

VARIATION IN Thalassia testudinum SEEDLING GROWTH RELATED TO  
GEOGRAPHIC ORIGIN

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## ABSTRACT

Seedling of Thalassia testudinum Banks ex König from the Florida Keys, Biscayne Bay, and Tampa Bay exhibited distinctive morphogeographical growth patterns under a variety of culture conditions. Root and leaf blade growth was greatest for the southern, Florida Keys population and lowest for the northern, Tampa Bay population; Biscayne Bay seedlings exhibited intermediate growth rates. Leaf widths correlated with growth patterns of cultured seedlings and patterns in the indigenous habitats. Responses to nutrient enrichment were inversely related to shoot growth rates, such that the northern population exhibited the greatest increase in growth, while the southern population responded the least; Biscayne Bay seedlings were again intermediate. These patterns suggest physiological acclimatization of T. testudinum populations to their indigenous environments, a characteristic which may be important when selecting seed stock for revegetation projects. A culture technique utilizing biodegradable compressed peat containers was examined. This method has potential for development of nursery stock and aiding in the anchorage of transplanted seedlings.

## INTRODUCTION

Seagrass beds play an important role in coastal marine ecosystems (Wood, Odum, and Zieman, 1969; den Hartog, 1977). Recognition of this importance, and the fact that seagrasses are subject to severe impacts by man's activities in the coastal zone (Thayer, Wolfe, and Williams, 1975), has led to the development of a variety of revegetation methodologies (Knight, Knutson, and Pullen, 1980). Revegetation studies involving the tropical-subtropical species Thalassia testudinum Banks ex Konig have utilized plugs, sods, individual shoots, seeds and seedlings (Kelly, Fuss, and Hall, 1971; Eleuterius, 1974; Phillips, 1974; Thorhaug, 1974; van Breedveld, 1975; Lewis and Phillips, 1980a). The apparent success of several large scale revegetation projects in Biscayne Bay, Florida, utilizing seeds and seedlings (Thorhaug, 1974, 1979), indicates that the use of seed material may be the most suitable method for mitigation projects involving T. testudinum. Lewis and Phillips (1980b) recently proposed that seed material might reduce the cost of seagrass mitigation if large numbers of seeds are periodically available. Compared to destructive transplanting techniques, such as sod or plug removal, the apparent nondestructive nature of collecting seeds suggests that this method should be examined in detail. We stress apparent since the impact of sexual reproduction in maintenance of established beds is unknown.

When considering seed stock for mitigation projects, the possibility of genetic fixing in local strains may be important (Odum, 1971). Geographically separated T. testudinum populations exhibit ecoplastic limits that are adaptive to local conditions (McMillan, 1978, 1979; McMillan and Phillips, 1979). Responses to the influence of habitat

include variation in leaf length and width (Phillips, 1960; Zieman, 1974) and variable reproductive patterns (Grey and Moffler, 1978; Moffler and Durako, unpublished data). In this regard, transplanting seeds or seedlings from remote locations may result in failure or poor success since they lack this factor compensation. The possibility of pathogen introduction utilizing nonlocal seeds also suggests that indigenous seed stock would be preferable for revegetation projects.

Seagrass research at the Florida Department of Natural Resources Marine Research Laboratory has involved studies on transplant techniques (Darovec et al., 1975; van Breedveld, 1975) and aspects of the reproductive ecology and physiology of T. testudinum in Florida (Moffler, 1976; Grey and Moffler, 1978; Moffler, Durako, and Grey, 1981). The aim of these investigations is an understanding of this important resource and the development of economically feasible and environmentally safe techniques for successful restoration of destroyed T. testudinum meadows. This paper reports on the differences in growth patterns of seedlings collected from three locations in Florida, representing a latitudinal gradient. Preliminary results of a novel transplanting system will also be discussed.

#### MATERIALS AND METHODS

Fruits, seeds and seedlings of Thalassia were collected along the Atlantic shoreline of Grassy Key in the Florida Keys (FK) and Matheson Hammock in Biscayne Bay (BB) on 12 August 1980; and along the Sunshine Skyway causeway and Mullet Key in Tampa Bay (TB) on 13 and 14 August. The material was transported to the laboratory cooled in ice chests. Seeds were excised from fruits within one day of collection. Seeds and

seedlings were surface sterilized for 10 min in a 5% sodium hypochlorite-seawater solution and held in separate aquaria filled with synthetic seawater (Instant Ocean) at 32 ppt salinity until needed for culture experiments.

### Tube Cultures

Tube cultures utilizing marine agar as a substratum were initiated in order to monitor leaf and root growth of seedlings. Four treatments (4 replicates each) were tested using 60 ml culture tubes with 20 ml agar and 30 ml of liquid media. The two liquid media were Instant Ocean and NH-15 (modified from Gates and Wilson, 1960) at 32 ppt; the two types of agar were nutrient agar composed of 6 g/l phytagar in 32 ppt NH-15 and marine agar composed of 6 g/l phytagar in 32 ppt Instant Ocean. The four treatments were combinations of the two types of agar and liquid media: Nutrient agar - Instant Ocean, Nutrient agar - NH-15, Marine agar - Instant Ocean, and Marine agar - NH-15. Seedlings from the three sites were again surface sterilized for 10 min followed by three one minute rinses in sterile synthetic seawater and placed in the culture tubes. All transfers were performed using a laminar flow hood and aseptic techniques. Larger culture tubes (80 ml) were tested to determine of the size of the tubes affected growth. These tubes contained 25 ml of nutrient agar and 45 ml of Instant Ocean. Illumination was provided by Duro-Test Vita Lites on a 14:10 L:D cycle and the temperature was held between 22-29°C.

### Pot Cultures

Seedlings were also grown in 5 x 5 x 12.7 cm plastic pots filled with an autoclaved mixture of builders' sand and aragonite shell hash (1:1). Three replicates from each population were placed in two aquaria:

one containing 71 l synthetic seawater (Instant Ocean) and the second containing 71 l synthetic seawater with von Stosch's enrichment (von Stosch, 1964). Salinities of both tanks were maintained at 32-34 ppt and temperatures were held between 24-26°C with a 14:10 L:D photoperiod cycle.

The remaining seedlings were planted in Peat Pellets (Jiffy-7) and placed in aquaria containing Instant Ocean. Light and temperature conditions were the same as above. After three months in culture, the rotted seedlings were transplanted randomly within a 1 x 1.2 m quadrat in Boca Ciega Bay, and monitored monthly for survival and growth.

### Growth Measurements

Leaf blade growth for all treatments was calculated by measuring the length and width of the leaf blades to obtain a total leaf blade area. Root lengths in the culture tubes were visually estimated using a metric scale placed behind the tube. Leaf and root growth rates of the three populations were compared using analysis of covariance and leaf widths were compared using t-tests, significance was determined at  $P < 0.05$ .

## RESULTS

Tube cultures provided a unique opportunity to monitor both leaf blade and root growth without disturbing the seedling (Figure 1). Seedlings growth in the tube cultures indicated populational differences (Figure 2). Clear patterns were not exhibited in response to nutrient enrichment of the agar or liquid media (Table 1), so responses were examined with respect to geographic origin only. Biscayne Bay (BB) seedlings had the greatest total root length (Figure 2b), due to the production of the greatest number of roots per seedling (Table 1). When

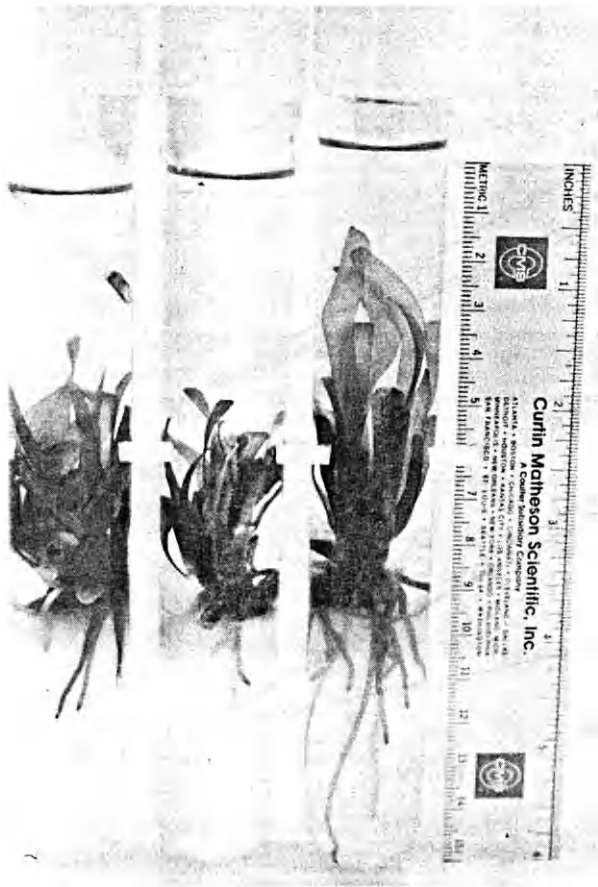


Figure 1. Thalassia testudinum seedlings in tube cultures.

growth of individual roots was considered, root lengths of the FK population increased the most, followed by BB seedlings with the TB population producing the least growth. Some restriction of root growth was evident in the 60 ml tubes and was also indicated by the significantly greater root lengths that were attained by seedlings in the 80 ml tubes (Table 1). In contrast, leaf blade growth was lower for all populations in the larger tubes. In both cultures leaf growth showed an inverse relationship with latitude of origin; the southern FK population exhibited the greatest growth, the northern TB population showed the least growth and BB seedlings were intermediate (Figure 2a).

Growth studies on the 60 ml tube cultures were terminated after 3 months due to contamination of the FK cultures by bacteria, the fungus

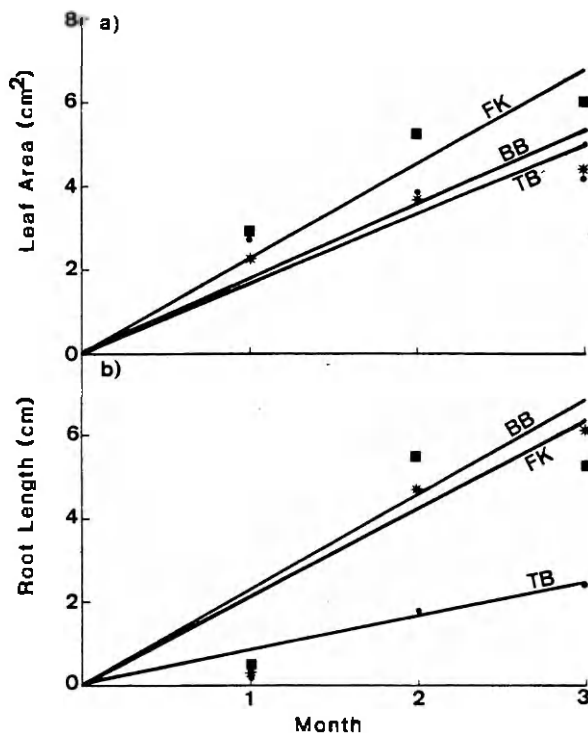


Figure 2. Leaf (a) and root (b) growth of *Thalassia testudinum* seedlings from Tampa Bay (dots), Biscayne Bay (stars) and the Florida Keys (boxes) in 60 ml tube cultures ( $y=a+bx+cx^2$ ).

Figure 3. Leaf blade growth of *Thalassia testudinum* seedlings from Tampa Bay (dots), Biscayne Bay (stars) and the Florida Keys (boxes) in a) von Stosch's nutrient enrichment medium and b) Instant Ocean ( $y=a+bx+cx^2$ ).

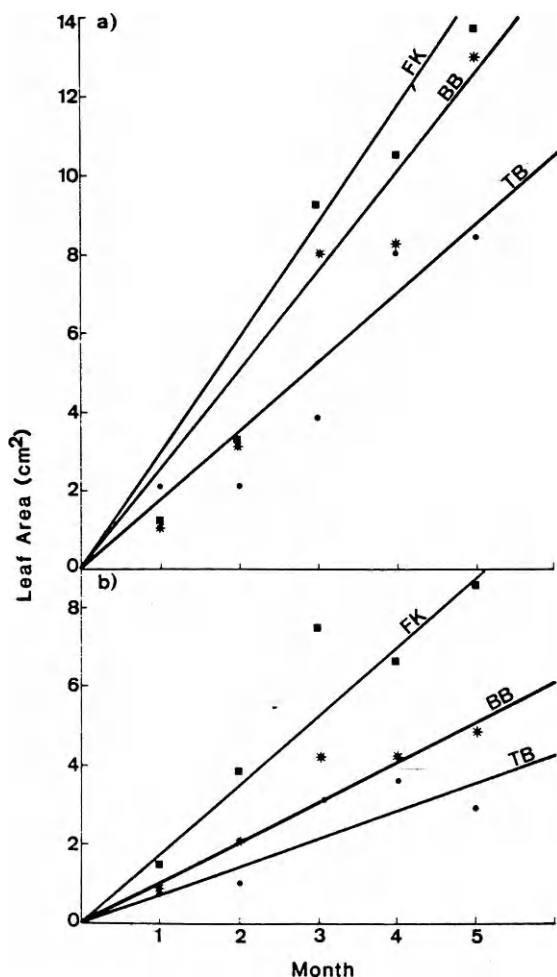




Table 1. Root and leaf blade growth of *Thalassia testudinum* seedlings in agar/seawater cultures after three months.

Treatment	Total Root Length (cm)			Leaf Area (cm <sup>2</sup> )		
	Tampa Bay	Biscayne Bay	Florida Keys	Tampa Bay	Biscayne Bay	Florida Keys
<u>60 ml tubes*</u>						
NH-15/N.A.	0.95	5.65	4.03	4.32	4.03	6.42
I.O./N.A.	2.48	7.00	6.35	4.48	4.05	6.27
NH-15/M.A.	2.52	6.40	4.75	4.29	4.13	4.58
I.O./M.A.	2.75	5.52	6.00	3.36	5.20	7.00
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Mean	2.18	6.14	5.37	4.18	4.35	6.04
Root#/sdling	2.50	3.81	2.47			
Length/root	0.87	1.61	2.21			

80 ml tubes

I.O./N.A.	3.27	12.10	14.98	3.18	4.21	4.50
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\*I.O.=Instant Ocean; N.A.=Nutrient Agar; NH-15=Nutrient enriched seawater; M.A.=Marine Agar. Values represent the mean of 4 replicates.

Table 2. Leaf growth rates of *Thalassia testudinum* seedlings to laboratory cultures.

Treatment	Growth Interval (months)	Shoot Growth Rates (cm <sup>2</sup> /mo)		
		Tampa Bay	Biscayne Bay	Florida Keys
<u>Tube cultures</u>				
60 ml tubes	3	1.38	1.47	2.07
80 ml tubes	3	1.08	1.40	1.50
<u>Pot cultures</u>				
Instant Ocean	5	0.72	1.04	1.78
von Stosch's	5	1.76	2.53	2.94
Peat Pellets	3	0.93(1.70)*	1.78(2.52)	1.72(2.80)

\*Numbers in parentheses indicate a growth index value where:

$$\text{Growth Index} = \frac{\text{mean leaf area / seedling}}{\text{mean leaf \# / seedling}}$$

Lindra thalassiae Orpurt et al. and high levels of Leucothrix mucor Oersted (Dr. Jack Fell, pers. comm.). All of the 60 ml tube cultures had some contamination evident, but only the FK cultures succumbed. The contamination in the FK cultures appeared as a red growth on the seedlings and in the agar. None of the 80 ml FK cultures exhibited this red contaminant and in general, these cultures appeared less contaminated.

Leaf growth of seedlings in the plastic pots repeated the inverse relationship between growth and latitude (Figure 3). Seedlings cultured without nutrients added had lower growth rates than the tube cultures, but the addition of nutrients increased growth rates significantly (Table 2). Responses to nutrient enrichment were inversely related to the levels of growth, such that the TB seedlings exhibited the greatest increase (244%), BB seedlings were intermediate in their response (242%) and the FK population showed the least increase (165%). Nutrient enrichment initiated a phytoplankton bloom which was controlled using diatom filtration.

Leaf growth rates of seedlings in the peat pellets was intermediate between the plastic pot cultures with and without nutrient additions (Table 2). Biscayne Bay seedlings had a slightly higher total leaf area than the FK seedlings, due to a greater number of leaves per seedling, but the growth rates of individual leaf blades (growth index) repeated the patterns of the other treatments. After 3 months in culture, many of the seedlings had roots that protruded through the webbing of the pellets and all were firmly anchored (Figure 4). The seedlings were transplanted in Boca Ciega Bay in November to an area that had supported seagrasses prior to dredging associated with intracoastal waterway maintenance. When transplants were examined one month later it



Figure 4. Thalassia testudinum seedling in peat pellet after 3 months in laboratory culture.

was clear that this site was still unsuitable for revegetation using T. testudinum because of high sedimentation rates and associated turbidity. Only three seedlings were still alive, the remainder were coated with sediments. Additionally, the site appeared to have been physically disturbed. In March several peat pellets were retrieved and it was observed that they had maintained their integrity after being submerged for over 6 months.

Leaf blades were generally widest for the FK population and narrowest for TB seedling, with BB seedlings exhibiting intermediate widths (Figure 4). The differences in leaf widths between treatment were not statistically significant and indicated that nutrient enrichment had little effect on this morphological characteristic, so increases in leaf area in response to fertilization could be attributed to leaf elongation.

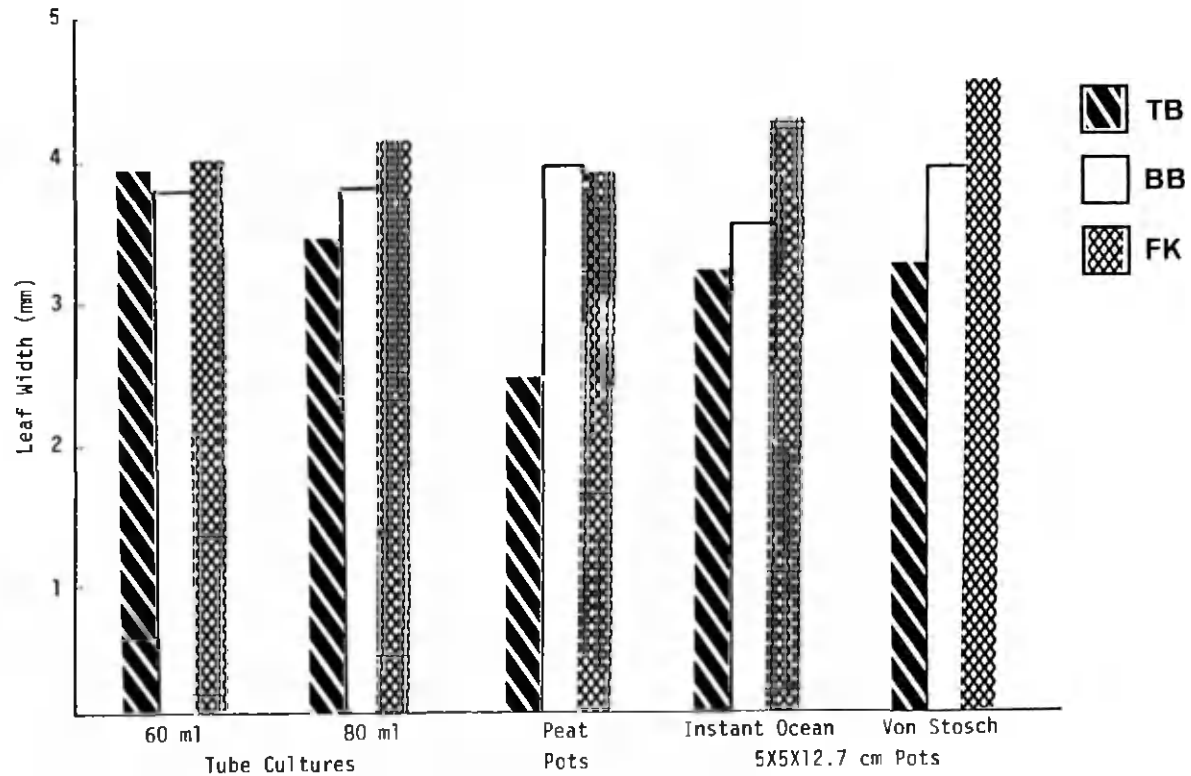


Figure 5. Leaf blade widths of *Thalassia testudinum* seedlings from Tampa Bay (TB), Biscayne Bay (BB) and the Florida Keys (FK) after 3 months in culture.

## DISCUSSION

This study demonstrates that Thalassia testudinum seedlings from three locations, representing a latitudinal range of only 320 kilometers, exhibit distinctive physiological and morphological features under controlled conditions. Previous investigations have shown leaf widths and cold tolerances to be distinctive in widely separated T. testudinum populations (McMillan, 1978, 1979). Morphological variation in leaf width indicates a response to environmental conditions (Phillips, 1960; Zieman, 1974), and maintenance of these patterns under controlled conditions suggests that the limits are genetically modulated (McMillan, 1978). Differing root and leaf growth patterns exhibited by seedlings from Tampa Bay (TB), Biscayne Bay (BB) and the Florida Keys (FK) also suggests genetic differences. Species with wide geographical ranges almost always develop locally adapted ecotypes (Odum, 1971), yet seedlings of T. testudinum from diverse habitats show little variation in isozymes (McMillan, 1980). This lack of isozyme variation contrasts with the differentiation found within a single population of Spartina patens (Silander, 1979), and between populations of Uniola paniculata (David Crewz, pers. comm.).

Root growth in the culture tubes was similar to that reported for T. testudinum seedlings growing in situ (Thorhaug, 1974). The absence of differential growth in response to nutrient partition suggest either that agar provides an adequate nutrient source over short periods or that seedlings contain sufficient nutrients to support 3 months growth, a supposition that is not supported by the responses in the plastic pot cultures. The smaller 60 ml culture tubes evidently reduced root elongation, and may have increased the deleterious effects of contamination.

Increased root growth in the 80 ml culture tubes, with accompanying reduced leaf growth, indicates that T. testudinum allocates a greater proportion of its biomass below ground when growth is not restricted. Dawes and Lawrence (1980) proposed that this improves the anchoring ability of the plant, while Barko and Smart (1978) suggested that this characteristic improves survival in infertile habitats by increasing the absorptive and/or storage capacity. The adaptive significance of the BB population's greater root production is puzzling since this population does not exist in a high energy area where anchorage would be a problem, although it may represent an adaptation to the bedrock substrate which is common in Biscayne Bay (Zieman, 1972; Wanless, 1976).

Infection patterns provided further evidence of populational differences in tube cultures. Florida Keys seedlings were the most contaminated and harbored differing contaminant species than the TB and BB seedlings. The pathogenic nature of contamination in culture systems may be due to stresses of the artificial conditions which are conducive to disease development (Fell, 1976). Yet, the possibility of introducing non-indigenous pathogens when transplanting seedlings in situ must be considered. Surface sterilization alone was not effective in eliminating contaminants. This treatment coupled with antibiotics may reduce the possibility of pathogen introduction when transplanting non-local seedlings, and should be considered in pilot mitigation projects.

Leaf blade growth and the widths of leaves in laboratory treatments also showed an inverse relationship with latitude of origin. McMillan (1978) suggested that differing leaf widths of geographic variants of T. testudinum indicate an ecotypic response, but as stated previously this has not been confirmed in isozyme studies (McMillan,

1980). Nutrient enrichment significantly increased leaf blade growth in the shell/sand plastic pot cultures. Fertilization might be used to facilitate establishment and coverage at revegetation sites so less material is initially needed. It is interesting that growth responses to nutrient enrichment moderated populational distinctions. Different I. testudinum populations may have similar photosynthetic or growth capacities, but they are attained under conditions related to their indigenous environments. This phenomenon has been observed in several marine algal species (King and Schramm, 1976; Durako and Dawes, 1980).

Anchoring seagrass transplants has proved to be a major problem in revegetation work. The number of anchoring and propagation techniques that have been developed reflects the magnitude of this problem (see bibliography by Knight et al., 1980). Phillips (1980) recommended plugs as the single most important method of transplantation, since many seedlings are lost in the field. However, the plug method is very destructive to the source beds since I. testudinum is slow to propagate into disturbed areas (Godcharles, 1971). This may cause more harm than good if plugging is done on a large scale (Steller, 1976). If a suitable anchoring method were available for use with seedlings a nondestructive revegetation program might be developed. Seedlings become firmly rooted in the peat pellets within 3 months and exhibited leaf growth comparable to the other treatments. The pellets provided anchorage and maintained their integrity in situ for over 6 months. No mortality was observed in laboratory peat pellet cultures. Since the transplant site was unsuitable for growth, the field results were inconclusive and more work needs to be done to test the suitability and cost effectiveness of the technique. Ease of sowing, transport and handling of the pellets and seedlings

suggests that they may offer a viable alternative to transplanting vegetative material.



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