NUTRIENT EFFECTS ON AUTOFRAGMENTATION OF

MYRIOPHYLLUM SPICATUM L.

THESIS

Presented to the Graduate Council of the

University of North Texas in Partial

Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Dian H. Smith, B.S.

Denton, Texas

August 1998

Smith, Dian H., <u>Nutrient effects on autofragmentation of *Myriophyllum*</u> <u>*spicatum* L.</u> Master of Science (Biology/Ecology), August, 1998, 33pp., 1 table, 7 figures, references 22 titles.

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Total nonstructural carbohydrate and biomass factors combine to indicate that Eurasian watermilfoil grown under low nitrogen environments directs more energy toward autofragment and stem production, whereas under high nitrogen environments more energy is directed toward stem and root crown development. Autofragmentation increased when nitrogen was reduced allowing colonization of new potential sites. Stem and root crown development increased when nitrogen levels were high allowing colonization of immediate area.

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INTRODUCTION

Myriophyllum spicatum L. (Eurasian watermilfoil) is a submersed aquatic macrophyte which is a nuisance to waterways of North America (Grace and Wetzel 1978, Grace and Tilly 1976). It was first documented in the United States in 1942 (Couch and Nelson 1985) and is currently established in 44 states throughout the continental United States (Florida Caribbean Science Center 1997). When this invasive weed colonizes a water system, it forms dense beds and thick canopies which competes with native plants for nutrients and shades them from light. Subsequently, plant diversity is reduced as native plants die back and Eurasian watermilfoil forms a monospecific stand (Madsen *et al.* 1991). Furthermore, water oxygen levels are reduced within these beds making the area inhospitable to oxygen dependent organisms such as fish and invertebrates (Honnell *et al.* 1993). Recreational use within these beds is reduced as well. Piloting boats through thick canopies is difficult, and swimmers can become entangled in stems. Additionally, during periods of senescence, large amounts of nutrients are released giving rise to algal blooms which can impart offensive odors and tastes to water.

Eurasian watermilfoil has three modes of reproduction: seed production, stolon production and fragmentation (Smith and Barko 1990). While large numbers of seeds are produced, this mode of reproduction is not the primary mechanism for the spread of the species (Smith and Barko 1990). Once an area

has been colonized, dense beds are formed by stolons, located in the upper few centimeter of the sediment, which extend from the parent plant. These stolons produce new plants and thus provide the species with a strategic mechanism for localized spread. Fragmentation is primarily responsible for the rapid dispersal of this species (Grace and Wetzel 1978, Aiken *et al*. 1979). There are two forms of fragmentation: allofragmentation and autofragmentation. Allofragmentation is the mechanical breakage of the plant stem by wave action, animals, boats and swimmers. Autofragmentation begins with the formation of adventitious roots on the upper 15 to 20 cm of stem apices before self-initiated abscission from the plant (Kimbel 1982). Field tests by Kimbel (1982) have shown that autofragments, which contain a higher level of total nonstructural carbohydrate than allofragments, are a more successful propagule than allofragments. Thus, Eurasian watermilfoil produces a vegetative clone which is high in carbohydrates and provides the species with an important mechanism for spread within a water body and between water bodies.

The phenology of Eurasian watermilfoil suggests that autofragmentation is linked to flowering and seed set (Madsen 1997) and occurs at the end of the growing season, when maximum biomass has been attained (Smith and Barko 1990). Peak biomass production may deplete the nutrient content of the environment and initiate autofragmentation. Consequently, alterations from optimum growing conditions, such as reduced bioavailability of nutrients, may induce the macrophyte to autofragment (Smart and Barko 1990).

Barko and Smart (1981) implicated potassium as a factor in the senescence of the species. Potassium is not readily accessible by macrophytes from sediments, but rather its primary source is water (Barko and Smart 1981). Competition for cation exchange sites occurs on the roots between nitrogen (as ammonium) and potassium, and ion selectivity favors the ammonium ion (Barko et al. 1986). Maximum reported potassium concentration in the tissues of Eurasian watermilfoil is 20 mg g^{-1} (Carpenter and Adams 1977) while the limiting plant growth concentration is calculated as 10 to 16 mg g^{-1} (Madsen et al. 1994).

Smart and Barko (1990) ascertained that biomass production was affected by nitrogen availability, and an increase in shoot fragments ensued under reduced nitrogen. According to Peverly (1979), the maximum concentration of nitrogen in the tissues of Eurasian watermilfoil is 35 mg g^{-1} and the limiting concentration is calculated as 12 to 22 mg g^{-1} (Madsen et al. 1994).

This investigation examined autofragment formation which appears to be triggered by a reduction in nitrogen and potassium content of the environment. The objectives of this study were to discern whether autofragment production by Myriophyllum spicatum L. would increase under environmental conditions of (1) low nitrogen concentrations, (2) low potassium concentrations, and (3) low concentrations of both nitrogen and potassium.

MATERIALS and METHODS

The study was conducted in circular 1,845-L fiberglass mesocosms, with

a diameter of 177 cm and a depth of 75 cm, located at the U.S. Army Corps of Engineers Lewisville Aquatic Ecosystems Research Facility (LAERF) in Lewisville, Texas (Latitude 33°04'45"N, Longitude 96°57'33"W). Pond sediment acquired from the LAERF and alum treated reservoir water originating from Lake Lewisville, Texas was used to establish an environment in which the macronutrient levels could be manipulated. Pond sediment had favorable ranges of texture, bulk density and organic matter for culturing rooted submersed aquatic plants. While submersed macrophyte growth in unamended pond sediment would be nitrogen and potassium limited, phosphorus would not be growth limiting (Smart et al. 1995). The alum treatment reduced phosphorous content of the water to attenuate algal growth which could have affected nutrients, light and water temperature. Aeration was provided via an air pump and air stones to maintain dissolved oxygen and carbon dioxide. Prior to the sixth week harvest, neutral density shade fabric was utilized to reduce solar irradiance to a maximal midday photosynthetically active radiation level of approximately 1,470 uE sec^{-1} m^{-2} (Smart and Barko 1990; Doyle, personal communication).

Four replicate mesocosms were utilized to study the effects of three treatments versus a control environment; consequently, the study was conducted in sixteen mesocosms with treatments randomly assigned. Since ambient amounts of nutrients were present in either sediment or water supply, the term "low nutrient" indicates that neither of the nutrient(s) under

consideration were amended into the treatment. Nutrient amendment in either sediment or water will be termed "high nutrient". In the control mesocosms, plants were grown in an environment low in nitrogen and potassium. In treatment mesocosms, plants were grown in environments high in nitrogen and/or potassium. One treatment received high nitrogen and low potassium; another received low nitrogen and high potassium; and the third treatment received both nutrients at high levels.

The primary source of nitrogen was the sediment in which Eurasian watermilfoil was grown. The sediment of the high nitrogen treatments was amended with NH_4Cl at a rate of 0.78 mg g^{-1} of nitrogen (N) in sediment (Smart and Barko 1990). Each growing container held 1,350 g of sediment; therefore, there was sufficient amount of nitrogen to support 30 g dry weight increase in biomass at a tissue concentration of 35.0 mg g^{-1}. The dry weight increase was estimated from maximum dry weight increase of Eurasian watermilfoil grown under optimum conditions during prior studies of similar length conducted in these mesocosms (Madsen, unpublished data).

Water was the primary source of potassium and accordingly high potassium treatments were established by amending reservoir water with dissolved KCl at a rate of 14.5 mg l^{-1} of potassium (K) in water. Water levels in the mesocosms were held at 57 cm so that each mesocosm contained approximately 1,403-L. Therefore, there was sufficient amount of potassium to support 30 g dry weight increase in biomass for 33 plants at a tissue

concentration of 20 mg g^{-1}.

Pond sediment was sterilized at 200°C for 24 h to eliminate competitive aquatic species. The sediment was amended with ammonium chloride according to the treatment schedule and deposited into plastic containers which retain approximately 1,350 g when filled to 1.5 cm from the top. One 20 cm long viable shoot apex of *M. spicatum* L., with an approximate dry weight of 0.16 g, was planted in each container. These propagules were harvested by hand from a culture pond at the LAERF. Gravel was added to the top of each container to help hold plant and sediment in place.

This study was conducted from mid-April through July (1996), which coincides with the active growth period of Eurasian watermilfoil when grown in Texas. While production of autofragments is not seasonal, their formation is associated with inflorescence production which occurs from spring through early summer with a peak incidence of flowering during the month of July. The length of the study was determined by the plant phenology, physical constraints of the mesocosms and water temperature which when greater than 30°C causes heat stress to most macrophytes (Madsen 1997).

An initial sampling of water, sediment and harvesting of plant tissues was conducted at the time of planting. Subsequently, plants were given three weeks to establish themselves. Since previous studies in Lewisville pond sediments experienced an approximate propagule mortality rate of 35% (Smart, personal communication), 135% of the plants and sediment required for the investigation

were prepared initially. During the third week, the contents of each treatment mesocosm were examined and vegetation culled so that each contained 20 containers and 20 healthy plants to complete the study. The second sampling harvest was conducted at the end of the third week of growth. Subsequent harvests were at three-week intervals, i.e., weeks 6, 9, and 12. The sampling harvests consisted of collecting water samples from each mesocosm, while sediment and plant tissue samples were harvested from four randomly selected containers within each of the four replicate mesocosm treatments.

Analyses of water, sediment and plant material were conducted from the samples collected from each harvest. Hydrolab[®] instrumentation was utilized *in situ* prior to collecting water samples to quantify the following variables: pH, dissolved oxygen, conductivity and temperature. Water samples were collected from each mesocosm immediately prior to the harvest. These samples were preserved and subsequently analyzed for alkalinity (as $CaCO_3$; SM#2320-B), nitrogen (as ammonia NH_3-N; SM#4500-NH_3-E), soluble reactive phosphorus (SRP; SM#4500-P-E), dissolved potassium (SM#3500-K-B), and dissolved inorganic carbon (SM#5310-B; APHA *et al.* 1995).

Sediment samples were collected and analyzed for exchangeable-nitrogen, dissolved potassium, and SRP content. Core samples totaling 120 ml of sediment were obtained from each treatment mesocosm (30 ml from each of the four containers harvested). The samples were collected in 150 ml Whirl-paks and refrigerated at 4°C prior to sediment extraction. Macronutrients were

extracted from the sediment utilizing 1N HCl and refrigerated at 4°C until analyzed (Bremner and Mulvaney 1982). Sediment extracts were analyzed utilizing the same analytical methods used on water samples.

The following variables were determined from collected plants: maximum plant length, and number of inflorescence, abscised autofragments and attached autofragments. All plant material from each plant from the sample harvest were collected and separated into component parts, i.e., inflorescence, abscised autofragment, attached autofragment, stem and root crown. After drying to a constant weight in a forced-air oven at 55°C for 48 h, plant components were weighed. Subsequently, plant tissues were ground to pass through a 0.5 mm screen, and a 250 mg sub-sample was subjected to a sulfuric acid/hydrogen peroxide block digestion (Allen et al. 1974). Due to limited plant tissue production, expense of procedure, and time involved in digestion and analyses of plant materials, samples taken from within a mesocosm were combined to produce one composite sample of plant component material. Therefore, four composite samples of each plant component from each treatment and the control were available for analyses. The analytical methods for nitrogen, potassium and phosphorus parameters, which follow the digestion procedure, were identical to those used on the water and sediment samples. Additionally, a sub-sample of plant component tissue was subjected to total nonstructural carbohydrate (TNC) analysis (Swank et al. 1982).

In a second study, three stem apices were planted (1 stem container[-1])

and quarantined by a screen barrier within each mesocosm to examine the time sequence from initiation of adventitious roots until abscission. Autofragments were tagged with numbered cable ties at the first appearance of adventitious roots. These plants were monitored and data collected twice a week.

A third study was conducted to discern the effect of nutrient treatments on the prospects of future expansion. During a peak period of abscission, autofragments from the three quarantined plants of each mesocosm which had broken away from the parent plant were collected. Three additional mesocosms of high nutrient levels were utilized to plant a random sample of each of the four treatment's abscised autofragments. The mesocosms were divided into four sections by a screen barrier so as to separate the autofragments originating from the different nutrient treatments. The autofragments were allowed to establish plants and grow for eight weeks. These plant were then harvested and weighed.

Chi-Square contingency and goodness of fit analyses tested for statistical significance between autofragment production of the four treatments. Corrections for discontinuity were applied as needed. Biomass and total non-structural carbohydrate means, which were obtained by multiplying the biomass of each component by the TNC content to compare allocation of carbohydrates to plant component by treatment, were analyzed for statistical significance and separated into statistically distinct groups using 1-way parametric ANOVA with Tukey comparisons of means tests. Total non-structural carbohydrate (TNC), free sugar, and starch concentrations as mg g^{-1} of dry weight were analyzed

using 1-way parametric ANOVA with Tukey comparisons of means tests. An alpha of 0.05 was used as the standard for interpreting results. Procedures for the statistical analyses followed Zar (1984).

RESULTS

High nitrogen amended mesocosms began the investigation with sediment concentrations which ranged from 0.54 ± 0.013 mg NH_3-N g^{-1} to 0.55 ± 0.036 mg NH_3-N g^{-1} sediment (Figure 1A). Plants grown in the low nitrogen regime mesocosms utilized the ambient amount of nitrogen in the sediment which ranged from 0.03 ± 0.005 mg NH_3-N g^{-1} to 0.04 ± 0.010 mg NH_3-N g^{-1} sediment.

Plants grown in high nitrogen environments had tissue nitrogen content ranging from 30 ± 6.2 mg NH_3-N g^{-1} to 32 ± 5.2 mg NH_3-N g^{-1} tissue at the initial harvest and continued to have tissue concentrations above 20 mg NH_3-N g^{-1} throughout the week 12 study. Low nitrogen regime plants began the study with a tissue nitrogen content ranging from 20 ± 4.3 mg NH_3-N g^{-1} to 23 ± 3.0 mg NH_3-N g^{-1} tissue. Nitrogen content of low nitrogen regime plants declined by the third week of growth and ranged from 10 to 14 mg NH_3-N g^{-1} tissue during the remainder of the study (Figure 1B).

High potassium treatments were established by amending reservoir water with dissolved KCl. High potassium treatments began the study with potassium

concentrations which ranged from 20.88 ± 0.25 mg l^{-1} to 21.03 ± 0.26 mg l^{-1} water

(Figure 1C). Low potassium treatments utilized ambient amounts found in the

reservoir water which ranged from 6.77 ± 0.09 mg l^{-1} to 6.86 ± 0.05 mg l^{-1} water.

No difference in the tissue concentrations was observed between the

plants grown in high versus low potassium regimes. Plants began the study with

tissue mean potassium contents from 13 ± 1.8 mg g^{-1} to 15 ± 1.7 mg g^{-1} tissue

regardless of environments (Figure 1D). Tissue concentrations remained within

the limiting condition range (10 to 16 mg g^{-1}) through the sixth week of growth.

Potassium concentrations increased to above limiting conditions by week nine

and although dropping slightly remained at or above the limiting conditions

through the twelfth week of growth.

Autofragment frequency. A total of 2,328 Eurasian watermilfoil

autofragments (attached and abscised) were collected from 384 plants grown

during this investigation. The observed frequencies among the four treatments

were significantly different from an expected 1:1:1:1 frequency distribution ($p <$

0.001;Table 1) .

A highly significant difference from an expected 1:1 frequency distribution

was observed in the autofragment production among the nitrogen treatments (p

< 0.001). Plants grown under low nitrogen conditions (Figure 1B) produced a

total of 1,616 autofragments while plants grown in the high nitrogen environment

produced only 712 autofragments.

There was no significant difference observed in autofragment production

from an expected 1:1 frequency distribution between the potassium treatments

(0.90 > p > 0.75). Low potassium treated plants (Figure 1C) produced a total of

1,156 autofragments whereas plants grown in an environment high in potassium

produced 1,172 autofragments.

Differences in the number of autofragments produced by the four nutrient

regimes became significantly different by the sixth week harvest at which time

the plants grown under low nitrogen levels were producing five times as many

autofragments as those plants grown in the high nitrogen treatments (p < 0.001;

Figure 2A). The largest difference in autofragment production was noted during

the ninth week harvest. A total of 892 autofragments were collected from the

sixteen mesocosms during that harvest with 778 autofragments collected from

low nitrogen treated mesocosms while 114 autofragments were collected from

the high nitrogen treatments (p < 0.001). By the twelfth week harvest, plants

were becoming heat stressed as the water temperature had risen to

approximately 30°C at 0800 reading. Heat stress has been associated with plant

and leaf senescence of Eurasian watermilfoil when grown in shallow southern

ponds (Madsen 1997). This heat factor could alter autofragment production, so

the study was concluded on the twelfth week harvest and the remaining eight

plants were collected from each mesocosm. The number of autofragments

collected from the twelfth week harvest showed a significant contingency

between the treatments. Plants grown in the high nitrogen, high potassium and

low nitrogen, low potassium environments produced significantly more

autofragments than the other two treatments (p < 0.001).

The total number of autofragments collected during the study does not represent total potential production over a 12 week interval. Total potential autofragment production per plant over a 12 week period when grown in a high nitrogen environment was calculated to be 9 ± 3.7 autofragments compared to a plant grown in a low nitrogen environment which had the potential for producing 13 ± 4.3 autofragments (ANOVA, p = 0.318).

Initiation until abscission. Results from the second investigation indicated that autofragment attachment to the parent plant persisted for up to 57 days of the 68 day observation period (Figure 3). Plants grown in high nitrogen environments abscised 12% or less of their tagged autofragments, while plants grown in low nitrogen environments abscised from 22% to 50% of their tagged autofragments. Similar results were observed during the major segment of this study where plants grown in high nitrogen environments abscised from 9% to 11% of their autofragment production while plants under the low nitrogen regimes abscised 29% to 42% (Figure 2B,C). Within ten days of an autofragment initiating its adventitious roots, approximately 10% of the autofragments produced in high nitrogen regimes had broken away from the parent whereas 16% of the low nitrogen, low potassium treated and 38% of the low nitrogen, high potassium treated plants had abscised their autofragments.

Biomass allocation. Once planted stem apices had sufficient time to become established plants and produce root systems, significant differences in

biomass production between nutrient regimes became apparent (Figure 4). By the sixth week, mean biomass produced by low nitrogen treated plants was significantly greater than that produced by the high nitrogen treatments (p = 0.0005). This trend continued throughout the investigation.

Differences in biomass were primarily due to autofragment and stem production (Figure 4C,D). By the sixth week, high nitrogen treated plants were producing from 0.08 ± 0.079 g to 0.16 ± 0.127 g of autofragment biomass whereas the low nitrogen treated plants were producing 0.6 ±0.14 g to 0.8 ± 0.31 g (p = 0.0007). The disparity increased by the ninth week such that high nitrogen treated plants were producing 0.2 ± 0.15 g to 0.3 ± 0.14 g while low nitrogen treated plants produced 1.49 ± 0.638 g to 1.52 ± 0.432 g of autofragment biomass (p = 0.0004). During that same harvest, differences in stem biomass were significantly separated by high and low nitrogen treatment (p = 0.0007). Plants grown in the high nitrogen environment produced 3.0 ± 0.54 g to 3.4 ± 0.71 g of stem biomass whereas plants grown in the low nitrogen environment produced 6.16 ± 1.6 g to 6.24 ± 0.743 g.

Carbohydrate Allocation. The proportion of free sugars, starch and the sum of the two termed total nonstructural carbohydrates (TNC) varied between plant components (Figure 5). This study found that Eurasian watermilfoil translocated high amounts of carbohydrates to the inflorescence. Free sugars ranged between 112 mg g^{-1} and 156 mg g^{-1} of inflorescence dry weight with an

equivalent amount of starch until seed formation when starch concentrations reached as high as 203 mg g^{-1}. High concentrations of free sugars and starch were observed in autofragments as well. Free sugars of the autofragments ranged between 73 mg g^{-1} and 154 mg g^{-1}. Starch content was equivalent to the free sugar content when the first autofragments appeared but steadily increased to as high as 359 mg g^{-1} of autofragment dry weight by the twelfth week harvest. Free sugar concentrations in the stems ranged from 18 mg g^{-1} to 104 mg g^{-1} with generally an equivalent amount of starch. Root crowns had a free sugar concentration range of 96 mg g^{-1} to 158 mg g^{-1} while 43 mg g^{-1} to 114 mg g^{-1} of root crown dry weight was starch.

Significant differences in the TNC content within plant tissues between high nitrogen and low nitrogen treatments were observed early in the investigation. By the sixth week harvest, high nitrogen treated plants had significantly higher TNC dry weight in their autofragments ($p = 0.0021$) and stems ($p = 0.0006$) as free sugars and starch, and root crowns during the third week harvest ($p = 0.0001$) as starch. By the ninth week of growth, although TNC had increased from the prior harvest, no significant differences were recognized between treatment regimes for autofragment, stem and root crown components ($p = 0.15$, 0.65, and 0.72 respectively). By the twelfth week, low nitrogen treated plants had significantly higher TNC in all plant components (inflorescence $p = 0.0007$, autofragment $p = 0.0273$, stem $p < 0.0001$ and root crown $p = 0.0002$) as starch concentrations of the low nitrogen treated plants were significantly

higher than the content of the high nitrogen treated plants.

Significant differences in TNC as grams per plant were observed between the high and low nitrogen regimes by the ninth week of growth (Figure 6A). Low nitrogen treated plants were producing from 1.4 ± 0.24 g to 1.7 ± 0.33 g TNC per plant compared to those grown in the high nitrogen environments which were producing from 0.5 ± 0.09 g to 0.6 ± 0.29 g TNC per plant ($p = 0.0001$). By the twelfth week of growth, the difference in TNC production had increased between the nitrogen treatments as low nitrogen plants produced from 3.9 ± 0.39 g to 4.3 ± 0.83 g of TNC per plant compared to high nitrogen plants which produced from 1.8 ± 0.36 g to 1.9 ± 0.29 g TNC per plant ($p < 0.0001$). The most significant differences during these two harvests were attributable to the amount of TNC allocated to autofragment and stem production.

A comparison of the TNC allocation between plant components collected during the ninth week, when the TNC content per plant component was the same for all treatments, revealed that high nitrogen treated plants were allocating approximately 65% of their TNC production to stems, 20% to root crowns and 15% to autofragments. In contrast, low nitrogen treated plants allocated approximately 49% of their TNC production to stems, 10% to root crowns and 40% to autofragments.

Autofragment Viability. A sampling of self-initiated abscised autofragments from parent plants which had been grown in the four nutrient

regimes were collected and planted in three mesocosms having the high nitrogen and potassium environment. After eight weeks of growth, no significant difference in biomass production was observed (total biomass $p = 0.68$, inflorescence $p = 0.30$; autofragment $p = 0.71$; stem $p = 0.71$; root crown $p = 0.70$) (Figure 7).

DISCUSSION

Eurasian watermilfoil produced approximately 2.25 times as many autofragments when the parent plants were grown in environments low in nitrogen (1,616 autofragments) compared to plants grown in high nitrogen environments (712 autofragments). A ten week concurrent study confirmed that plants grown in low nitrogen environments not only had higher incidents of autofragment formation, but further indicated that low nitrogen parent plants abscised at least twice as many of their autofragments and did so within a shorter period after initiation of the autofragment's adventitious root system. Additionally, more biomass was produced by the low nitrogen plants which was primarily attributable to greater biomass production directed toward the development of autofragment and stem components.

Analysis of the carbohydrate content of the plant components revealed that plants grown in different environments allocated stored energy to different mechanisms of dispersal. Photosynthesis, which produces carbohydrates, takes place in the upper stems. When production is in excess, carbohydrates are

translocated as sugar to the lower stem, root crown, autofragment and inflorescence. Once the metabolic needs of the plant have been met, these free sugars are converted for long-term storage as starch. The inflorescence of Eurasian watermilfoil consumes the plant's carbohydrate production for the development of flowers and subsequently seeds for the next generation. Starch stored in the lower stem and root crown initiate rapid regrowth of the upper stems when above-ground biomass has been damaged, such as during periods of senescence, overwintering, herbivory or the like. Autofragments store starch to provide Eurasian watermilfoil with a vegetative propagule for the spread of the species.

High nitrogen treated plants translocated and stored higher amounts TNC to autofragment, stem and root crown components early in the development of the plant. Once the planted stem fragments became established plants, the TNC contained within each of the plant components was equivalent in samples grown in either high or low nitrogen environments.

Comparison of the plant's TNC in grams of each plant component to the total produced by all components revealed that Eurasian watermilfoil grown in high nitrogen environments allocated most of the TNC production to stem and root crowns. Concentrating energies toward stem and root crown production provide these plants with a high energy supply for regrowth as well as providing the energy to develop stolon growth for expansion into the immediate area.

Plants grown in low nitrogen environments directed most of their energies

toward the production of autofragments and stems. Increasing the number of stems, increases the number of autofragments that can be produced. Autofragments provide the species with a mechanism for intermediate distance spread so that this vegetative clone can leave the current, unfavorable environment and spread to more favorable areas within and between water bodies.

Regardless of the environment in which Eurasian watermilfoil was grown, autofragments were highly vigorous having stored sufficient amounts of TNC so that the plants produced from them were not significantly different in biomass production after eight weeks of growth.

The nitrogen level of an environment in which *Myriophyllum spicatum* L. is grown will determine which of its mechanisms of dispersal will receive most of this invasive exotic's energy. While seed production has never been shown to be an important mechanism for spread, colonies expand locally via stolon production while autofragments provide a mechanism for intermediate distance spread. When grown in environments low in nitrogen, significantly more of the colony's energy is directed toward the production of autofragments. In contrast, Eurasian watermilfoil grown in high nitrogen environments directs most of its energy toward localized spread by storing high amounts of carbohydrates in the stem and root crown components. This provides the plant with energy for regrowth and stolon production.

Eurasian watermilfoil begins its growing season early when water

temperatures are too low for many North American native species. It competes with the natives for nutrients and vigorously grows to the surface and shades natives from light. When grown in low nitrogen environments, this vigorous growth pattern is augmented by an increased production of autofragments. These autofragments, which remain buoyant for extended periods, are transported away from the colony by currents, wave action, and human intervention (e.g. boats) before sinking to the sediment and establishing new plants. Eurasian watermilfoil has an ecological advantage by beginning its growing season earlier than many natives, and when nitrogen availability is limited, accelerating production of autofragments. This increased production of autofragments enhances the probability that these vegetative propagules will leave the current unfavorable habitat and spread to a more suitable one early enough in the growing season to compete with natives in other areas of the water body.

One goal of lake management is to have a healthy, diverse community of aquatic life which enhances water quality. Invasion by Eurasian watermilfoil may quickly outcompete native species and form dense monocultures which defeats this goal. By understanding the phenology of this aggressive weed and knowing which of its propagules will be utilized under known conditions, lake managers increase their ability to control the spread and dispersal of Eurasian watermilfoil in a timely and cost effective manner.

In order to maximize control regimes, such as herbicide treatments, lake

managers of oligotrophic lakes or water bodies with sediment interstitial nitrogen levels of less than 1 mg NH_3-N l^{-1}, should conduct applications early in the formation of autofragments. By doing so, they will avoid the autofragments breaking away from the parent plants and spreading to other areas of their lake or to other water bodies.

Table 1. Total number of Eurasian watermilfoil autofragments (attached and abscised) collected from each mesocosm during the study period from each of the four nutrient regimes.

Treatment	High Nitrogen High Potassium	High Nitrogen Low Potassium	Low Nitrogen High Potassium	Low Nitrogen Low Potassium
Quantity collected from replicate mesocosms:				
Mesocosm 1	115	44	175	225
Mesocosm 2	117	36	260	215
Mesocosm 3	105	107	142	291
Mesocosm 4	85	103	173	135
Totals	422	290	750	866
sample size (n)	4	4	4	4
Mean (SD)	105.5 (14.6)	72.5 (37.7)	187.5 (50.64)	216.5 (63.95)

Figure 1. Mean nutrient levels of environment and tissue concentrations for the four nutrient regimes during the twelve week study. Bars indicate standard deviation from mean (n=4). A) Concentration of nitrogen within sediments; B) Concentration of nitrogen in plant tissue per treatment; C) Concentration of potassium within water; D) Concentration of potassium in plant tissue per treatment.
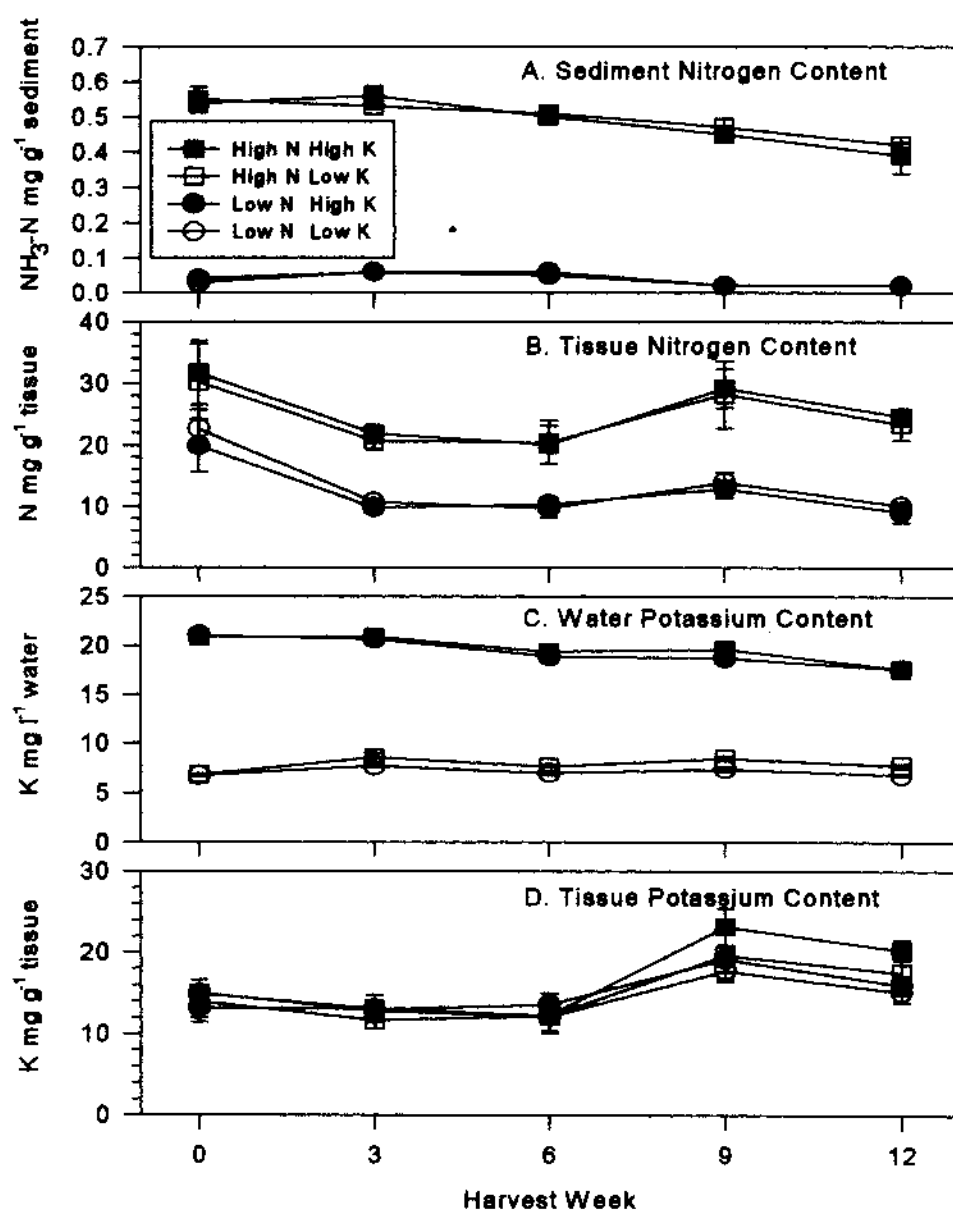
Figure 2. Mean and standard deviation frequency of autofragments collected

per mesocosm during the twelve week study from each nutrient regime (n=4). A)

Total number of autofragments collected; B) Total number of autofragments

which had abscised from the parent plant; C) Total number of autofragments

which were attached to parent plants.
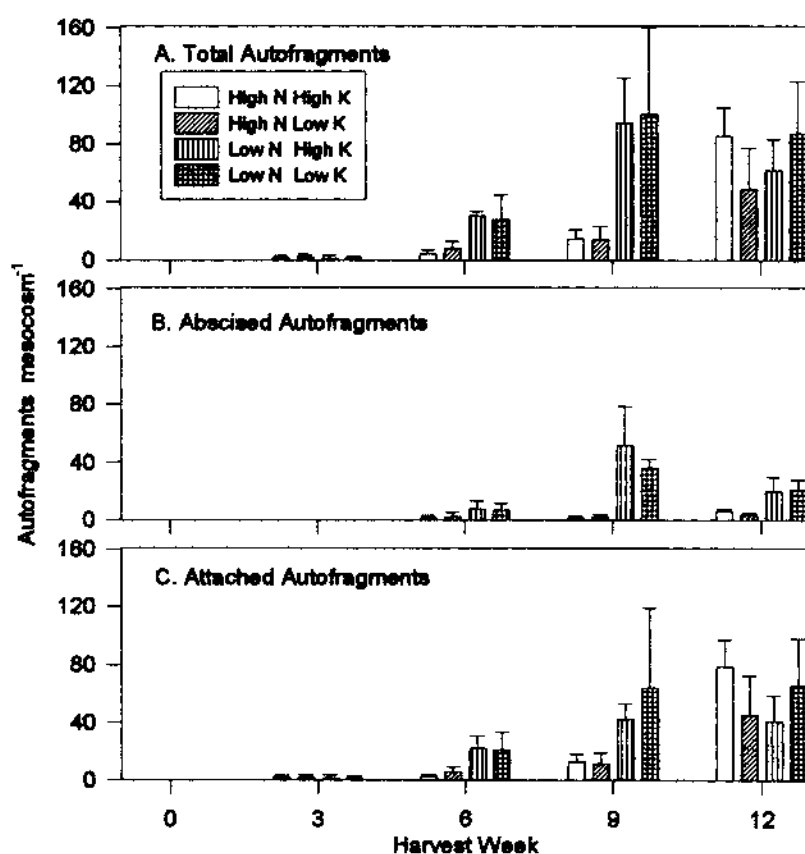
Figure 3. Number of days mean percent of autofragments persisted on parent plants, and number of days mean percent of autofragments abscised from parent plants. Bars indicate standard deviation from the mean (n=4). A) Autofragment fate in high nitrogen, high potassium environment; B) Autofragment fate in high nitrogen, low potassium environment; C) Autofragment fate in low nitrogen, high potassium environment; D) Autofragment fate in low nitrogen, low potassium environment.
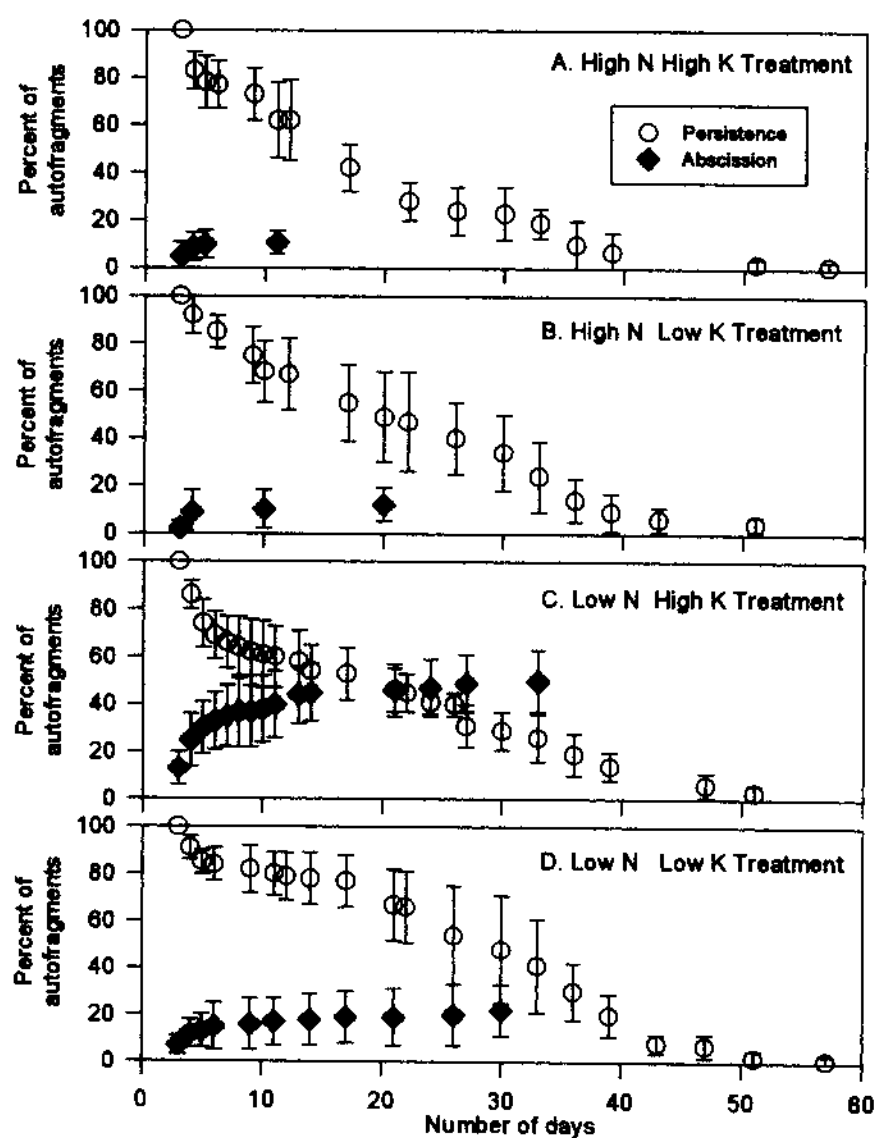
Figure 4. Mean biomass production in grams per plant for the four nutrient regimes over the twelve week investigation. Bars indicate standard deviation from the mean and letters indicate statistically distinct groups (n=4). A) Total biomass in grams per plant; B) Inflorescence biomass in grams per plant; C) Autofragment biomass in grams per plant; D) Stem biomass in grams per plant; E) Root crown biomass in grams per plant.
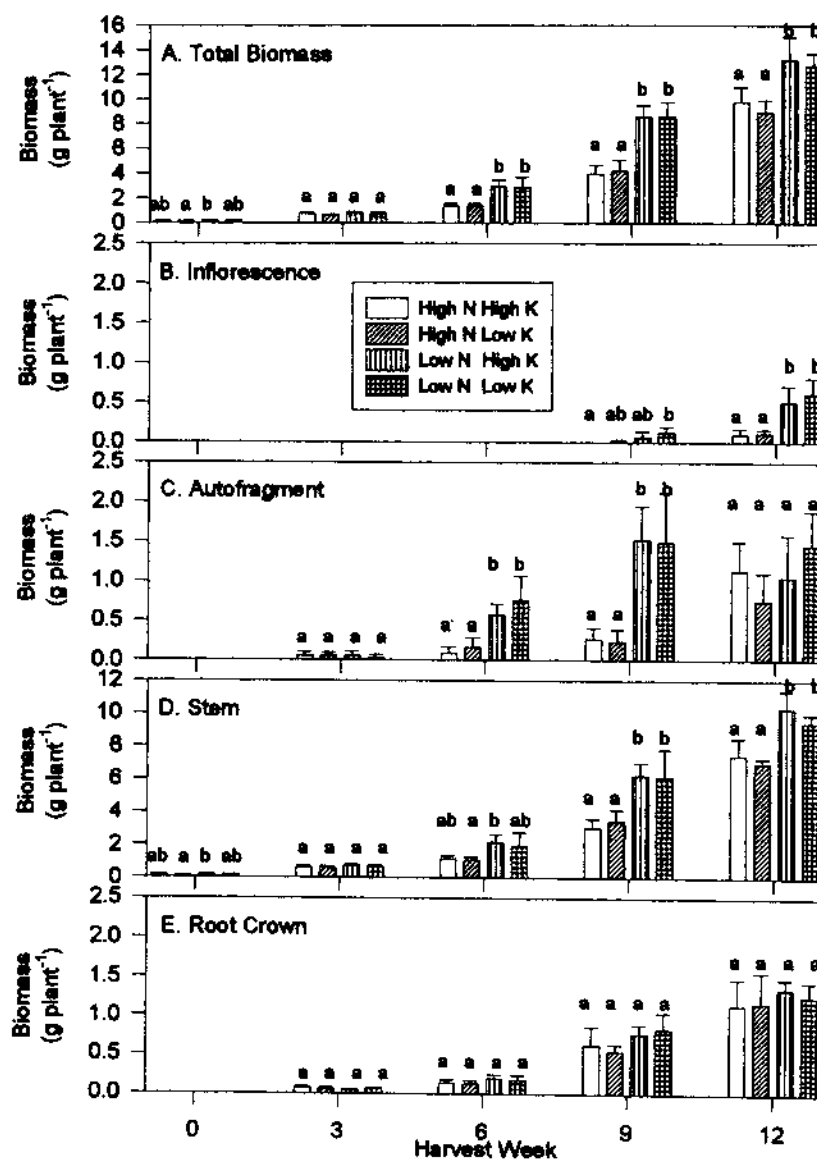
Figure 5. Mean total nonstructural carbohydrates (TNC) in milligrams per gram of plant component dry weight per nutrient regime. Bars indicate standard deviation from the mean and letters indicate statistically distinct groups (n=4). A) TNC as mg g^{-1} inflorescence biomass; B) TNC as mg g^{-1} autofragment biomass; C) TNC as mg g^{-1} stem biomass; D) TNC as mg g^{-1} root crown biomass.
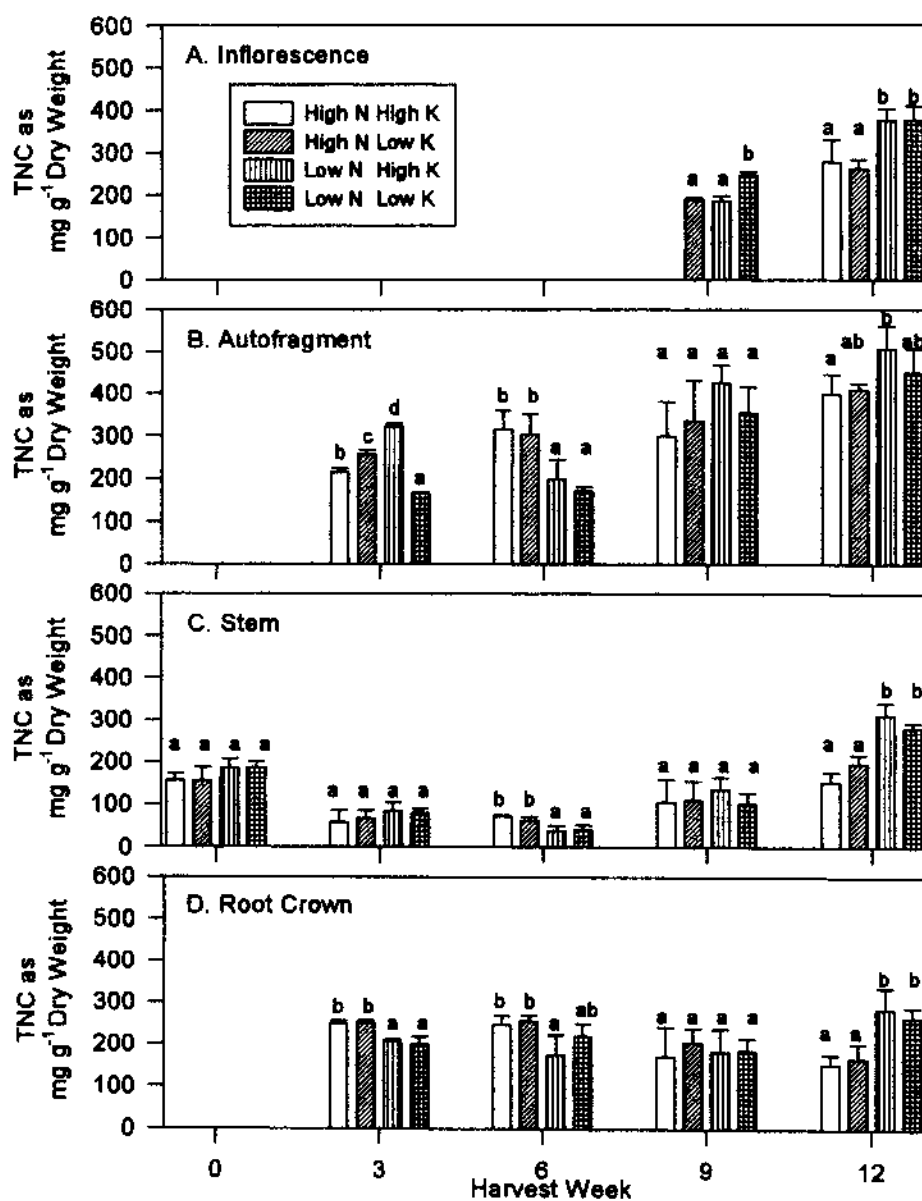
Figure 6. Mean total nonstructural carbohydrates (TNC) in grams per plant and for each plant component per nutrient regime. Bars indicate standard deviation from the mean and letters indicate statistically distinct groups (n=4). A) TNC in grams per plant; B) TNC in grams contained in the inflorescence biomass; C) TNC in grams within autofragment biomass; D) TNC in grams contained in stem biomass; E) TNC in grams within root crown biomass.
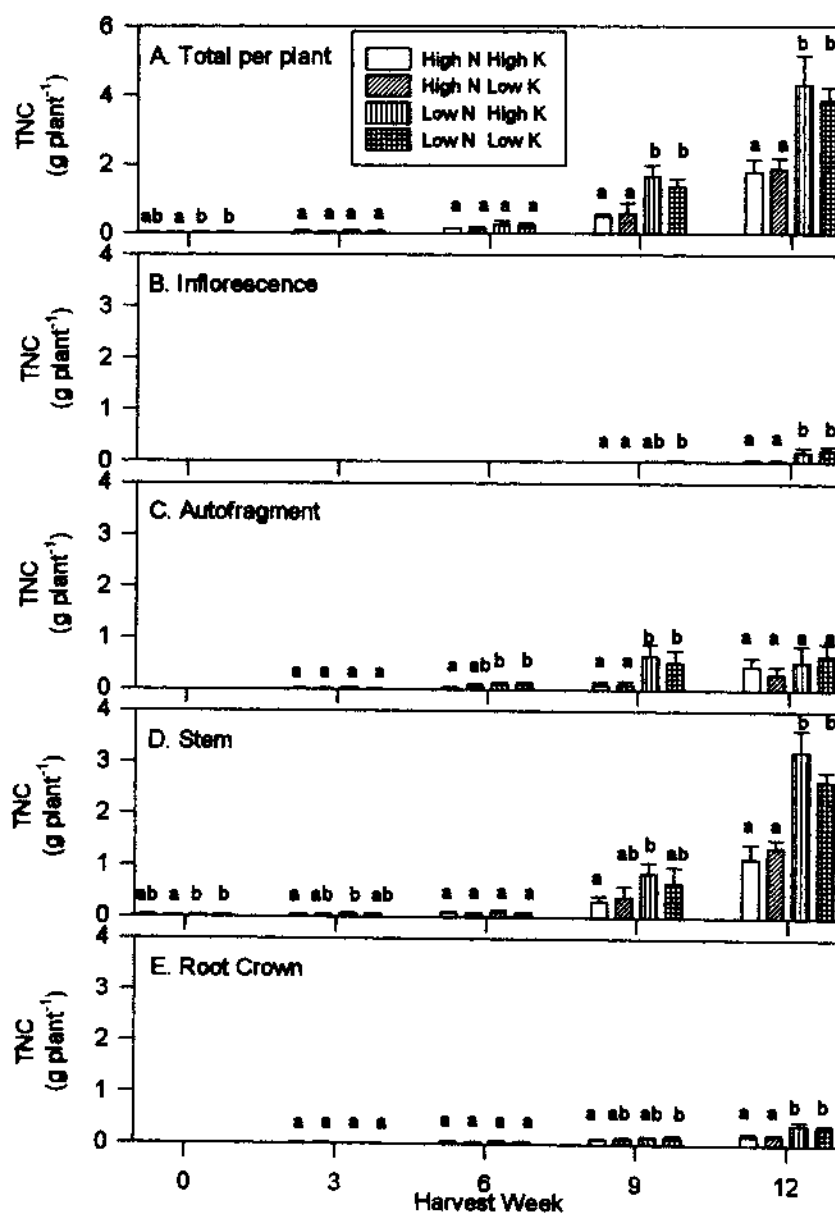
Figure 7. Mean biomass production in grams per plant for plants established

from autofragments produced by the four nutrient regimes and grown for eight

weeks in a high nitrogen, high potassium environment. Bars indicate standard

deviation from the mean and letters indicate statistically distinct groups (n=3). A)

Total biomass in grams per plant; B) Inflorescence biomass in grams per plant;

C) Autofragment biomass in grams per plant; D) Stem biomass in grams per

plant; E) Root crown biomass in grams per plant.
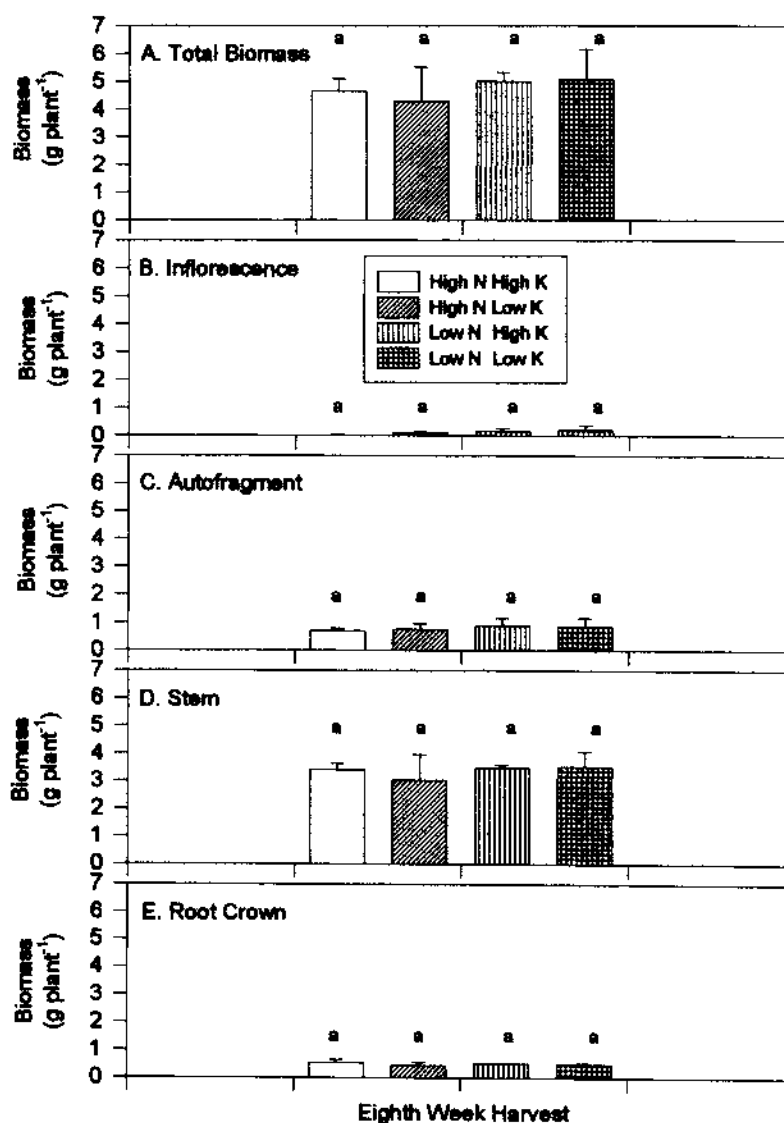
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