Title
Combating Arundo donax and Other Rhizomatous Aquatic and Estuarine Nuisance Grasses By Exploiting Their Ecophysiological Characteristics

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INTRODUCTION

Successful invasions by rhizomatous grasses.

During the past 3–4 decades, parallel expansions of populations of non-indigenous rhizomatous grasses have occurred in aquatic and estuarine habitats around the nation. Among these expansions are *Spartina alterniflora* invasions in the salt marshes of the Pacific Northwest (Callaway and Josselyn, 1992), take-over of large sections of the riparian ecosystems in Southern California by *Arundo donax* (Bell, 1997) and *Phragmites australis* invasions in the upper regions of salt marshes in the mid-Atlantic states (Hellings and Gallagher, 1992).

The east coast salt marsh grass *Spartina alterniflora* (smooth cordgrass) has been invading west coast salt marshes from San Francisco Bay through Washington since the early 1970's (Callaway and Josselyn, 1992). Although *S. alterniflora* can produce seeds that are spread by water movement, it spreads mainly through the expansion of its underground rhizomes. In Washington salt marshes, *S. alterniflora* is large compared to other salt marsh species, and it is altering ecosystem structure by affecting benthic structure and species diversity (Sayce, 1988). Likewise, in San Francisco Bay, *S. alterniflora* displaces the native wetland plants, as well as eelgrasses and algae.

In the mid-Atlantic region, *Phragmites australis* (common reed) has invaded many wetlands (Hellings and Gallagher, 1992), and replaced much of the upper marsh vegetation, that was characterized by *Spartina patens* and *Distichlis spicata*. The 2–3 m tall stems die back at the end of the growing season, but the densely grown shoots (up to 200 shoots·m⁻² (Granéli, 1989)) remain in place, affecting sedimentation patterns, surrounding less tall vegetation, and use of the marsh by mammals and birds.

The rhizomatous grass *Arundo donax* (giant reed) that in the riparian ecosystems of Southern California has expanded into large, self-sustaining populations, has become an ecological and economical pest. The populations expand through the distribution of vegetative propagules, in the form of stem and rhizome fragments by the rivers.

Currently the expansions of rhizomatous grasses like these are combated with mechanical and/or chemical methods. Both these approaches have pros and cons, and their application is affected by the local environmental conditions, funding, and available manpower (Bell, 1998).
The overall goal of this project was to increase the knowledge of the ecophysiology of the internal processes targeted by, or the ecological processes resulting from control efforts, and to facilitate conveyance of this type of information and knowledge to agencies and individuals engaged in control effort of rhizomatous grasses in aquatic and estuarine habitats.

The physiological process that we will focus on is allocation of photosynthates to different parts of the plant and the role of internal nitrogen in this process, in order to determine the most effective time for herbicide application.

MATERIALS AND METHODS

Allocation of photosynthates to different parts of the plant and the role of internal nitrogen in this process.

*Spartina alterniflora* growth analysis experiment – natural nitrogen deprivation.

Seedlings of *Spartina alterniflora* were purchased from Pineland Nursery, New Jersey in March 2001. Average initial dry weight of the seedlings was $3.0 \pm 1.64$ g. The seedlings were rinsed and placed in tap water for a week. Each of 102 randomly selected seedlings was then placed into a 10-liter (L) plastic container filled with 3 L of quarter strength Hoagland nutrient solution (Smart and Barko, 1980). A diaphragm air pump supplied a continuous airflow into the solution through air stones. Every two days during the 17-week experiment, water lost to evaporation was replaced with de-ionized water. The initial electrical conductivity of the nutrient solution was 0.70 mS. We maintained a relatively constant conductivity in the nutrient solution by using an YSI 30 conductivity meter to take the measurement and adding enough concentrated (100x) nutrient solution to bring the conductivity back to the original 0.70 mS. Plants were expected to disproportionately take up nitrate ions from the nutrient solutions, thus causing deprivation of nitrate in the solution. This greenhouse experimental model simulated what occurs naturally in the interstitial water in salt marsh soils during the growing season. The nitrogen supply in *S. alterniflora* marsh varies seasonally with relatively low nitrogen concentration at the end of the growing season in the fall (Gallagher et al., 1980; Gallagher, 1983). Every week the plants were harvested from six buckets. The plants were rinsed in tap water and separated into green leaf blades, senesced leaf blades, stems, roots, rhizomes, and rhizome tip tillers. Rhizome tip tillers are the green photosynthetic rhizome tips that curve upward with only one to two cm tall and have not developed any leaf blades. All tissues were dried to constant weight at 60° C, and ground in a Wiley mill to pass through a 40-mesh screen. The total nitrogen and carbon content of the different tissues was determined with an elemental analyzer (Exeter Analytical E440). The Critical Nitrogen Contents (CNC) of all tissues were estimated as described by Bradley and Morris (1992).
Every month from June 2002 to March 2003, six mature leaf blades of the tall and the short *S. alterniflora* were collected from the Canary Creek Marsh near Lewes, Delaware. The total nitrogen and carbon content of the leaf blades were determined as described above.

*Phragmites australis* – growth analysis, with high and ultra low nitrogen treatments. Between April and October 2003 one hundred small *Phragmites australis* plants generated from rhizomes of plants grown from seed collected in a tidal salt marsh in Lewes, Delaware, were cultured hydroponically in individual five gallon buckets under controlled nitrogen conditions. At the outset of the experiment plants had a mean height of $52.43 \pm 13.57$ (S.D.) centimeters and a mean dry weight of $0.4632 \pm 0.34$ (S.D.) grams. Nutrients were initially supplied to all plants in the concentrations present in one-half strength Hoagland solution. After 10 weeks the usual nutrient solution was replaced with a Hoagland solution lacking nitrates in one half of the replicate plants in the experiment.

All 100 buckets were randomly positioned on a single bench with florescent lighting overhead. The lights were left on 16 hours per day over the course of the whole experiment. A diaphragm air pump was used to supply a continuous flow of air into the solution of each bucket through plastic tubing attached to aquarium airstones. Each bucket contained 5 liters of nutrient solution at the outset of the experiment. As plant size increased and with it evapotranspiration rates, it became clear that a larger volume of liquid was needed.

After 11 weeks all remaining buckets in the experiment were switched to 10 liters of half strength Hoagland solution with or without nitrogen, depending on which treatment each plant had been assigned. The electrical conductivity of the solutions upon their creation is $1.09 \pm 0.02$ mS with nitrates and $0.28 \pm 0.01$ mS for the solution lacking nitrates.

The aerated nutrient solution was monitored for evaporation and lost water volume was replaced with fresh mill-Q filtered water. After the switch to the 10L volume the conductivity of the nutrient solution (YSI-30 conductivity meter) was measured regularly, as a proxy for nutrient levels in each solution. To provide the plants a constant supply of nutrients, drops in conductivity, which we attribute to nutrient uptake by the plants, were compensated with the addition of concentrated (100x) Hoagland solution. Concentrated nutrient solution was added to the solutions as described above for *Spartina alterniflora*.

As plants were harvested on a prearranged schedule, they were rinsed with deionized water to remove any nutrient solution from the outside of the roots. The height, the length of stolons (when present), the number of stems, and the number of leaves was recorded for each plant. Plants were separated into green leaf blades, senesced leaf blades, stems (with surrounding leaf sheaths), roots, rhizomes, rhizome tip tillers, stolons (horizontal stems), and side shoots. Tissues were dried at 60°C to a constant weight and the dry weights of each tissue were recorded for each plant.
The nitrogen and carbon content of the different tissues was determined with an elemental analyzer (Exeter Analytical E440). The CNCs of all tissues were estimated as described above.

RESULTS

Allocation of photosynthates to different plant parts and the role of internal nitrogen in this process.

*Spartina alterniflora*

In the hydroponic culture described earlier, the *S. alterniflora* seedlings that were precultured in deionized water showed an initially slow, but increasing growth, until the growth is reduced near the end of the experiment at week 12 (fig. 1). Specifically, the growth of leaves and stems stopped (fig. 2), as the plants exhaust the N supply from their nutrient solution (fig. 3), and the leaves’ internal N (on a carbon basis) decreases down to their CNC (fig. 4). Before the leaves reached their CNC at the end of week 11, rhizome biomass showed a slow increase to $1.93 \pm 0.257$ g. After the leaves reached their CNC in the last five weeks of the experiment, approximately 6.11 g was added to the rhizome biomass. While at CNC, internal leaf N content was seen to increase above CNC for a short period, after nutrient additions to the culture solution. In this period, the leaves showed slight biomass increase as well (fig. 2 and 4). As the rhizomes grow, new roots and tillers develop at many of their internodes, and the biomass of these tissues show a significant increase (fig. 2), that is related to the increase of *S. alterniflora* rhizome length.

![Figure 1: Total *Spartina alterniflora* biomass (g dry weight) over 17 weeks of hydroponic culture in which nutrients were added in response to lower electrical conductivity reading due to nutrient uptake by the plant. Error bars represent S.E.M.](image)
Figure 2: *Spartina alterniflora* tissue biomass (g dry weight) over 17 weeks of hydroponic culture as described for figure 1. Error bars represent S.E.M.

Figure 3: Total nitrogen remaining in the nutrient solution (g N) over 17 weeks of hydroponic culture in which nutrients were added in response to lower electrical conductivity reading due to nutrient uptake by the *Spartina alterniflora* plant using the solution. Error bars represent S.E.M.
The leaves that were collected from tall and short *S. alterniflora* in the DE salt marsh, showed differences in the seasonal pattern of the internal N content (N/C) (fig. 5). At the start of the growing season in March, the leaf N/C ratio in the tall *S. alterniflora* is high at almost 0.06 g N/gC. In the short *S. alterniflora* the N/C was lower than both the tall *S. alterniflora* from the same marsh, and the *S. alterniflora* in the hydroponic greenhouse experiment early in their growing season (fig. 4). The internal N/C ratio in the leaves of the short *S. alterniflora* does not change much during the entire growing. In the second half of the growing season, the internal leaf N/C ratios in the tall *S. alterniflora* leaves decreases and becomes indistinguishable from the N/C ratio in the short *S. alterniflora*’s leaves.
Figure 5: Seasonal pattern of leaf nitrogen content (g N/g C) of tall and short *Spartina alterniflora* collected in a Delaware salt marsh. Error bars represent S.E.M.

*Phragmites australis*

This project is still in progress, and the results are still somewhat preliminary.

The preliminary results show that the N supply to the plant affected 1) the overall growth of the plants, and 2) the allocation of growth over the different tissues.

After half of the plants in the experiment were transplanted to a nutrient solution **without** N, and the other half received renewed half strength Hoagland solution (**with** N), differences in growth and biomass allocation between the groups were observed (fig. 6). Overall biomass accumulation (including senesced leaves) and total living biomass (excluding senesced leaves), consistently show slightly less overall growth by the plants without nitrogen.
Figure 6: *Phragmites australis* total and separate tissue biomass (g dry weight), over 21 weeks of hydroponic culture, with high N availability in the first 11 weeks. After 50% of the plants were moved to a nutrient solution without N (open symbols), the remaining 50% continued to receive N (solid symbols). Error bars represent S.E.M.
The difference between the growth patterns of the two groups was most pronounced in the growth of the green leaves and the stems. These variables show a reduction in the nitrogen-deprived plants when compared to the plants that continue to receive nitrogen, especially the leaves (fig. 6). In addition to the seemingly smaller amount of aboveground plant material, the leaves of the nitrogen-deprived plants were a much lighter color green, and leaf senescence was more common for these plants (fig. 6). As a result of this combination of responses, the nitrogen-deprived plants appeared much smaller than those that continued to receive nitrogen. At the time of reduced leaf growth by the no-nitrogen plants, the internal N content in the nitrogen-deprived plants dropped significantly below the N content in the leaves of the N-supplied plants (two-way ANOVA, p < 0.001) (fig. 7). This was observed through both the carbon-based and the dry-weight based determination of the tissue N content. The lowest mean N/C ratio was 0.038 ± 0.001, and % N dropped below 2%, to 1.69 ± 0.052%, at the time that the N content in the leaves of the control plants was lowest as well, with N/C = 0.077 ± 0.004 and N = 3.446 ± 0.167%.
The nitrogen-starved *P. australis* had two tissues that did not grow less than on the nitrogen supplied plants; the roots and rhizomes. The roots appeared to grow more when deprived of nitrogen than when nitrogen was present (control group). No significant difference could be determined from the harvests to date, but the *P. australis* plants that will be harvested later, are expected to show significantly larger root growth among the plants stressed by low to no nitrogen availability, than among the plants that were supplied with nitrogen. For the other belowground tissue type, the rhizomes, there was no difference in growth between the two treatment groups (ANCOVA, p > 0.010). Like with the roots, nitrogen deprivation did not negatively affect rhizome growth by developing *P. australis* plants.

Figure 7: *Phragmites australis* green leaf nitrogen content on a carbon basis (N/C = gN/gC), and on a dry weight basis (%N = gN/g total dry weight) over 21 weeks of hydroponic culture, with high N availability in the first 11 weeks. After 50% of the plants were moved to a nutrient solution without N (open symbols), the remaining 50% continued to receive N (solid symbols). Error bars represent S.E.M.
For the no-nitrogen plants, the period of highest root and rhizome growth coincided with the reduced leaf and stem growth, and the low leaf N content.

As with the tall *S. alterniflora* discussed earlier, leaf N content of *P. australis* in the field was at its highest value at the start of the growing season (N/C = 0.090 ± 0.003), and decreases as the growing season progressed (fig. 8). At this time the *P. australis* field sampling study is still in progress, but the August 2003 sample N/C ratios are 0.0745 ± 0.002, a value that is comparable to leaf N/C ratio in the nitrogen supplied *P. australis* in our experiment. In these months in Canary Creek marsh the *P. australis* is not yet nitrogen limited.

![Seasonal pattern of leaf nitrogen content](image)

**Figure 8:** Seasonal pattern of leaf nitrogen content on a carbon basis (N/C = gN/gC), and on a dry weight basis (%N = gN/g total dry weight) for *Phragmites australis* collected in a Delaware brackish marsh. Error bars represent S.E.M.
DISCUSSION

During nitrogen deprivation the growth of mature green leaves was limited or non-existent, due to lack of mobile nitrogen. Both the function and growth of leaf tissues have a high requirement for nitrogen. Each functional leaf cell will need to contain a minimum amount of nitrogen for both photosynthetic pigments and enzymes such as chlorophyll and Rubisco, and a full complement of DNA and nucleic acids for RNA production. Once the tissue nitrogen has been diluted to the lowest functional level, also referred to as the Critical Nitrogen Content (CNC), each cell will only contain this minimum amount of cellular nitrogen allowing it to function at maintenance level. Therefore these cells will not contain enough nitrogen to produce another complement of DNA and enzymes, preventing them from dividing and the tissue to grow. During internal nitrogen limitation (leaf N/C at CNC) when leaf biomass did not increase anymore, photosynthesis was not reduced in *Ipomoea batatas* (sweet potato) (Wijte et al., 1997). Photosynthesis continued after the leaves have reached their CNC and the external nitrogen was depleted. The carbohydrates produced cannot be stored in the leaf tissue since further input of carbon compounds into leaf cells would lower the nitrogen to carbon ratio below their CNC, thus interfering with cell function. Instead of having been incorporated into the leaves, these carbohydrates were translocated to the sinks that are the rhizomes, roots, and for *S. alterniflora*, the rhizome tip tillers.

This *S. alterniflora* study has shown that growth of that species’ vegetative reproductive structures, the rhizomes, occurs after the internal nitrogen content in the leaves is too low to allow for growth of the leaves. The manufacturers of systemic herbicides advise application of their product when there is substantial translocation to the belowground tissues of the plant. For the control of *S. alterniflora* to be effective, a systemic herbicide has to be carried in the phloem stream to the belowground permanent structures for winter survival and spring regrowth, the rhizomes and the associated rhizome tip tiller. Most rhizome growth (76%) and nearly all growth of rhizome tip tillers occur when the leaf N content has reached its CNC. It is obvious therefore that most photosynthate transport and incorporation in the rhizomes and rhizome tip tillers occurs at the time of low leaf N/C ratios, and not before. We can expect substantial ‘delivery’ of the active ingredient in systemic herbicides which are carried in the phloem, such as glyphosate, to the target tissues would occur at the time of low leaf N/C. Therefore, it may be beneficial to take leaf N/C ratios into consideration when determining the timing of systemic herbicide applications.

The field sampling in this study showed that the leaf N/C ratio of short *S. alterniflora* that grow away from the creek banks is almost always at its CNC level, while the leaf N/C ratio of the taller plants that grow on the creek banks showed a high level at the beginning of the growing season, and a decrease with time (and growth) not unlike that of the plants in our greenhouse study. This indicates that our experimental conditions were a reasonable mimic of creek bank salt marsh conditions. Additionally, it indicates that N availability in the fine grained, and anoxic marsh sediment away from the creek banks is significantly different from that in more coarse grained creek bank sediments, through which, rather than over which, the flooding water moves during tidal movements. The
low oxygen availability in this marsh sediment may interfere with the uptake of the nutrients, or the nitrogen content of this soil may be lower since exchange between the interstitial soil water and the periodically overlaying nutrient rich sea/marsh water will be limited due to the dense and fine-grained nature of the soil.

In a series of studies by our laboratory that observed the role of leaf N content in the allocation of growth on multiple species, the *P. australis* study was the first in which we controlled the N concentration in the hydroponic nutrient solution. Continuous high concentrations were maintained for all plants until the 11th week of the experiment, at which time the N was removed from the nutrient solution for half of the remaining plants. The growth of the plants in both the no-nitrogen and the nitrogen supplied groups showed the importance of external and internal N on the allocation of growth in *P. australis* plants. It was interesting that even after the removal of the external N, the plants increased their living biomass by 39 g dry weight, which was almost as much as the 49 g biomass increase of the plants supplied with N.

The biomass increase of the plants without external N was most likely supported by the pool of internal N in the plant. At the time the external N supply was removed, the N content in the plants was above their CNC. The critical nitrogen content of a tissue is defined as the lowest amount of nitrogen in tissues that will allow for growth the growth of that tissue. Total plant biomass increased after the removal of the external N, which was evidence that photosynthesis must have continued. As more carbohydrates are produced and incorporated into the plant without an external supply of new nitrogen, the internal N/C ratio in the tissues will decrease. As described earlier, among the different tissues, leaves have the highest CNC, and will therefore reach this CNC earlier than tissues with lower CNC values. When the leaves reached their CNC, they could no longer incorporate more C into their own tissues, since this would reduce their N/C below the CNC and interfere with their function (photosynthesis). At this point two scenarios are possible; one is that photosynthesis could shut down, and the other is that all the carbohydrates produced in the leaves could be transported to other tissues of the plant. Sweet potato (*Ipomea batatas*) leaves with N contents at CNC showed the same maximum photosynthesis rate as was observed when their N content was significantly below their CNC, and the leaf biomass was still increasing (Wijte, 1997). For rhizomatous plants, the rhizomes have always been considered a sink for excess carbohydrates. The results of our *P. australis* study to-date suggests that the roots, as well as the rhizomes are tissues in which ‘excess’ carbohydrates can be incorporated.

Experiments on storage root crops, strongly suggest that potassium, which causes phloem vessels to be wider, increases the storage root biomass (Geiger and Conti, 1983, Bourke, 1985). Wider phloem vessels would allow for more effective transport of photosynthates away from the leaves where they are produced, where they cannot be incorporated anymore. Thus avoiding a build-up of these photosynthesis products in their production vessel. A study by Arp (1991) showed that photosynthetic acclimation (reduction of photosynthesis rates at high CO$_2$ levels) originally attributed to the high CO$_2$ treatment, occurs if the plants are completely root and rhizome bound. The time until this drop in photosynthesis rate occurred was directly related to the diameter of the pots in which the
experimental plants were grown (Arp, 1991). This photosynthesis rate reduction was not observed in field grown plants without limitations on root and rhizome growth (Arp, 1991, Ainsworth et al., 2003). If the plants physically do not have more room to grow roots and rhizomes, no new roots or rhizomes can be available to receive the translocated photosynthates, the photosynthesis products will indeed accumulate in the production vessel (the leaf cell), and production will be lowered to a minimum maintenance level.

With a N/C ratio of 0.00869 ± 0.00037 rhizome CNC is 4.37x smaller than that of the leaves (0.037982 ± 0.00110), the rhizomes can store 4.37 times as much C per molecule or gram of N in their tissues than the leaves can. At this time, the root N/C ratios have not been analyzed yet. Based on the growth of the roots of the no-nitrogen plants, especially when compared to that of the nitrogen supplied plants, we expect the N/C ratio of the roots to be (relatively) low as well.

The picture, based on our results to-date, of growth allocation in P. australis and the role of leaf N content suggest that rhizome and root growth continues after leaf N content has significantly decreased and the amount of senesced leaf tissue is almost as much as the amount of (light) green leaves. Since the carbohydrates that ‘fueled’ this biomass increase were produced by photosynthesis and translocated from the leaves, the active ingredient of systemic herbicides applied at this time would still be transported to these roots and rhizomes, that are the target of the herbicide treatment.

In summary:
Growth by the belowground structures that are the target for systemic herbicides, such as roots, rhizomes, and for S. alterniflora the rhizome tip tillers, occurs after leaves tissues have reached their CNC, even if the aboveground tissues of the plant look less than vigorous.

LITERATURE CITED


THESES SUPPORTED
Christiana Chen (Summer 2003)
• Growth allocation of *Spartina alterniflora* under nitrogen limitation.

Laura Bedinger (planned for Summer 2004)
• The role of tissue nitrogen in allocation of growth in *Phragmites australis*, common reed.