Biologically based treatments for the production of loblolly pine.

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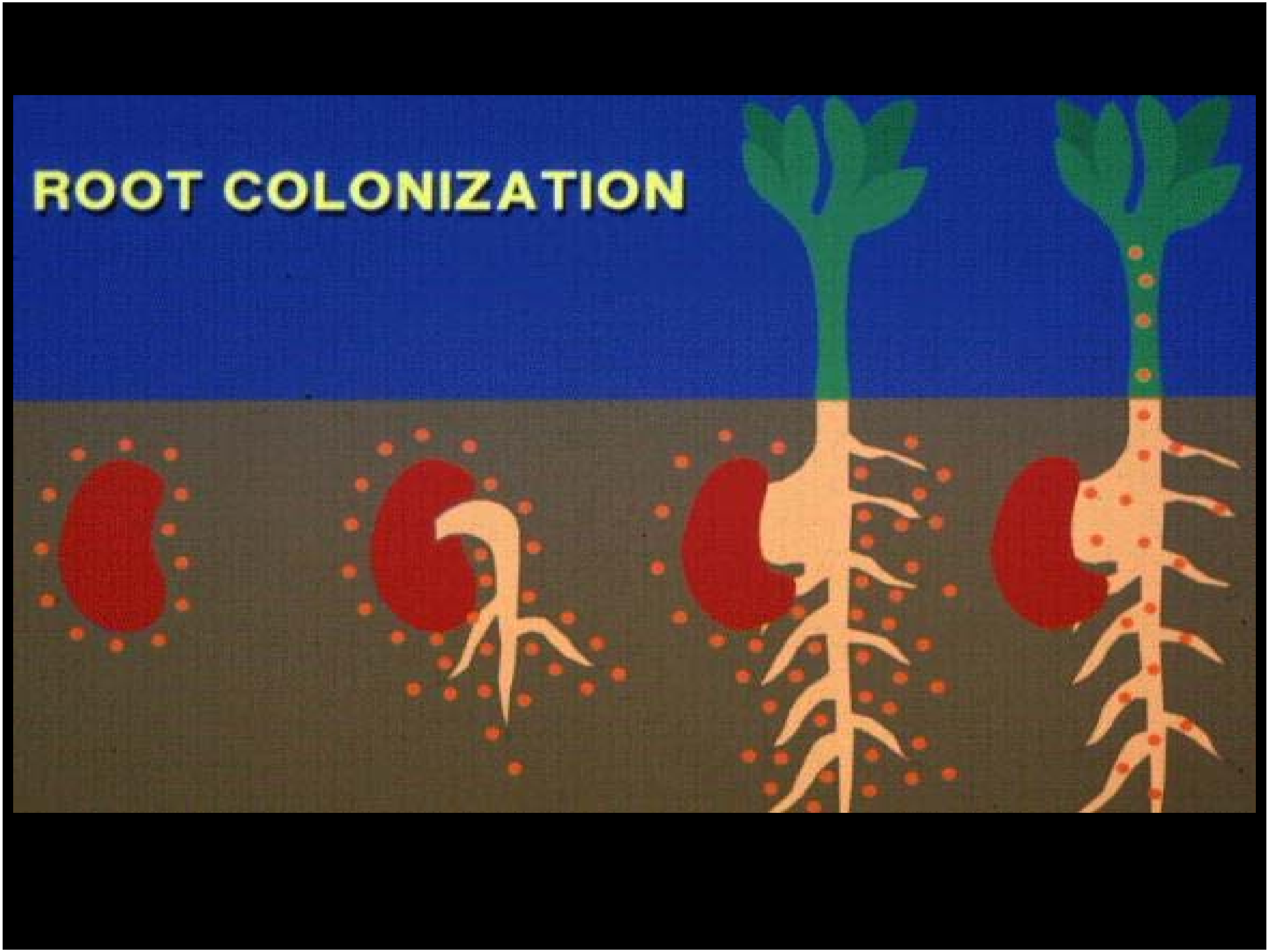
Biological Control

Agents

- Bacteria – PGPR
- Fungi
- Mycorrhizae
- Soil amendments

Discovery & Identification

- **Plant growth-promoting rhizobacteria**
  - **naturally occurring asymbiotic soil bacteria that have the capability to aggressively colonize plant roots and stimulate plant growth**


ROOT COLONIZATION

PGPR Activity - Biological Control

• Produce lytic enzymes	Mitchell 1961
• Siderophore production	Kloepper et al 1980
• Competition for nutrients	Suslow 1982
• Competition for root sites	Elad & Chet 1987
• Produce hydrocyanic acid	Schippers 1988
• Produce antibiotics	Weller 1988
• Induce systemic resistance	Liu et al 1996

Biological Control

- Wheat seed inoculated with three different bacteria sown in Fusarium infested soil

EPR - Emergence Promoting

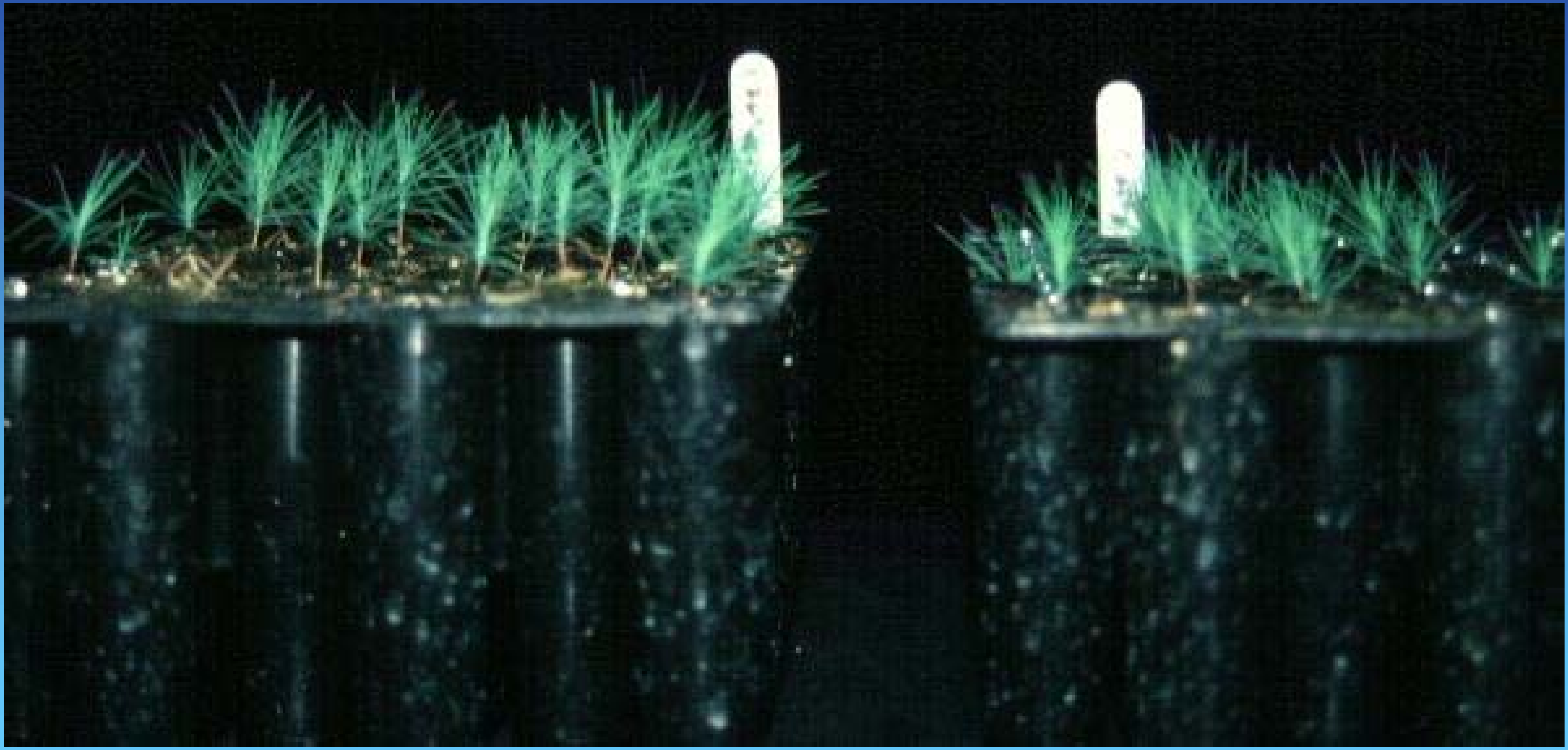
Commercially Available Products

Product	Bacteria	Target Effect
Kodiak	Bacillus subtilis	Growth promotion & Biocontrol of Rhizoctonia and Fusarium
Deny	Burkerholdia cepecia	Biocontrol of Fusarium & Pythium
Actinovate	Streptomyces lydicus	Biocontrol of Pythium, Fusarium & Rhizoctonia
YIB	Bacillus spp.	Root growth promotion
Epic	Bacillus subtilis	Growth promotion & Bio-control of Rhizoctonia and Fusarium

PGPR - Gymnosperm Research

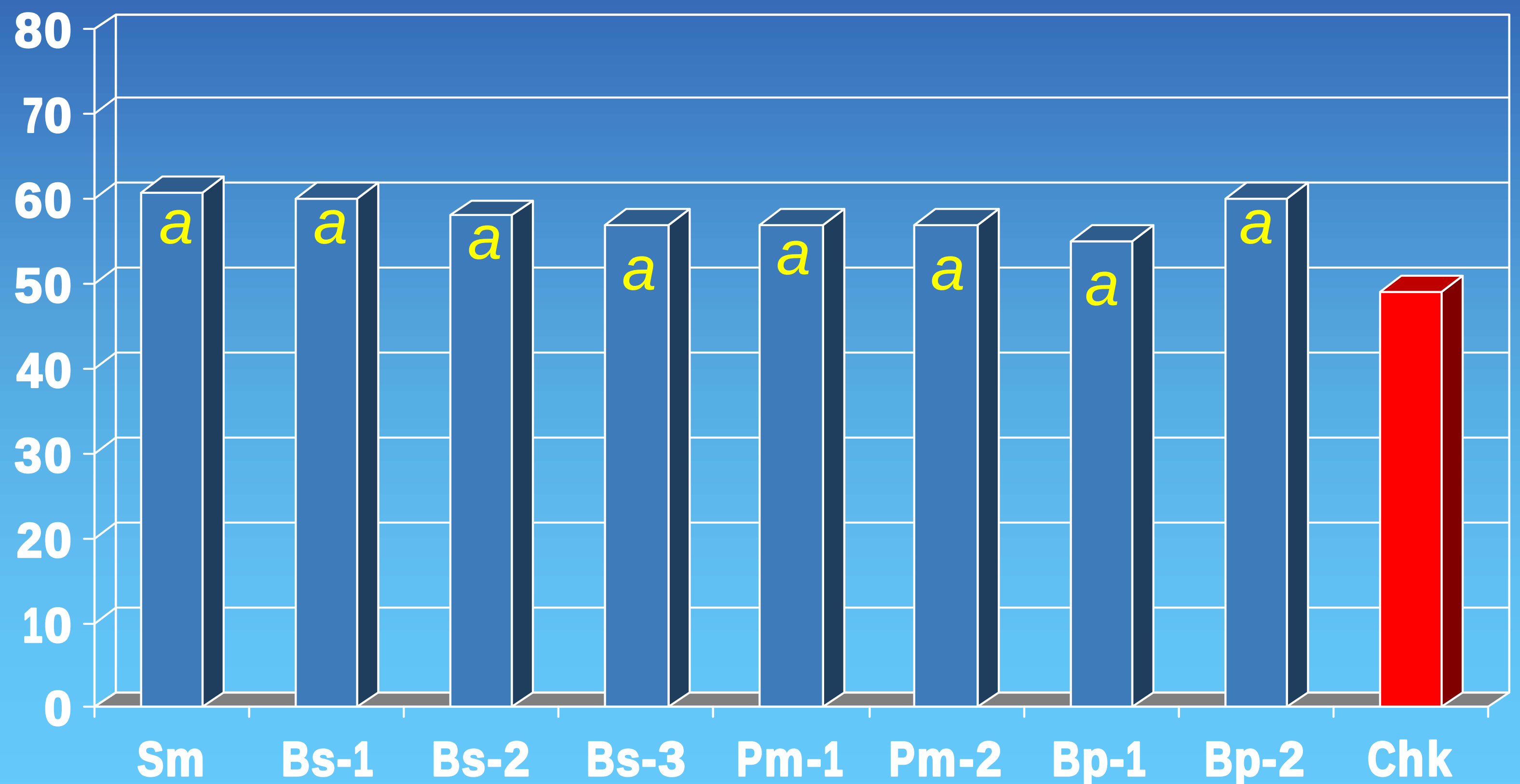
• *Pseudotsuga* sp.	Increased height 27%	Parker 1975
• *Pinus contorta*	Increased biomass 33%	Holl et al 1988
• *Picea glauca*	Increased height	
Picea mariana	and biomass 59%	Beall 1989
• *Pinus banksiana*	Increased biomass	Beall 1989
• *Pinus syvelstris*	Increased shoot length	Pokojska 1992
• *Pseudotsuga*	Increased biomass 68%	Holl 1992
• *Tsuga* sp.	Increased biomass 30%	Chanway 1995
• *Pinus taeda*	Increased biomass	
Pinus elliottii	Increased biomass	Enebak et al 1998

EPR - Emergence Promoting

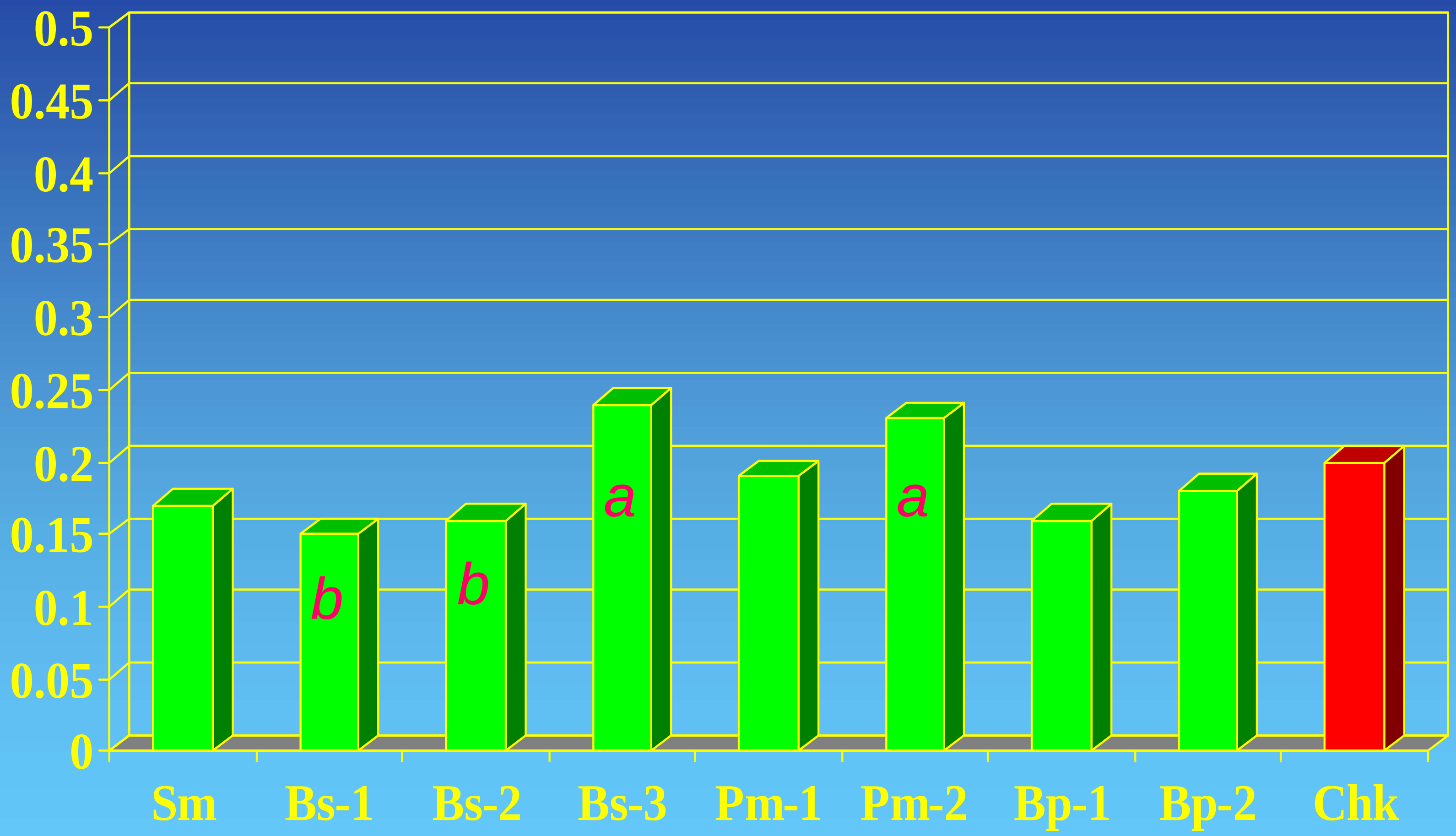


EPR - Emergence Promoting

Three week-old loblolly pine seedlings

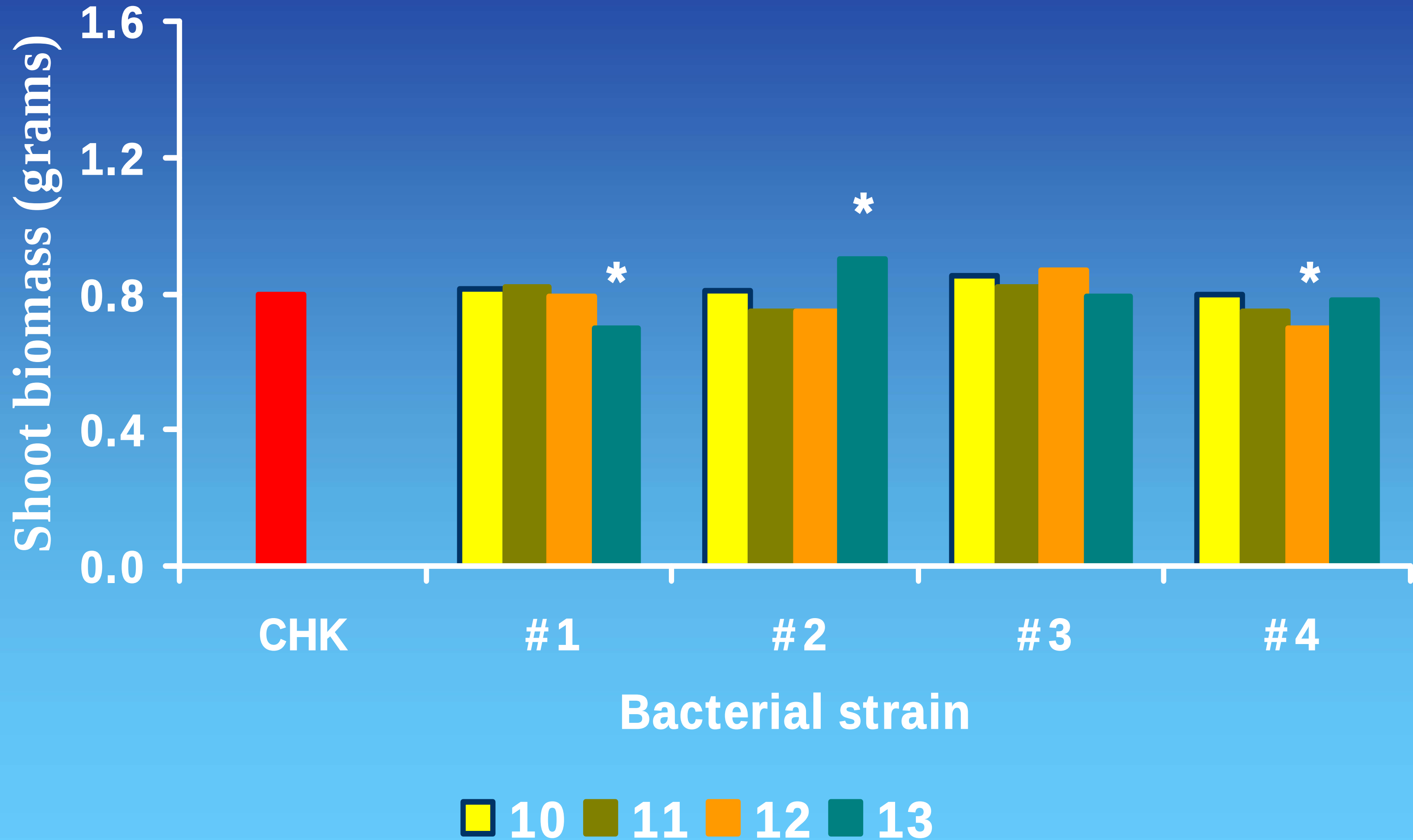


PGPR Research – Root Growth



Enebak, Wei and Kloepper – Forest Science 1998. 44:139-144

Effect of bacteria strain and dose on shoot biomass



Vonderwell & Enebak – Forest Science 2000. 46:437-441

Symbiotic Relationships

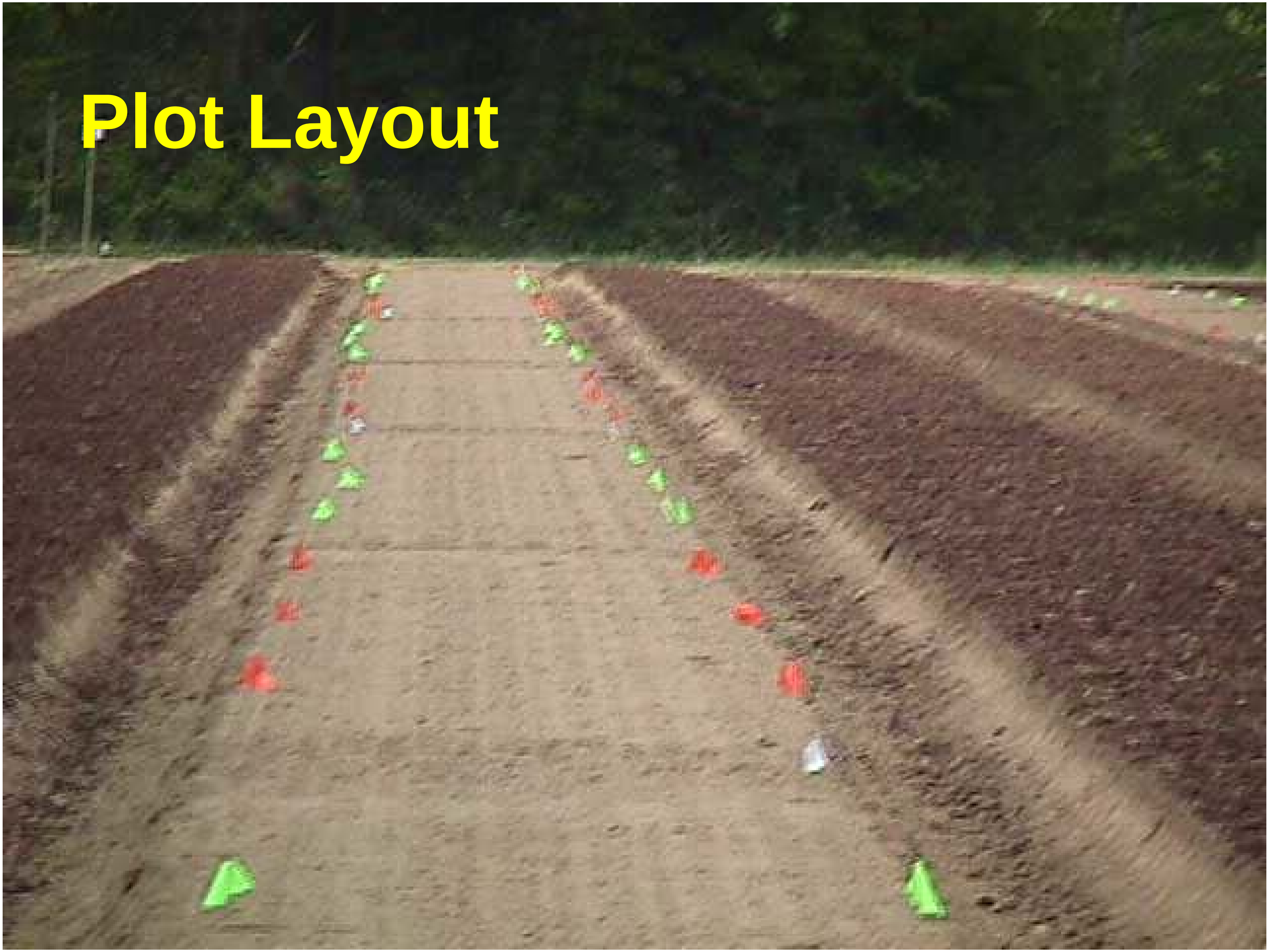
- Increase / Decrease ectomycorrhizae infection
- Increase / Decrease endomycorrhizae infection
- Alter species of mycorrhizae

Materials & Methods

- Soil was treated with various combinations of MB, Chloropicrin, Sectagon & Eptam
- Loblolly pine treated at the time of sowing with *Panebacillus macerens*
- 3 - 4 ft2 subplots installed over soil treatments as either treated seed or non-treated seed
- Seedlings counted over growing season
- At end of season 15 seedlings per subplot removed and growth characteristics measured
- 1998, 1999, 2000 at Flint River Nursery - GA
- 1998 at Hauss Nursery – AL
- 1999, 2000 at Carter Nursery - GA

Soil Treatments

Sowing seed


Plot Layout

Seedling Counting & Collection

Table 1. Mean seedling density, size and dry weight by seed treatment with *Paenibacillus macerans* for loblolly pine at Flint River GA in 1998, 1999 and 2000.

Year	Bacteria	Density (ft2)	RCD (mm)	Hgt (cm)	Root Wgt (g)	Shoot Wgt (g)
1998	No	**21.4**	**4.3**	**21.4***	**0.68**	**3.1**
	Yes	**21.8**	**4.2**	**19.7**	**0.72**	**3.0**
1999	No	**21.7**	**3.6**	**25.3***	**0.71**	**2.7**
	Yes	**22.7**	**3.6**	**24.5**	**0.66**	**2.6**
2000	No	**22.0**	**4.2**	**27.0**	**0.85**	**2.9**
	Yes	**22.4**	**4.1**	**26.6**	**0.81**	**2.9**

Table 2. Mean seedling density, size and dry weight treated with *Paenibacillus macerans* for loblolly pine at Hauss and Carter Nurseries in 1998, 1999 and 2000.

Year	Bacteria	Density (ft2)	RCD (mm)	Hgt (cm)	Root Wgt (g)	Shoot Wgt (g)
1998	No	**22.5**	**4.6**	**na**	**0.79**	**2.9***
	Yes	**23.8***	**4.4**	**na**	**0.72**	**2.7**
1999	No	**21.6***	**5.0**	**na**	**0.89**	**3.1**
	Yes	**19.4**	**5.2**	**na**	**0.96***	**3.4***
2000	No	**24.8**	**5.1**	**na**	**0.90**	**3.4**
	Yes	**25.4***	**5.1**	**na**	**0.90**	**3.3**

Summary – Bare Root Nurseries

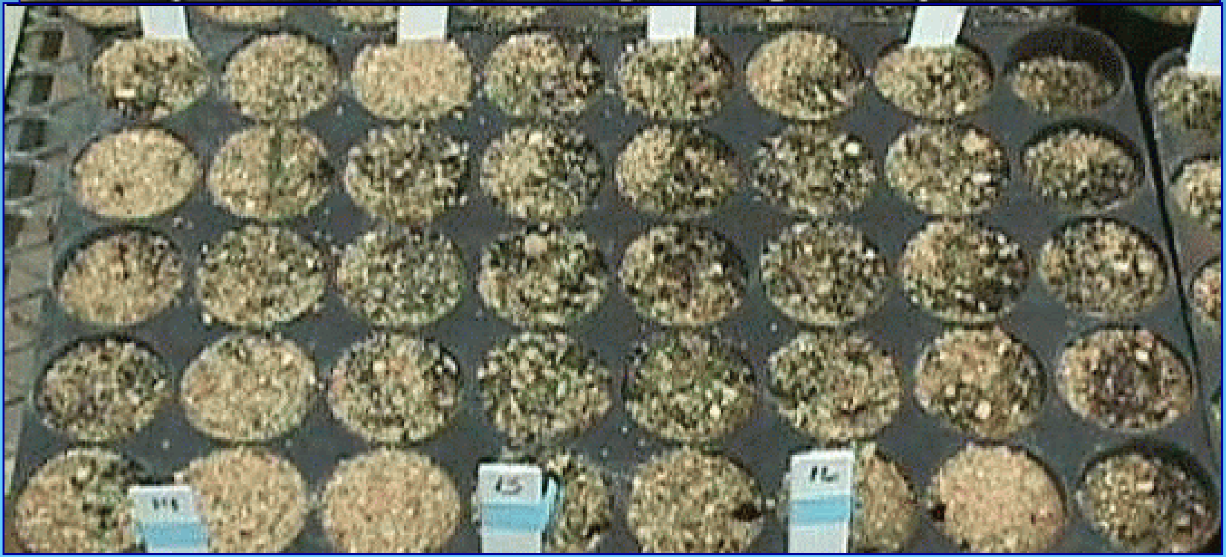
- Enhance seedling emergence
- Enhance some seedling growth
- Dose sensitive
- Nursery specific
- Species and family specific
- Fine tuning for nursery, species & family would take years

Biological - Fungi

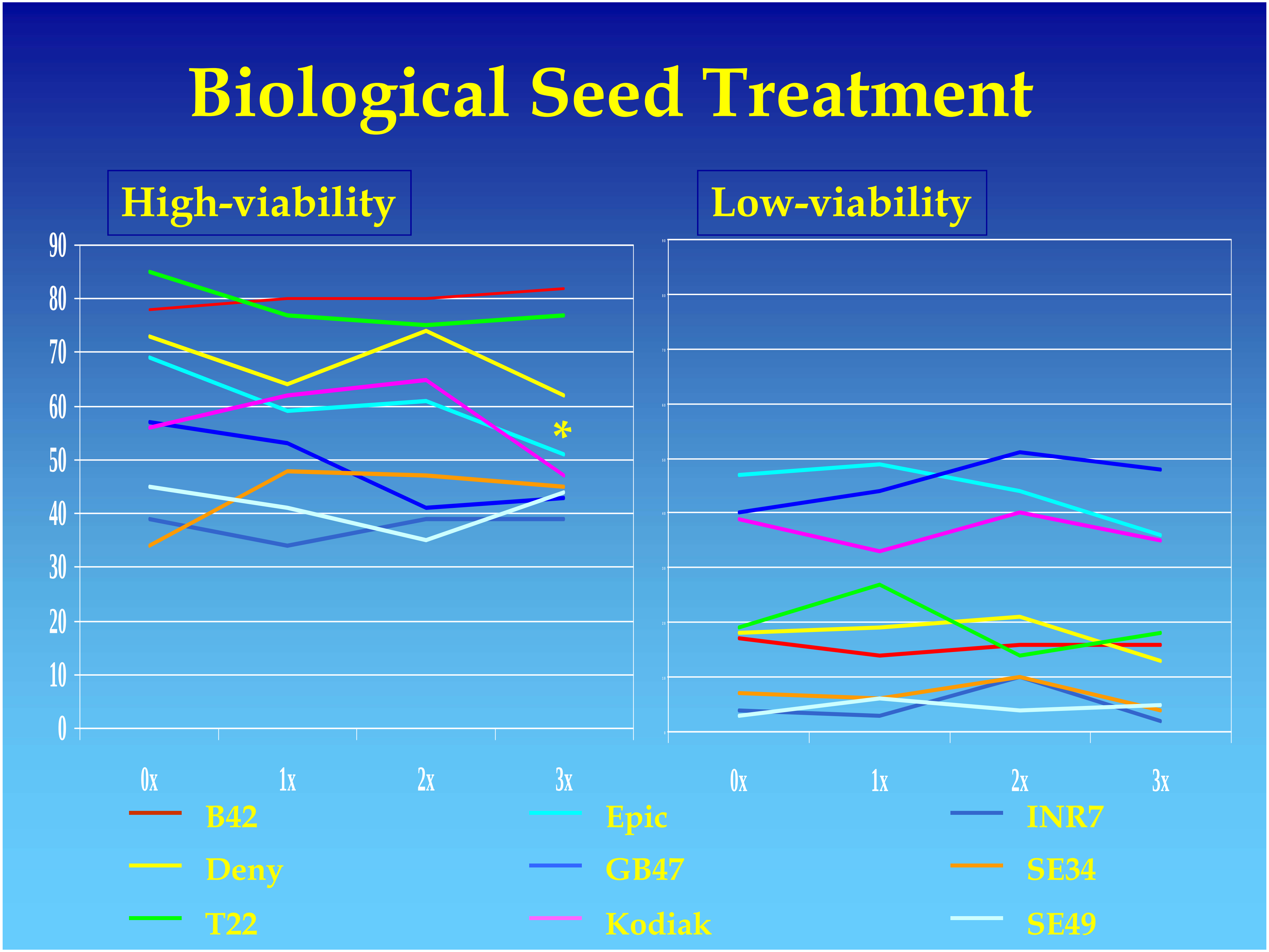
- T22®, and Deny® applied as pre-sowing treatments. Three rates and a control
- Each treatment applied to two types of seed:
  - high-viability seed: 92-95%
  - low-viability seed: 17-53%
- Sown in 5 x 8 container blocks.
- Total number of germinants and damped-off seedlings counted every other day for 8 weeks.

Fungal Seed Treatment

Pre-treatment high-viability seed lot



Pre-treatment low-viability seed lot



Biological Seed Treatment
High-viability
Low-viability
90
80
70
60
50
40
30
20
10
0
*
0x
1x
2x
3x
0x
1x
2x
3x
B42
Deny
T22
Epic
GB47
Kodiak
INR7
SE34
SE49

Summary & Conclusions

- Biological agents neither increased nor decreased longleaf seed germination.

- Five fungicides found to inhibit *Fusarium* sp commonly associated with longleaf pine seed.
- Benlate® and Manzate® treatment resulted in a 10% increase in percent germination.

Biological - mycorrhizae

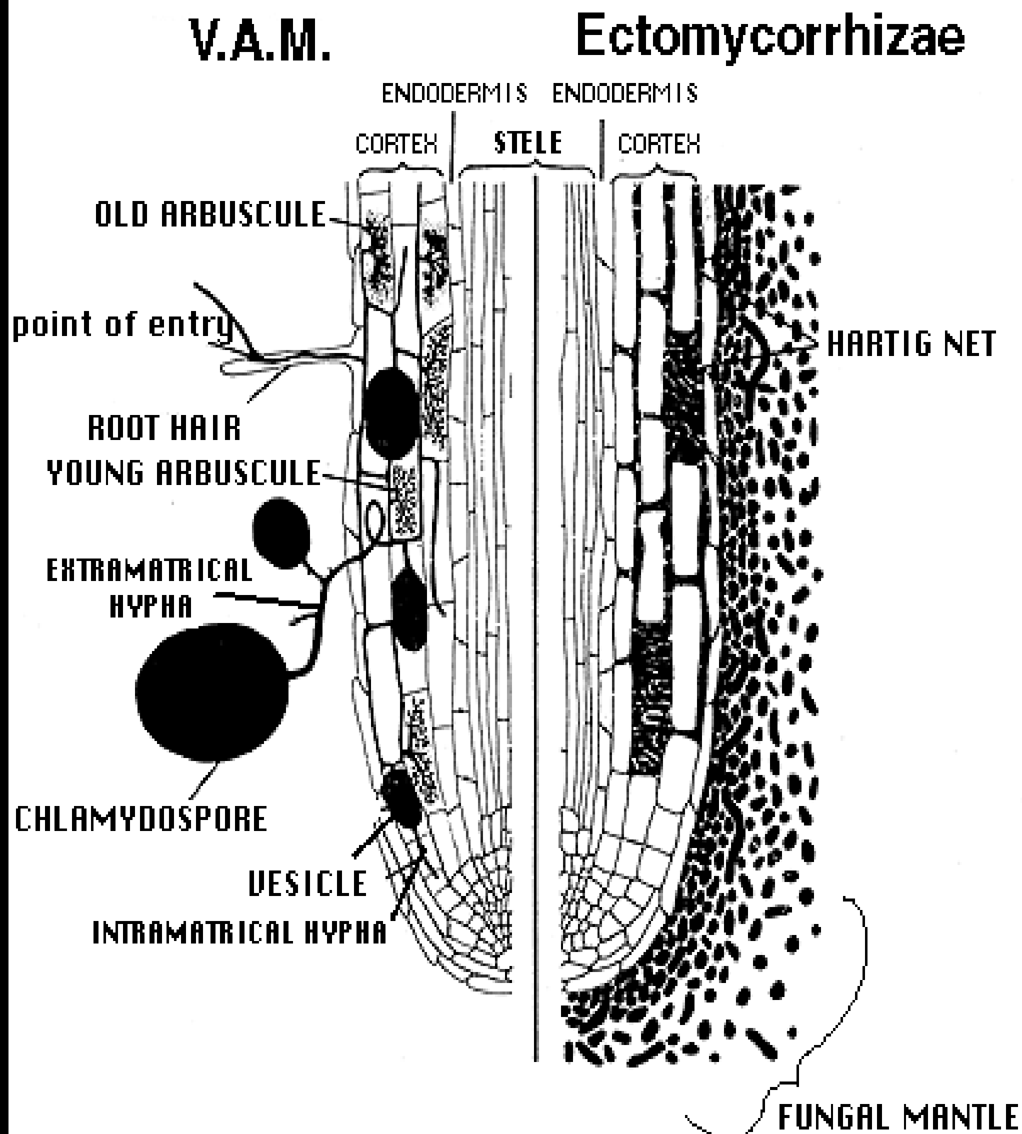
- **Mycorrhizae: A Greek word that means Root - Fungus. Much research has shown that mycorrhizae are a critical ingredient to the survival of forest trees**.
- Symbiotic relationship. Both tree and fungus benefit.
- Tree benefits from increased root area for absorption of nutrients and water.
- **Fungus benefits because it receives food from the tree's roots.**

Ectomycorrhizae (outside)

- Produces a fungal mantle
- Roots tend to be “forked”
- Spread via spores in the wind
- Found on many conifer species
- Found in many bare-root and container systems

Endomycorrhizae (inside)

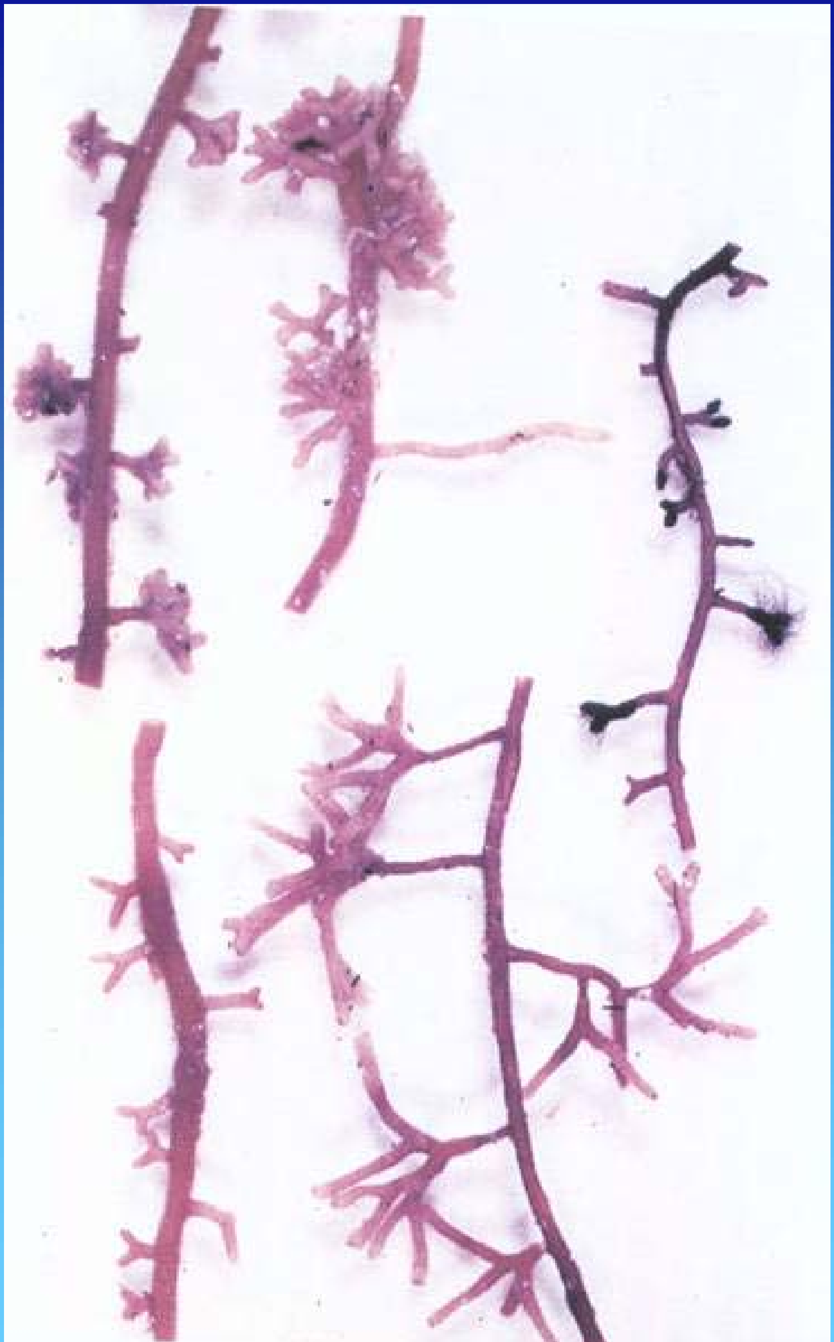
- No visual difference externally
- Produce swellings, or vesicles, on plant roots
- Spread via infected roots
- Found on many hardwoods and cover crops



DIAGRAMMATIC REPRESENTATION
OF THE TWO TYPES OF MYCORRHIZAE

Mycorrhizae

- Selectively absorb and accumulate certain nutrients, especially Phosphorus
- Solublize and make available non-soluble minerals
- Keeps feeder roots functional longer
- “Protects” feeder roots from soil pathogens
- Result in forking of fine roots

Thelephora terrestris

Most common ecto in nursery soils.

Spread via spores from neighboring fields.

Not unusual to have 100% colonization.

‘Smothering’ fungus.

Can be quickly replaced by native mycorrhizae after out-planting.

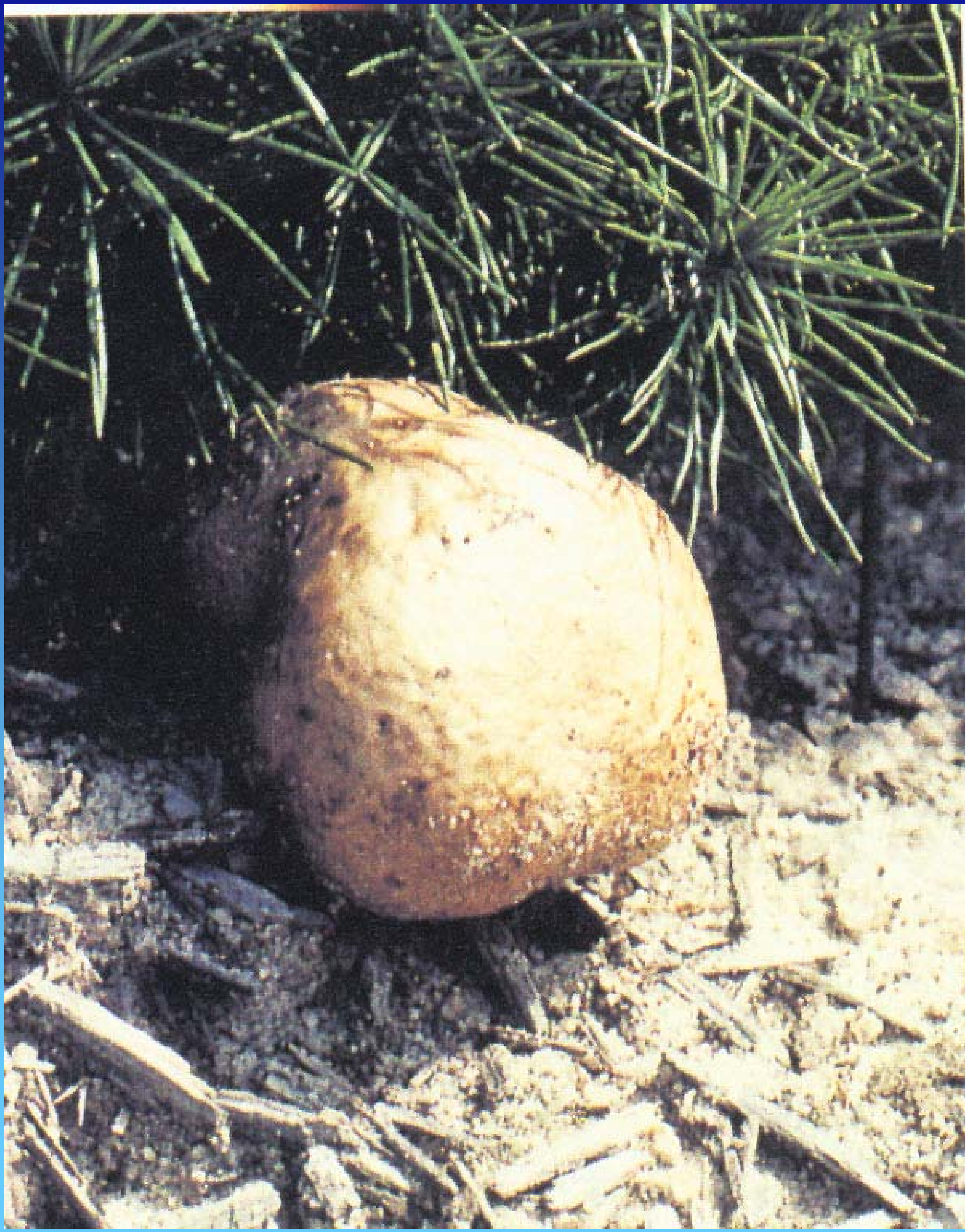
Pisolithus tinctorius
also known as Pt

Second most common ecto

Has been shown to increase survival of seedlings after out-planting on harsh sites.

Not easily spread. Needs vegetative mycelia.

Easily replaced after outplanting.



Mycorrhizae

- Except for purposes of ‘market forces’ or EXTREMELY harsh sites, the addition of fungal inoculum to either nursery soils or container systems to “increase mycorrhizae” and consequently seedling survival, is not necessary with respect to growing conifer seedlings in the southern United States.

Soil Amendments

- Bark – Conifer / Hardwood
- Green manures
- Pulp mill waste
- Saw dust
- Chicken House waste
- Compost

Biologicals

- In the competitive business such as forest-tree nurseries, the lack of a consistent response of a biologically based practice for the control of a target pest (insect, pathogen, weed) makes their wide-spread use significantly limited.

- Test on small areas over a period of a few years under normal conditions before adopting wide scale use.