Propagation and Production of SAV transplant stock for ecosystem restoration

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Why have we lost our SAV?

Man-made systems (reservoirs)

Natural systems

- **Disturbance**
  Eutrophication, watershed development, storms, etc
- **Displaced by nonindigenous species**
  hydrilla, Eurasian watermilfoil
- **Nonindigenous animals**
  Common carp, nutria, grass carp, Canada geese
- **Management actions**
  Dredging, herbicides

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If we restore environmental conditions (water quality) will SAV recover?

Dependence on seed bank / propagule bank

Obstacles:

- **Biotic disturbance**
  - Nonindigenous species
- **Physical disturbance**
  - Wind, waves, erosion, loss of substrate
- **Water level fluctuations**
  - Common in multipurpose reservoirs

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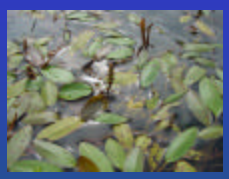
If replanting is required, what propagules should we use?

Seed?

- **Availability**
- **Viability**
- **Germination cues**
- **Storage**
- **Slow establishment**



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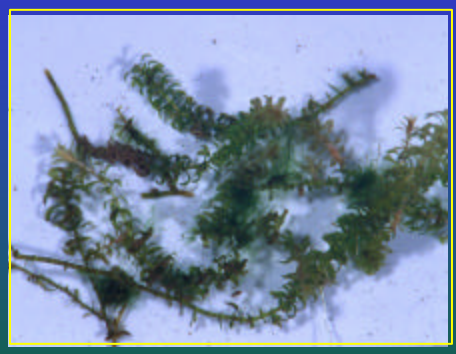
If replanting is required, what propagules should we use?

Fragments?

- Buoyancy
- Limited reserves
- Nonindigenous species



fragments

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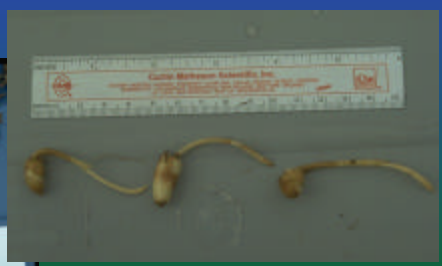
If replanting is required, what propagules should we use?

Tubers / winterbuds?

- Availability, buoyancy, handling



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If replanting is required, what propagules should we use?

Bare-root transplants?

- Availability, buoyancy, handling, nonindigenous species

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If replanting is required, what propagules should we use?

Nursery-grown plants

- Robust, mature plant; transportable with well-developed shoots and roots contained in a sediment matrix that is readily incorporated into the bottom substrate

Quickest, hardiest, most likely to succeed

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So, where do you get nursery-grown SAV transplants?

Commercial growers?

- Limited availability

"Grasses in classes", etc?

- Limited availability

Grow your own?

- You control species selection, timing, etc.

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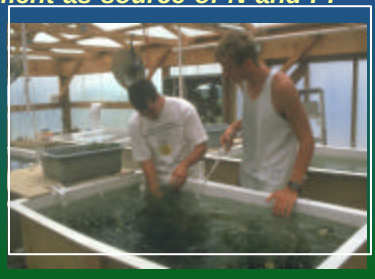
So, how do you grow SAV transplants?

Based on techniques developed in the early 1980's

- Smart and Barko, 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquatic Botany 21:251-263.

Depends on the use of natural sediment as source of N and P.



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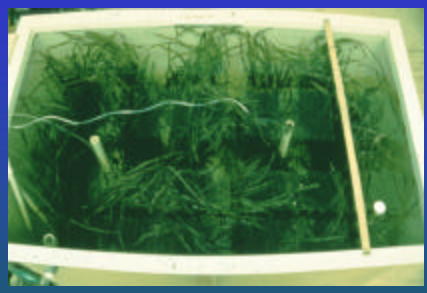
Plant Growth Requirements

- Light
- Temperature
- Nutrition
  - Sediment / Water
- Photosynthetic carbon source



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Plant Growth Requirements: Light

- Greater than 50% of full sunlight detrimental
  - ❑ 33% or 50% neutral-density shade fabric
- Clear water (no phytoplankton blooms)
- Greater than 12:12 photoperiod advantageous
- Difficult to provide adequate artificial light on large scale
- *Most economical and efficient production during spring, summer, and fall in outdoor facilities*

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Plant Growth Requirements: Temperature

Optimum for many species near 28C

- **Range: 25-30C**
- **Protect from hard freeze in winter**

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Plant Growth Requirements: Nutrition

Sediment requirements

- **Rooted SAV derives much of its N and most of its P from sediment**
- **P in water grows algae**

The *sediment* should have a high fertility and an ability to retain P

fine-textured, mineral (not organic) sediment

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Plant Growth Requirements: Nutrition

Water requirements

- Rooted SAV derives much of its N and most of its P from sediment
- P in water grows algae

The *sediment* should have a high fertility and an ability to retain P

alum-treated water is clear and P-free

tap water must de-chlorinated

a 1-2 cm layer of aquarium gravel over the sediment can help reduce P release

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Plant Growth Requirements: Nutrition

Water requirements (cont'd)

- Many species of SAV have a high requirement for K in the water column

may need to occasionally monitor K concentration and add as needed

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Plant Growth Requirements: Photosynthetic carbon source

Water requirements

- The concentration of free CO_2 in most freshwaters is low, particularly at pH levels above 8.3
- Many species of SAV utilize and benefit from bicarbonate
- Many species have a requirement for Ca in solution

While aeration can help replenish CO_2 taken up in photosynthesis, this does not eliminate the need for bicarbonate and Ca. pH should be monitored and alkalinity should be checked occasionally. If alkalinity declines, Ca may need to be added as well.

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Plant Propagule Production Requirements: Containers

Must be easily transported

- Plastic nursery pots, 3 to 4" diameter
- Weakly-rooted species might benefit from peat liners
- Held in trays to prevent tipping

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Plant Propagule Production Requirements: Tanks

Containers must be easily accessible

- Constructed of wood and lined
- Concrete or fiberglass raceways
- Rubbermaid tanks
- 50 – 100 cm water depth
- Flat bottom
- Water supply

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Plant Propagule Production Maintenance Requirements

Weed control

- Monocultures are easier

  One tank – one species

  If fragments can spread, they will

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Plant Propagule Production Maintenance Requirements

Pest control

- Watch for insect damage and deal with it early
- Snails can be a problem occasionally
- *Gambusia* (mosquito fish)

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Plant Propagule Production Maintenance Requirements

Algae control

- Prevention is easier than control

Water exchange (with alum-treated water)

Filtration (sand or DE filters)

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Plant Propagule Production Maintenance Requirements

Water quality maintenance

- Rapidly growing plants profoundly alter water chemistry

Partial water exchanges to maintain alkalinity, Ca, and K

Filtration if needed for turbidity

Aeration (air lifts) for mixing, gas exchange

Consider CO_2 augmentation for high production systems

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Plant Propagule Production Maintenance Requirements

Sediment nutrient depletion

- Rapidly growing plants can quickly deplete sediment N

Fertilize sediments with NH_4 prior to planting

Add N to sediments as needed

Add N sparingly to water (<1 mg N/L) - use caution

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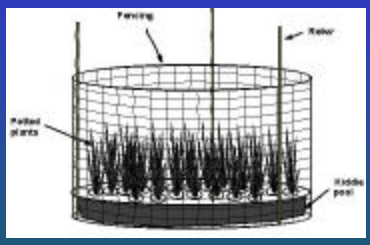
In-lake Plant Propagule Production: An Alternative to an Off-site Facility

- Use sediment from the site
- Kiddy pools or "floats"
- Protect from disturbance
- *Pre-conditions plants to WQ conditions at restoration site*
- *Minimizes transportation and labor*



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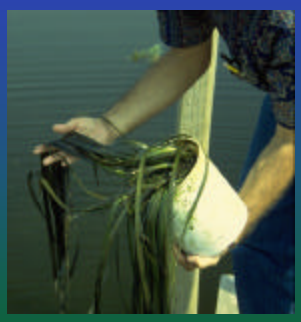
Plant Propagule Production

The end result



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Handbook for Ecosystem Restoration

- **Growth characteristics**
  Growth form, reproduction, perennation
- **Range**
- **Use and habitat**
- **Culture**
  Propagule, light, container, substrate, fertilization, depth, comments

Vallisneria americana

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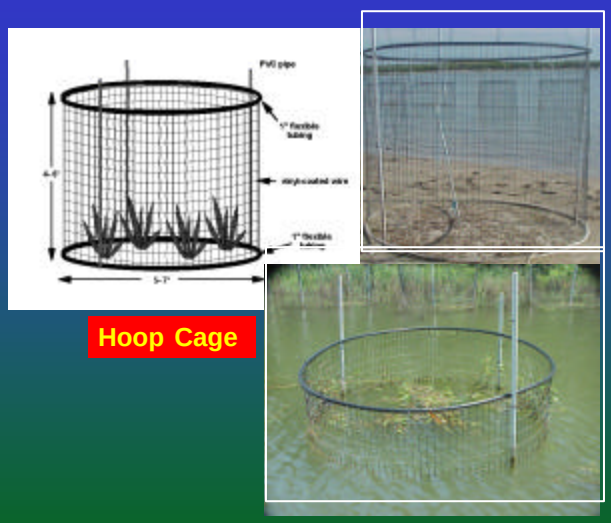
Handbook for Ecosystem Restoration

- **Field planting**
  - Propagule
  - Season
  - Substrate
  - Water depth
  - Exclosure type
  - Comments



Hoop Cage

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Propagation and Production of SAV

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Applications and Limitations of Micropropagation for the Production of Underwater Grasses

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Micropropagation – the manipulation of small quantities of axenic plant material ranging from simple cells to stem pieces under conditions favorable to the formation of new plants.

Related Terms – Tissue culture – Cell culture – Axenic culture

Examples of Agronomic Plants Propagated by Micropropagation

Boston Fern	Rhododendron	Strawberries
African Violets	Mountain Laurel	Potatoes
Tulips – Lilies	Apples	Perennial Corn

Advantages of Micropropagation

1) No seasonal constraints

2) Large numbers of plants produced

3) Inexpensive

4) Plants are axenic and disease free (specific techniques)

5) Plants are clones

Disadvantages of Micropropagation

1) Plants are clones

2) Some specialized training requirements

3) What to do with all the plants produced

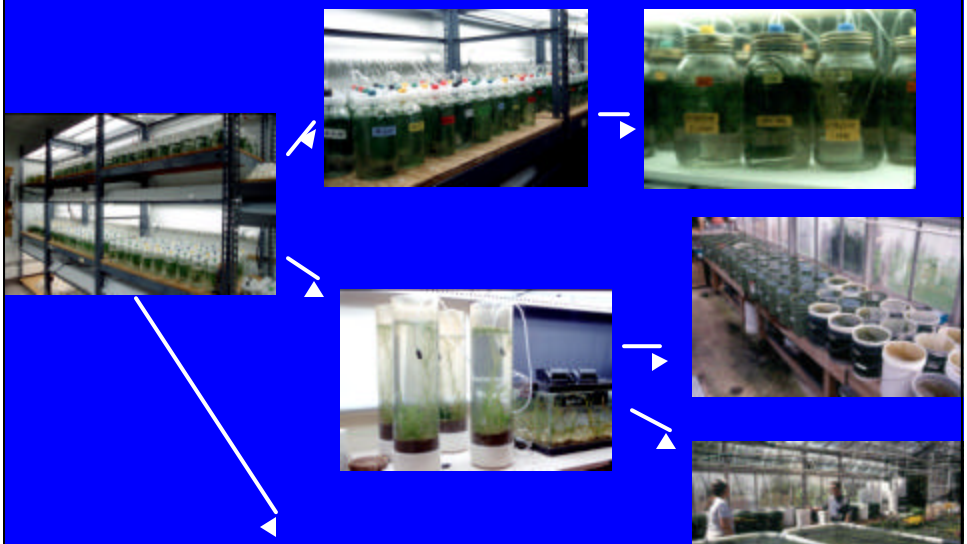
4) Transitioning to field sites

Procedural Requirements for Developing a Micropropagation System

1) **Species Selection** – Desirable ecotypes – Value – Demand – Applications
2) **Explant Choices** – Sterile – Semi-sterile – Meristems
3) **Disinfestation of Explants** – Bacteria – Fungi – Algae
4) **Development of Propagation Media** – Minerals – Carbohydrates – Plant growth regulators
5) **Media Refinement**
6) **Development of Growth Media** – Minerals
7) **Development of a Transition Protocol** – Lab – Greenhouse - Field

Application of Micropropagation to Submersed Aquatic Plants

- **Physiological studies of plant growth and development**
- **Contaminant dose/response studies – chemical ecology**
- **Bioassays of sediment and water**
- **Education/demonstration projects**
- **Plant production for field establishment**



Transplant Applications

(continued next page)

Transplant Applications

Costs for Basic Propagation Facility

1) Laboratory

•Autoclave	$6,000
•Laminar Flow Hood	$5,000
•Culture Room	$9,000

2) Propagation Cost/1000 Multi-stemmed Transplants

•Media	$ 22
•Culture Tubes	$ 48
•Labor	$ 160
	$ 230

3) Preparation for Field Establishment

•Containers	$ 30
•Labor	$ 160
	$ 190

Total Production Costs	$ 420/1000	$0.42/plant

Challenges for using Micropropagation for Production of Submersed Aquatic Plants

- Limited species – Little success with seagrasses
- Sporadic demand for quantities of plants
- Short planning horizons for field applications
- Ill-defined project objectives
- Significant gaps in basic plant physiology

This work was supported by the Maryland Port Administration with special thanks to Mr. Nathaniel Brown

Bay Grasses in Classes Overview

- Students learn the importance of SAV while growing different species in their classroom.
- Participate in restoration effort
- Create plant stock for restoration activities

  Since 1998-

  ~ 28,000 students participated

  ~ 2,300 m^2 of wild celery and sago pondweed planted at 8 sites

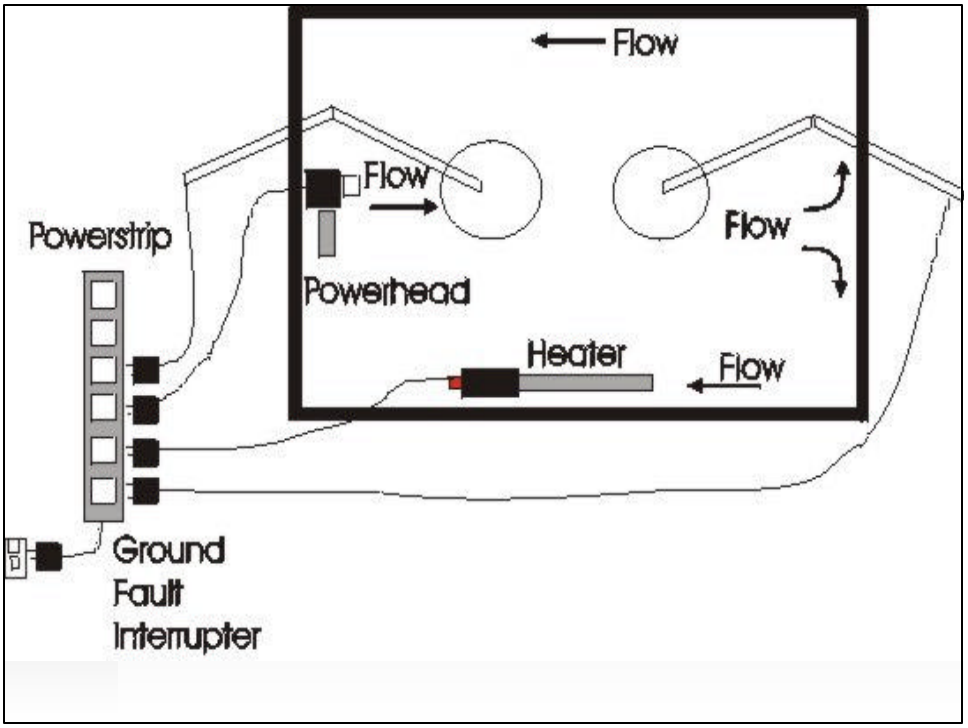

Susquehanna River
Piney Run Reservoir
Baltimore
Rocky Point Park
Chester River
Washington D.C.
Corsica River
Choptank River
Centreville
Marshyhope Creek
Nanticoke River
Patuxent River
Pocomoke River
Potomac River
Solomon's Island

Materials: Total List for 2 growth chambers

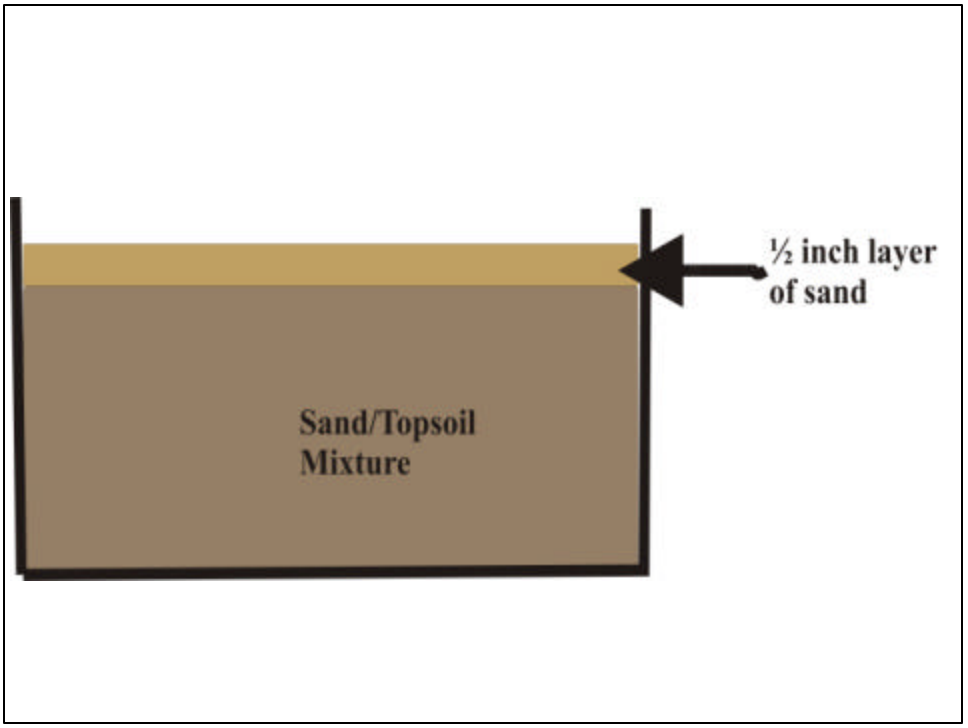
2 - growth chambers
- 2 - sponge filters
- 2 - powerheads
- 4 - incandescent light bulbs (60 watt)
- 4 - light shrouds (swing arm desk lamp)
- 2 - power strips with surge protectors
- 2 - ground fault interrupters (GFI)
- 2 - thermometers
- 2 - submersible aquarium heaters
- 1 - pH test kit

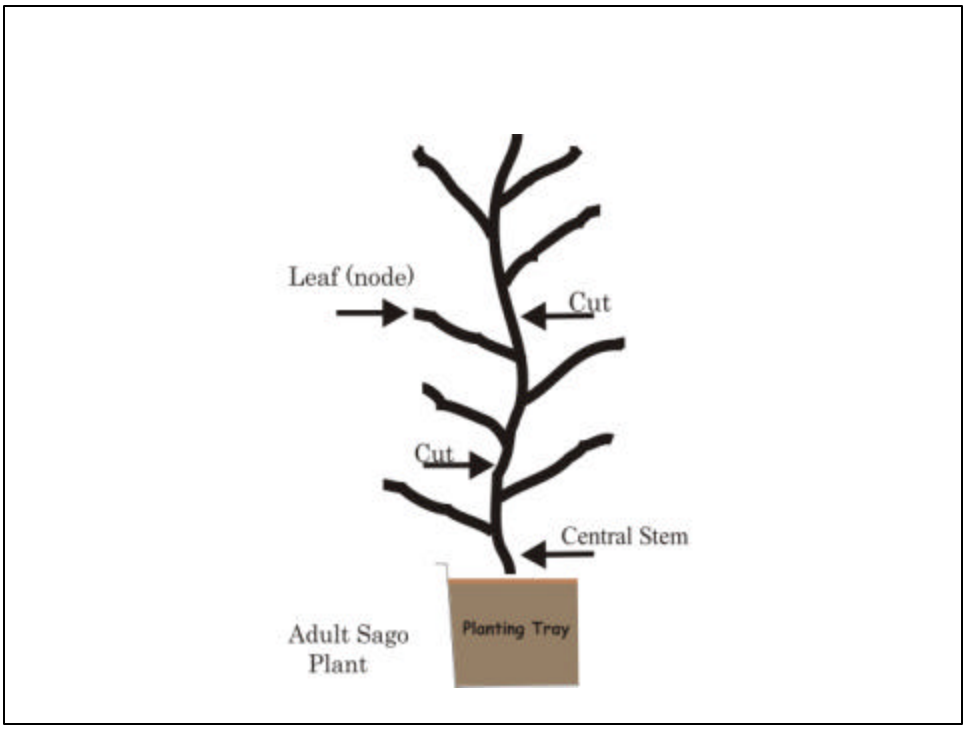
1 - nitrate test kit
- 6 - planting trays
- 1 - foam sheet
- 1 - bag of topsoil (40 pounds, lower organic content than potting soil)
- 1 – bag sand

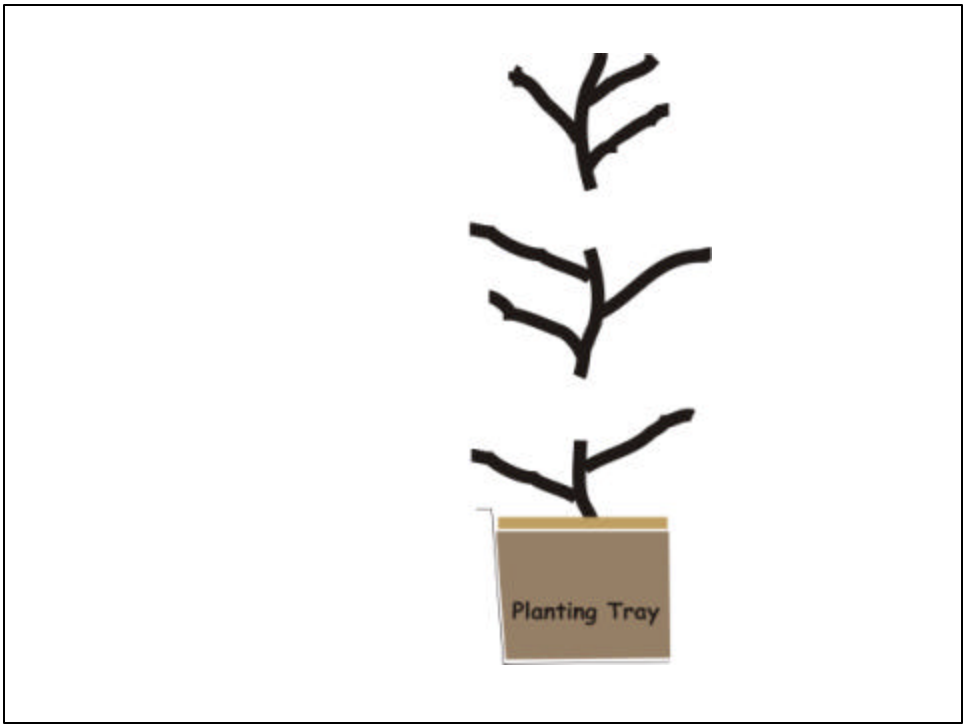

Grasses in Classes


Flow
Flow
Powerstrip
Powerhead
Flow
Heater
Flow
Ground
Fault
Interrupter

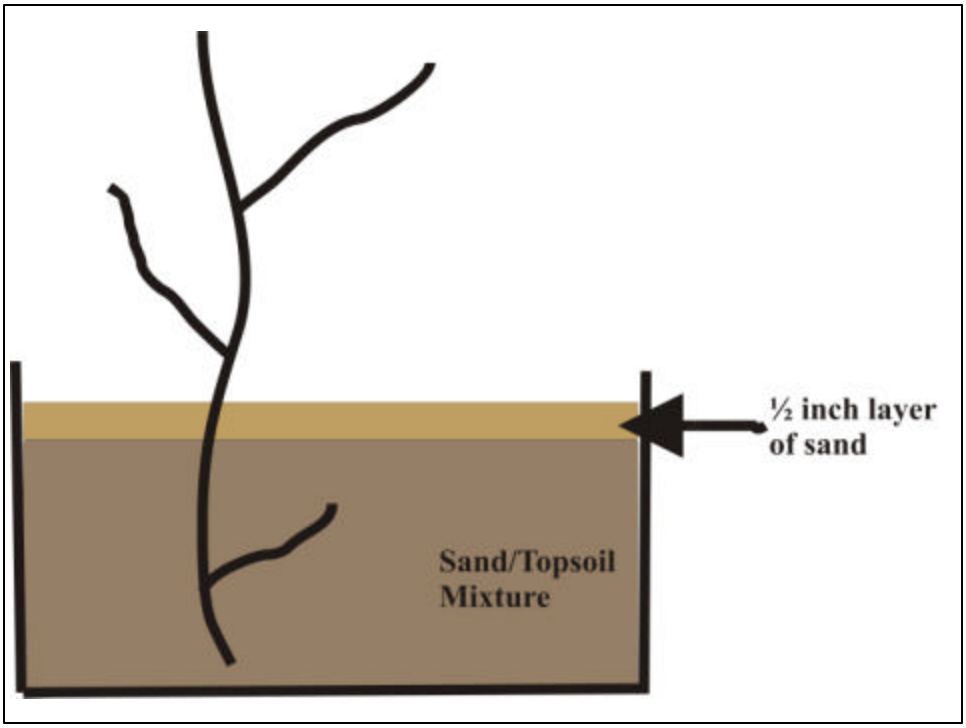

NATURE'S PRIDE
TOP SOIL
Net Wt. 40 Lbs. (18.1 Kg)
QUIKRETE
ALL-PURPOSE
SAND


½ inch layer of sand
Sand/Topsoil Mixture


Leaf (node)
Cut
Cut
Central Stem
Adult Sago Plant
Planting Tray


Planting Tray


Sago Plant
After Cutting


½ inch layer
of sand
Sand/Topsoil
Mixture

Tips for Micropropagation

- 84 degrees- lower temps grow too slow, but higher temps create algae problems
- Keep it short- as the plants get too long, they will brown and lose leaves
- Keep tanks about chest high
- Plants will keep in refrigerator after micropropagation for weeks