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# Experimental Effects of Lime Application on Aquatic Macrophytes: 2. Growth Response versus Treatment Time and Lime Concentration

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**PURPOSE:** This research investigated the effects of applying lime (as calcium hydroxide; $Ca(OH)_2$) either early or later in the life cycle on the growth, survivorship, and reproductive success of Sago Pondweed (*Stuckenia pectinatus*) in an outdoor experimental mesocosm setting.

**BACKGROUND:** In addition to controlling sediment phosphorus in aquatic systems, lime ($CaCO_3$ and $Ca(OH)_2$) applications have been shown to be effective in both suppressing submersed macrophyte growth and changing species composition in a variety of ponds, small lakes, canals, and dugouts (Babin et al. 1992; Chambers et al. 2001; Prepas et al. 2001a, 2001b). The mode of action for controlling growth is believed to be temporarily induced inorganic carbon limitation of photosynthesis at high pH. Symptoms include pigmentation loss and arrested biomass development after treatment (Chambers et al. 2001, James et al. 2005). Other factors such as reduced light availability due to precipitation of calcite may also be involved in growth suppression. The lime dosage and exposure time requirement to suppress growth in this manner are not precisely known as dosage has primarily been targeted at phosphorus removal from the water column via precipitation with calcite rather than at macrophyte control. Another uncertainty is when to apply lime during the plants' life cycle for most effective control. The objectives in Phase 2 of this research were to examine the effects of various lime application dosages during early and later stages of growth on the survivability of a test plant, sago pondweed, grown in experimentally controlled mesocosms.

**METHODS:** Experimental lime application rates ranged between 0 and 1000 mg $L^{-1}$ (Table 1) to overlap and extend beyond concentration ranges that have been used in field experiments (typically 10 to 275 mg $L^{-1}$; Prepas et al. 2001a, 2001b; Reedyk et al. 2001; Chambers et al. 2001; Rattei 2004). Commercially-obtained propagules (Kester's W.F.G. Nurseries, Omro, Wisconsin) of sago pondweed (*Stuckenia pectinatus* (L.) Boerner) were germinated in the laboratory and one sprouted plant was transplanted into a polyethylene container (16 cm wide x 16 cm deep x 22 cm height) filled with homogenized sediment (obtained from Eau Galle Reservoir, WI; see James et al. (2005) for a description of sediment characteristics) to a depth of 10 cm. Eight replicate containers were planted for each treatment (study design described below) for a total of 56 containers. An additional 16 replicate planted containers were assembled for determination shoot and root biomass and leaf chlorophyll concentration at the time of lime application (see methods below). All planted containers were incubated in outdoor clear fiberglass mesocosms (i.e., one mesocosm per treatment; 1.2-m diam x 1.2-m height; 1400-L capacity). Natural lighting was controlled with a 30-percent shade cloth deployed 2 m above the mesocosm surface. All mesocosms were filled with locally obtained tap water prior to the start of the experiment (Ca = 57 mg • $L^{-1}$; Conductivity = 422 µS; Mg = 28 mg • $L^{-1}$; $NO_2NO_3$-N = 0.2 mg • $L^{-1}$; K = 0.8 mg • $L^{-1}$; Na = 1.6 mg • $L^{-1}$; $SO_4$ = 21 mg • $L^{-1}$; pH = 7.8). Circulation pumps (Beckett Versa Gold G90AG; 0.34 $m^3$ $min^{-1}$) provided water circulation in each tank during the entire study. Inorganic carbon chemistry was not altered by bubbling air into the mesocoms; thus, equilibration between atmospheric and aqueous phases of $CO_2$ occurred via diffusional processes.

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**Table 1**
**Experimental Treatments and Lime Application Concentrations**

| Treatment | Days After Germination | Plant Replicates per Mesocosm | Lime Concentration | |
|---|---|---|---|---|
| | | | (mg $L^{-1}$) | (mg $m^{-2}$) |
| 1 | Control | 24[1] | 0 | 0 |
| 2 | 12 | 8 | 250 | 305 |
| 3 | 12 | 8 | 500 | 610 |
| 4 | 12 | 8 | 1000 | 1220 |
| 5 | 42 | 8 | 250 | 305 |
| 6 | 42 | 8 | 500 | 610 |
| 7 | 42 | 8 | 1000 | 1220 |

[1] Eight containers each were harvested at day 12 and day 42 after germination to determine biomass at the time of lime treatment during early and later stages of the growth cycle.

Commercially obtained lime (as $Ca(OH)_2$) was applied as a slurry to experimental mesocosms by mixing the appropriate dry powder mass (as grams of $Ca(OH)_2$) for each intended concentration with 8 L of tap water, then dispersing it evenly over the surface of the mesocosms. For experiment 1, lime was applied to mesocosms 12 days after germination (7 June); plants were allowed to grow for 72 days after treatment. For experiment 2, plants were allowed to grow for 42 days (i.e., shortly after flowering) before lime application (9 July), then an additional 42 days after treatment. All plants from both experiments were harvested on 18 August 2004 (total growth period = 84 days). The study was conducted between 26 May and 18 August 2004.

Shoot and root fresh and dry biomass were determined for each plant container at the end of the study. For shoot biomass, a 10-cm sprig was removed, weighed, and frozen for analysis of leaf chlorophyll. The remaining shoot material was briefly soaked in a 1 N hydrochloric acid solution to remove any calcium carbonate (calcite: $Ca(CO_3)$) deposits on the plant, gently rinsed in tap water, and dried at 90 $^oC$ for dry mass determination. Roots sieved from the sediment were dried for below-ground biomass determination (root material was not pretreated with 1-N HCl). Leaves (~0.1 – 0.5 g fresh mass) removed from the apical portion of the frozen sprig were extracted in a 50:50 solution of DMSO (dimethyl-sulfoxide) and acetone before fluorometric determination (Welschmeyer 1994) of leaf chlorophyll. Leaf chlorophyll was expressed as mg $g^{-1}$ leaf dry mass using correction factors to account for the percentage of fresh mass that represented $Ca(CO_3)$ and a $Ca(CO_3)$-free fresh mass:dry mass ratio. Shoot and root biomass and leaf chlorophyll at the time of lime application 42 days after germination were also estimated using procedures described above. Leaf chlorophyll was not measured for newly sprouted shoots (i.e., 12 days after germination).

Throughout the study, in situ temperature, pH, dissolved oxygen, and conductivity were monitored in each mesocosm at 2- to 3-day intervals using a Hydrolab Surveyor 3 that was calibrated against known buffers and Winkler titrations. Integrated water column samples were collected for the determination of total alkalinity (expressed as mg $CaCO_3$ $L^{-1}$) as titration with 0.02 N sulfuric acid to an end-point of pH 4.5 (APHA 1998). Free $CO_2$ and bicarbonate, carbonate, hydroxide alkalinity at 25 $^oC$ were estimated by calculation based on ionization constants (American Public Health Association(APHA) 1998).

## RESULTS:

**Experiment 1.** Before lime application, mean pH and total alkalinity in the mesocosms were 8.19 (± 0.03 S.E.) and 202 mg $L^{-1}$ (± 2 S.E.), respectively. pH increased over the course of the study to a maximum of 9.8, while total alkalinity declined to ~ 100 mg $L^{-1}$ in the control mesocosm due to net removal of $CO_2$ via photosynthesis (Figure 1). Bicarbonate accounted for most of the total alkalinity in the control mesocosm throughout the study period (Figure 2a). Free $CO_2$ concentrations were negligible throughout the study, as pH was generally above 8.0. In the mesocosm treated with 250 mg lime $L^{-1}$, pH increased to ~ 10.1 and remained greater than 10 for only 4 days (Figure 1). It steadily declined over the next 22 days to ~ 9.3 and fluctuated between 9.3 and 9.9 until the end of the study. Total alkalinity declined abruptly in this mesocosm as an apparent consequence of calcium supersaturation and precipitation as calcite, resulting in overall declines in bicarbonate alkalinity from pretreatment levels (Figure 2b). A bicarbonate alkalinity minimum of 22.7 mg $L^{-1}$ was observed 3 days after treatment. The concentration increased thereafter; however, it did not recover to control or pretreatment levels (maximum post-treatment bicarbonate alkalinity concentration = 43.1 mg $L^{-1}$).

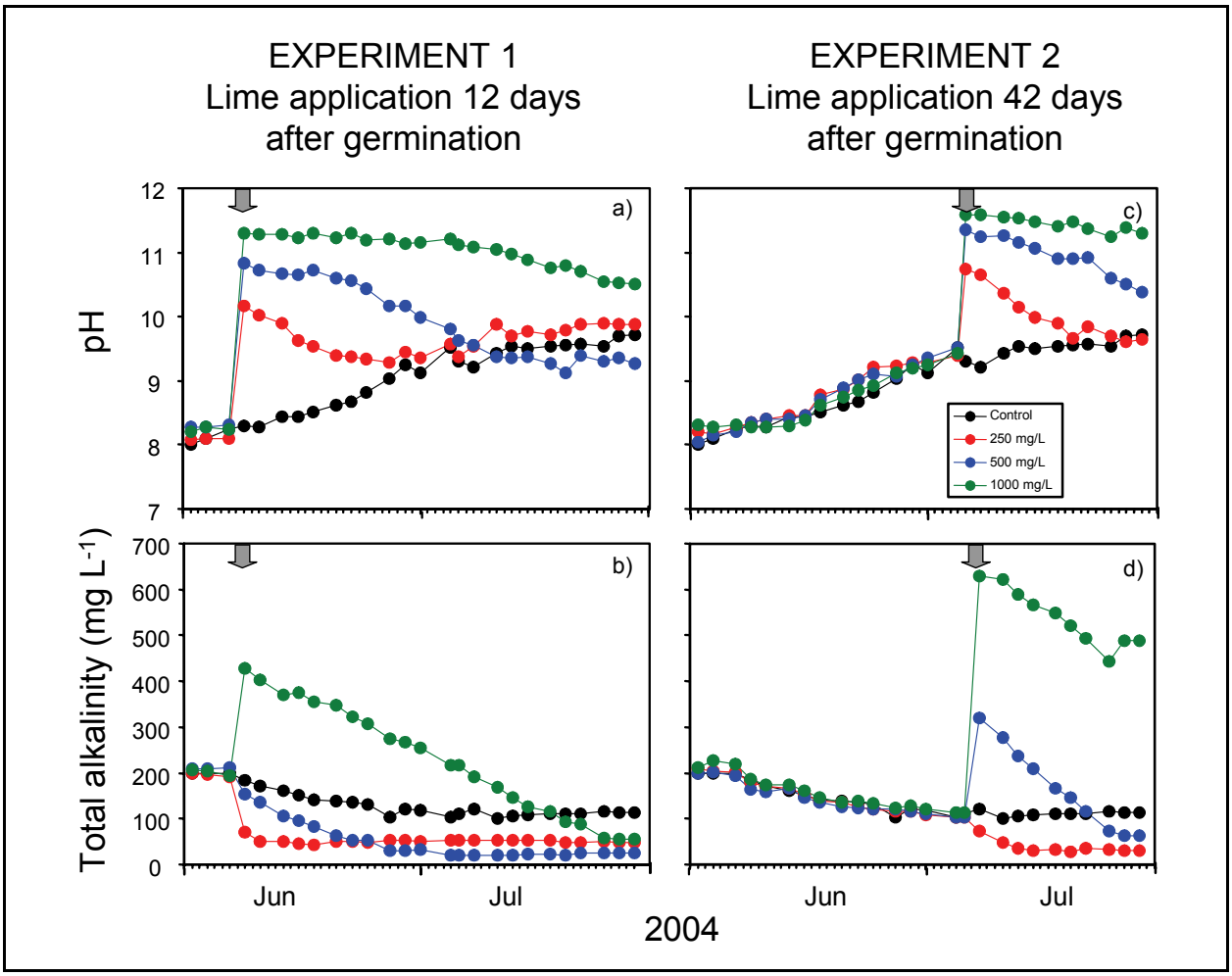


Figure 1. Variations in pH and total alkalinity in control and treated mesocosms after lime application (arrow denotes the day of treatment)

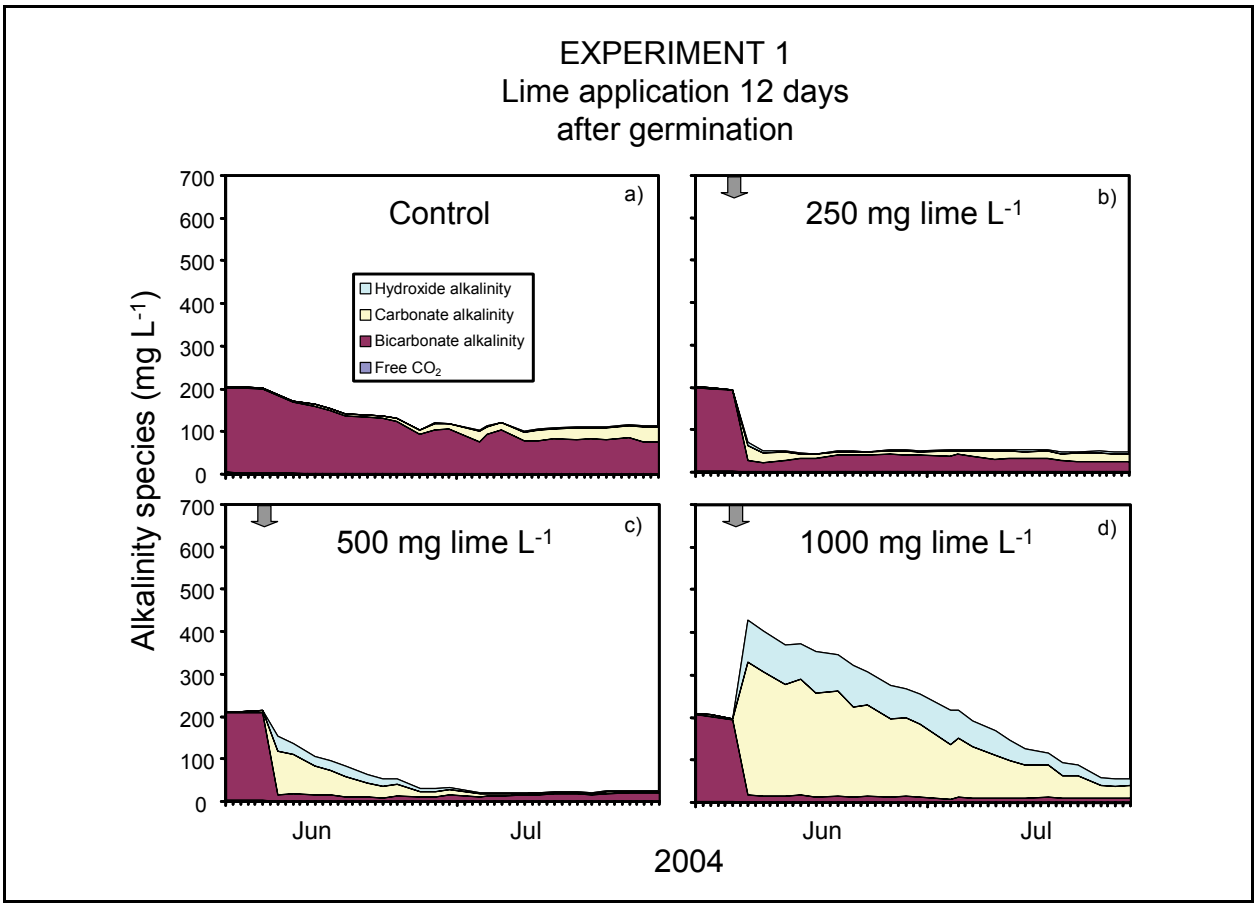


Figure 2. Variations in mean pH and alkalinity species for the (a) control (i.e., no lime application) mesocosm and mesocoms treated with (b) 250 mg lime $L^{-1}$, (c) 500 mg lime $L^{-1}$, and (d) 1000 mg lime $L^{-1}$ for sago pondweed treated with lime 12 days after germination (arrow denotes the day of treatment)

In the mesocosm treated with 500 mg lime $L^{-1}$, pH increased to a peak of 10.8 and was above 10.3 over a 16-day period after treatment (Figure 1). It declined steadily and reached control values by July 6 (29 days after treatment). Total alkalinity decreased below control values after treatment with 500 mg lime $L^{-1}$, indicating calcite precipitation. Bicarbonate alkalinity decreased substantially after lime application, while carbonate and hydroxide alkalinity increased, reflecting pH increases above 10.3 and a shift in equilibrium to carbonate dominance. Bicarbonate alkalinity remained below approximately 20 mg $L^{-1}$ for 51 out of the 72 post-treatment days (Figure 2c).

In the mesocosm treated with 1000 mg lime $L^{-1}$, pH increased to a peak of 11.3 and was above 10.3 throughout the entire post-treatment phase of the study (Figure 1). In contrast to the other lime treatments in experiment 1, total alkalinity increased to greater than 400 mg $L^{-1}$ shortly after application of 1000 mg lime $L^{-1}$ and was composed primarily of carbonate and hydroxide alkalinity (Figure 2d). Bicarbonate alkalinity declined rapidly after lime application as a result of precipitation with calcium and a shift in equilibrium at high pH. Total alkalinity declined in a linear pattern from its peak between 8 June and the end of the study, reflecting declines in carbonate and hydroxide alkalinity. It decreased below control levels by 21 July. Bicarbonate alkalinity remained low throughout the post-treatment period (mean 12.1 mg L-1 ± 0.5 S.E.).

At the time of lime application on 8 June, shoot and root biomass were very low at 0.06 g and 0.24 g, respectively. At the end of the study (17 August; 72 days after treatment), mean shoot and root biomass were significantly lower in treated mesocosms, relative to the control means, and there was a trend of decreasing biomass as a function of increasing initial lime concentration applied to the mesocosms (Figures 3a and 3b; ANOVA, Statistical Analysis System (SAS) 1994). Although mean shoot and root biomass were suppressed in the mesocosm treated with 250 mg lime $L^{-1}$, plants had recovered from the initial treatment and had grown considerably relative to plants exposed to the higher lime concentration treatments. In contrast, mean shoot and root biomass were much lower in the mesocosms treated with 500- and 1000-mg lime $L^{-1}$ compared to the control means. Mean plant biomass was negligible in the mesocosm treated with 1000 mg lime $L^{-1}$, and plants appeared to be dead at the time of harvest.

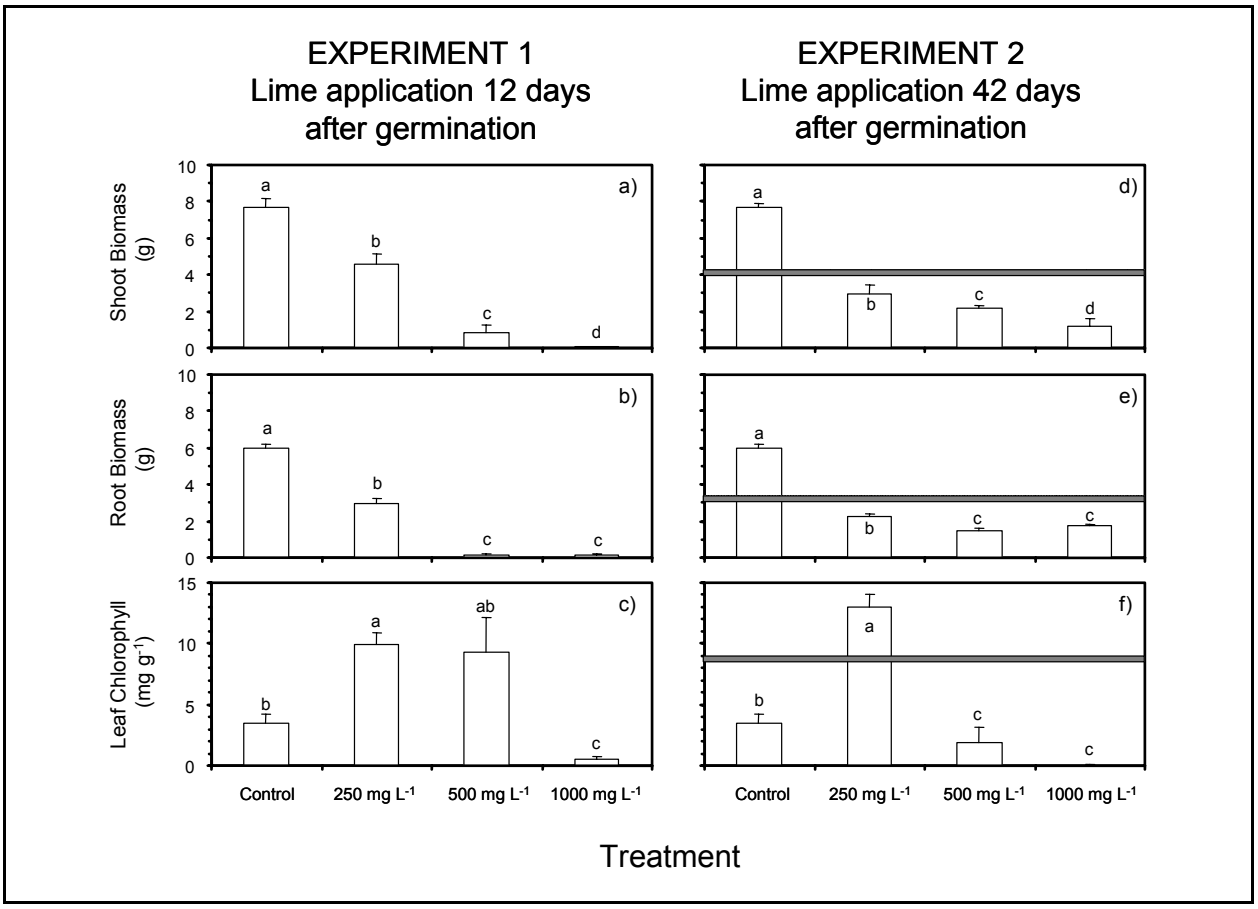


Figure 3. Variations in mean shoot and root biomass and leaf chlorophyll content as a function of lime application concentration for experiments 1 and 2. Horizontal gray line indicates the mean at the time of treatment for experiment 2. Shoot and root biomass were negligible at the time of treatment in experiment 1 (leaf chlorophyll was not measured at the time of treatment). Letters indicate significant differences among means at the 5-percent level or less (ANOVA; SAS (1994))

All plants treated with a lime application exhibited initial pigment loss. Stems and leaves were white in appearance. Further growth after the time of lime treatment occurred via either new auxiliary buds on blanched tissue or from the root stock, primarily for plants treated with 500-mg lime $L^{-1}$ or less. At the time of harvest, mean leaf chlorophyll concentrations were greatest for plants subjected to the 250- and 500-mg lime $L^{-1}$ treatments (Figure 3c). Even though mean shoot and root biomass were very low for plants subjected to the 500-mg lime $L^{-1}$ application, noticeable recovery occurred in the form of new buds that developed by mid-August near the base of the plants or from the roots. Mean leaf chlorophyll concentrations were very low for plants subjected to the 1000-mg lime $L^{-1}$ treatment, reflecting the lack of shoot and root growth.

Plants treated with 250 mg lime $L^{-1}$ flowered and formed tubers in similar numbers and size as plants grown under control conditions (Figures 4a and 4b). No flowering or tuber production occurred for plants treated with lime application concentrations greater than 250 mg $L^{-1}$.

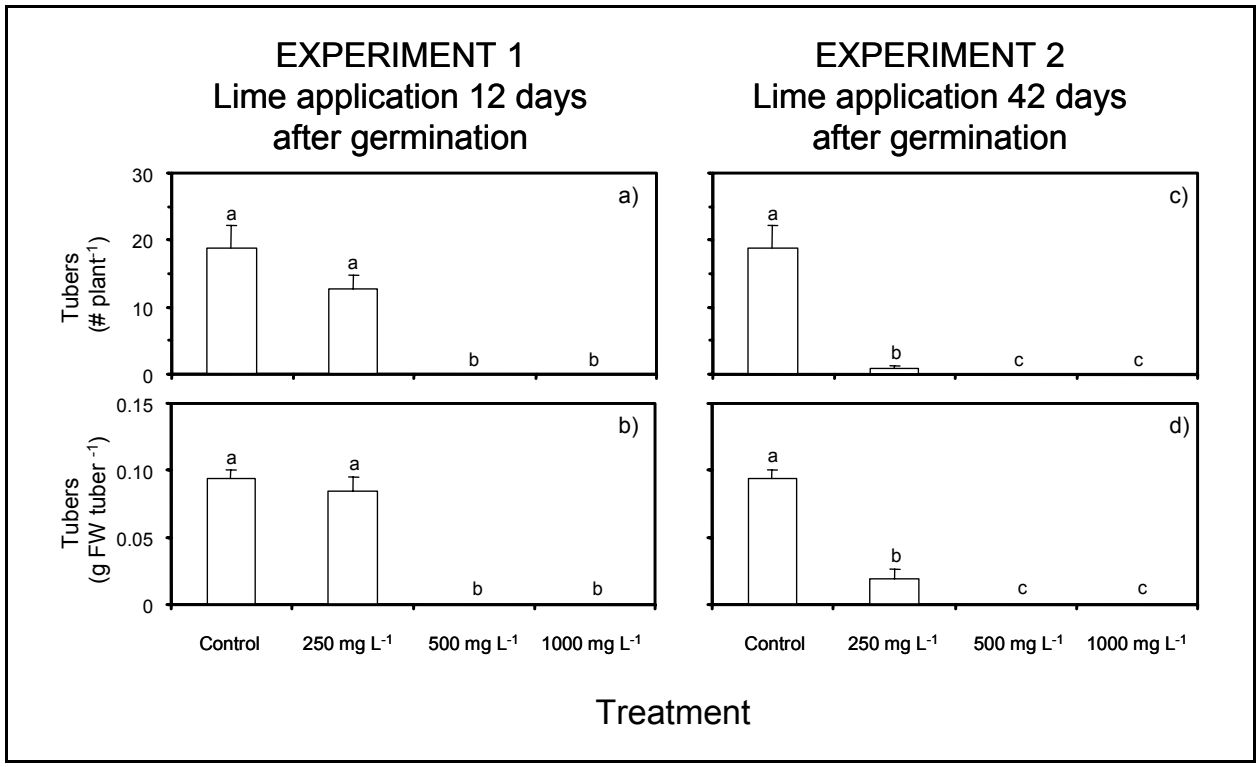


Figure 4. Variations in mean tuber number and fresh mass as a function of lime application concentration for experiments 1 and 2. Tubers were not detected at the time of treatment for either experiment. Letters indicate significant differences among means at the 5-percent level or less (ANOVA; SAS (1994))

**Experiment 2.** Mean pH was higher (9.46 ±0.03 S.E.), while mean total alkalinity was lower (105.5 mg $L^{-1}$ ± 2.9 S.E.), immediately prior to lime application in July, versus patterns observed in experiment 1. These differing initial conditions, and higher water temperatures, led to more exaggerated increases in pH and total alkalinity in the mesocosms treated with lime in July (Figures 1c and 1d). In the mesocosm treated with 250 mg lime $L^{-1}$, pH was above 10.3 for 6 days. It was above 10.3 for 22 days and 42 days in mesocosms treated with 500- and 1000-mg lime $L^{-1}$, respectively. pH declined to control levels within 14 days in the mesocosm treated with 250-mg lime

$L^{-1}$. However, pH levels in the mesocosms subjected to 500- and 100-mg lime $L^{-1}$ remained above control levels throughout the treatment phase of the study.

Total alkalinity increased to greater than 600 mg $L^{-1}$ after treatment with 1000-mg lime$L^{-1}$ in July, contrasting with the much lower concentration spike that was observed for this same application rate in experiment 1 (Figure 1). Total alkalinity concentrations also increased initially in the mesocosm treated with 500-mg lime $L^{-1}$ in July. They then declined steadily, falling below control levels 16 days after treatment. Similar to experiment 1, total alkalinity declined rapidly in the mesocosm treated with 250-mg lime $L^{-1}$. For all lime-treated mesocosms, bicarbonate alkalinity declined to minimum values shortly after treatment (Figure 5). Bicarbonate alkalinity remained at negligible concentrations throughout the post-treatment phase in the mesocosms treated with 500- and 1000-mg lime $L^{-1}$. It was below 20 mg $L^{-1}$ for 19 days following treatment and increased slightly by the end of the study in the mesocosm subjected to 250 mg lime $L^{-1}$; however, it never recovered to pre-treatment or control levels.

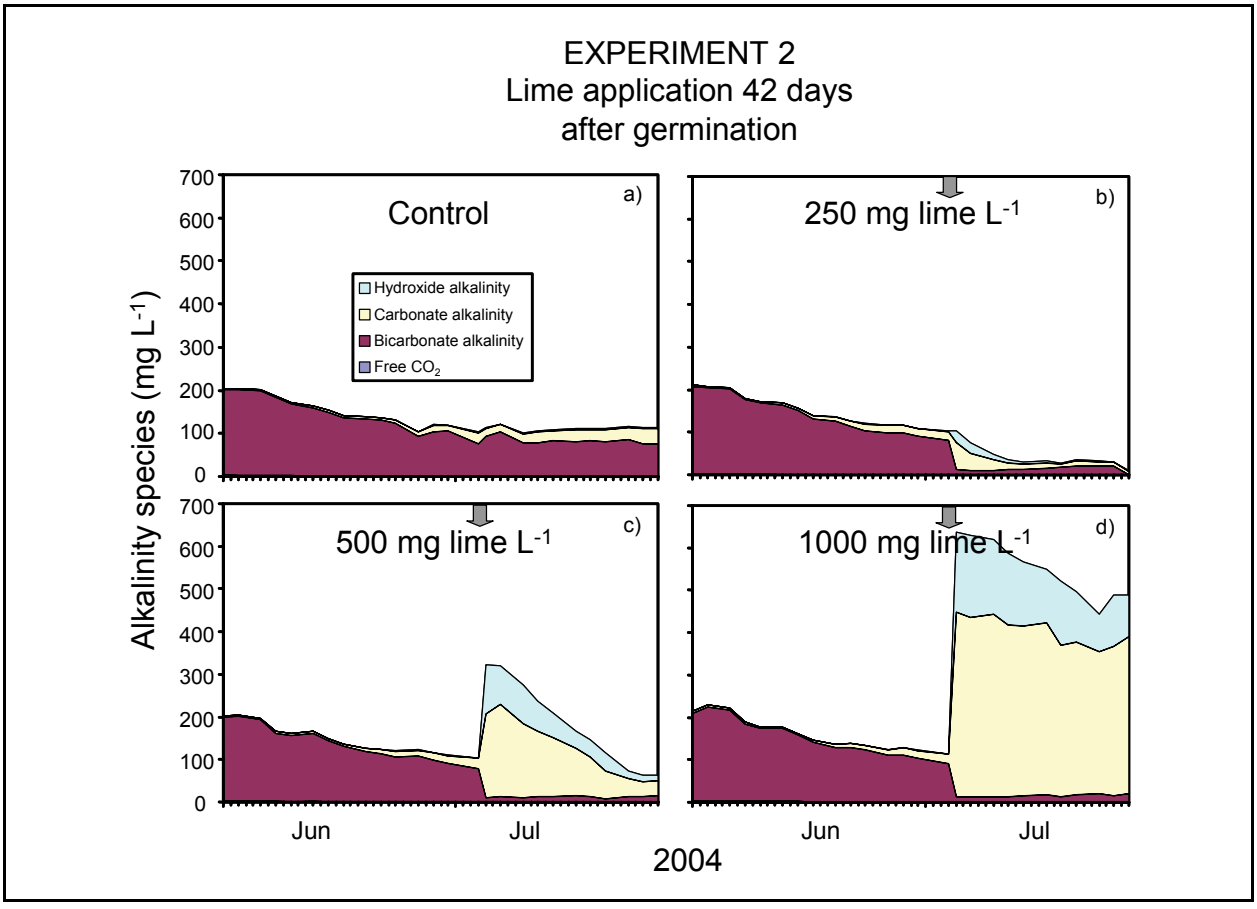


Figure 5. Variations in mean pH and alkalinity species for the (a) control (i.e., no lime application) mesocosm and mesocoms treated with (b) 250-mg lime $L^{-1}$, (c) 500-mg lime $L^{-1}$, and (d) 1000-mg lime $L^{-1}$ for sago pondweed treated with lime 42 days after germination (arrow denotes the day of treatment)

Comparable to patterns observed in experiment 1, plants treated with lime quickly lost coloration and turned white. At the end of the study, mean shoot and root biomass were significantly greater in the control versus treated mesocosms (Figures 3d and 3e; ANOVA, SAS (1994)). Mean biomass levels in the control mesocosm were also significantly greater at day 84 versus day 42 (i.e., day of lime application; ANOVA; SAS (1994)), indicating growth during the treatment period. In contrast, both mean shoot and root biomass were significantly lower at the end of the study versus at the time of treatment for all plants treated with lime in experiment 2 (ANOVA; SAS (1994)). These patterns suggested that growth had stopped in connection with lime treatment and some overall net tissue biomass loss occurred during the treatment phase.

Although net tissue loss occurred, some new auxiliary bud germination was observed on plants subjected to 250-mg lime $L^{-1}$. This new growth was reflected in the high leaf chlorophyll levels observed at the end of the study (Figure 3f). However, no new growth was observed for plants treated with greater than 250-mg lime $L^{-1}$ and mean leaf chlorophyll was much lower for these treatments versus those in the control or the 250-mg lime $L^{-1}$ treatment (ANOVA; SAS (1994)).

Tuber formation was suppressed and the tuber mass was significantly lower for plants treated with lime relative to plants grown in the control mesocosm (Figures 4c and 4d). Tuber development was not observed at the time of lime application in July (42 days after germination). At the end of the study, plants grown in the control mesocosm had produced a mean 19-tuber $plant^{-1}$ (± 3.4 S.E.) with an average mass of 0.094 g fresh mass $tuber^{-1}$ (± 0.007 S.E.). In contrast, plants subjected to 250-mg lime $L^{-1}$ developed few, if any, tubers and were significantly smaller in mass relative to plants grown in the control mesocosm. No tuber formation was observed for plants treated at higher lime concentrations.

**Synthesis of Relationships Between Lime Concentration, Inorganic Carbon Availability, and *S. pectinatus* Growth Suppression.** The effects of lime dosage concentration on mesocosm pH shortly after application (i.e., $pH_{final}$ = 2 days after lime application) were dependent on the initial pH and total alkalinity of the system at the time of treatment (Figure 6). $pH_{final}$ increased in a curvilinear pattern as a function of increasing lime dosage concentration over a range of initial pH and total alkalinity concentrations. $pH_{final}$ was also higher as a function of higher initial pH and lower initial total alkalinity at the time of lime application for a given lime concentration category due to differences in the carbonate buffering capacity at the time of treatment. Thus, 250-mg $L^{-1}$ lime additions to mesocosms resulted in a greater $pH_{final}$ when the lime was applied at an initial pH of 9.3 versus when it was applied at an initial pH of 8.3.

Total alkalinity and various alkalinity species responded differently as a function of lime application concentration and initial pH and total alkalinity conditions (Figure 7). For instance, the 250-mg lime $L^{-1}$ application drove the $pH_{final}$ up to only approximately 10.1 and bicarbonate alkalinity fell to 22.7 mg $L^{-1}$ when it was applied at an initial pH of ~ 8.3. When applied at a higher initial pH, this same lime concentration drove the pH up to approximately 10.7 and bicarbonate alkalinity declined to 8.5 mg $L^{-1}$. At lime concentrations greater than 250 mg $L^{-1}$, total, carbonate and hydroxide alkalinity concentrations increased substantially above initial concentrations as $pH_{final}$ increased above approximately pH 10.8. As shown earlier (see above), *S. pectinatus* pigment loss and growth suppression occurred when $pH_{final}$ increased above approximately 10.3 for at least 6 days. Thus, the

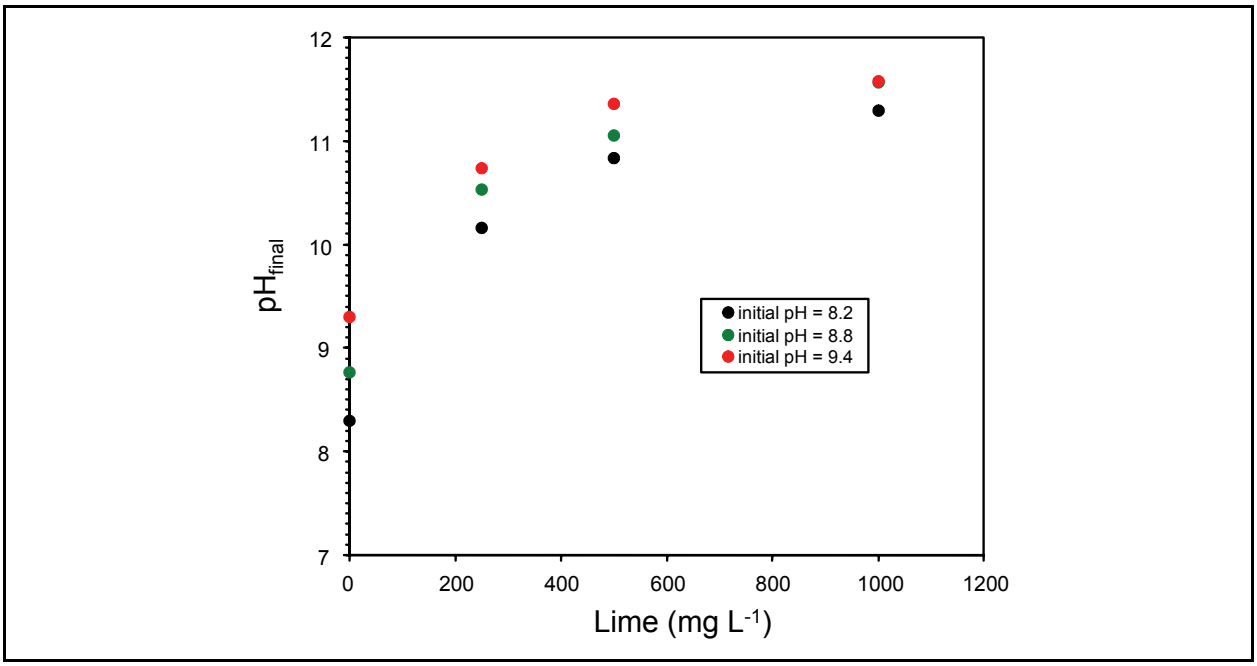


Figure 6. Variations in pH after treatment ($pH_{final}$) as a function of lime application concentration. Lime was applied at different initial pH levels. The green circles represent data collected in 2003 (James et al. 2005), while the red and black circles represent data collected in 2004

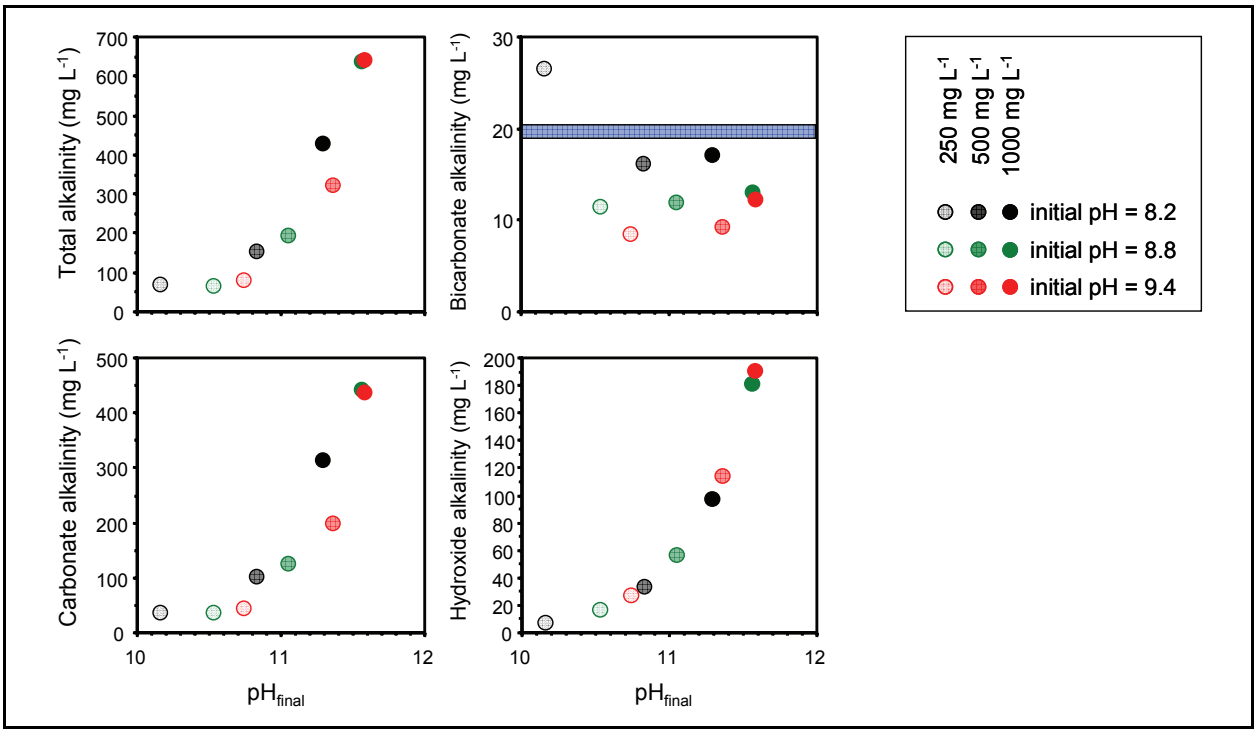


Figure 7. Relationships between the initial pH of the system (i.e., before treatment), the pH 2 days after treatment ($pH_{final}$), and the concentration alkalinity species for different lime application concentrations. Bicarbonate alkalinity concentrations below the horizontal blue bar coincided with growth and tuber development suppression. The green circles represent data collected in 2003 (James et al. 2005), while the red and black circles represent data collected in 2004

lime-induced $pH_{final}$ range that suppressed *S. pectinatus* growth without causing an excessive increase in carbonate and hydroxide alkalinity (and, therefore, total alkalinity) appeared to range between approximately 10.3 and 10.8.

**DISCUSSION:** Results indicated that lime application suppressed *S. pectinatus* growth and arrested tuber development under certain dosage and exposure regimes. Growth suppression symptoms appeared to be associated with lime-induced increases in pH to approximately 10.3 or greater for at least 6 days (i.e., experiment 2 – mesocosm treated with 250 mg lime $L^{-1}$) and a decline in bicarbonate alkalinity. In particular, at higher lime application concentrations (i.e., >250 mg $L^{-1}$), plant growth and tuber production were significantly suppressed in conjunction with relatively long periods of low bicarbonate alkalinity. Although the mode of action is not precisely known, results suggested that lime application indirectly affected plant growth and tuber production by driving the system toward inorganic carbon-limitation by inducing co-precipitation of bicarbonate as calcite and shifting equilibrium toward carbonate dominance at high pH.

Submersed macrophytes favor free $CO_2$ but in moderately hardwater alkaline (pH range of 8 to 9) systems, bicarbonate ($HCO_3^-$) is the dominant form of inorganic carbon. Submersed macrophytes have evolved mechanisms to use this source (Madsen et al. 1993, McConnaughey and Whelan 1997). Adding hydrated lime increases pH and drives inorganic carbon equilibrium toward calcite formation and depletion of $CO_2$ and $HCO_3^-$ as follows:

$$Ca(OH)_2 = Ca^{+2} + 2\ OH^-$$

$$Ca^{+2} + 2HCO_3^- = Ca(HCO_3)_2$$

$$Ca(OH)_2 + CO_2 = CaCO_{3\ (solid)} + H_2O$$

$$Ca(OH)_2 + Ca(HCO_3)_2 = 2CaCO_{3\ (solid)} + 2\ H_2O$$

As pH increases above approximately 10.3 (carbonate-bicarbonate equivalence point) most of the inorganic carbon is in the form of carbonate ($CO_3^{2-}$) and $Ca^{+2}$ solubility is low, causing photosynthetic inhibition in most submersed macrophytes (McConnaughey 1998). Results suggested that driving equilibrium toward $CO_3^{2-}$ dominance, low $Ca^{+2}$ solubility, and a low $HCO_3^-$ threshold concentration was critical in growth suppression of *S. pectinatus*.

Plant response in mesocosms treated with 250 mg lime $L^{-1}$ (both experiments) provided further insight into probable lime dosages required to impact *S. pectinatus* growth and reproduction. In experiment 1, treatment with 250 mg lime $L^{-1}$ did not appreciably suppress growth or production of tubers. Although bicarbonate alkalinity initially declined in this mesocosm after treatment, it apparently did not fall below growth-limiting concentration levels and gradually increased (although not to control levels) over the remainder of the growing period as $CO_2$ diffused back into the system. In contrast, this same lime application concentration had a greater impact on plant growth and tuber production, relative to controls, when applied at higher initial pH (and temperature) and lower alkalinity in experiment 2. pH increased to greater than 10.3 as a result of lime application, bicarbonate alkalinity decreased to less than 20 mg $L^{-1}$ for 19 days following treatment, and plants lost pigmentation. The system was not driven toward high carbonate and hydroxide alkalinity under

this treatment because pH never exceeded approximately 10.8, and total alkalinity declined due to precipitation of calcite. These patterns suggested that a lime-induced lower threshold of bicarbonate alkalinity was required to impact plant growth and tuber production and dosage should be adjusted to meet that threshold level. For *S. pectinatus*, the bicarbonate alkalinity lower threshold level was less than approximately 20 mg $L^{-1}$.

Conversely, new *S. pectinatus* growth occurred when bicarbonate alkalinity concentrations increased above this threshold level after lime application. Although stressed macrophyte tissue did not recover, new growth in the form of auxiliary buds occurred for plants treated with 250-mg lime $L^{-1}$ in conjunction with bicarbonate alkalinity increases above a minimum threshold level of approximately 20 mg $L^{-1}$ after lime application. These patterns indicated that plants can initiate new growth as inorganic carbon availability increases above limiting levels after a lime application.

The minimum exposure time required to control plant growth and reproduction under inorganic carbon limiting conditions is not yet known. In experiment 1, plants treated with 500-mg lime $L^{-1}$ were exposed to threshold-limiting bicarbonate alkalinity levels for 51 days (out of a total of 74 days of lime exposure) and exhibited some minor new growth by the end of the study period, as indicated by high mean leaf chlorophyll. However, flowering and tuber production were completely suppressed, indicating control of next year's growth. Plants treated with 250-mg lime $L^{-1}$ in experiment 2 were exposed to these threshold levels for only 19 days and exhibited new tissue growth. However, even with some new growth recovery, tuber production was significantly suppressed in both cases, which has implications for longer term plant control. It appeared that lime application may be most beneficial in controlling plant growth, flowering, and tuber formation versus acting as an herbicide.

Used as a growth inhibitor, lime application at a certain stage in the plants' life cycle could provide certain advantages to plant management success. However, relatively long exposure times (i.e., on the order of weeks) at high pH and low bicarbonate alkalinity appear to be required to control growth and reproduction. Lime treatment early in the plants' life cycle would appear to suppress plant growth at low biomass levels and halt flowering and tuber production. Treatment later in the plants' life cycle would be most effective in controlling next year's growth by suppressing seed and propagule development. For the latter treatment case, propagation control may be achievable over a lower exposure time period if the lime is applied just before the onset of flowering and tuber development.

Impacts of potentially high pH and alkalinity on other biota need to be carefully considered in dosage estimation and use of lime to control macrophyte growth and reproduction. The 500-mg lime $L^{-1}$ treatment in early June and the 250-mg lime $L^{-1}$ treatment in early July were effective in suppressing growth and tuber production without causing an increase in total alkalinity and excessively high pH (maximum pH was less than10.8). At higher lime dosages, increases in pH above 10.8 were associated with very high alkalinity that is toxic to other biota. Since lime application alters acid-base equilibrium, jar tests and carbonate equilibrium models should be used to accurately predict dosage levels required to lower bicarbonate alkalinity to threshold-limiting levels without impacting total alkalinity.

More information is needed regarding the impacts of lime application on the growth of a variety of species in order to better assess the feasibility of using lime to control submersed macrophyte growth and reproduction. If lime-induced inorganic carbon limitation is the primary mechanism of growth suppression, lime dosage should be estimated based on shifting equilibrium toward threshold levels of $HCO_3^-$. Threshold levels may differ among species due to differences in photosynthetic compensation points (i.e., level at which inorganic carbon availability is too low for photosynthesis; Titus and Stone 1982). Perhaps some species are more susceptible to inorganic carbon limitation at higher compensation points than others, allowing for the possibility of lower lime application concentrations and lower pH maxima (i.e., less than10) that would not be as harmful to other biota. For instance, the findings of Chambers et al. (2001) suggested that some macrophyte species (i.e., *Myriophyllum excalbescens*) are more susceptible to lime and lower pH maxima than others for Canadian ponds. If so, selective control may be possible by adjusting lime concentration to induce inorganic carbon limitation at safe pH levels.

**SUMMARY:** Lime application affects $CO_2$-$HCO_3^-$-$CO_3^{2-}$ equilibrium by raising pH, causing the precipitation of bicarbonate and its removal as calcite, and driving equilibrium toward carbonate and hydroxide alkalinity. *S. pectinatus* growth completely ceased and plants lost pigmentation in conjunction with lime-induced increases in pH above approximately 10.3 and declines in bicarbonate alkalinity below approximately 20 mg $L^{-1}$. New plant growth in the form of auxiliary bud germination occurred in conjunction with increases in bicarbonate alkalinity above approximately 20 mg $L^{-1}$ after lime application. Application of too much lime shifted equilibrium toward production of hydroxyl ions and very high pH, resulting in total alkalinity levels that were unacceptable to other biota. Thus, results suggested that lime dosage rates need to be geared toward depletion of bicarbonate alkalinity without increasing total alkalinity, and proper dosage should be estimated using jar tests and equilibrium models. Since lime appeared to indirectly inhibit growth rather than act as a herbicide, application should be targeted toward preventing flower formation and tuber production to reduce the next year's growth. Uncertainties still exist regarding optimal exposure time required to effectively control propagation. Finally, the effects of lime application on other aquatic plants that have different metabolic pathways (i.e., C3 versus C4), inorganic carbon compensation points, and growth cycles need to be examined in order to develop sound management technologies for controlling nuisance macrophyte growth and internal P loading in aquatic systems.

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