1702 Cover ltr 8 March 2000

Cockroach Bay. Summary of Data for 1999

Results from the University of South Florida

By: Clinton J. Dawes
Department of Biology
University of South Florid
Tampa, Florida.

Research Assistant: Mr. John Andorfer
Department of Biology

INTRODUCTION

A proposal to study *Thalassia testudinum* Banks ex Konig (turtle grass) regrowth in Cockroach Bay (CRB) was approved by the Environmental Protection Commission of Hillsborough County E.P.C. in October 1996. The three year project began 1 April 1997 and terminated 31 August, 1999. This report summarizes the apical meristem induction studies on turtle grass carried out by the University of South Florida from 1 April 1997 to 31 August 1998 (see previous reports) and details the studies carried out from 1 August 1998 through 30 November 1999.

FIELD STUDIES

Field studies involved nursery experiments begun in June 1997, 1998, and 1999. Each experiment involved the establishment of three field nurseries, two in CRB and one in Tampa Bay.

1997 Nurseries. Recovery Area 4 (RA-4; Hole-in-the Wall) and RA-2 (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 west of channel C in RA-2. Each nursery was surrounded by turtle grass and located in sandy areas. Details of site selection, dates, plant growth regulator (PGR) treatments, and planting techniques are given in the August 1997 report.

All sites contained 40 transplants of turtle grass that had two short shoots per rhizome. In addition, eight transplants with a single short shoot per rhizome were also used for a total of 48 plantings per nursery. All survivors were collected on 3 August 1998 and overall survivorship was low (16%). The primary survivors were plants treated with auxin only (Treatment 2). All of

the survivors produced one or two new rhizomes with apical meristems and new short shoots. Further, all of the new rhizomes were produced from the short shoot and not from the old rhizome. The conclusion was that a more highly controlled planting procedure is required to increase survival before detailed PGR experiments could be carried out in the field.

1998 Nurseries. Based on laboratory culture experiments carried out during the winter monsths of 1997 and 1998, the nurseries established in 1998 included the use of pots and sterile sand to support early production of roots and new apical meristems. The goal was to compare the success of turtle grass with or without pots with transplants that are directly planted in the field nurseries along with the effect of fertilizer and the presence of an apical meristem. The experiments compared fertilization immediately after transplantation versus a delay of 1 month to determine level of root production. In addition, the importance of the apical meristem in growth and use of pots were examined.

Experimental nurseries were initiated at 3 sites described in the 1997 Nursery experiment. Details of site selection, dates, plant growth regulator treatments, and planting techniques are given in the August 1998 report. The experiment was expanded to 7 treatments (n = 8) for a total of 56 plants per nursery. Six sets of 8 double short shoot rhizomes lacked an apical meristem, while two sets had apical meristems. Treatments were fertilized at the time of planting in May and August 1998. All plants were removed on 12 May 1999 with 54% survival overall (90 out of 168 plantings). The highest survival was in RA-4 (85%), the most protected site. Although only 2 of the 39 plants with apical meristems produced new apices (5%), these plants were responsible for 86 new short shoots. In contrast, the 51 double short shoots that lacked an apical meristem produced 55 new rhizome tips (108%; Fig. 2). The study showed the following: (1) Use of plastic pots was not effective and resulted in a high level of anaerobiosis in the containers; (2) Presence of an apical meristem inhibited production of new meristems; (3) Fertilization did not affect survival; and (4) Site protection (e.g. RA 4 site) was the overriding factor in survival.

1999 Nurseries. Based on the 1998 field and aquaria experiments, the 1999 studies used cardboard boxes that would disintegrate after burial and tested the ability of young and old short shoots to produce new meristems. Nurseries were selected at RA-4, inside the mouth of RA-2

(Channel 2C), and outside the mouth of RA-2 in Tampa Bay. The nurseries were established on 21 May 1999 (ambient temperature: 26 °C, salinity: 36 ppt). Each nursery had 4 boxes with 8 double short shoot rhizome segments, 4 of which were young (originally with an apical meristem) and 4 old (at least 4 short shoots from the growing tip). Treatments included (1) sand, (2) fertilizer, (3) top soil, and (4) fertilizer and top soil. A fifth treatment at RA4 used Dip'n Grow © (Astoria-Pacific Inc), a hormone mixture (1%IBA, 0.5% NAA in a 20:1 ratio and 5 min soak). All boxes were harvested on 24 November.

The survival rate of double short shoot units was 75%; of which 21% were young units and 54% were old units. Thus survival was significantly higher than in the previous 2 nursery experiments. Further, survival was again highest in the most protected site, RA-4 (83%) compared to channel 2C of RA-2 (63%) and the Tampa Bay site outside of Channel 2C (63%). As noted in the previous nursery experiments, new short shoots and apical meristems were produced exclusively from short shoots and not the rhizome. Also, production was almost completely by old rather than young plants (Fig. 1-6). Short shoot production was highest (12) in the sand plus fertilizer treatment (Fig. 1-3) even though the treatment at RA-4 had been mostly lost due to erosion. Overall, 41 new short shoots were produced, with almost all occurring at the Tampa Bay site.

Except for 1 double short shoot, production of new rhizome meristems (Fig. 4-6) was by old double short shoots with the highest number in the Tampa Bay site (Fig. 6). The highest production of meristems occurred in treatment 2 using sand and fertilizer, while the use of top soil (treatment 3) resulted in significantly lower production. In conclusion, the experiment demonstrated the effectiveness of degradable pots with sand and fertilizer for survival and production of new meristems and short shoots. In addition, the older double short shoot units are the ones of choice for production of new apical meristems.

LABORATORY STUDIES.

Aquaria experiments with turtle grass were added during the winter months of 1997-98 and 1998-99 because field studies were limited to spring through early fall (April through October), the period of seagrass growth. Aquaria studies allowed use of short term experiments (e.g. 2-3 months) to test ideas for the field nursery experiments.

Aquaria Experiment 1. The goal was to examine the effects of fertilizer and plant growth regulators (PGR's) on rhizome meristem induction of turtle grass. Details of the experiments and results are in the 1998 report. The two-month experiment was successful with 98% survival. All plants appeared healthy and producing new blades. However there were no outstanding significant differences between treatments (fertilizer +/- PGR's) with regard to new blades or root biomass. Further, the three levels of fertilizer did not result in different responses. However the number of replicates (3) was low in this study and thus, statistical analysis was limited. By contrast, the apical meristem biomass indicated significant new rhizome growth in a short time.

Aquaria Experiment 2. The goal of this study was to continue testing the effects of PGR's on induction of roots and rhizome meristems in turtle grass. The experimental design and detailed results are in the 1998 report. Although replication was increased to 8, there were no significant differences ($P<0.5$) between use of the PGR's kinetin, auxin plus kinetin, or Rootone compared with control plants in terms of leaf mass, root biomass, or number of new roots. Again, survival was high (95%), the plants appeared healthy, with all plants producing new leaves. Although fertilizer levels did not appear to affect the outcome of Aquaria Experiment 1, the lack of nutrient addition may have affected the overall 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.

Aquaria Experiment 3. The tank experiment tested the null hypothesis that addition of fertilizer (8 g Forestry Tablets: 22N-8P-2K) or a commercial PGR (Rootone) will not affect the growth or production of rhizome meristems of turtle grass.

Four 55.5 L tanks with commercial silica sand were established and illuminated by 8 fluorescent bulbs on 7 October 1998. Each tank contained 16 double short shoots without apical meristems, 8 treated with Rootone powder and 8 untreated. Two tanks contained 4 Forestry Tablets that were evenly distributed in the sand. The experiment ran until 17 February 1999 (19 weeks) when the plants were harvested to determine rhizome growth, production of meristems, and roots. Salinity, using distilled water to maintain 30 ppt, and temperature were monitored weekly.

Within 2 weeks the two tanks with fertilizer tablets showed growth of algae on the walls, sediment, and seagrasses so that cleaning at least once a week was required. Tank 2 became cloudy after about 5 weeks even though the flow-through filters were changed every 2-3 weeks. All of the plants treated with Rootone in Tank 4 died. All other plants showed new blade production and appeared healthy. A minimum of one new rhizome meristem developed from an existing short shoot with the largest number of new rhizome apical meristems in Tank 1 (2 from controls, 5 from those treated with Rootone; Fig. 7). The amount of new rhizome biomass varied widely between treatments, suggesting that meristem initiation occurred throughout the 19-week experiment (Fig. 8). The number of new short shoots ranged from 1 (Tanks 1,2,3) to 3 (Tank 1) with no correlation to Rootone treatment (Fig. 9). Dry weights of the new short shoots also showed wide variations supporting the observation that initiation occurred throughout the experiment.

The original double short shoots and their rhizome showed no pattern in terms of weight changes (Fig. 10). Leaf weight (Fig. 11) was highest in Tank 1(unfertilized; both control and Rootone treatments) and lowest in Tank 2 (fertilized) where an algal bloom occurred during the experiment. Short shoot weights (Fig. 12) and rhizome weights (Fig. 13) were not significantly different, although Tank 2 again showed the lowest level. Root weight (Fig. 14) was significantly higher in Tanks 1 and 3 (without fertilization) for both control and Rootone treated double short shoots, which supported the findings for Aquaria Experiments 1 and 2. In conclusion, Aquaria Experiment 3 does not indicate fertilizer or Rootone is effective.

Aquaria Experiment #4. The null hypothesis tested was that high concentrations of selected PGR's (auxins, cytokinins) will not influence production of rhizome meristems in *Thalassia testudinum*. The use of high concentrations of PGR is based on propagation of bamboo (Sekar *et al.*, 1998. J. Amer. Bamboo Soc. 12:30-36).

In the aquaria room, six 37 L tanks were filled with commercial silica sand and illuminated by 8 fluorescent bulbs (12 h photoperiod) beginning on 29 October 1998. Each tank had 1 Forestry Fertilizer Tablet broken and distributed in the sand and each treatment consisted of 5 double short shoots without apical meristems. All soakings were for 6 h in 15 ppt salinity the day after collection and cleaning (plants were heavily epiphytized and contained sediment). The

treatments were:

1 and 2. Tank 1 Front: control. Back: 0.3g L^{-1} NAA.

3 and 4. Tank 2 Front: 0.3 g PAA L^{-1}. Back: PAA + 0.03 g L^{-1} 2iP.

5 and 6. Tank 3 Front: 0.3g L^{-1} NAA + 0.03 g L^{-1} zeatin. Back: NAA + 0.03 g L^{-1} 2iP.

7 and 8. Tank 4 Front: 0.3g L^{-1} PAA + 0.03 g L^{-1} BAP. Back: PAA+0.03 g L^{-1} zeatin.

9 and 10. Tank 5 Front: 0.3 g L^{-1} PAA + NAA. Back: 0.3 g L-1 NAA + 0.03 g L^{-1} BAP.

11 and 12. Tank 6 Front: 0.03g L^{-1} 2iP. Back: 0.03 g L^{-1} BAP.

Although only one Forestry Tablet was used at the beginning of the experiment, a light to heavy algal bloom occurred in all tanks. The experiment was terminated on 1 March 1999 (17.5 weeks) and the roots and blades from each short shoot were separated, dried, and weighed. All plants survived (100%), although a number of the older short shoots showed very limited root growth. There were no significant differences in blade or root production between older and younger short shoots. Further production of the two short shoots did not differ significantly between PGR treatments. The control showed the highest blade growth while the PGR treatment NAA + Zeatin showed the highest root growth in terms of dry weight material.

No rhizome meristems were produced, although 17 new short shoots developed from existing ones (Fig. 15). Of the 17 new short shoots, 4 developed from the younger and 13 from the older ramet in the double short shoot units Most unusual, 8 of the new short shoots were produced from 4 original second (older) short shoots, a production that had not been previously observed in any of the tank studies, and something that is rare in our observations of field plants. As shown in Figure 15, the highest number of new short shoots occurred after treatment with NAA alone (4) and PAA + BAP (4); the other hormones showed lower productions: NAA + Zeatin (1), PAA alone (2), PAA + 2iP (2), and BAP (2). In conclusion, there was a unique response by the plants to extremely high levels of 11 combinations of PGR's with the production of new short shoots, but not rhizome apical meristems. Turtle grass is sensitive to PGR's and thus we believe that some combination should result in rhizome initiation.

Aquaria Experiment #5. The experiment tested whether the PGR gibberellic acid (GA_3), with or without nitrate ($NaNO_3$), will induce rhizome meristems from old or young short shoots. Short shoot age was determined using the number of leaf scars (Fig. 16). Four 20-gallon (55.5 L)

tanks were used. The plants for each treatment (6 double short shoots, 3 old and 3 young) were planted in trays in sterile, washed sand. Each tank contained three trays and all trays from tanks 2 and 4 were exposed 80 μM NO_3 for 4 h each week and flushed with seawater before returning to the tanks. From each tank, one tray was exposed to 0.05 mg L^{-1} GA_3 (gibberellic acid) and a second tray to 5.00 mg L^{-1} GA_3.

Double short shoots were collected and planted in the tanks on 26 March and harvested on 7 July 1999 (15 weeks). Eighty percent or better of all double short shoots survived (Fig. 17) and 30 of the 32 new apical meristems were produced by old short shoots (Fig. 18). There were no significant differences in number of new apical meristems produced between plants treated with low concentrations of GA_3 and the controls (13 each), while only 6 short shoots produced apical meristems when treated with the high concentration. In summary, there was no evidence that GA_3 treatments were effective and that production of an apical meristem is limited to older short shoots.

Aquaria Experiment #6. The null hypothesis was that there will be no effect of the PGR gibberellic acid (GA_3, 0.05 mg L^{-1}) or nutrients ($NaNO_3$, 80 μM) on apical meristem development regardless of weekly exposures. Four 10-gallon (37 L) tanks were used and 4-5 double short shoots were planted in the silica sand in trays similar to Aquaria Experiment #5. Every week each tray was removed and the plants exposed to one of the following four treatments for 4 hours: (1) A control with filtered seawater in which only 6 double short shoots with apical meristems were placed. (2) A tray with 5 double short shoots pulsed with $NaNO_3$. (3) A tray containing a single double short shoot with an apical meristem and 4 without that were exposed to GA_3. (4) A tray with 4 double short shoots lacking an apical meristem that were exposed to $NaNO_3$ and GA_3.

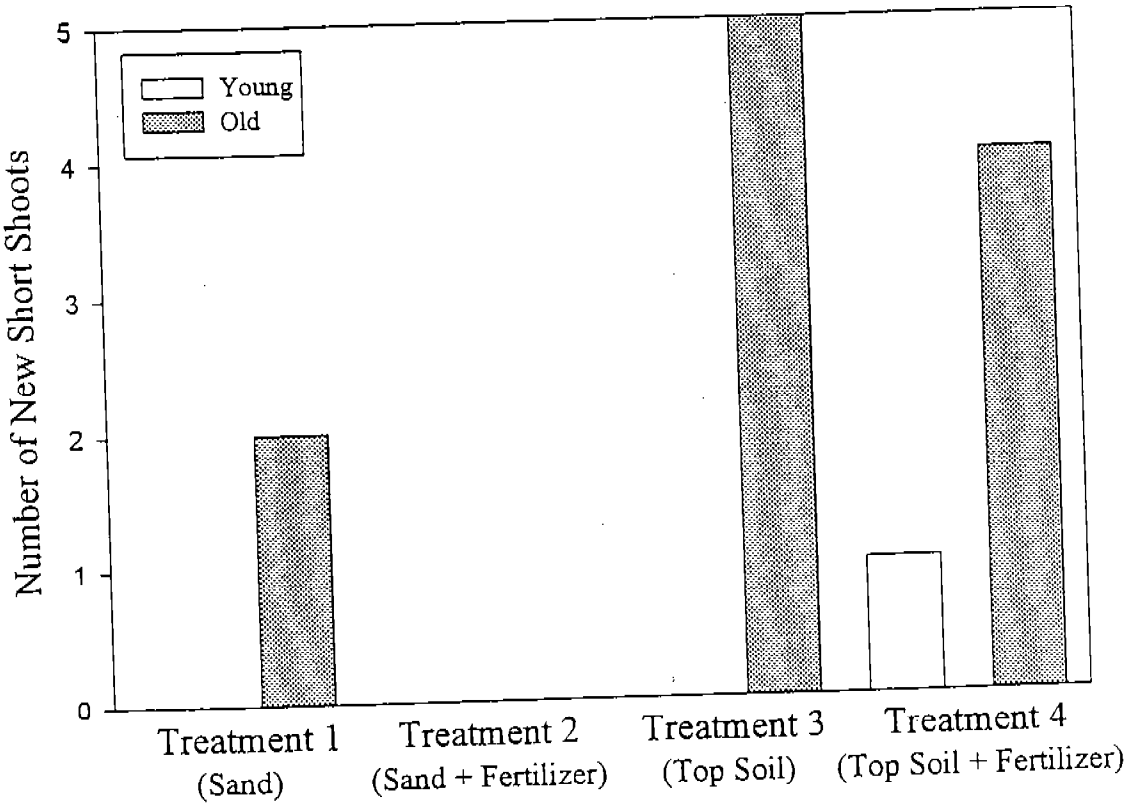
The experiment began on 29 March 1999 and nutrient pulsing was discontinued after the initial week due to plankton growth in tanks 2 and 4. Plants were harvested 13 July 1999 (15 weeks) and all showed new blade growth and 100% survival. Plants having an apical meristem did not produce another. There were no significant differences in apical meristem production between use of nutrients or GA_3. The number of new short shoots was low, with two of the existing apical meristems becoming short shoots when they extended out of the sand. In summary, the presence of an apical meristem appears to inhibit production in the other short shoot

and the use of GA_3 was ineffective for induction of meristems.

SUMMARY

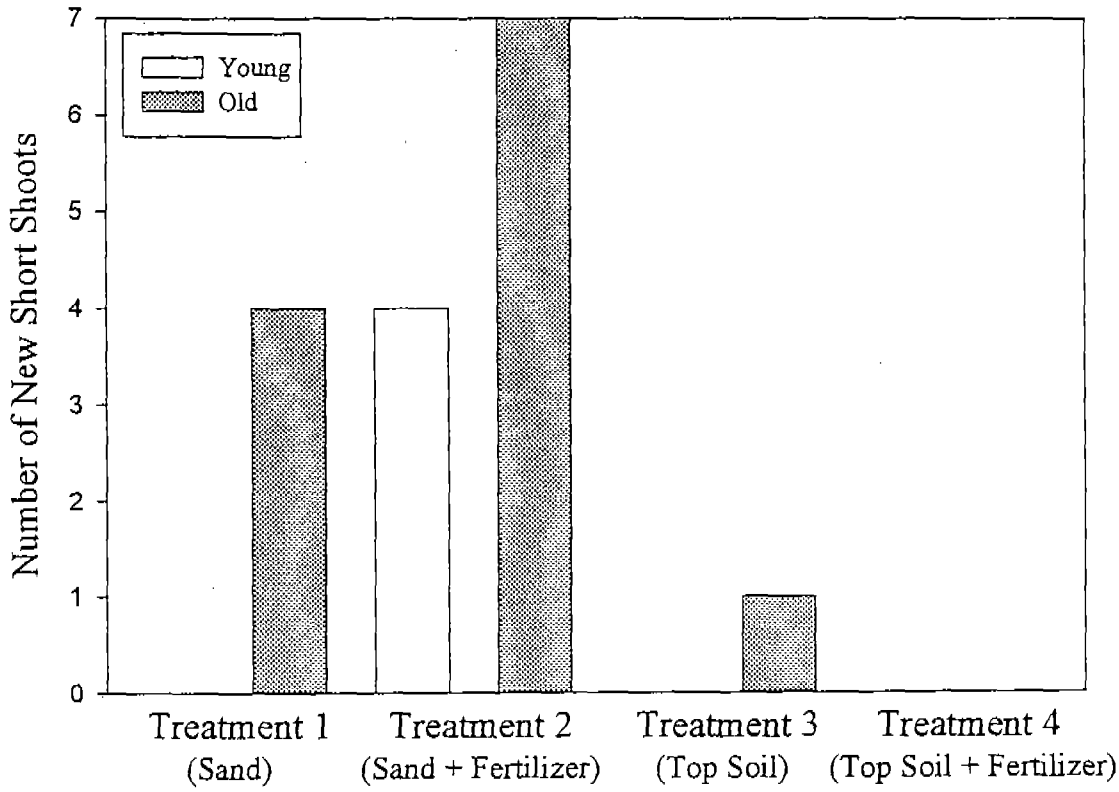
A number of developmental aspects of turtle grass growth were demonstrated in the nursery and aquaria experiments carried out from 1997 through 1999. First, the original roots are non-functional and die after transplantation and new roots are produced from the short shoots within two months. In addition, nutrients suppressed root development. Thus, any transplantation must take into account the delay in rhizome growth due to root formation. Second, root and rhizome meristem production is by the short shoot, not the 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. Thus, there is a need to establish standard techniques for turtle grass transplantation. Fourth, use of plant growth regulators (PGR's) does affect short shoot and rhizome meristem production. However, the correct combination of PGR's is not clear. Fifth, older short shoots are the primary sites of new short shoots and apical meristems. Just how old a short shoot must be is not clear and may reflect its ability to store reserve nutrients. Sixth, the presence of an apical meristem inhibits the production of a new one regardless of short shoot age. In conclusion, it is recommended that further studies should be carried out to improve survival rates in the field and induction of rhizome meristems and these should include use of disposable pots and other treatments with PGR combinations.

Short Shoot Production



Recovery Area 4

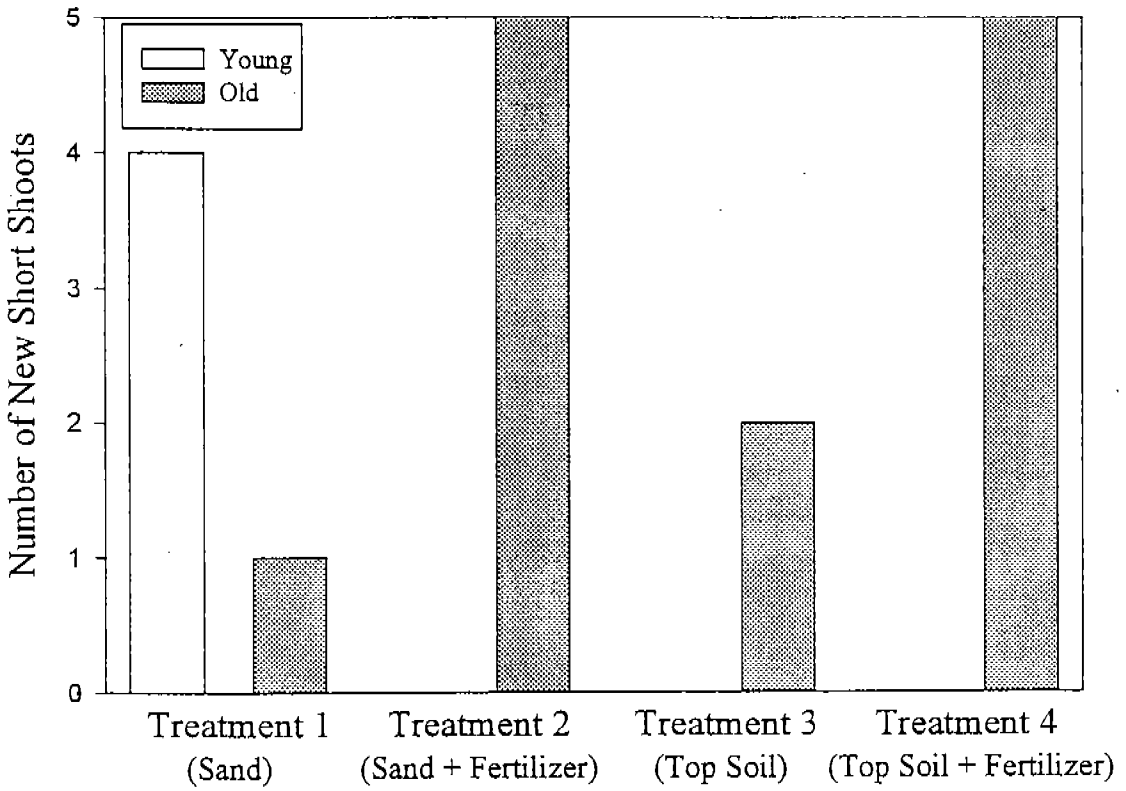
Short Shoot Production



Recovery Area 2C

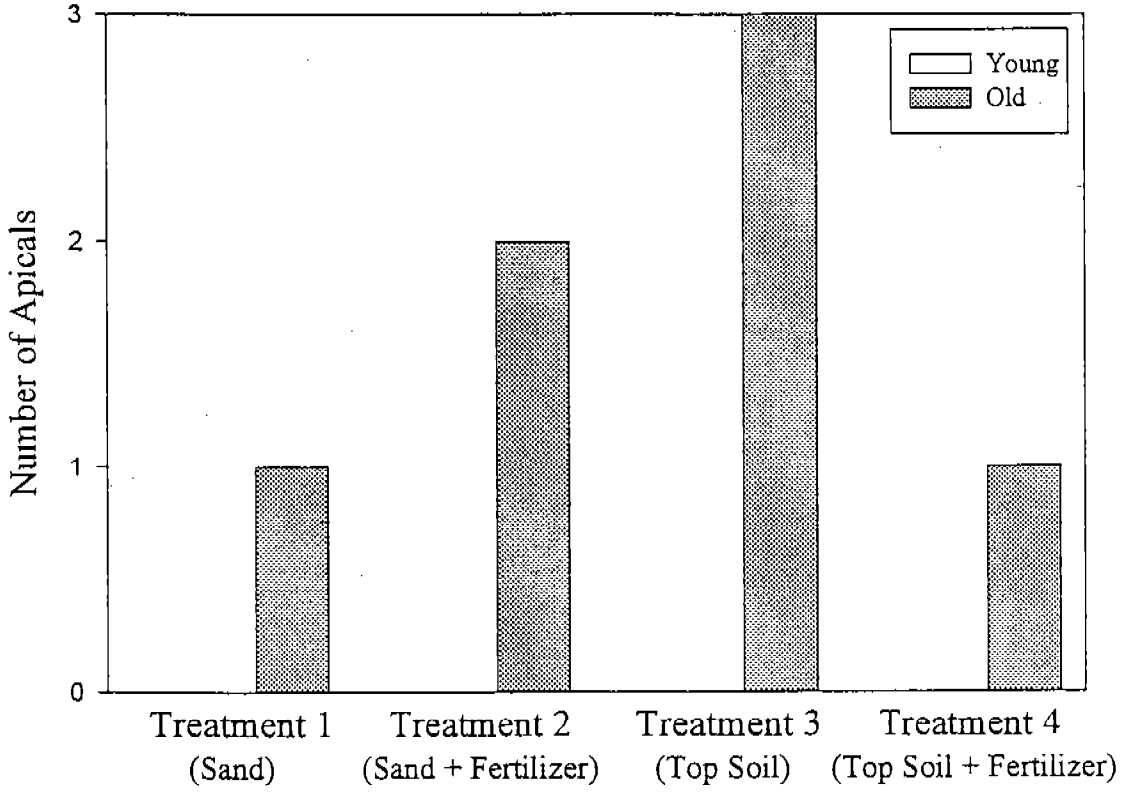
1706

Short Shoot Production



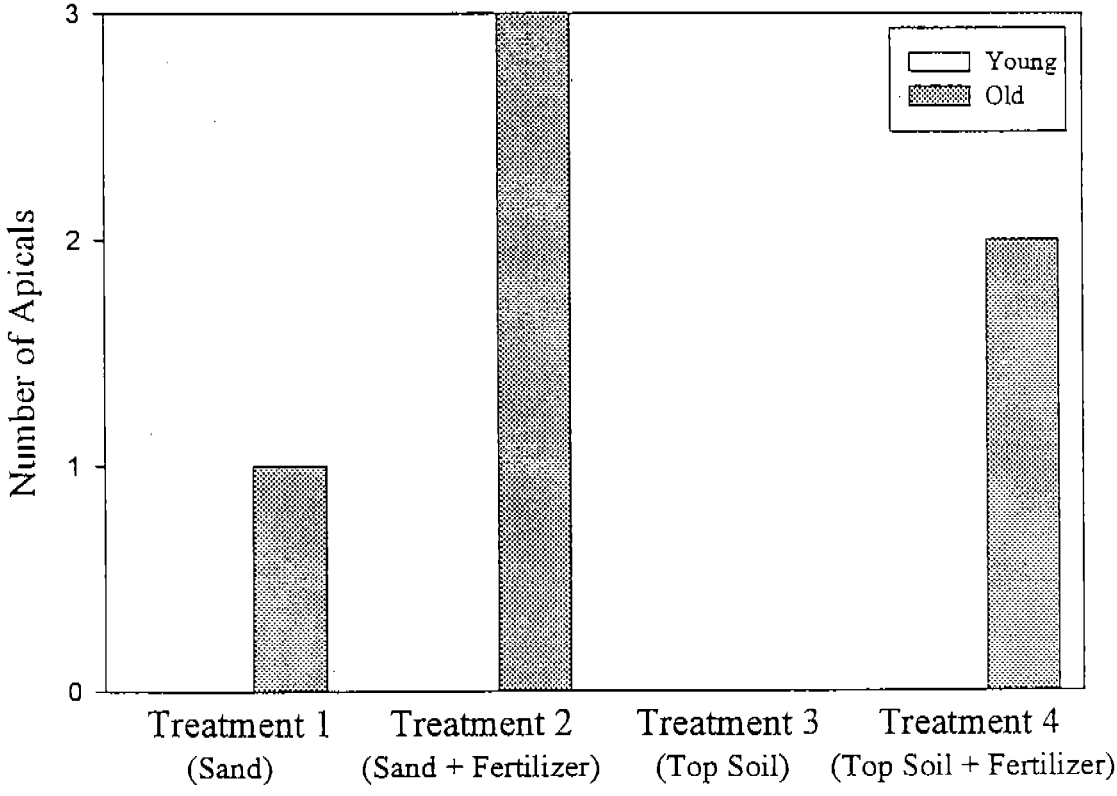
Tampa Bay

Apical Production



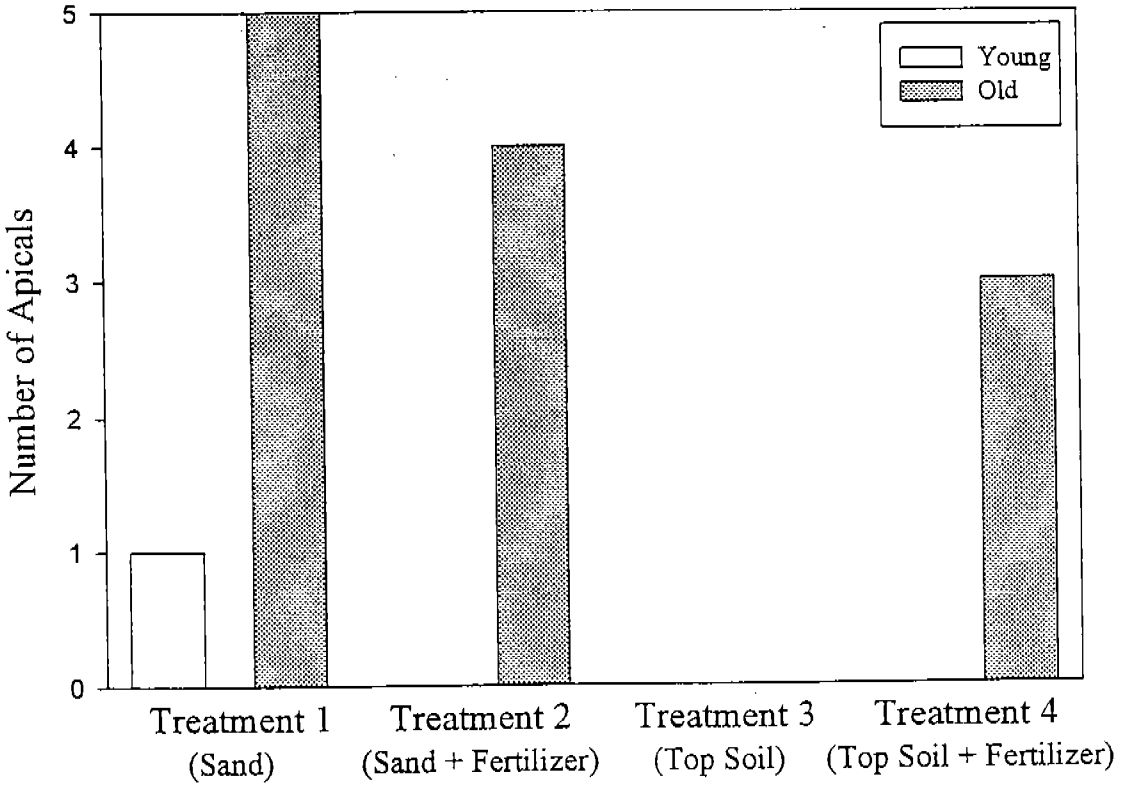
Recovery Area 4

Apical Production

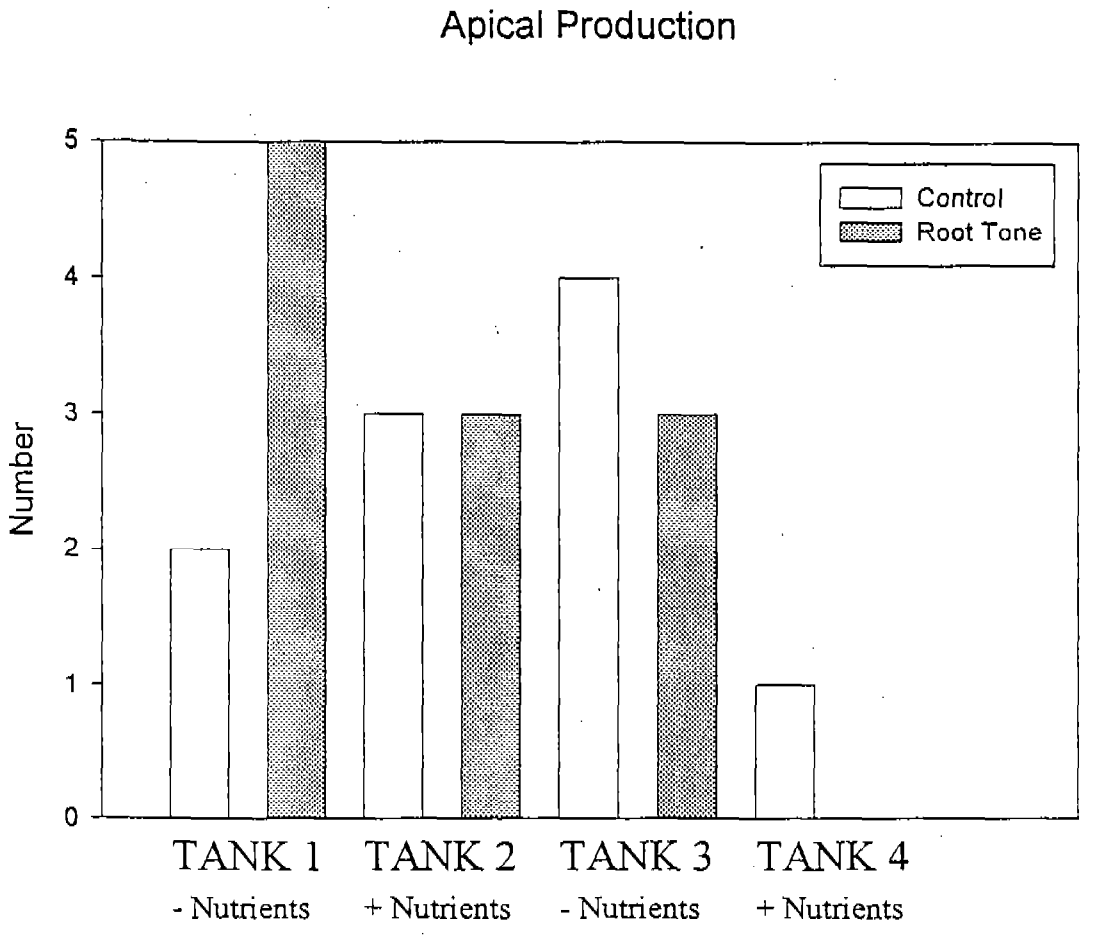


Recovery Area 2C

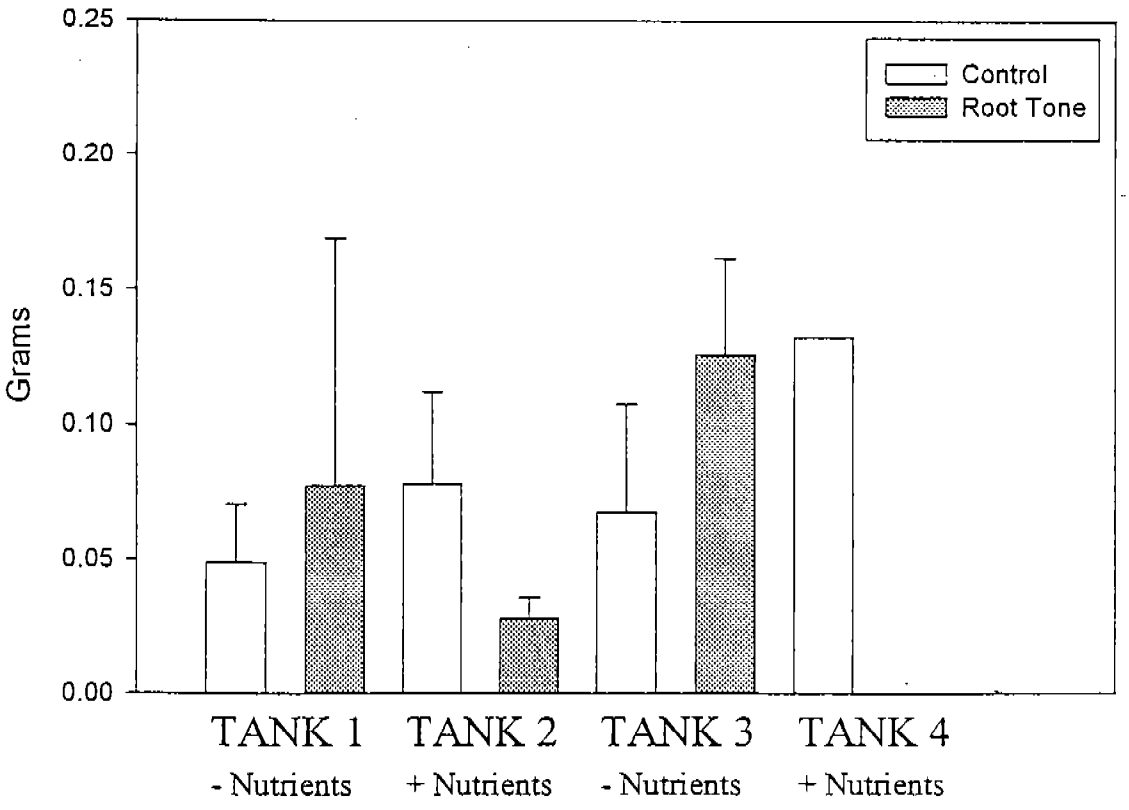
Apical Production

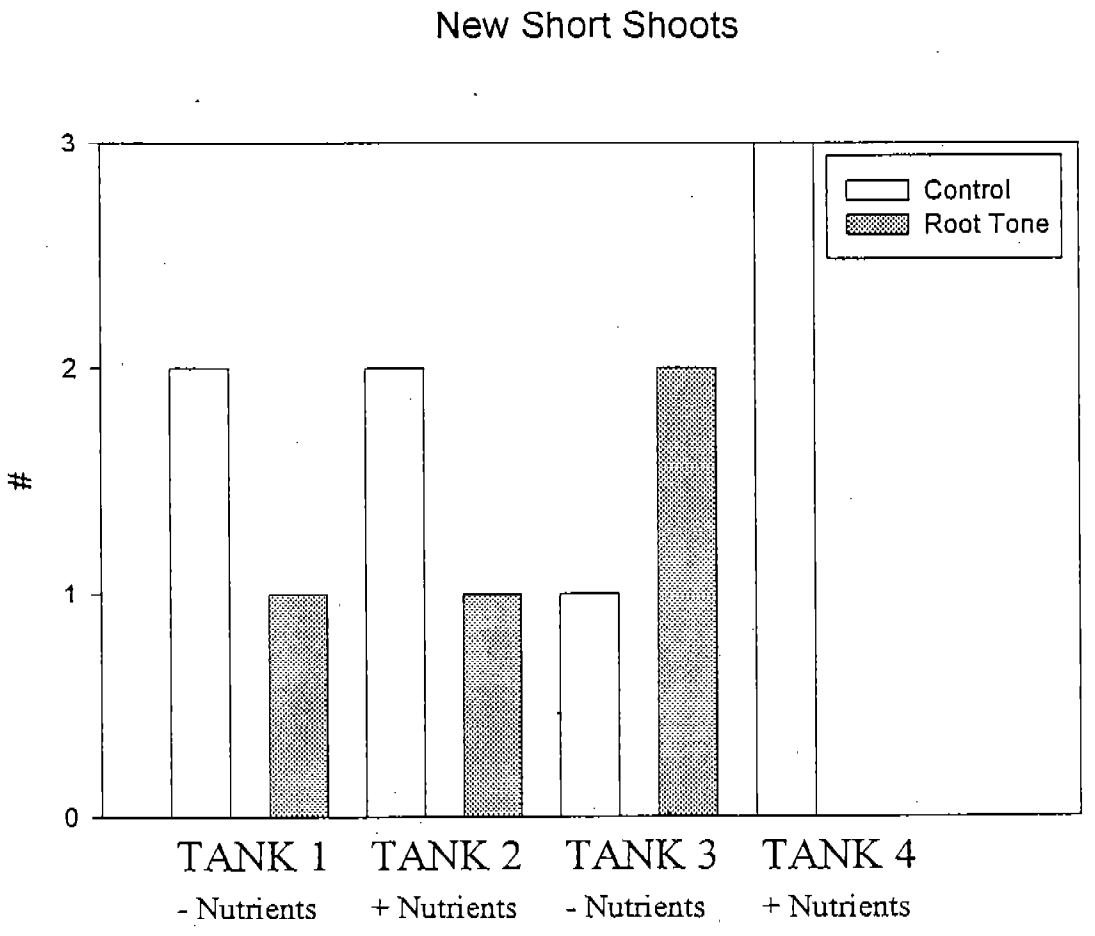


Tampa Bay

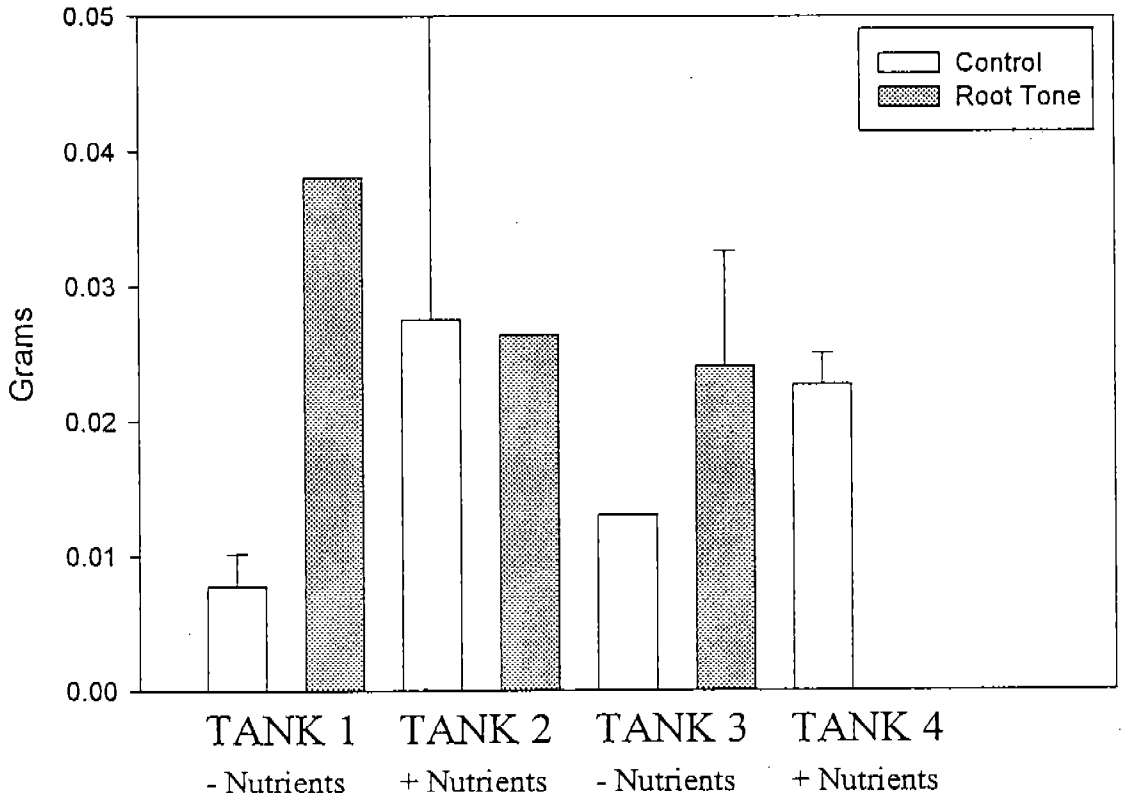

Apical Production
Number
5
4
3
2
1
0
Control
Root Tone
TANK 1
- Nutrients
TANK 2
+ Nutrients
TANK 3
- Nutrients
TANK 4
+ Nutrients

Apical wt.

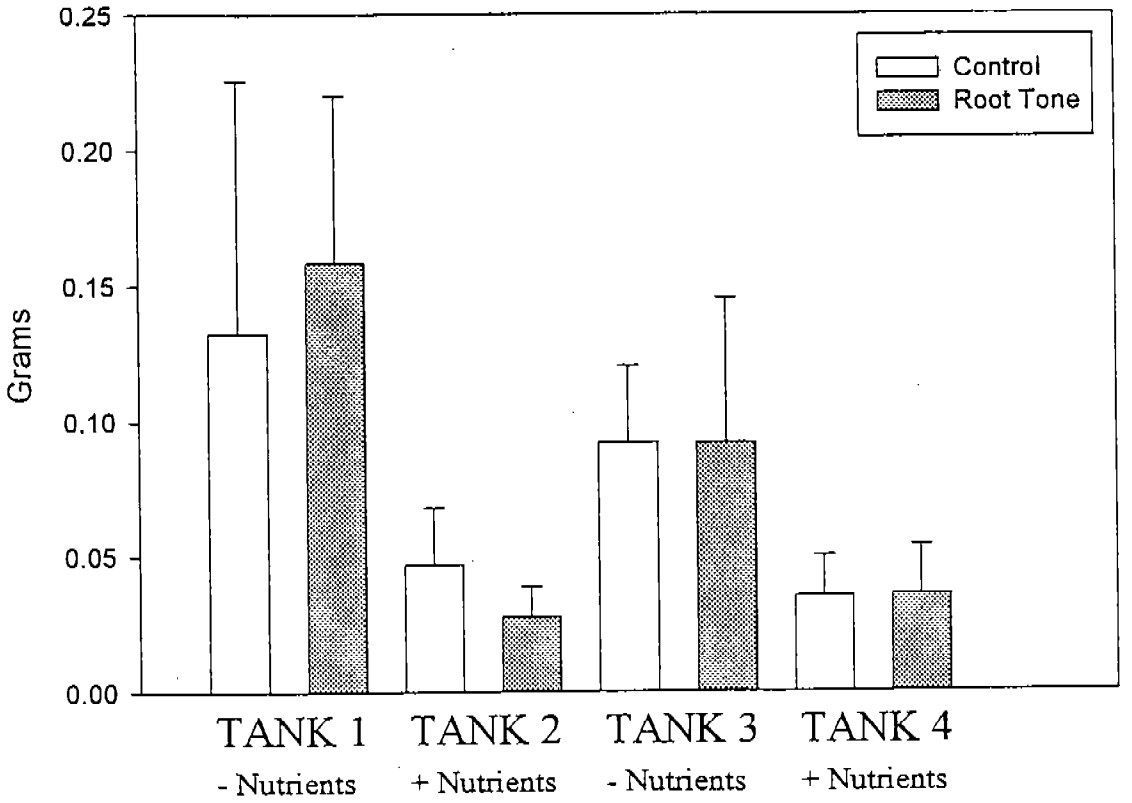

0.25
0.20
0.15
0.10
0.05
0.00
Grams
Control
Root Tone
TANK 1
- Nutrients
TANK 2
+ Nutrients
TANK 3
- Nutrients
TANK 4
+ Nutrients


New Short Shoots
3
2
1
0
#
Control
Root Tone
TANK 1
- Nutrients
TANK 2
+ Nutrients
TANK 3
- Nutrients
TANK 4
+ Nutrients

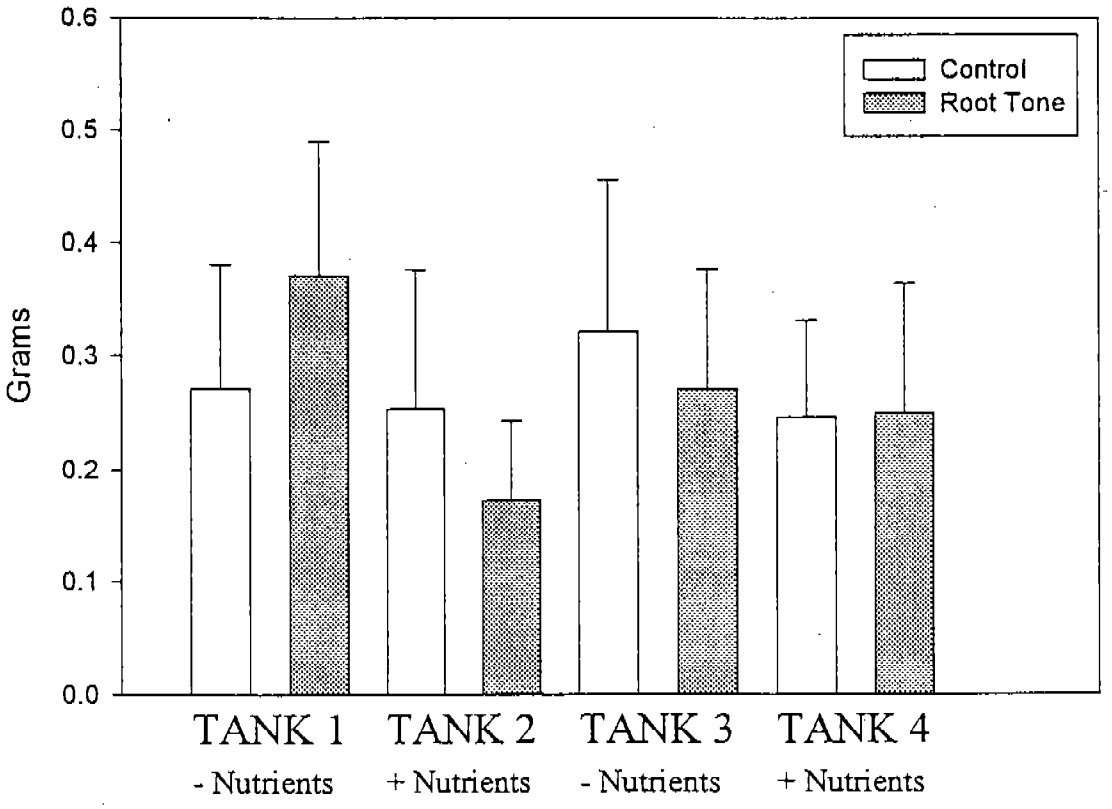
New Short Shoot wt.



Leaf wt.

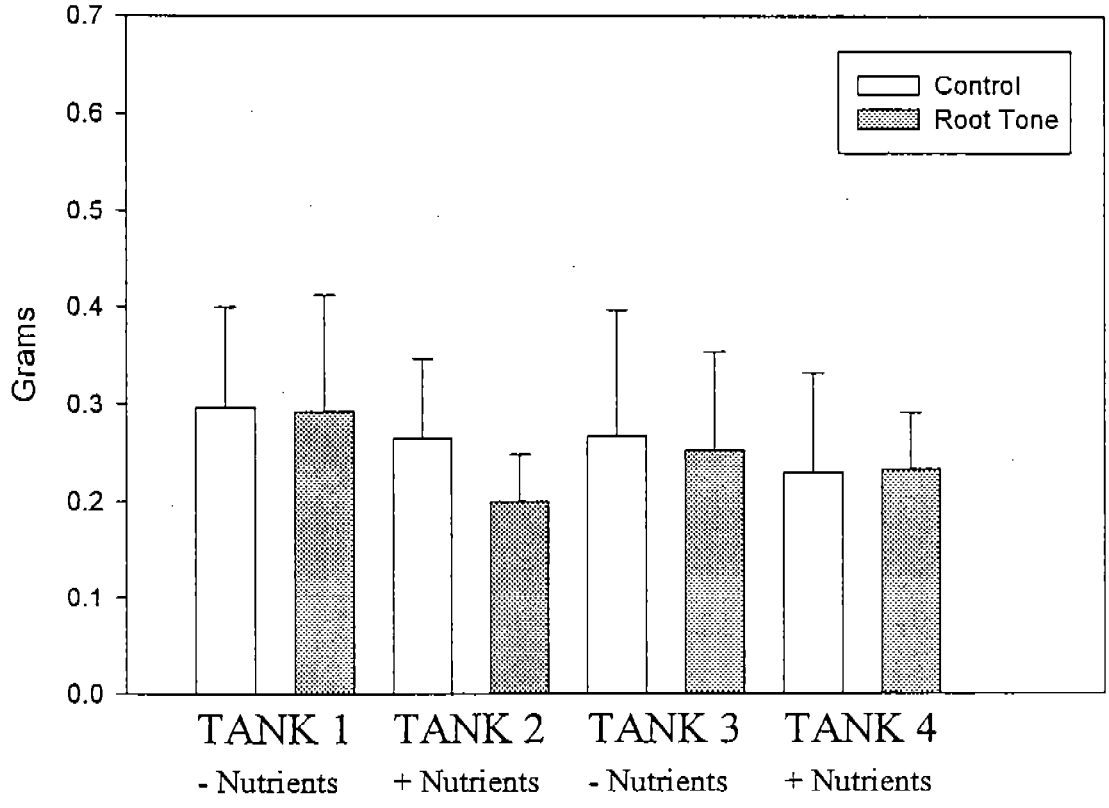

0.25
0.20
0.15
0.10
0.05
0.00
Grams
Control
Root Tone
TANK 1
- Nutrients
TANK 2
+ Nutrients
TANK 3
- Nutrients
TANK 4
+ Nutrients

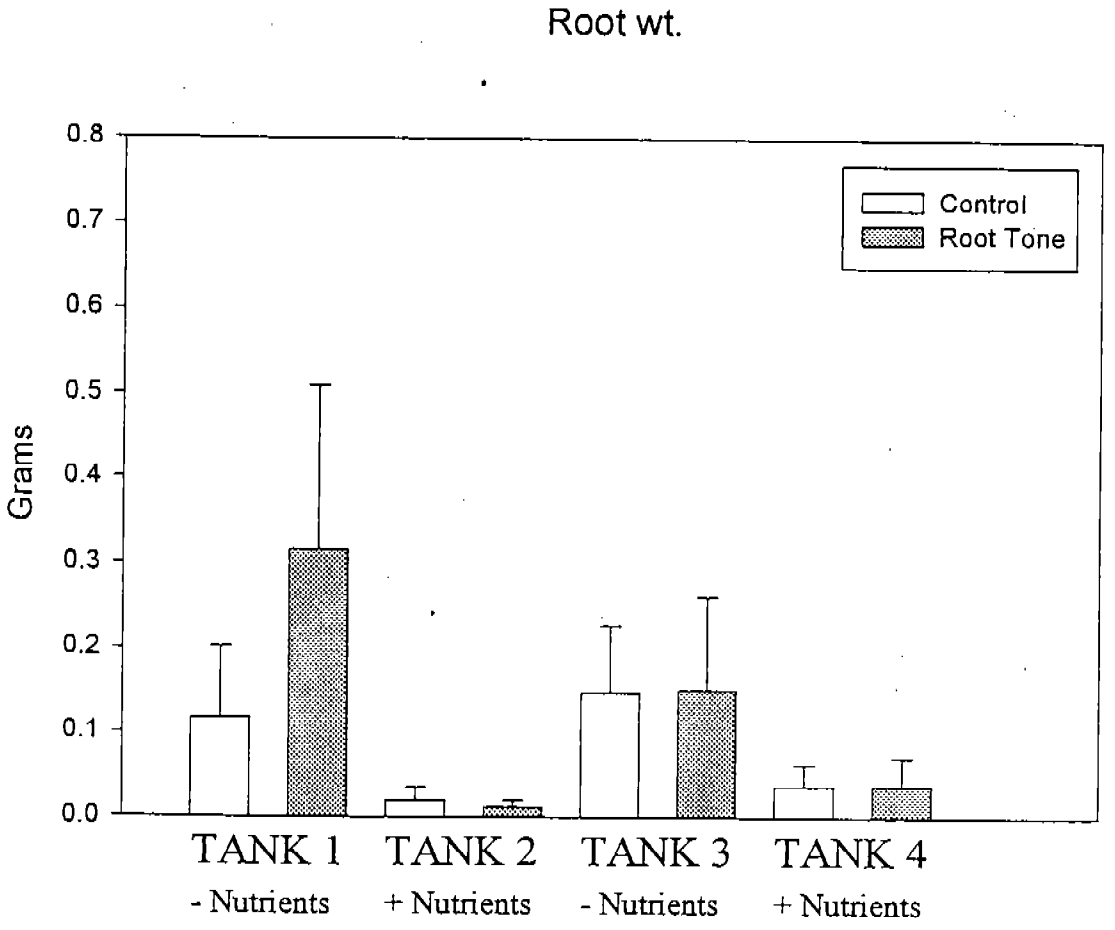
Short Shoot wt.

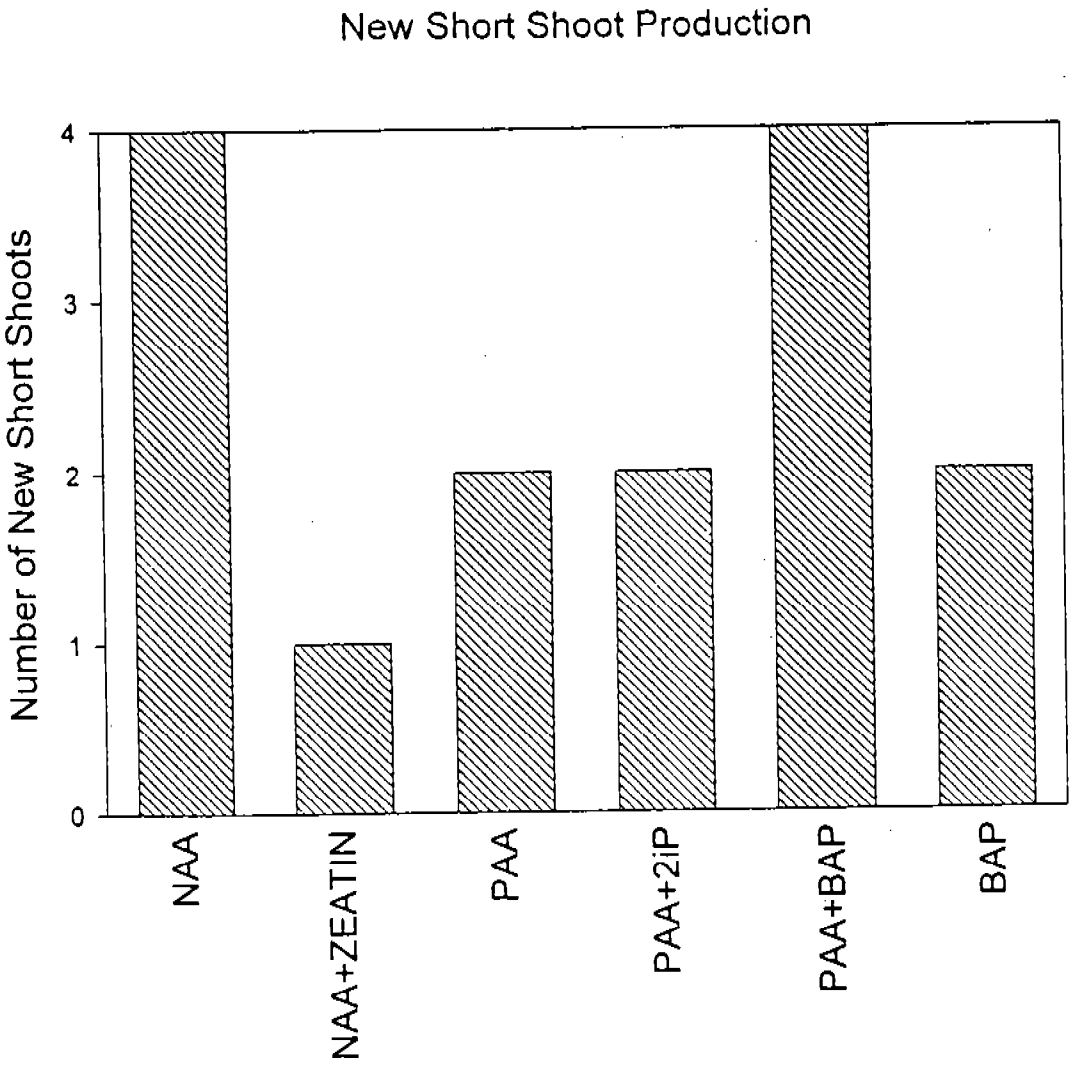


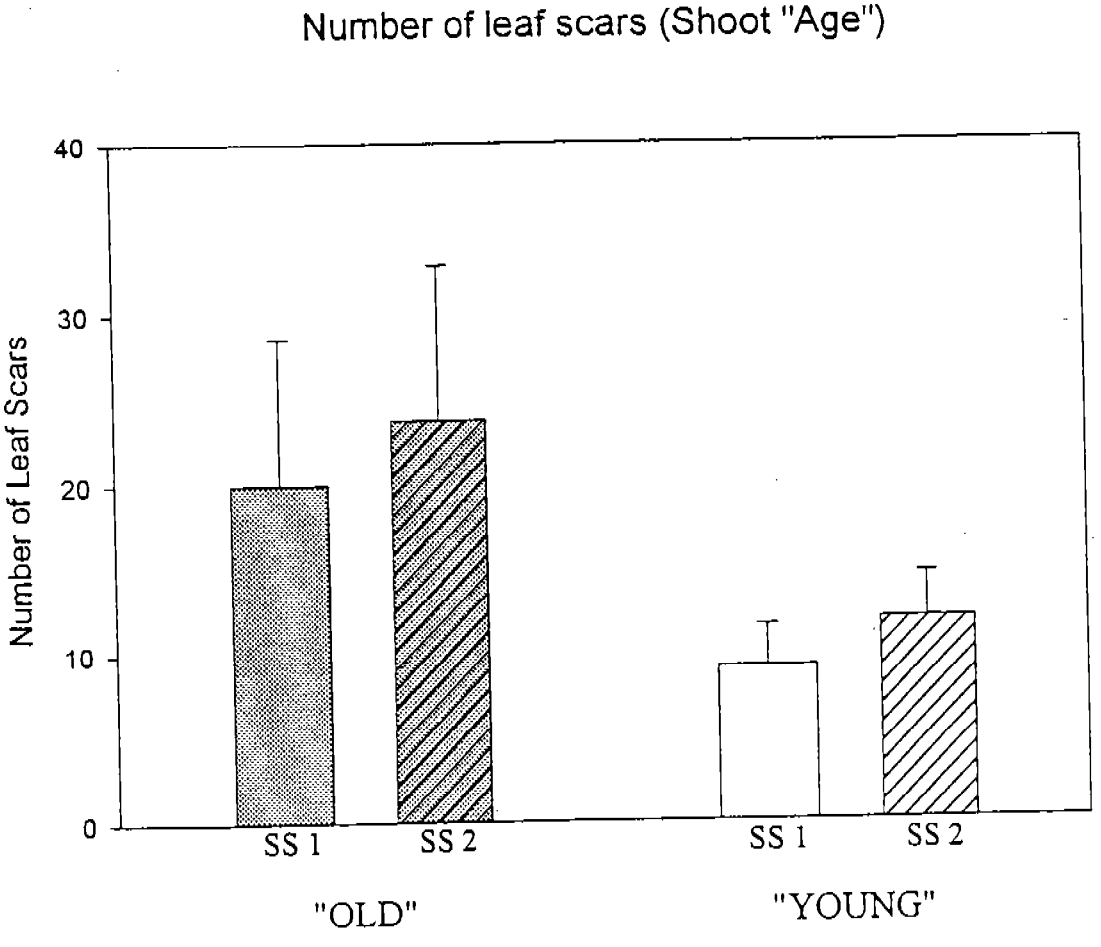
170c

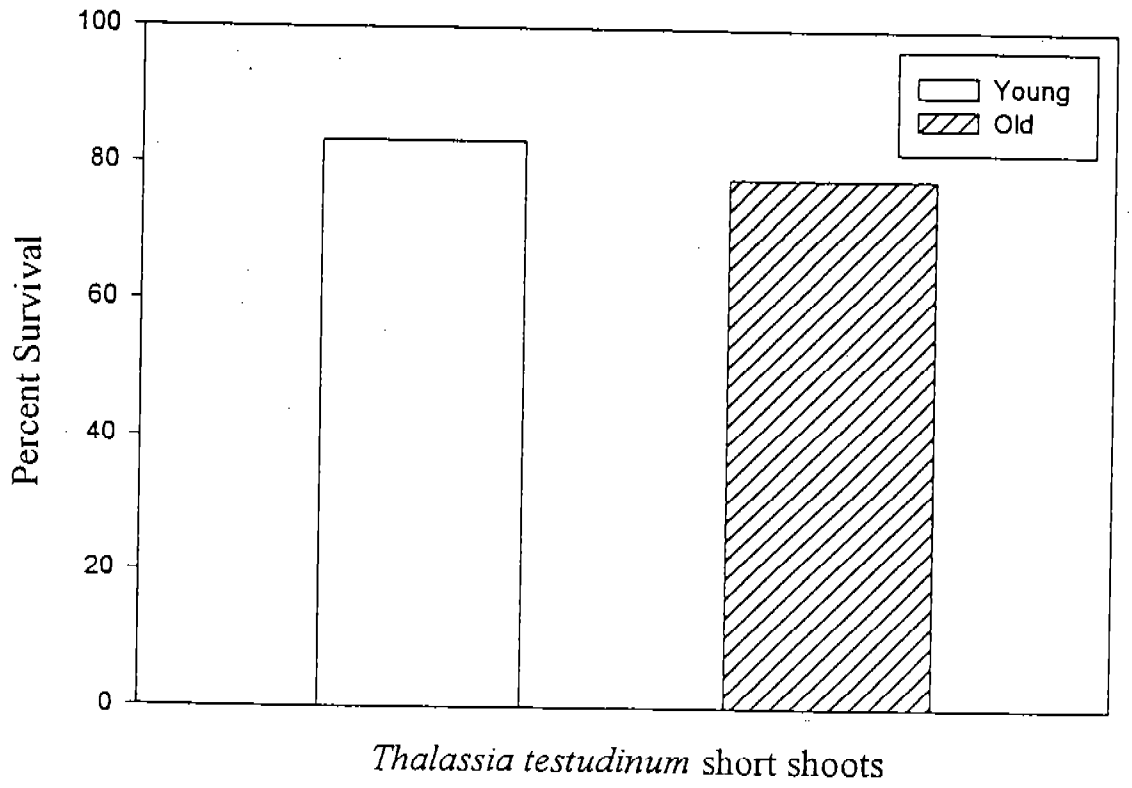
Rhizome wt.

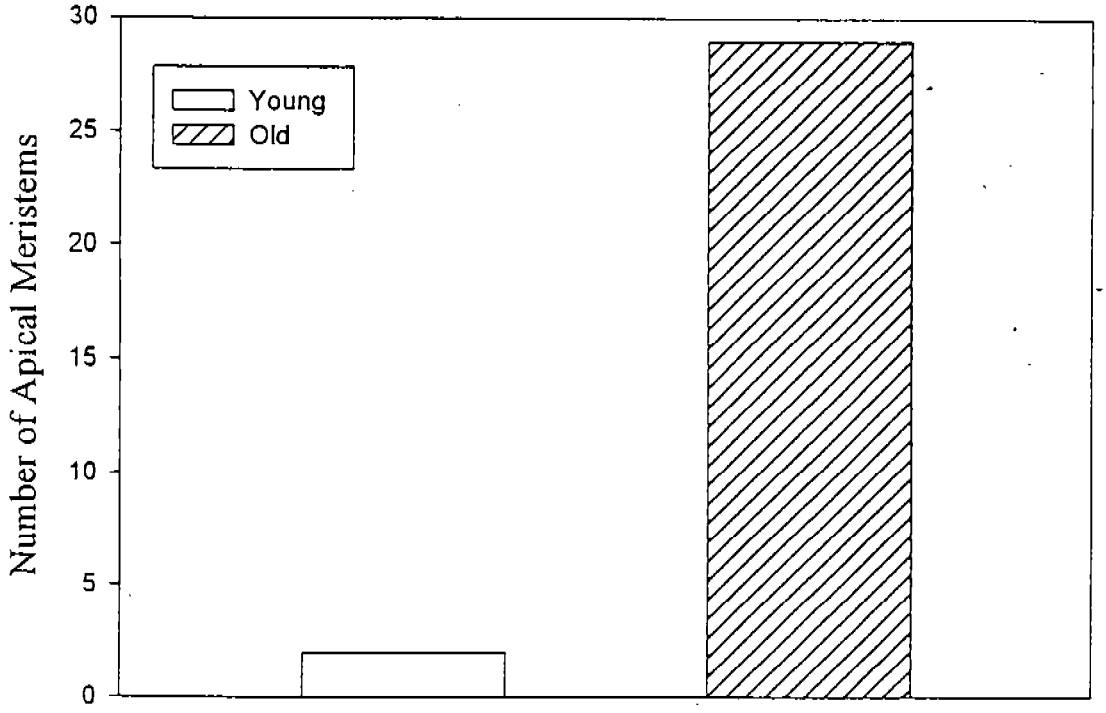



Root wt.
Grams
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
Root Tone
TANK 1
- Nutrients
TANK 2
+ Nutrients
TANK 3
- Nutrients
TANK 4
+ Nutrients


New Short Shoot Production
Number of New Short Shoots
0
1
2
3
4
NAA
NAA+ZEATIN
PAA
PAA+2iP
PAA+BAP
BAP


Number of leaf scars (Shoot "Age")
Number of Leaf Scars
40
30
20
10
0
SS 1
SS 2
SS 1
SS 2
"OLD"
"YOUNG"


100
80
60
40
20
0
Percent Survival
Young
Old
Thalassia testudinum short shoots



Thalassia testudinum short shoots