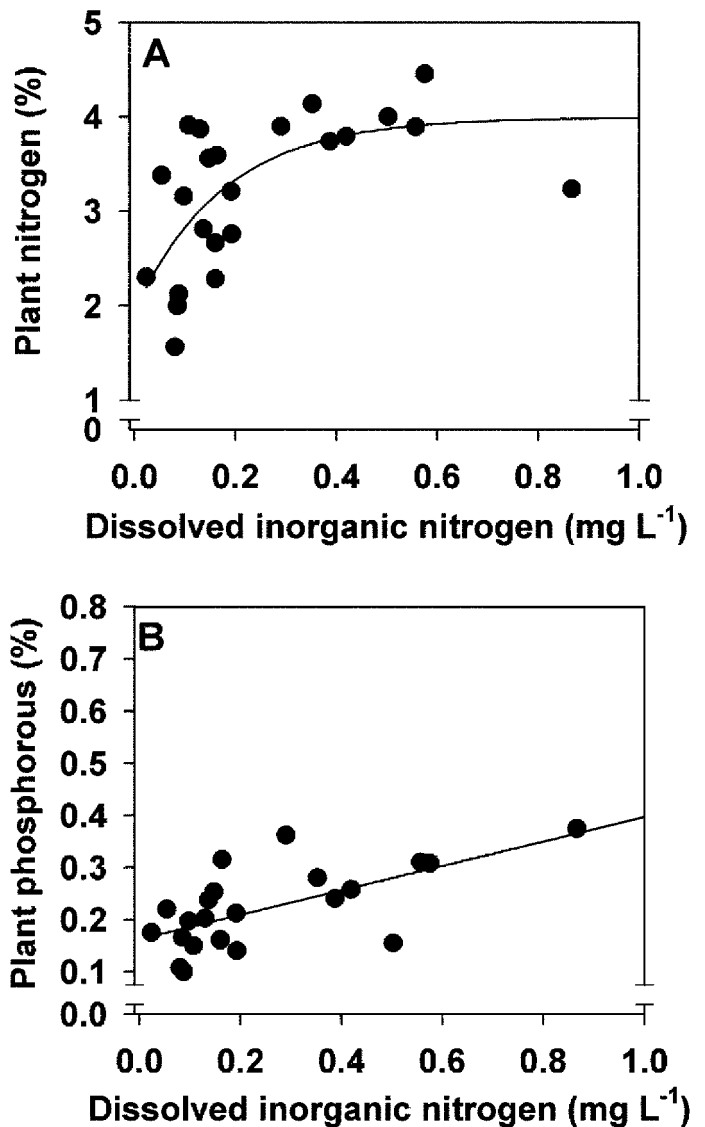

Figure 1. Correlations between water dissolved inorganic nitrogen (DIN) concentrations and plant N and P contents across 10 sites in the Rio Grande Valley, sampled once or repeatedly in 2003-2004, and five sites elsewhere in coastal Texas sampled in June 2004 (n = 22 site sampling events). A, Association between DIN and plant N. B, Association between DIN and plant P. Lines represent exponential plateau (A) and linear (B) regression analyses.

ax) was apparent in the DIN-plant P association ($R^2 = 0.35$, coefficients significant at P < 0.001). Because samples were taken from diverse field sites at different times under varying environmental conditions, the regressions are only suggestive of trends, and coefficients are not presented. Waterhyacinth plants growing in nutrient solutions or sewage effluent readily absorb N in either the $NH4^+$ (Tucker 1981, Moorhead et al. 1988) or $NO3^-$ (Musil and Breen 1977) ionic forms (Gopal and Sharma 1981, Wilson et al. 2005). Figure 1A suggests that plant N content was not affected at field sites at which water DIN concentrations exceeded about 0.4 mg L^{-1}. However, in studies in which water nutrient levels and plant ages were controlled, plant N responded positively in a linear

manner up to 1 mg L⁻¹ DIN (Wilson et al. 2005), or 25 mg L⁻¹ DIN (Reddy et al. 1989). As shown in Figure 1B, increases in DIN can positively influence P content in cultivated plants (Reddy et al. 1989), especially when water P levels are high relative to N (Shiralipour et al. 1981). Water SRP concentration was not correlated to plant N and P in field plots ($P > 0.05$), in contrast to past studies in controlled systems (Ornes and Sutton 1975, Sato and Kondo 1981, Reddy et al. 1990, Jayaweera and Kasturiarachchi 2004). The association between SRP and plant P may be weak relative to DIN and plant N (Gossett and Norris 1971, Wooten and Dodd 1976) due to variation in water N:P ratios (Gopal and Sharma 1981), excretion of P from roots (Dunigan et al. 1975), and the lower levels of water phosphorous required by waterhyacinth for growth and nutrient uptake saturation, as compared to nitrogen (Wilson et al. 2005).

DIN and SRP were not canonically or individually correlated to plant density, or to root or shoot biomass ($P > 0.05$). Increases in water N and P lead to increased plant biomass in tank and sewage pond studies (Gopal and Sharma 1981, Reddy et al. 1989, 1990, Jayaweera and Kasturiarachchi 2004), but in the present study, these associations were likely affected by variation across field sites in the frequency and intensity of mechanical, chemical and biological control, environmental disturbance caused by flooding and frosts, and the resulting changes in the ages of plant populations. For example, plant N and P storage and plant productivity are highest in frequently harvested cultures (Reddy and D'Angelo 1990). The ability of waterhyacinth to respond to increased water nutrients through absorption and biomass production decline as plants age (Xie et al. 2004) and shoot density increases (Reddy et al. 1989, Wilson et al. 2005).

Herbivory by biocontrol agents can reduce plant N and P (Center and Van 1989, Center et al. 1999, Heard and Winterton 2000). While water DIN and SRP were not correlated to damage by larval or adult waterhyacinth weevils ($P > 0.05$), plant N and P were canonically correlated to adult weevil damage on the two youngest unfurled leaves ($F = 5.01$, df = 4, 34, $P = 0.003$, $n = 21$ sampling events). Plant N content was negatively associated with weevil damage on the youngest unfurled leaf (Figure 2) ($r = -0.69$, $n = 21$, $P < 0.001$) and the trend was linear ($R^2 = 0.50$, coefficients significant at $P < 0.001$). Plant N production and/or storage likely decreased in response to weevil damage on these leaves (Center and Van 1989).

Water Nutrients at On and Off-river Sites

Seasonal and disturbance-related changes in water nutrients could, in part, explain the lack of strong correlations of nutrients to plant biomass in the field. Across four sampling times, water DIN and SRP varied seasonally, as in past studies (Gossett and Norris 1971, Center and Spencer 1981), with the highest values occurring in December 2003 samples. DIN concentrations at on-river sites (mean ± SE in mg L⁻¹; 1.96 ± 0.15) was 2.6-fold higher than at off-river sites (0.75 ± 0.61 mg L⁻¹) at that time ($F = 8.58$, df = 1, 7, $P = 0.02$). SRP concentrations were 2.2-fold higher on the river (0.92 ± 0.06 mg L⁻¹) than off of the river (0.41 ± 0.32 mg L⁻¹) ($F = 6.01$, df = 1, 6, $P = 0.05$). At the five on-river sites, DIN decreased by 76% and
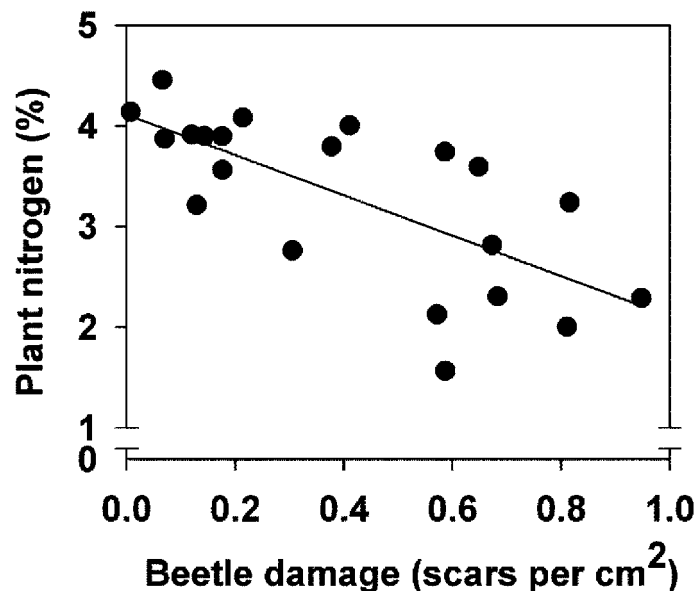

Figure 2. Correlation between waterhyacinth weevil leaf scar density and plant N ($n = 21$ site sampling events). The line represents a linear regression analyses.

SRP decreased by 68% by May 2004, but water nutrients were still higher on the river than off (DIN, $F = 62.8$, df = 1, 7, $P < 0.001$; SRP, $F = 11.0$ df = 1, 7, $P = 0.01$). Water nutrients often increase in winter due to reduced waterhyacinth growth and uptake (Tucker and Debusk 1983), but in an infested Mexican reservoir, nutrients were highest in the spring (Mangas-Ramirez and Elias-Gutierrez 2004). Six and nine months after plant removal, DIN and SRP at sites on the Rio Grande may have been positively affected by plant decay or the loss of plants that would have absorbed nutrients, but nutrient loading and water exchange factors unrelated to waterhyacinth removal were likely more important, and were not studied here. Specific effects of plant shredding and herbicide application are more likely to be observed in restricted water bodies such as reservoirs, due to the release of nutrients from sunken, decaying plants (Mangas-Ramirez and Elias Gutierrez 2004).

Site Disturbance, Nutrients and Biological Control

Water DIN concentrations were 1.8-fold higher at five relatively disturbed sites (canals and creeks) (mean ± SE; 0.216 ± 0.075 mg L⁻¹) than at five sites with relatively low disturbance levels (0.117 ± 0.018 mg L⁻¹) and plant N contents were 1.3-fold higher (3.21 ± 0.35 and 2.52 ± 0.37 percent dry weight, respectively), but these differences were not significant ($P > 0.05$). Water SRP and plant P also did not vary according to disturbance category. In controlled environments, high water flow increases waterhyacinth nutrient uptake over short time frames (Rogers and Davis 1972). Disturbance caused by frequent harvesting of tanks also increases uptake, and alters the balance between NH_4^+ and NO_3^-, though not total DIN (Reddy and D'Angelo 1990). In the present study, shoot biomass was 3.5-fold higher at the

relatively disturbed sites (Figure 3), although this difference was not statistically significant because of the large degree of variation in shoot biomass among individual sites. Sites disturbed by plant removal often support larger and more nutritious surviving plants (Center et al. 1999). As in the case of water nutrient associations with biomass, variation in the age and stress states of individual populations at the time of sampling may have obscured the benefits of disturbance for biomass. The density of scars caused by waterhyacinth weevils feeding on youngest unfurled leaves was 3.1-fold greater at sites with relatively low disturbance than at disturbed sites (F = 11.4, df = 1, 8, P = 0.01) (Figure 4A). Necrosis scores related to infection by *C. piaropi* on oldest leaves were 1.4–fold higher at undisturbed sites (χ^2 = 4.59, df = 1, P = 0.03) (Figure 4B). Consistent with these findings, waterhyacinth plants invading mechanically-controlled sites often have little weevil damage (Center and Durden 1986). Waterhyacinth weevil scarring and *C. piaropi* fungal necrosis accumulated on leaves at low disturbance sites (Center et al. 1999, Moran 2004a), aided by the positive correlation between weevil scarring and fungal necrosis (Moran 2004b) and the link between *C. piaropi* fungal necrosis and low plant growth (Charudattan et al. 1985). Because of the negative association between weevil scarring and plant N (Figure 2), weevil damage could lead to declines in plant N and ultimately biomass at low disturbance sites over time. These reductions in plant N content could eventually have a negative impact on weevil reproduction (Heard and Winterton 2000), but weevils can exert control impacts even at low plant N levels (Center et al. 2005). As in past studies in South Texas (Moran 2004a), damage by biocontrol agents was too low to clearly limit plant biomass.

This study showed that some of the water nutrient-plant nutrient associations occurring in cultured waterhyacinth plants also occur in dynamic, variable field infestations. The
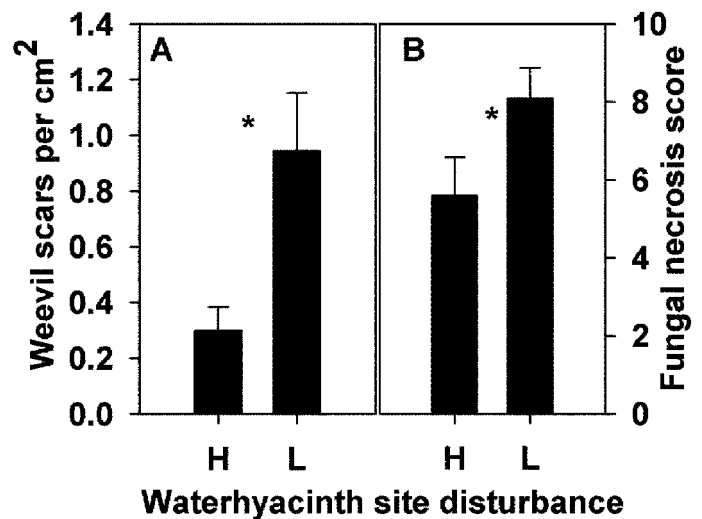

Figure 4. Influence of waterhyacinth disturbance level ('H' = high, 'L' = low) on (A) weevil scars per cm² area on the youngest unfurled leaf and (B) fungal necrosis scores on the oldest leaf. Each value is the mean (±SE) of five sites (A) or 10 sampling squares (B). Asterisks indicate a significant difference in one-way ANOVA (for weevil scarring) or maximum-likelihood estimation (for fungal necrosis scores).

role of the negative weevil scarring-plant N association in controlling waterhyacinth growth is complex, because artificial and natural disturbance affects the prevalence of damage by weevils and may influence water and plant nutrients and biomass. Plant growth, artificial and natural removal, waterhyacinth weevil development and reproduction, and the presence of other biocontrol agents such as *C. piaropi* all likely interact in determining the efficacy of control.

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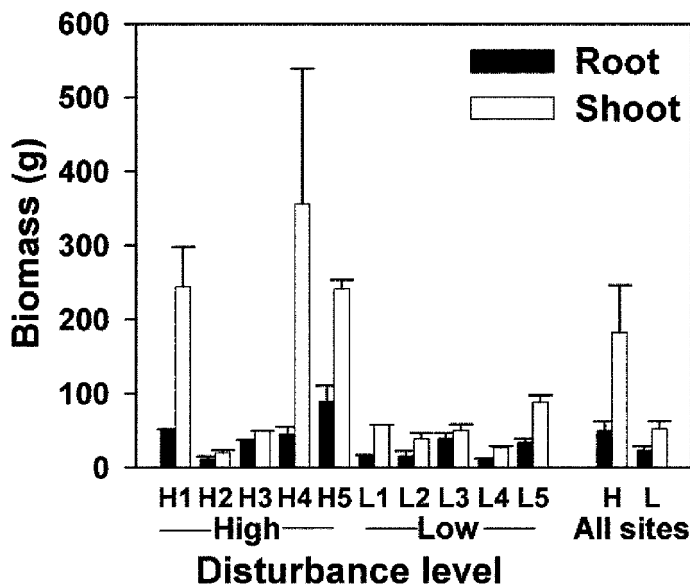
Figure 3. Root and shoot biomass (mean ±SE) of waterhyacinth collected from 10 populations classified into two groups of high ('H') or low ('L') disturbance levels. Means across all five sites in each group are shown on the right.

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