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Cockroach Bay. Summary of Data for 1998

Results from the University of South Florida

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INTRODUCTION

A proposal to study *Thalassia testudinum* Banks ex Konig (turtle grass) regrowth in Cockroach Bay (CRB) was approved by the Environmental Pollution Commission of Hillsborough County E.P.C. in October 1996. The study is for a three year period and began on 1 April 1997. This report summarizes activities on the induction of apical meristems carried out by the University of South Florida during the second year, from 1 August, 1997 through to 31 August 1998. The aerial photographic monitoring and some restoration experiments on *T. testudinum* has been covered by Dr. J. Nicholas Ehringer, Hillsborough Community College.

FIELD STUDIES

The field studies now include analysis of the June 1967 nurseries as well as the establishment of three nurseries for turtle grass in 1998. In 1997 and 1998, two experimental nurseries were established in Cockroach Bay (CRB) and one in Tampa Bay.

1997 Nurseries. Recovery Area 4 (RA-4; Hole-in-the Wall) and RA-2C (near channel C in RA 2 on the edge of Tampa Bay) were selected for the two nursery areas within CRB. The Tampa Bay site was located about 200 m off RA-2. Each nursery was surrounded by turtle grass and located in sandy areas with propeller cuts traversing them. Details of site selection, dates, and planting techniques are given in the August 1997 report. All sites contained 40 transplants of turtle

grass consisting of 2 short shoots per rhizome and 8 transplants with a single short shoot per rhizome (total of 48 plantings per nursery). Treatments of transplanted turtle grass for induction of rhizome apical meristems included: (1) a control group with no plant growth regulator application, (2) 0.2% auxin (naphthalene acetic acid, NAA), (3) 0.1% cytokinin, (4) 0.2% NAA + 0.02% cytokinin, (5) 0.1% NAA + 0.01% cytokinin, and (6) commercial rooting hormone (Green Light Rootone: 0.2% 1-naphthaleneacetamide) containing a fungicide (4.04% Thiram).

The nurseries were monitored on 4 Sept. and 17 Dec. 1997; all survivors were collected on 3 Aug. 1998. Survivorship was low. There was 29% survival at the Tampa Bay nursery by 17 Dec. and no survivors in Aug. It appeared a boat had anchored or moved around in the nursery. There were 81% and 67% survivals in the RA's 4 and 2C sites respectively by 17 Dec. 1997 and 15% and 19% survivorship in Aug. 1998 respectively. The primary survivors (6 of 8 in RA 4, 3 of 8 in RA 2C) were plants treated with auxin only (Treatment 2). All 18 of the survivors produced one (4 individuals) or two (14 individuals) new rhizomes with apical meristems and new short shoots. In all cases, the new rhizome was produced from the short shoot and not from the old rhizome. The average new rhizome length of the 18 survivors was 9.0 cm (± 0.6 S.E.).

1998 Nurseries. Based on laboratory culture experiments (see below) the nurseries established in 1998 involved use of pots and sterile sand to support early production of roots and new apical meristems. The goal is to compare the success of *Thalassia testudinum* with or without pots with transplants that are directly planted in the field nurseries along with the effect of fertilizer and presence of an apical meristem. Because turtle grass roots do not survive transplantation, it appears that new root production requires a period of at least 1 month. Thus, this experiment compares fertilization immediately after transplantation vs a delay of 1 month to determine level of root production. In addition, the importance of the apical meristem in growth is examined as well as the effect of pots.

Experimental nurseries were initiated at 3 sites described in Field Expt. 1. Sites were selected on 13 May, planted on 25 June, and fertilized on 3 Aug. 1998 using plants collected in Greater Cockroach Bay. The site in RA 4 was moved across the channel to RA 3 due to extensive erosion, while the sites at RA 2C and Tampa Bay were located on the north side of the channel. The experiment was expanded to 7 treatments (n = 8) for a total of 56 plants per nursery. Six sets of 8

plants are double short shoot-containing rhizomes without an apical meristem. while two sets have apical meristems. Treatments at each of the three sites are: 1. Control with no pot, fertililizer. or apical meristem; 2. No pot, fertilizer initiated after 1 month, no apical meristem; 3 No pot, fertilization at time of planting, no apical meristem; 4. In a pot, fertilization at time of planting. no apical meristem; 5. In a pot, no fertilization, no apical meristem; 6. No pot, fertilization at time of planting, with an apical meristem; 7. No pot, no fertilization, with an apical meristem. Fertilization is by the addition of two Forest Tablets (NPK: 20-8-2) added every 8 weeks to the designated treatments throughout the growing period (June through October). Pot treatments consisted of plastic trays. Each pot holds the 8 replicate double short shoot rhizomes of a single treatment. Silica sand was used as the substrate for all plantings. This was done by excavating a hole with a bottomless pot, placing in the plants, adding silica sand, and removing the pot. Each treatment of 8 plantings was marked with a stake with a metal tag. Fertilization was done on the potted plants on 13 May and all treatments to be fertilized (2,3,4,6) after establishment were done on 3 August 1998. The sites will be monitored during the fall and winter and survivors will be collected in the summer of 1998.

LABORATORY STUDIES.

In addition to the proposed field studies, we carried out two 2 month+ culture experiments during the academic year 1997-98 in order to better understand regrowth of transplanted *Thalassia testudinum*. The use of tank experiments was attempted because our field studies are necessarily limited to spring through early fall (April through October) because of the slowdown of growth by turtle grass in the winter. Aquaria studies, if successful would allow shorter term experiments (e.g. 2 months) to select for optimal transplanting conditions in the field.

Aquaria Experiment 1. The goal was to examine the effects of fertilizer and plant growth regulators on rhizome meristem induction of *Thalassia testudinum*. The study was initiated on 7 November 1997 and terminated 13 January 1998. Rhizomes with double short shoots and no apical meristems were taken from inner CRB when the field salinity was 12 ppt and water temperature 18 °C. The plants appeared stressed (limited blade development). Three 20 gallon aquaria were used and three levels of fertilizer (8 g Forestry Tree Tablets: 22N-8P-2K); were possible (1, 2. or 3

tablets per tank = L, M, H in Figures 1-3). Hormone concentrations were $10^{-7, -5, -3}$ M NAA. and control (n = 6 double short shoot units treatment^{-1}; 2 tank^{-1}). Lanolin paste was used to carry hormones, applied to scraped side of each short shoot and cut ends of rhizome of all 72 plants. Plants were grown in the 3 aquaria (20 gallon, 20 cm silica sand, 8 plants per row, randomly mixed). Salinity was maintained at 26-28 ppt, temperature at 26 to 28 °C, and irradiance with a 16 h photoperiod under 200 µmols photons m^{-2} s^{-1} (measured daily). Using three tanks the number of replicates was low (n = 3) for each treatment.

The 2+ month experiment was successful in that there was 98% survival with all plants appearing healthy and producing new blades. However there were no outstanding significant differences between treatments with regard to new blades (Fig. 1) or root biomass (Fig. 2). Further levels of fertilizer did not appear to affect the same factors (Fig. 1-3) but "n" was low in this study. By contrast, the apical meristem biomass (Fig. 3) indicated a significant new rhizome growth in a short time.

Aquaria Experiment 2. The goal of this study was to continue testing the effects of hormones on induction of roots and rhizome meristems in *Thalassia testudinum*. Again, the tank studies were intended to guide the single, yearly field nursery study. The number of replicates was increased by reducing the number of treatments in order to determine significant differences.

The experiment was initiated on 26 February 1998 and terminated on 22 April. Rhizomes having two short shoots and no apical meristem were taken from Cockroach Bay where the salinity was 15 ppt and temperature was 19 °C. Each tank had 24 double short shoots so that all three 20 gallon tanks held a total of 72 plants. Each tank was designed as a replicate with 3 rows of 8 plants each. The four treatments were randomized in each row so that there were two replicates of each treatment per row increasing n to 18 plants per treatment. Tanks contained 20 cm clean silica sand without fertilizer. Salinity was maintained at 26 ppt and temp 27 to 28 °C, 16 h photoperiod at 200 µmols photons m^{-2} s^{-1} (measured daily). The four treatments (n = 8 treatment^{-1}) were: (1) Control (Lanolin paste applied to scraped short shoots), (2) With cytokinin (10^{-5} M Kinetin in lanolin paste), (3) With auxin and cytokinin (10:1 ratio of NAA: Kinetin in lanolin paste), and (4) with Rootone commercial rooting agent (0.2% NAA + 4.04% Thiram). In the Rootone treatment, the double short shoot rhizomes were placed in a Ziplock bag with the powder and shaken, then in the air for 5 min

before planting to allow powder to dry.

The results did not show any significant ($P<0.5$) differences between use of plant growth regulators kinetin, auxin plus kinetin, or Rootone vs the control in terms of leaf mass (Fig. 4), root biomass (Fig. 7) or number of new roots (Fig. 6). Again survival was high (95%) and this may reflect the limited length (2+ months) of the experiment. Plants were healthy and showed a high production of new leaves (Fig. 5). Although fertilizer levels did not appear to affect the outcome of the first aquaria experiment, perhaps the lack of any nutrient addition affected the over all response of the plants in the second experiment. In support of these findings, there was a much lower production of rhizome meristems when compared with Aquaria Experiment 1.

SUMMARY

The two aquaria experiments, although not showing significant differences in treatments did demonstrate three developmental aspects of *Thalassia testudinum* that must be considered in creating nurseries. First, the original roots die after transplantation and new roots are produced within 2 months from the short shoots. Thus transplantations must take the delay in root formation into account. Second, root and rhizome meristem production occurs on the short shoot, not the transplanted rhizome. In fact the original rhizome appears to function only in terms of supplying nutrients to the growing short shoots. Third, the apical meristem can develop rapidly upon transplantation if conditions are correct. This last point gives support to the need to establish standard techniques for turtle grass transplantations.

Based on these results, and the low survivorship of the first field experiment, we are focusing on methods to induce rapid production of roots to support induction of new apical meristems. Further we are concentrating the induction efforts on the short shoots of turtle grass.

ACKNOWLEDGMENTS

Mr. John Andorfer is the Research Assistant on this project. Mr Andorfer prepared the solutions, helped carry out the plantings and will aid in the aquaria studies during this winter. We also wish to thank undergraduates from the University of Tampa, Mr. Nol Rizzuto and Mr. Joe Severson who helped in the plantings this summer.

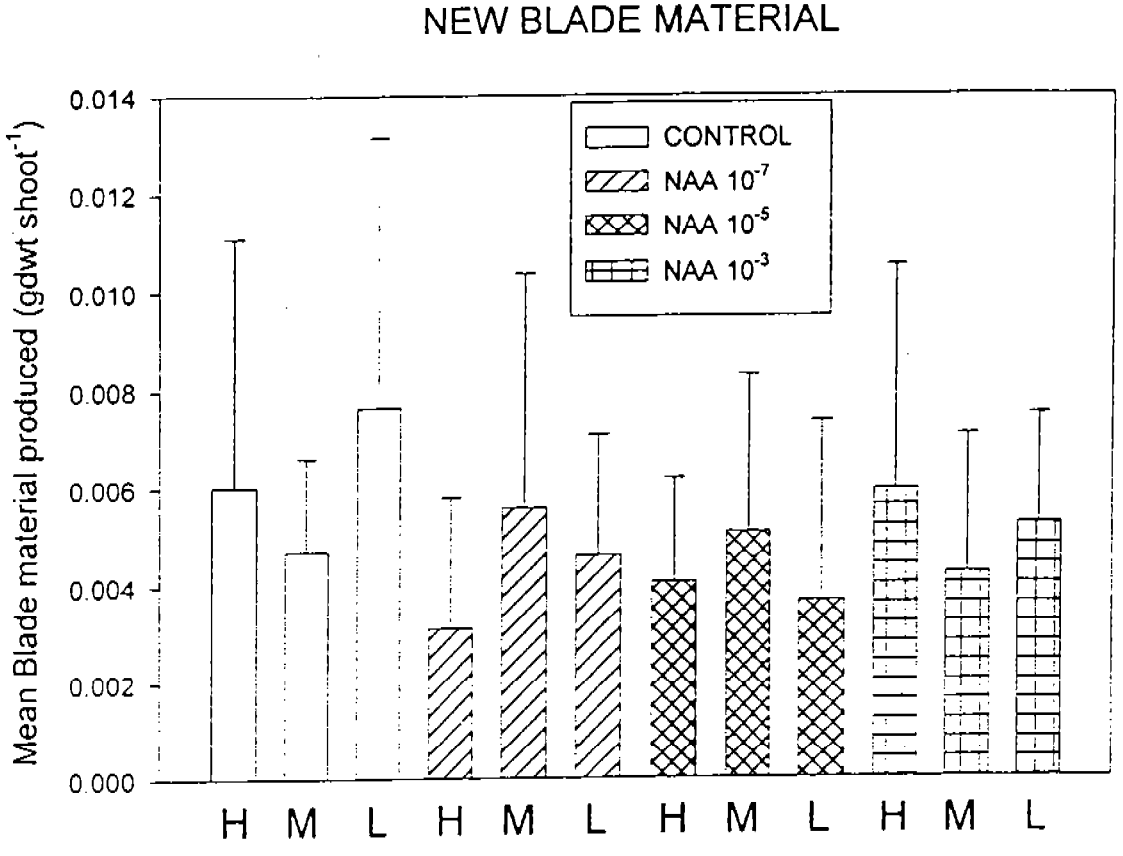
NEW BLADE MATERIAL
Mean Blade material produced (gdwt shoot^{-1})
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
CONTROL
NAA 10^{-7}
NAA 10^{-5}
NAA 10^{-3}
H M L H M L H M L H M L

Figure 1

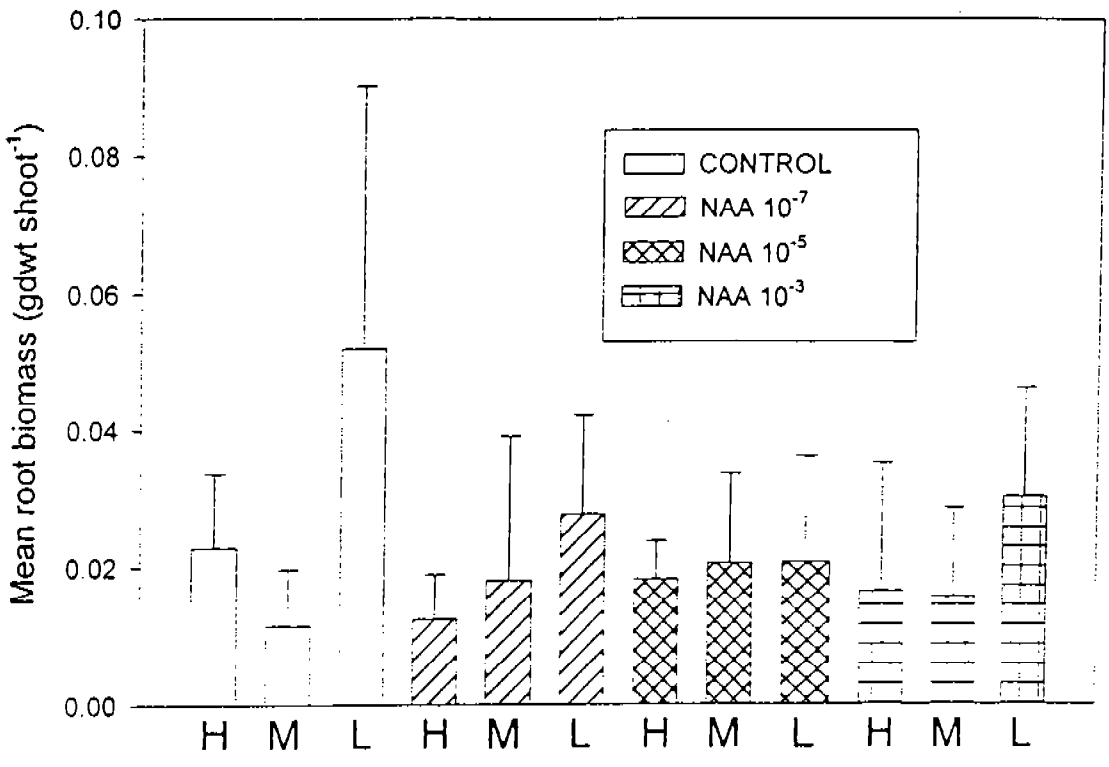
ROOT BIOMASS
Mean root biomass (gdwt shoot^{-1})
0.10
0.08
0.06
0.04
0.02
0.00
CONTROL
NAA 10^{-7}
NAA 10^{-5}
NAA 10^{-3}
H M L H M L H M L H M L

Figure 2

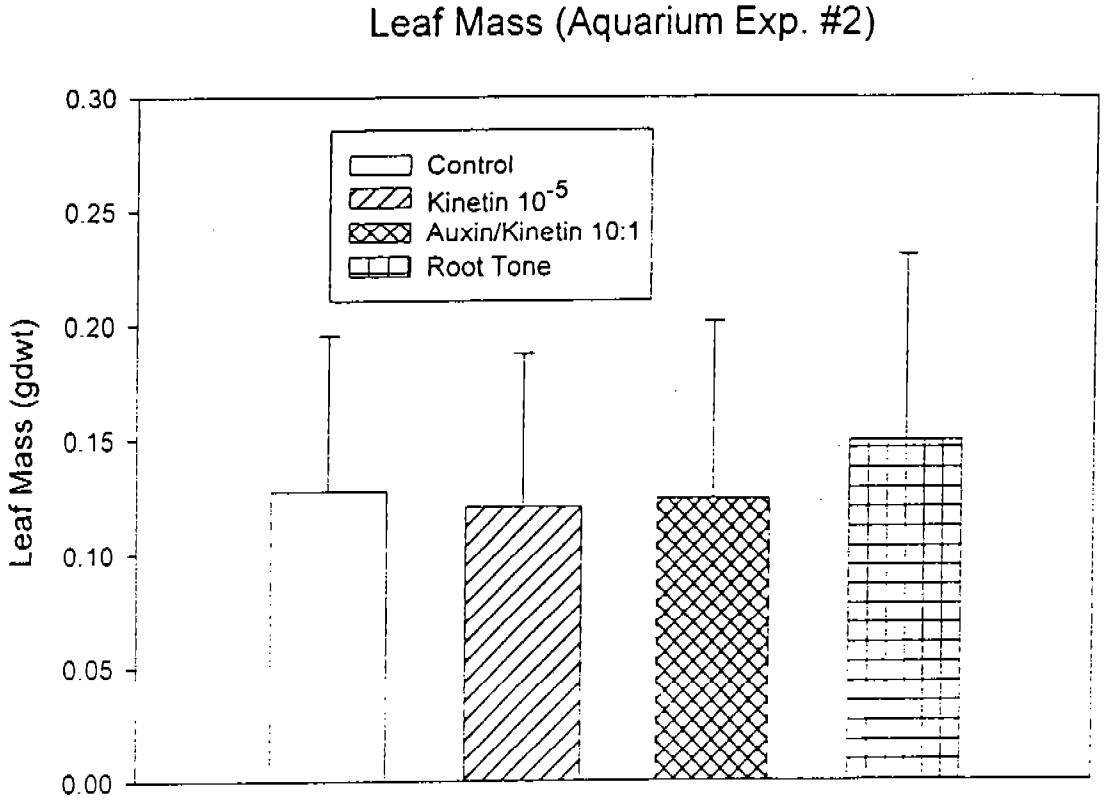
Leaf Mass (Aquarium Exp. #2)
Leaf Mass (gdwt)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Control
Kinetin 10-5
Auxin/Kinetin 10:1
Root Tone

Figure 4

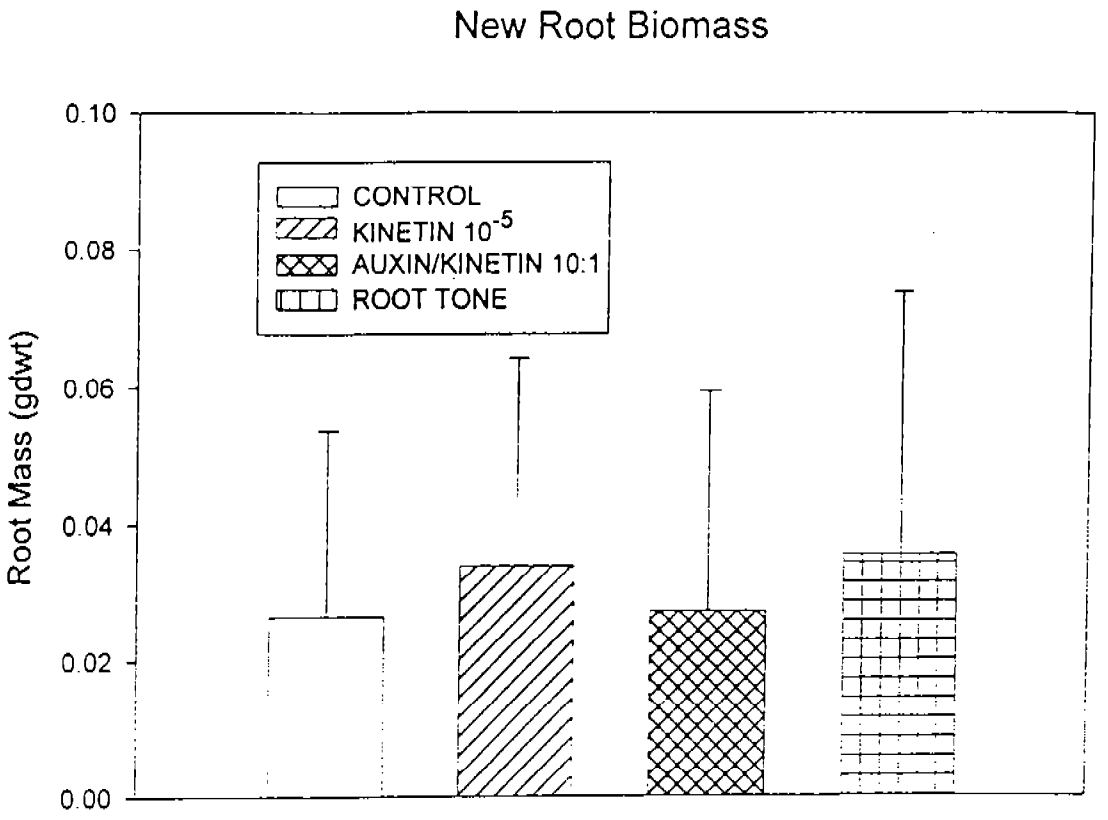
New Root Biomass
Root Mass (gdwt)
0.10
0.08
0.06
0.04
0.02
0.00
CONTROL
KINETIN 10-5
AUXIN/KINETIN 10:1
ROOT TONE

Figure 5