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# Do Tissue Carbon and Nitrogen Limit Population Growth of Weevils Introduced to Control Waterhyacinth at a Site in the Sacramento-San Joaquin Delta, California?

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## ABSTRACT

Waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), is a serious problem in the Sacramento Delta. Two weevil species (*Neochetina bruchi* Hustache and *N. eichhorniae* Warner) have been introduced as biological control agents. Factors such as weather, disease, predators, and plant quality affect growth and reproduction of insect herbivores. The purpose of this study was to test the hypothesis that nitrogen (N) in the tissue of waterhyacinth was not sufficient to support weevil growth and reproduction. Waterhyacinth at a site in the Delta (Whiskey Slough) were sampled at 2- to 3-week intervals in 1995, 1996, and 1997. Lamina samples were analyzed for tissue C and N. Tissue C varied less than either tissue N or the C:N ratio. Tissue N was greatest in the leaf lamina, followed by stem bases, and leaf petioles. Lamina tissue N was higher in spring and somewhat reduced in late summer and winter. The lamina C:N ratio was generally <15 after mid-May. Comparing tissue N levels for Delta waterhyacinth with a previous study relating weevil growth to tissue N indicates that tissue N should not limit growth and reproduction of either weevil species during spring and summer. Because it grows better on plants with high N content and because it has a greater impact on the growth of high N plants, *N. bruchi* may be a more effective biological control agent in the Sacramento Delta.

*Key words:* aquatic weeds, *Eichhornia crassipes*, tissue nitrogen, biological control, weed biology.

## INTRODUCTION

The invasive aquatic plant, waterhyacinth, is a serious problem in the Sacramento Delta (Anderson 1990). It grows abundantly in this ecosystem and its biomass interferes with pumps for agricultural and domestic water supplies, navigation, and recreational uses. It also affects water quality and prevents access to wetlands for desirable wildlife species.

Two species of weevils, *Neochetina bruchi* and *N. eichhorniae* (Coleoptera: Curculionidae) are used as biological control agents for waterhyacinth (Center and Van 1989, Van and Center 1994). These weevils feed upon the leaf lamina, petioles, and stem bases and reduce plant size by sufficiently damaging the leaves that they die. In the mid-1980s, both

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species were introduced into the Delta (Stewart et al. 1988). Subsequent sampling indicated that the weevils had become established. At a site along White Slough near Lodi, California *N. eichhorniae* was present at a mean density of 0.28 (S.E. = 0.09) weevils per plant (K. E. Godfrey, USDA Aquatic Weed Control Research Laboratory, Davis, CA, pers. comm.). In 2002, another researcher concluded that "weevils were much more common in the Delta than previously suspected," ranging in density from 0 to 12 *N. bruchi* per plant in an extensive survey (P. Akers, California Department of Food and Agriculture, Sacramento, CA, pers. comm.). However, the weevils have not had long-term impact on waterhyacinth abundance (Anderson 1990). Similarly, Jimenez et al. (2001) reported that 4 years after release of waterhyacinth weevils in Mexico, they had become established at five of seven release sites and that plants had numerous feeding scars on them, but there were no apparent reductions in waterhyacinth plant size, wet weight and number of plants per m$^2$ at these sites. Jimenez et al. (2001) employed extensive procedures to insure that the weevils they released were disease free. Hill and Olckers (2001) reviewed biological control efforts against waterhyacinth in South Africa and concluded that success had been variable. In a preceding paper, Center and Dray (1992) noted for Florida sites that the weevils provided substantial control but that ". . . consistent, reliable reductions at all sites have not resulted."

Many factors such as weather, disease, predators, and plant quality may affect growth and reproduction of insect biological control agents and thus their establishment and performance (Newman et al. 1998). Tissue N levels are especially important in this regard (Cram 1965a, b, Hilliard and Keeley 1984a, b, Mattson 1980, Room and Thomas 1985, Room et al. 1989, Hunt et al. 1993, Wheeler and Center 1996).

With respect to waterhyacinth, plant quality (especially tissue N) has been suggested as an important factor determining the growth, reproduction, and abundance of waterhyacinth weevils. Center and Wright (1991) reported that laboratory populations of waterhyacinth weevils preferred leaves with high N concentrations and presented data indicating that young leaves with tissue N concentrations approximately 3.6% or greater were fed upon more frequently than older leaves with tissue N levels between 2% and 3%. Center and Dray (1992) studied a number of field populations of *N. bruchi* in Florida and reported that the most reproductively active populations occurred on mature unstressed plants that they characterized as having the "highest quality." They noted that these populations were on waterhyacinth growing in areas where they presumably received high nutrient inputs from adjacent land and speculated that the "high quality plants" reflected the nutrient supplies available to them and in turn to the weevils. Center (1994) reported that waterhyacinth quality (especially leaf tissue N) profoundly influenced egg production by *N. eichhorniae,* being higher when weevils were raised on leaves with >4.5% leaf tissue N than on leaves with approximately 2% leaf tissue N. Heard and Winterton (2000) reared *N. bruchi* and *N. eichhorniae* on waterhyacinth with high (4.65%) and medium (2.93%) levels of leaf lamina tissue N. They reported that both species reduced growth of waterhyacinth with low tissue N, but *N. bruchi* had a significantly greater impact on growth of waterhyacinth with high tissue N than *N. eichhorniae*. Heard and Winterton (2000) attributed this greater impact on high N plants to quicker development, higher survival, and higher fecundity of *N. bruchi* on high N plants.

There are few reports of the N content of waterhyacinth in natural populations (Taylor and Robbins 1968, Boyd and Blackburn 1970, Parra and Hortenstine 1974, Gopal 1987) and there are no published data on tissue N levels for waterhyacinth growing in the Delta. The purpose of this study was to test the hypothesis that waterhyacinth tissue N was not sufficient to support weevil growth and reproduction.

## MATERIALS AND METHODS

Waterhyacinth plants growing in the Delta at Whiskey Slough which is located at approximately 37°56'N, 121°22'W, were sampled at 2 to 3 week intervals beginning November, 1995 through July, 1997. Sampling stopped when the plants were sprayed with herbicides in July, 1997. On most sampling dates, we collected ten waterhyacinth ramets; however, on a few dates six to nine ramets were collected and on one date 20 were collected. We collected the ramets by wading into the water near the shore using a rake. To minimize the likelihood of collecting multiple ramets from the same plant, we moved the sampling point 1 to 2 m to the side of the previous sampling point after each ramet was collected. Ramets were returned to the laboratory where they were dried for 48 h in an oven set at 55 C. Tissue sub-samples were collected from the leaf petioles and lamina for analysis of tissue C and N using a Perkin-Elmer model 2400 CHN analyzer with acetanilide as the standard. In 1997, we also analyzed sub-samples from the crown or stem base (i.e., the structure from which the petioles emerge).

In order to compare tissue N values for samples collected from plants at Whiskey Slough with previously published whole plant values, we calculated whole plant values for these plants by multiplying the N content of each plant part by the weight of that part to determine total N content in mg. We then determined total plant N by summing N for the various parts and dividing it by the total weight of the plants for the 1997 samples because these were the only samples that included data from stem bases. We then calculated linear regression equations relating total plant N content (%) to N content (%) of each plant part. The regression equation relating petiole N content to whole plant N content had the highest value for $R^2$ (0.95) and was highly significant ($P = 0.0002$). We used it (whole plant N = 0.554 + 0.926 × petiole N) to estimate whole plant N content for samples collected in 1995 and 1996. All statistical calculations were performed using SAS procedures (SAS 1999).

## RESULTS AND DISCUSSION

Tissue C and N content and C:N ratio varied during the course of this study (Figure 1). Averaged across all plant parts and sampling dates within the growing season, tissue C varied less (coefficient of variation (CV) = 7%) than either tissue N (CV = 50%) or the C:N ratio (CV = 65%). We calculated mean values and standard errors for N content of leaf lamina, petioles, and stem bases during the growing season.

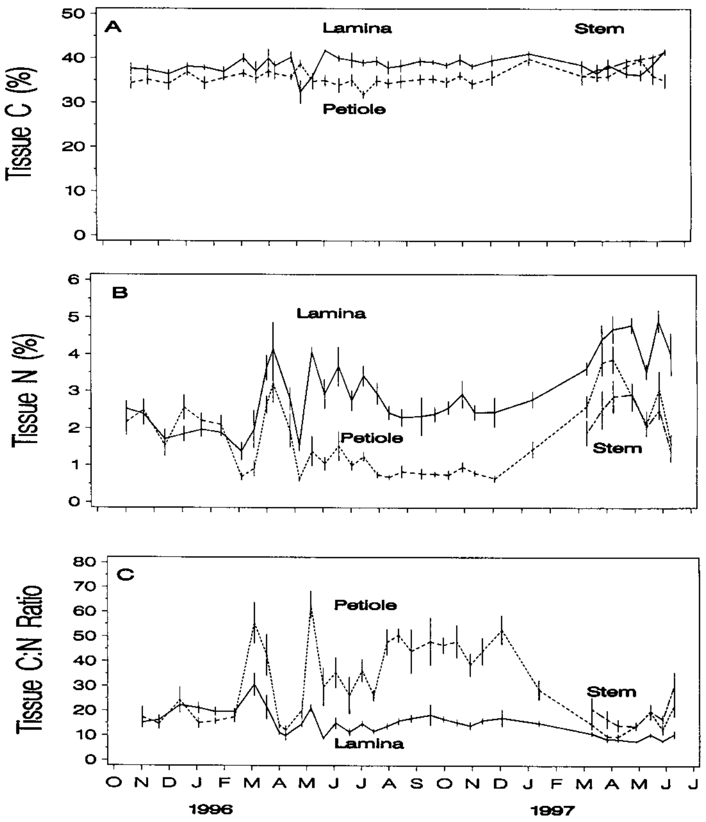

Figure 1. Tissue nutrients for waterhyacinth collected from Whiskey Slough in the Sacramento Delta from November, 1995 to July 1997: tissue C (A), tissue N (B), tissue C:N ratio (C). Plotted values are the mean ± the standard error.

Comparison of 95% confidence intervals indicates that tissue N was greatest in the leaf lamina (3.45 ± 0.8%, N = 153), followed by stem bases (2.53 ± 0.1%, N = 4 0), and lowest values were measured in leaf petioles (1.83 ± 0.9%, N = 153). Musil and Breen (1977) also reported that lamina tissue N (4.91%) was greater than in petioles (2.17%). The lamina C:N ratio was generally <15 after mid-May. Thus waterhyacinth leaf tissue was within the range of C:N ratios (≤17) believed to be favorable to aquatic herbivores (Russell-Hunter 1970, McMahon et al. 1974).

The available information on waterhyacinth tissue N content for natural populations is mostly from whole plant estimates. Tissue N levels varied from 0.14% to 10.5% for plants collected at various times from Sudan, Egypt, Florida, Alabama, Brazil, and South Africa (Gopal 1987). Parra and Hortenstine (1974) sampled waterhyacinth from 19 Florida lakes on four occasions (between June and December) in 1972. They reported single mean values for whole plants of 1.61 ± 0.50% for N and 34.9 ± 5.9% for C (mean ± standard deviation, N = 19). Taylor and Robbins (1968) collected waterhyacinths from Lake Alice, Florida and reported that whole plant mean N content was 1.5%. Based on the Whiskey Slough data from June to December, 1996 and 1997, mean values for all samples during this period for whole plant C and N were similar (given the variation in values) to those reported for Florida plants, being 36.8 ± 2.9% and 1.92 ± 0.98% (mean ± standard deviation), respectively. The slight disparity may be due to differences in the timing and frequency of sample collection between the two studies.

For Whiskey Slough plants, lamina tissue N was higher in spring during both 1996 and 1997. Tissue N values were somewhat reduced in late summer and winter. A similar pattern was reported by Boyd (1976) who found that for plants grown in cultures with controlled nutrient levels tissue N was greatest in June and decreased with growth to September, when the lowest tissue N values were measured. Boyd and Blackburn (1970) reported seasonal changes in waterhyacinth N content for a natural population collected from the "vicinity of Fort Lauderdale, Florida." They reported mean monthly crude protein for whole plants. We calculated N content from their data based on the relationship, crude protein = nitrogen × 6.25. Results are shown in Table 1 along with appropriate monthly means from the Whiskey Slough data. Seasonal changes in waterhyacinth N content for Whiskey Slough plants decreased as the plants aged. Thus, they were similar to the pattern reported by Boyd and Blackburn (1970) for Florida waterhyacinths. Although only mean values are available from the Florida plants, the 95% confidence intervals for the Whiskey Slough data suggest that with the exception of plants collected in April whole plant tissue N levels were actually lower than for plants from this Florida site.

Musil and Breen (1977) reported that tissue N varied within a waterhyacinth mat. Such differences may result in a mosaic of tissue N levels confronting insect herbivores. Thus it may be more informative to compare individual tissue N values than to consider only mean values. Thus, we compared tissue N levels in lamina relative to the high and medium levels of tissue N reported to affect weevil growth and reproduction by Heard and Winterton (2000). Two-thirds (67%) of all lamina tissue N levels measured during this study were greater than or equal to 2.93% (Heard and Winterton's medium level) and 18% were greater than 4.65%, the high tissue N evaluated by Heard and Winterton (2000). Based on these results, we reject the hypothesis that waterhyacinth tissue N for plants from the Sacramento Delta is not sufficient to support weevil growth.

These findings have implications for the establishment and success of weevil biological control agents at Whiskey Slough and in the Delta in general. Measured tissue N levels

TABLE 1. COMPARISON OF WATERHYACINTH MEAN MONTHLY VALUES FOR WHOLE PLANT N CONTENT FOR FLORIDA AND CALIFORNIA PLANTS.

| | N (%) | |
|---|---|---|
| Month | Florida[1] | California[2] |
| April | 3.52 | 3.35 (3.17 to 3.54, n = 120) |
| May | 3.76 | 2.55 (2.28 to 2.82, n = 94) |
| June | 2.91 | 2.29 (1.93 to 2.66, n = 46) |
| July | 2.51 | 2.01 (1.73 to 2.29, n = 60) |
| August | 3.10 | 1.55 (1.21 to 1.89, n = 26) |

[1] Florida data were estimated from Boyd and Blackburn, 1970.

[2] California values are based on samples collected in 1996 and 1997. Numbers in parentheses following California values are 95% confidence intervals and the number of samples (n).

for Whiskey Slough waterhyacinth, in conjunction with data presented by Heard and Winterton (2000) indicate that tissue N may not limit growth and reproduction of either weevil species in the Delta, especially during the spring or summer. Because it grows better on plants with high N content and because it has a greater impact on the growth of high N plants the findings of Heard and Winterton (2000) imply that *N. bruchi* would be a more effective biological control agent in the Sacramento Delta. Additional work on the interactions of waterhyacinth tissue N and weevil growth and reproduction under conditions closely resembling those found in the Delta is warranted. These findings imply that efforts to understand limitations on weevil population growth in the Delta would benefit from information on the impacts of weather, predators, or diseases (Chikwenhere and Vestergaard 2001), or their interactions with waterhyacinth tissue N.

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