Effects of *Pythium* and Cold Storage on the Survival of Southern Pine Seedlings

What's the Difference?

Container

- $180/1000 seedlings
- Intact root plug after lifting
- Higher survival
- Extended planting season (Sept-March)
- Storable throughout fall and winter

WHY?

Bareroot

- $80/1000 seedlings
- Exposed root system
- Lower survival
- Defined planting season, especially if stored
- Poor storability before mid-December

My Project Involved Answering….

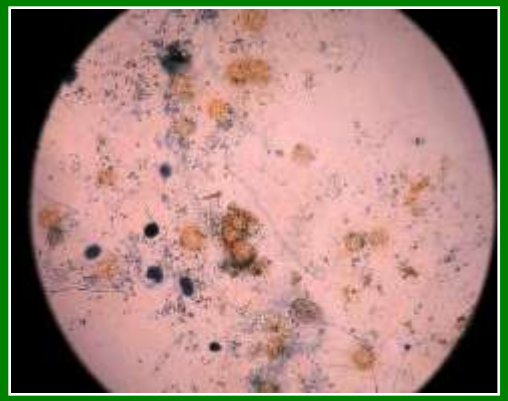
1. Why bareroot seedlings store poorly from October to mid-December?

AND

2. Why container-grown seedlings store better than bareroot seedlings?

A Theory to Explain Poor Bareroot Seedling Storability

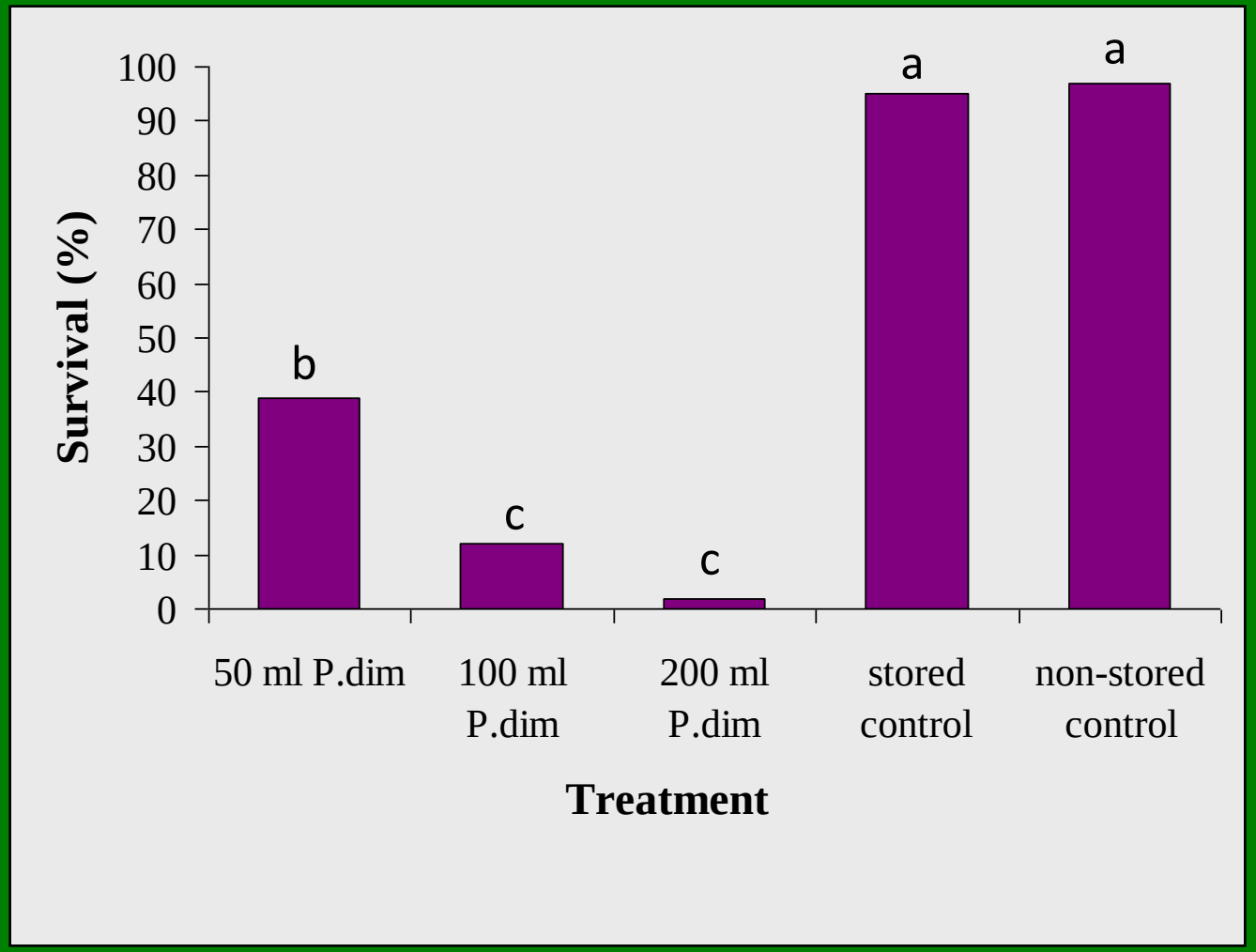
At lifting, bareroot seedling roots are torn and wounded.



Pythium infects the roots through the wound.

Pythium multiplies in the moist, cool storage conditions and causes seedling death after outplanting.

Pythium dimorphum reduced bareroot longleaf pine survival after cold storage.



Sun 1996

(1) Bareroot & Container Seedling Survival

- Study Objective:

  To inoculate loblolly, longleaf, slash, and shortleaf pine seedlings with *Pythium* prior to storage and determine the effect on seedling survival after outplanting.

Seedling Inoculations with *Pythium*

Seedling Survival After Cold Storage

Bareroot Longleaf (2008), Loblolly, & Slash (2009)

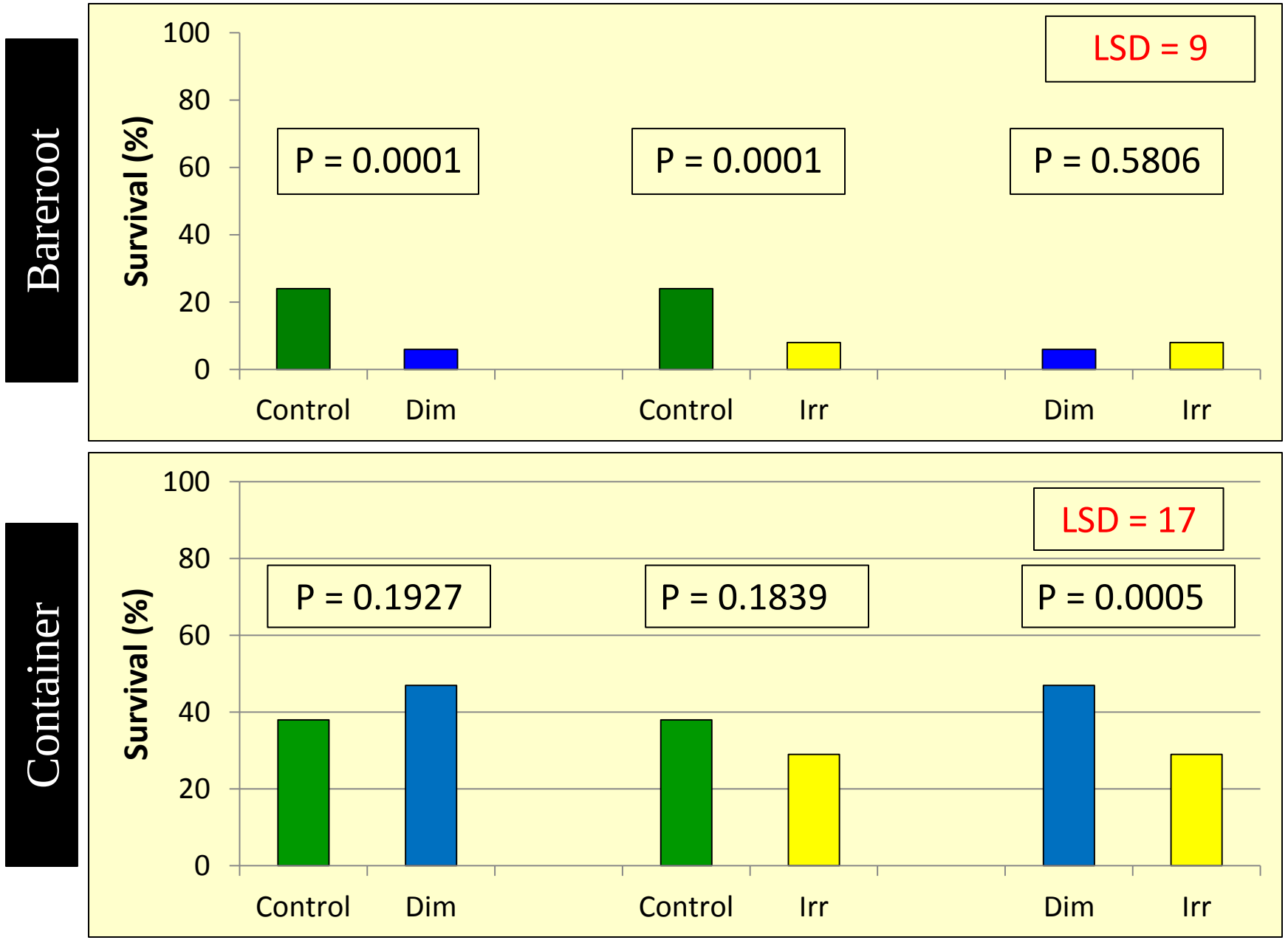
- Inoculated with *P. dimorphum* and *P. irregulare*
- Three levels: 50, 100, & 200 g
- Control dipped in water

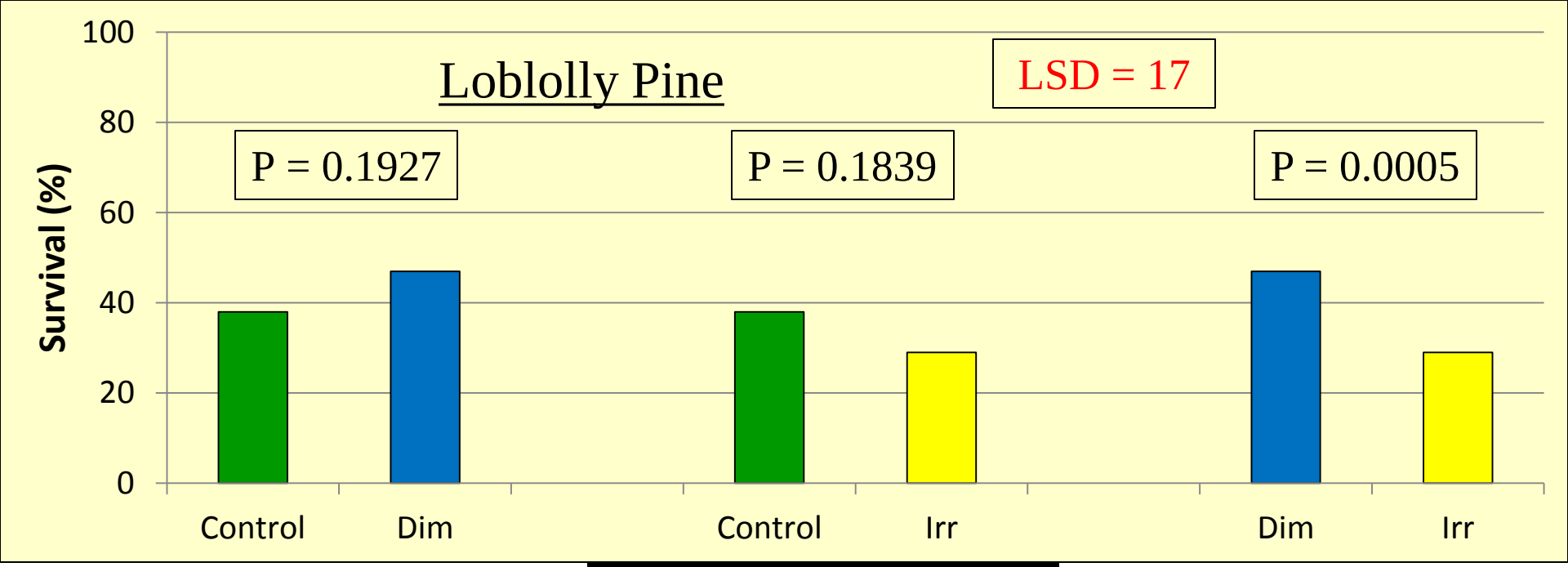
Container Longleaf (2008), Loblolly, Slash, & Shortleaf (2009)

- Inoculated with *P. dimorphum* and *P. irregulare*
- One level: 200 g
- Root plug wounded or not wounded
- Control dipped in water

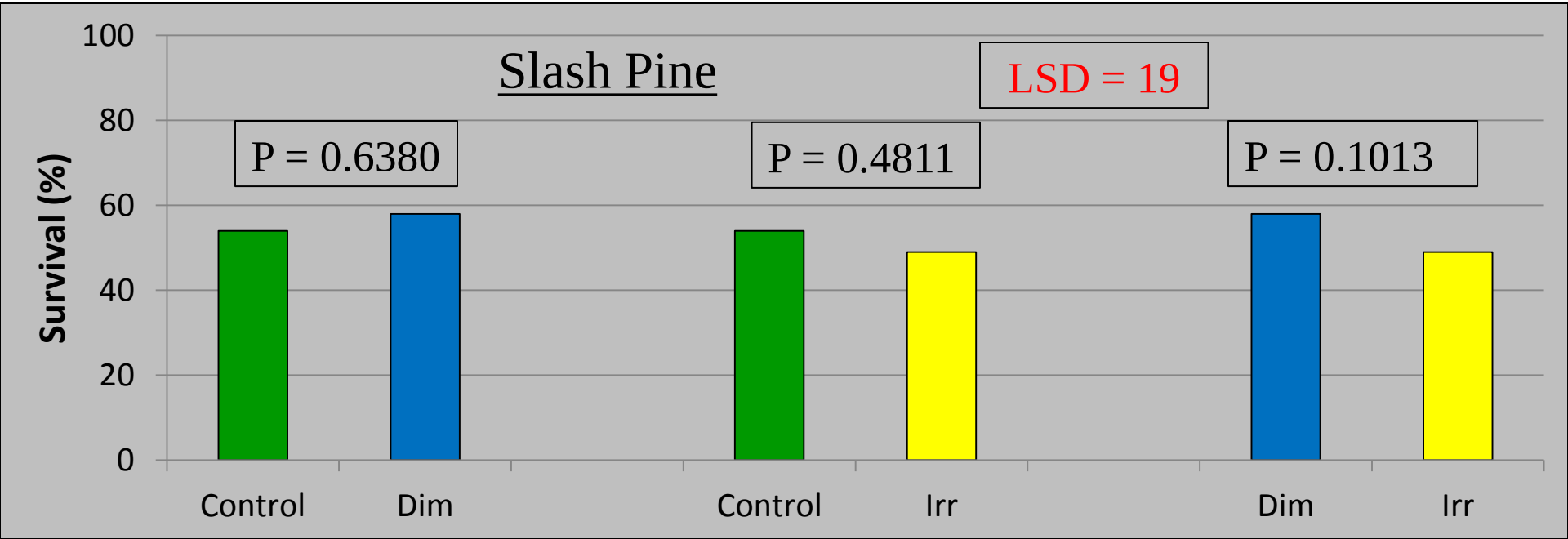
- Storage Periods: 4, 6, and 12 weeks (4-5°C)
- Survival: seedlings outplanted after each storage period and monitored for 6 months

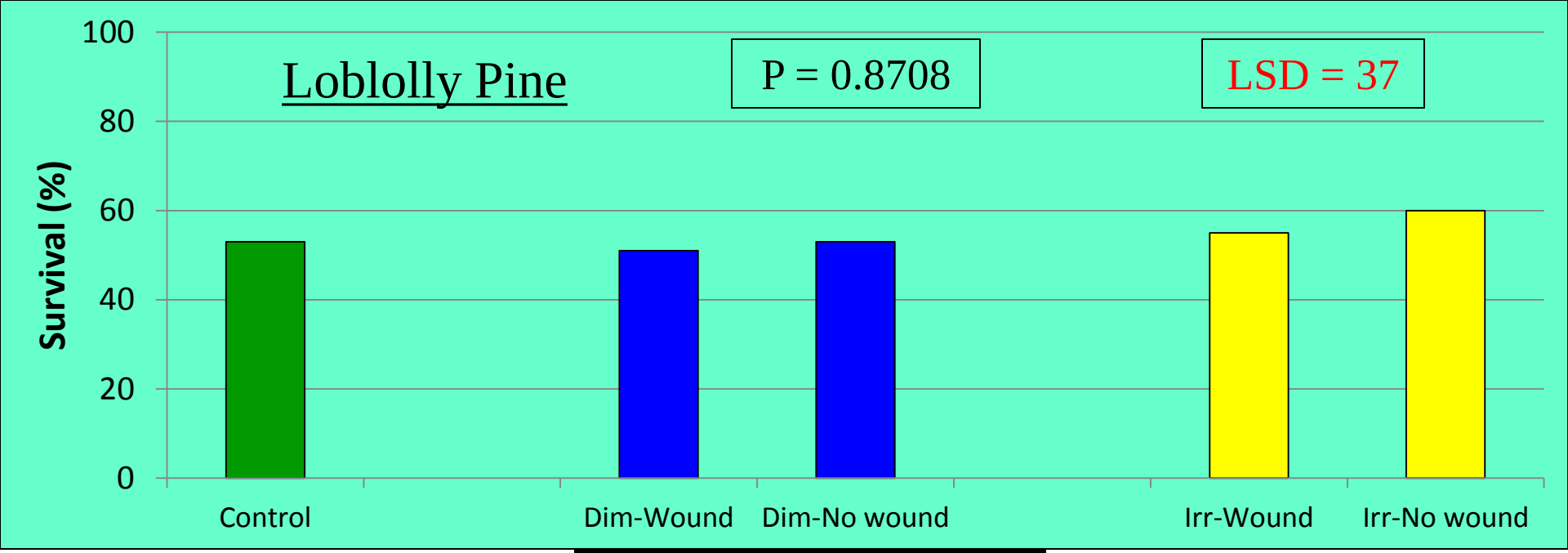
Longleaf Pine Survival-2008



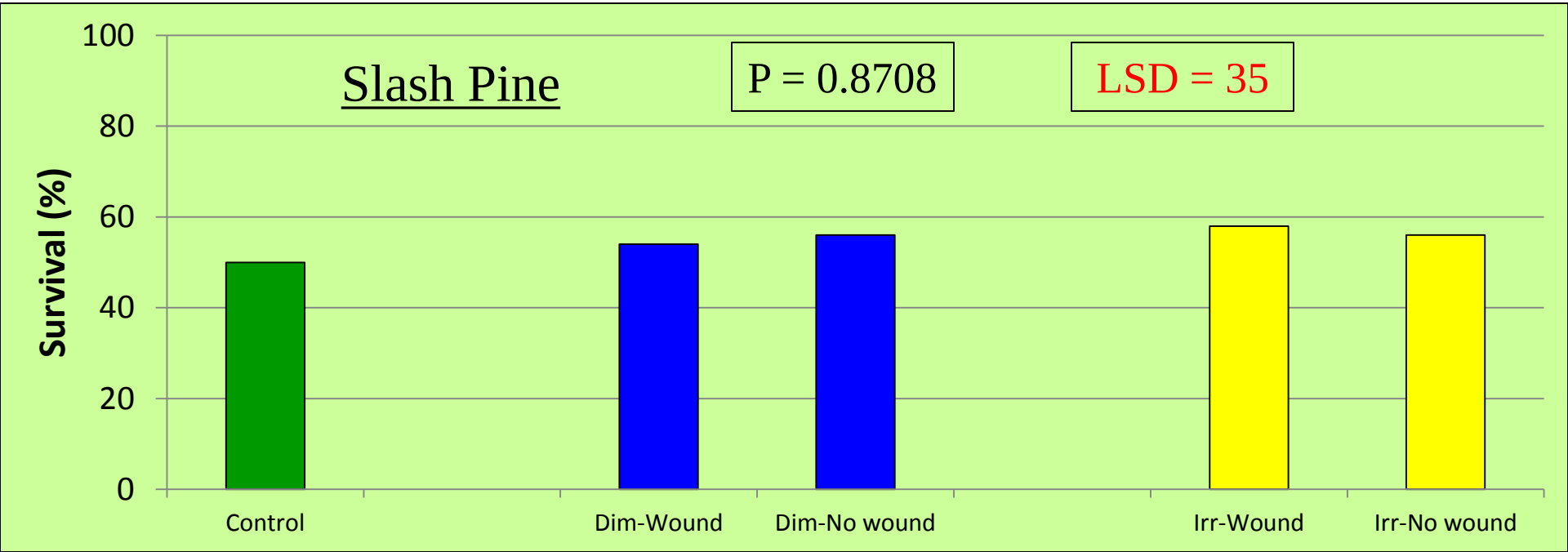


Bareroot Seedlings

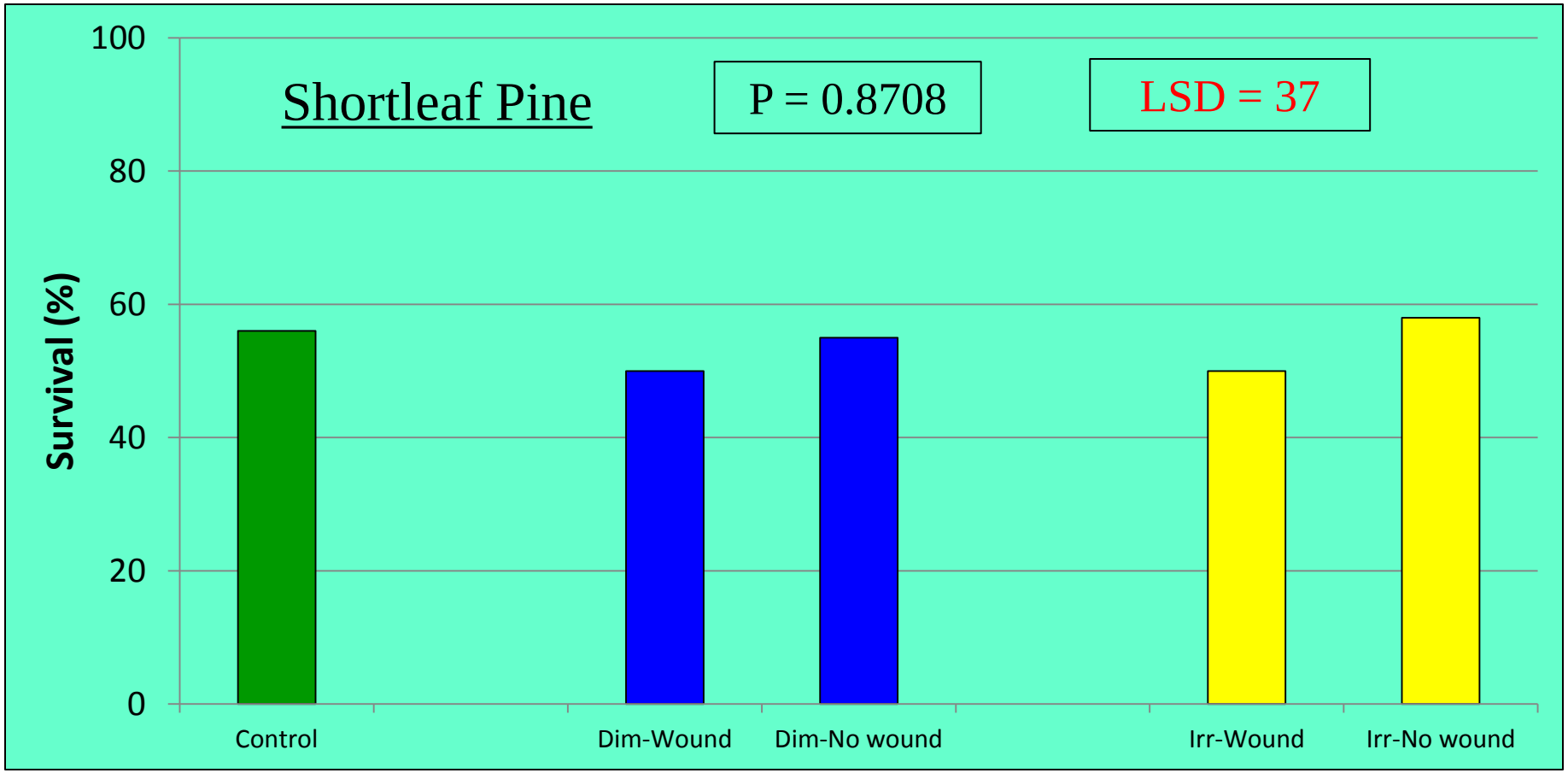




Container Seedlings



Container Seedlings



Conclusions

• Study Objective:

To inoculate loblolly, longleaf, slash, and shortleaf pine seedlings with *Pythium* prior to storage and determine the effect on seedling survival after outplanting.

- *Pythium* reduced bareroot longleaf pine survival.
- *Pythium* did not affect bareroot loblolly or slash pine survival.
- *Pythium* did not affect container-grown seedling survival

(2) Bareroot & Container Seedling Survival in Peat Moss

- Study Objective:

  To inoculate longleaf, loblolly, slash, and shortleaf pine seedlings with *Pythium* before storage in the presence of peat moss and evaluate seedling survival.

Antagonistic fungi present in peat moss may have a suppressive effect against *Pythium*

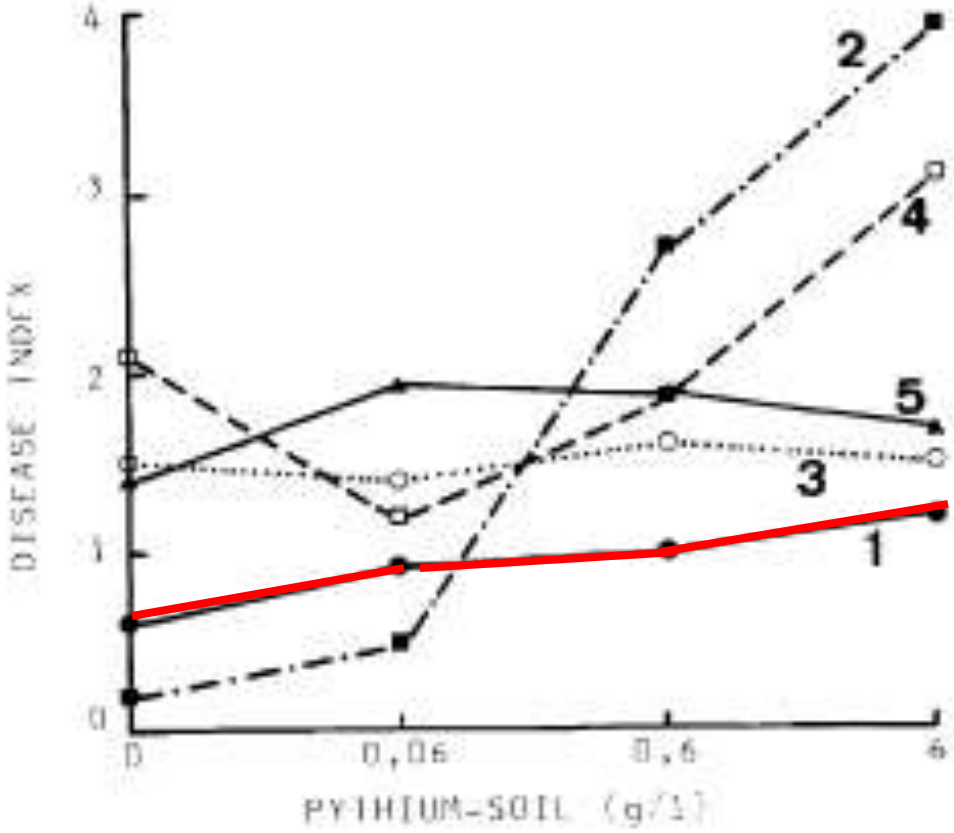


Figure 1 - Screening of 5 peat lots for their receptivity to P y t h i u m disease.

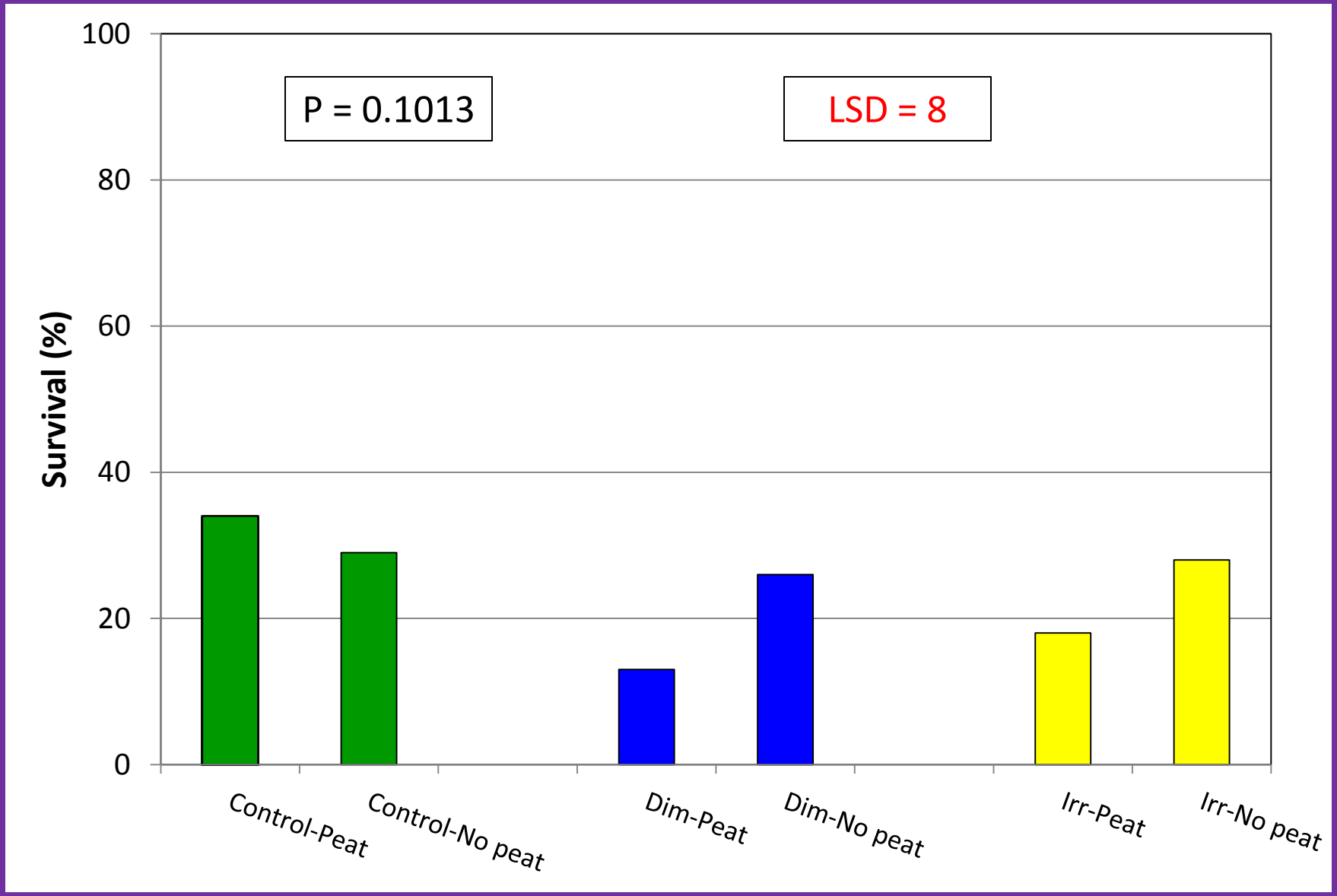
Wolffhechel 1988

Bareroot Loblolly Pine Survival

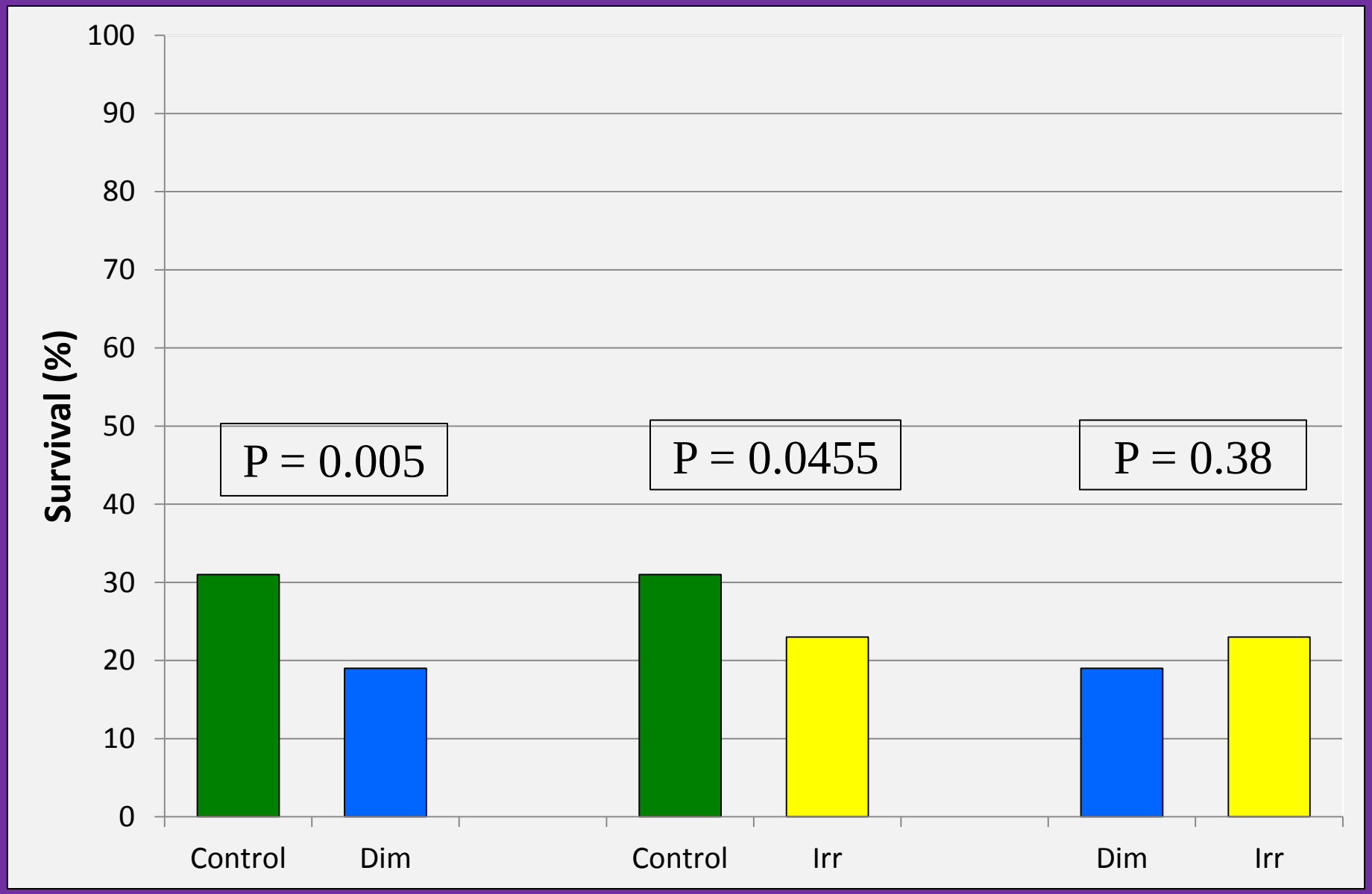
Experimental Design:

- Roots inoculated with 0 or 200 g of *P. dimorphum* and *P. irregulare*
- Packed in a peat-mix (70-80% sphagnum) or no peat-mix
- Storage periods: 4, 8,and 12 wks (6 trts; 4 reps of 30 seedlings/trt)
- Seedlings outplanted after each storage period and survival monitored for 4 months

Loblolly Pine Survival-*Pythium* and Peat Treatments



Loblolly Pine Survival-*Pythium* Treatments



Container Seedlings: Peat vs. Perlite

Pine Species

Loblolly, Longleaf, Slash, & Shortleaf Pine

Media

Peat moss or 100% perlite

Fungi

- *P. dimorphum* or *P. irregulare*
- 1 mL per cell

Root Wounding

Cut one side of the plug or not wounded

Cold Storage

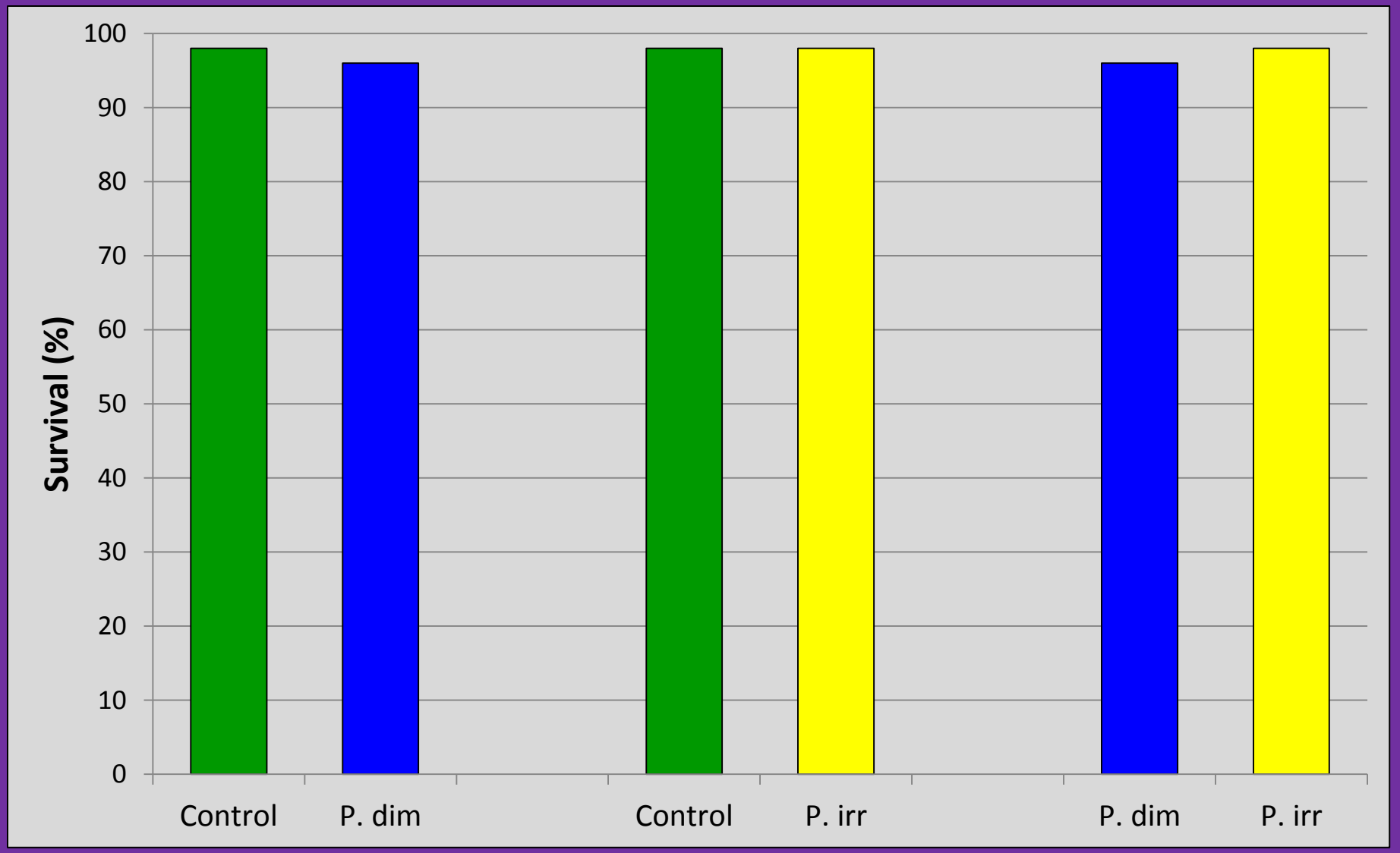
3, 6, or 12 weeks

Loblolly Pine Survival

P = 0.1900

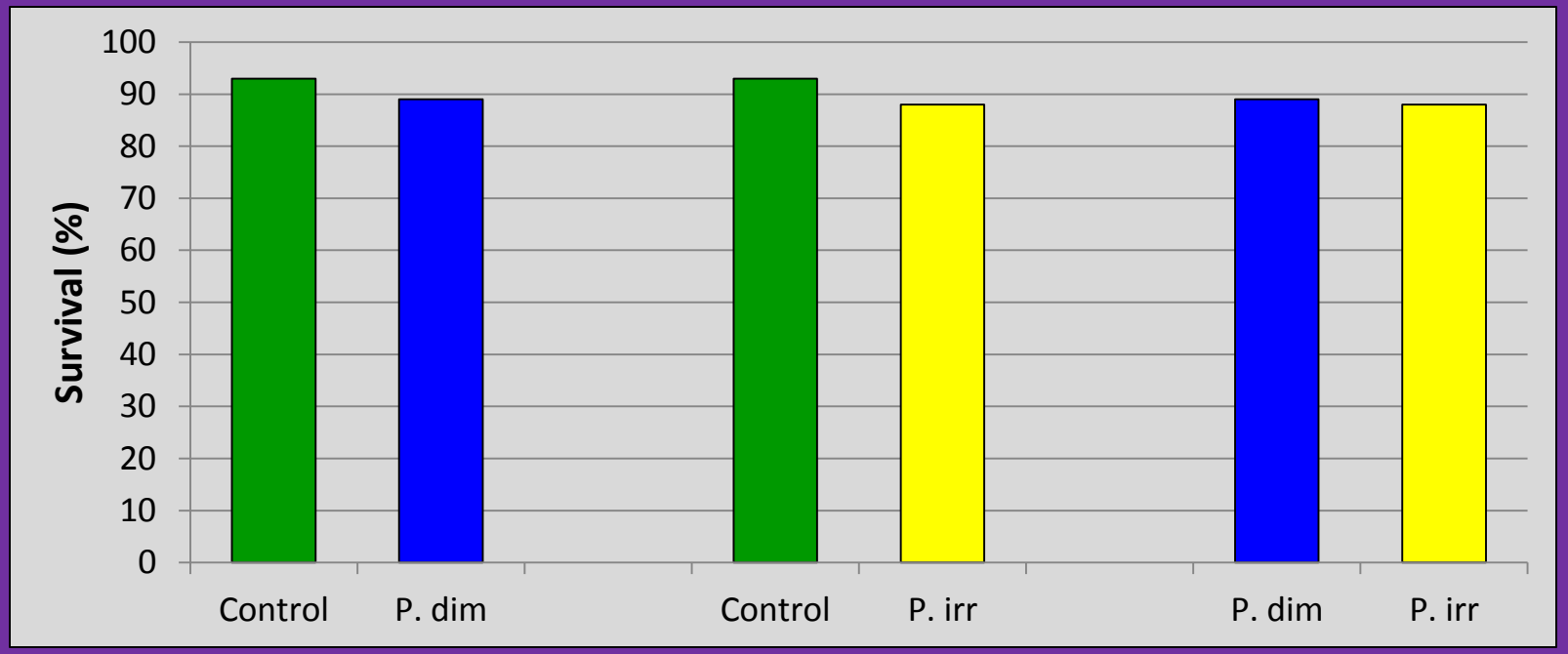
P = 0.6613

P = 0.3814



Longleaf Pine Survival

P = 0.1519 | P = 0.0270 | P = 0.4286



P = 0.0003

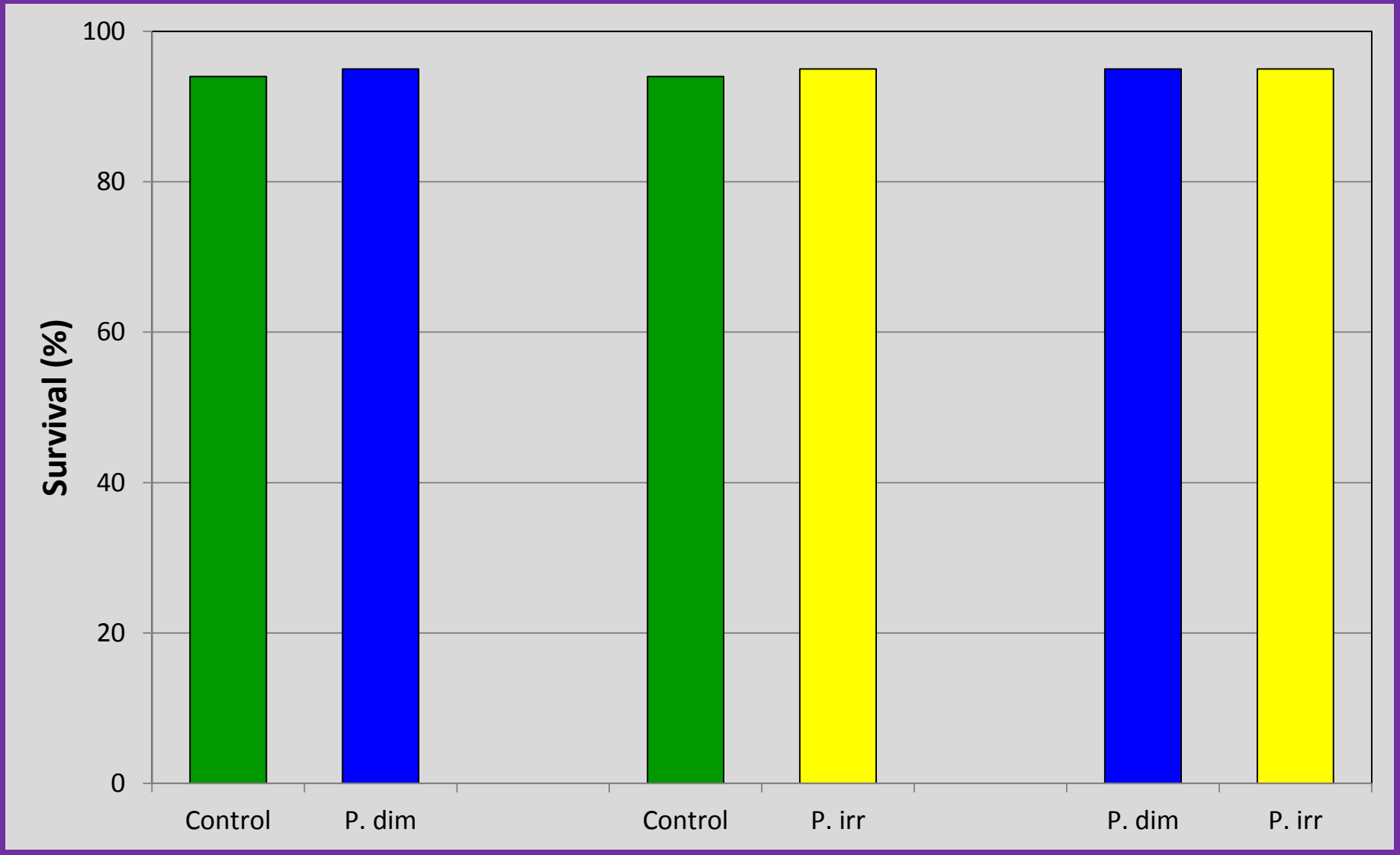
	Survival (%)					
	0 wks		4 wks		8 wks	
	Peat	Perlite	Peat	Perlite	Peat	Perlite
Control	96	100	96	90	82	95
P. dimorphum	71	98	98	95	90	88
P. irregulare	84	98	98	85	62	100

Slash Pine Survival

P = 0.7139

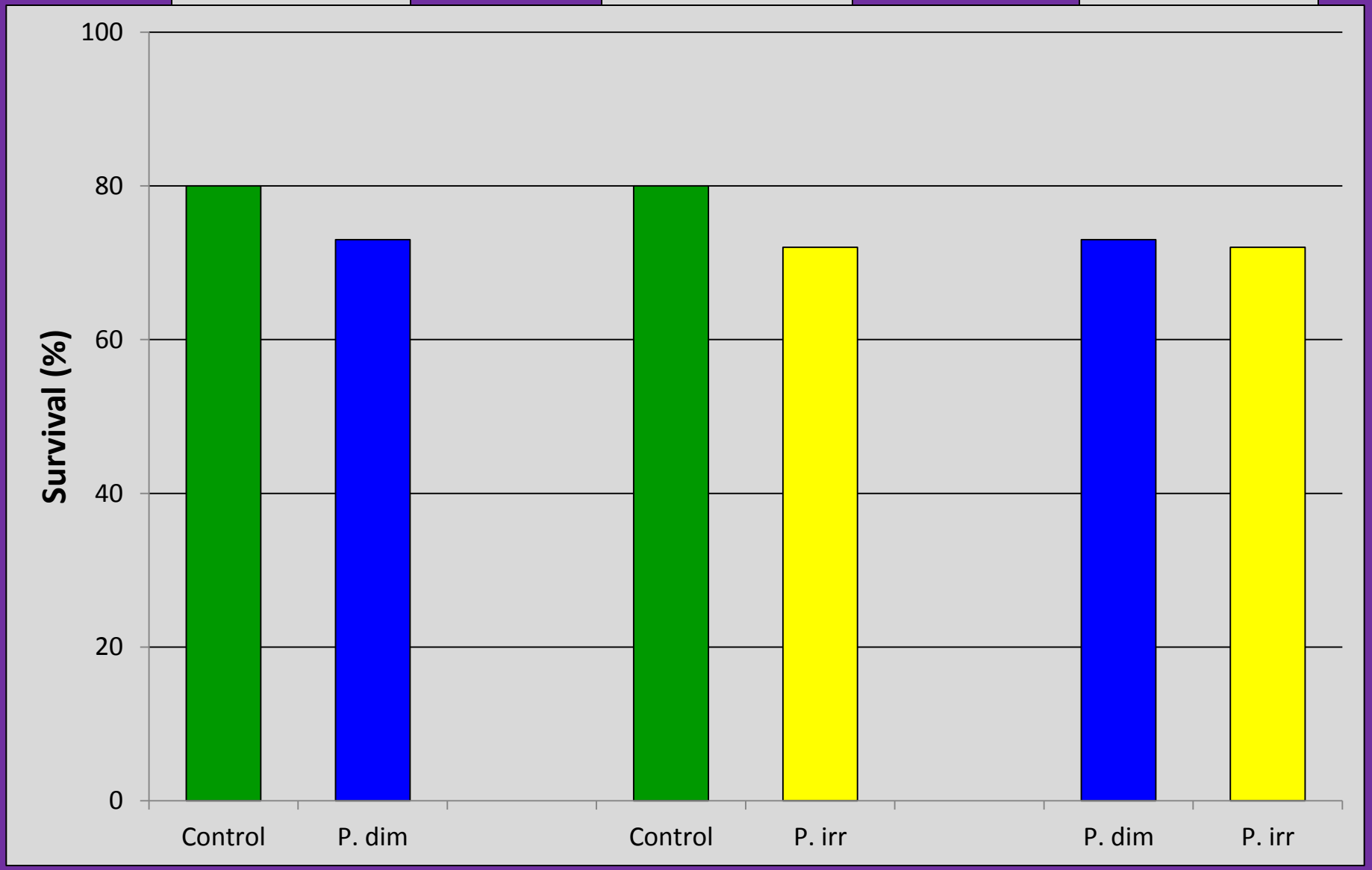
P = 0.5825

P = 0.8545



Shortleaf Pine Survival

P = 0.0794 | P = 0.0482 | P = 0.8195



Conclusions

• Study Objective:

To inoculate longleaf, loblolly, slash, and shortleaf pine seedlings with *Pythium* before storage in the presence of peat moss and evaluate seedling survival.

➢ The peat-mix used did not show an antagonistic effect against *Pythium*.

➢ *P. irregulare* reduced container longleaf pine survival in the peat-mix.

➢ *P. irregulare* reduced container shortleaf pine survival in perlite.

(3) Loblolly & Slash Pine Root Growth Potential

- Study Objective:

  To inoculate loblolly and slash pine seedlings with *Pythium* prior to storage and evaluate the effect on seedling root growth potential (RGP) and survival.

Loblolly and Slash Pine Root Growth Potential (RGP)

Experimental Design:

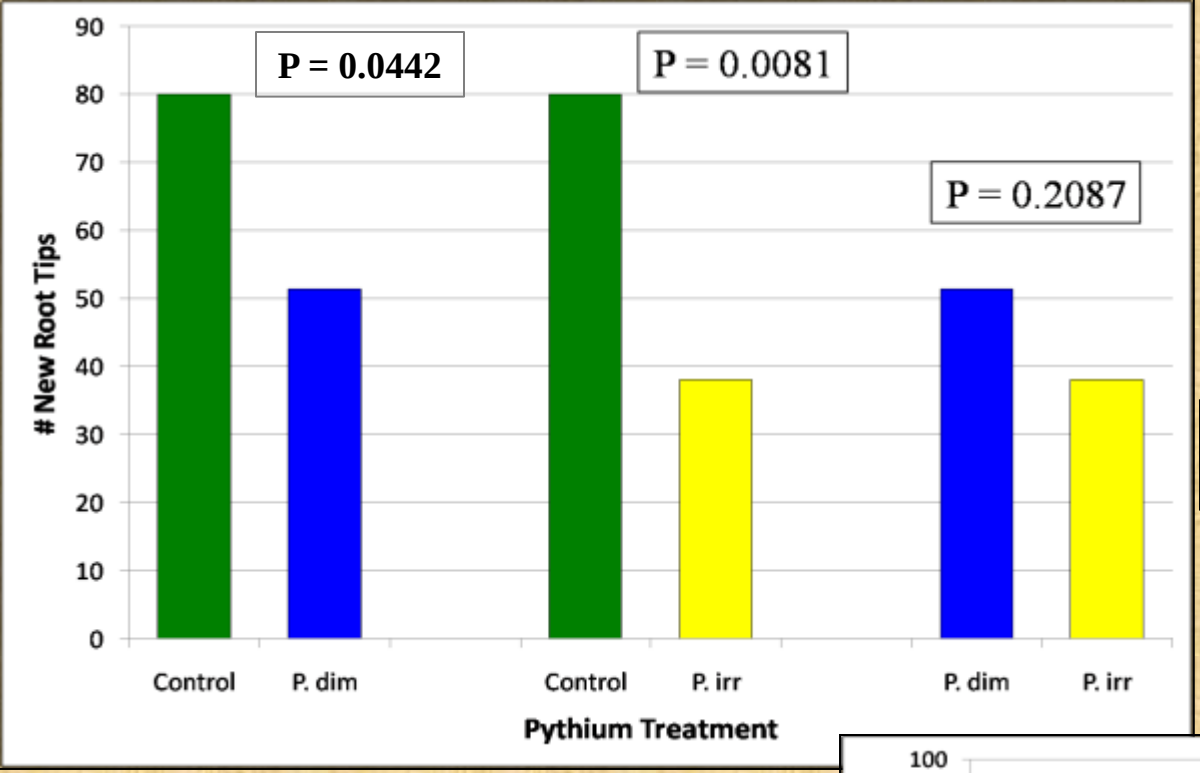
- Inoculated with *P. dimorphum* and *P. irregulare*
- Three levels: 0, 50, and 200 g
- Cold stored for 3 weeks in plastic bags at 4-5°C
- Seedlings placed in aerated aquariums for 28 d
- 3 reps of 15 seedlings/treatment

Measurements:

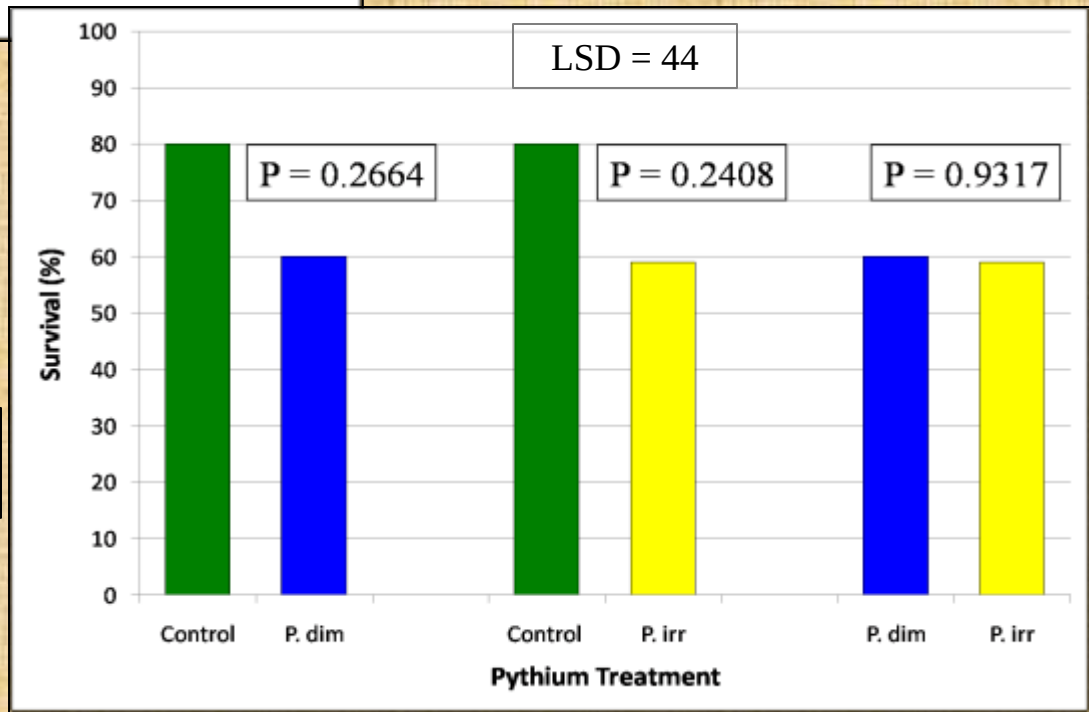
- Root length, diameter, volume, surface area, & number of new white tips measured using a scanner and software (WinRhizo)
- RCD on day 1 and 28
- All seedlings were outplanted on day 34 to monitor survival for 4 months

Loblolly Pine

New Root Tips

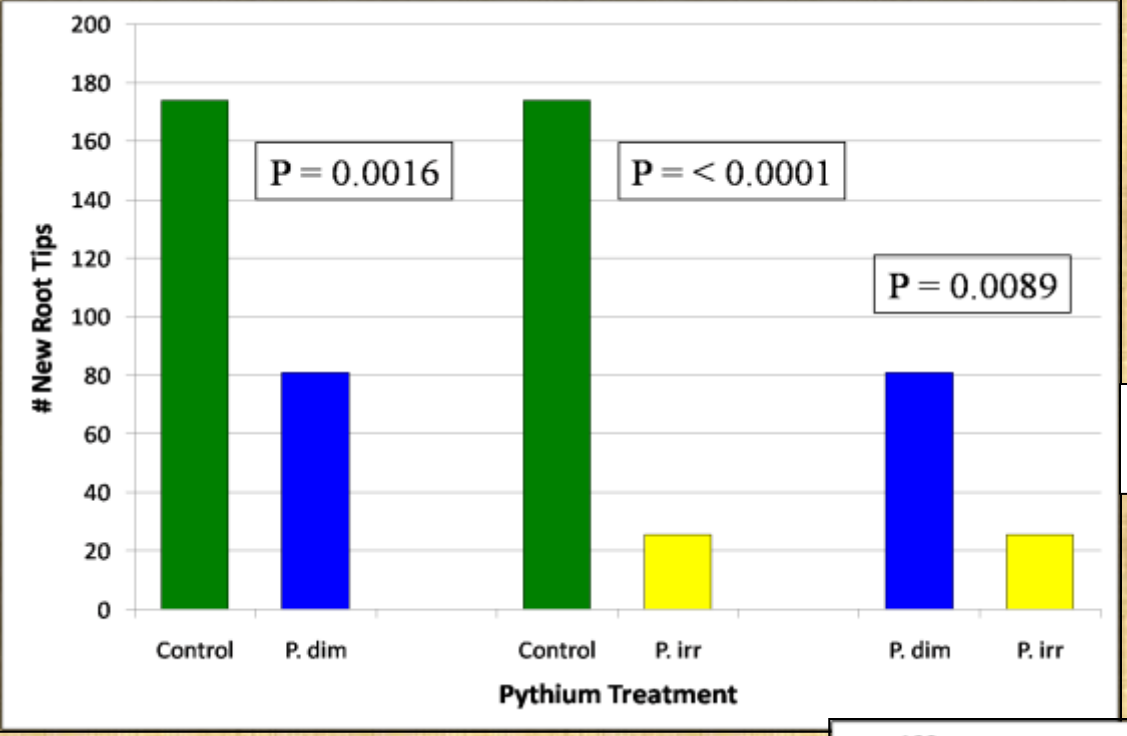


Survival

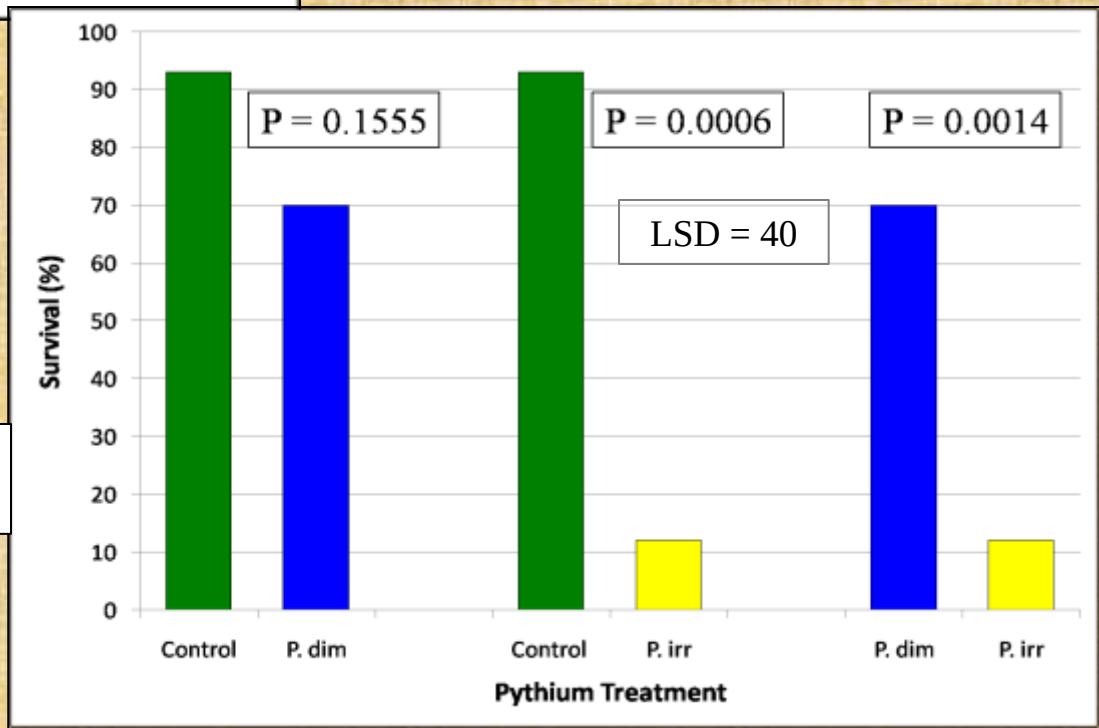


Slash Pine

New Root Tips



Survival




Slash
Control


Slash
Irr50

Conclusions

- Study Objective:

  To inoculate loblolly and slash pine seedlings with *Pythium* prior to storage and evaluate the effect on seedling root growth potential and survival.

➢ *Pythium* reduced loblolly pine RGP but survival was not affected.

➢ *Pythium* reduced slash pine RGP and survival.

(4) *Pythium* Soil Surveys

- Study Objective:

  To collect bareroot nursery soils in the fall (Nov) and winter (Jan) to determine if time of year affects *Pythium* populations.

Nursery Soil Surveys

- Soil collecting kits were mailed to 28 nurseries:

Nov 2008/Jan 2009 (Year 1) & Nov 2009/Jan 2010 (Year 2)

- Soil samples were assayed for *Pythium* spp. on selective media (PARP)

Methods

- 1 g of soil mixed in 100 ml of water/agar mixture
- 1 ml (13 drops) was placed onto PARP media

- 10 plates (10 ml of soil mixture) was assayed per nursery sample

- *Pythium* was quantified as the number of colony forming units (CFUs) per mg of soil.

Survey Results

Year 1	CFUs/mg soil	CFUs/mg soil
	November 2008	January 2009
Total	122,000	41,000
Mean of (+) Nurseries	15,000	5,000

P > T = 0.5774

Year 2	CFUs/mg soil	CFUs/mg soil
	November 2009	January 2010
Total	104,000	110,000
Mean of (+) Nurseries	12,000	8,000

P > T = 0.3492

Pythium Soil Surveys

- Study Objective:

  To collect bareroot nursery soils in the fall (Nov) and winter (Jan) to determine if time of year affects *Pythium* populations.

➢ Year 1-*Pythium* levels were numerically higher in the fall.

➢ Year 2-*Pythium* levels were numerically higher in the winter.

Nurseries without *Pythium*:

Year 1: 19/35

Year 2: 18/39

(5) Proline®-Amended Agar

- Study Objective:

  To determine if Proline-amended agar has an effect on the growth and survival of *Pythium dimorphum*, *Pythium irregulare*, and *Botrytis cinerea*.

Proline® Amended Agar

Proline® has shown promising results for controlling fusiform rust, but could it also control *Pythium* or *Botrytis*?

- Fungal tested: *P. dimorphum*, *P. irregulare*, and *B. cinerea*
- Proline® amended to potato dextrose agar (PDA):

A. Control (no Proline®)

B. ¼ label rate (1.25 fl oz/ac)

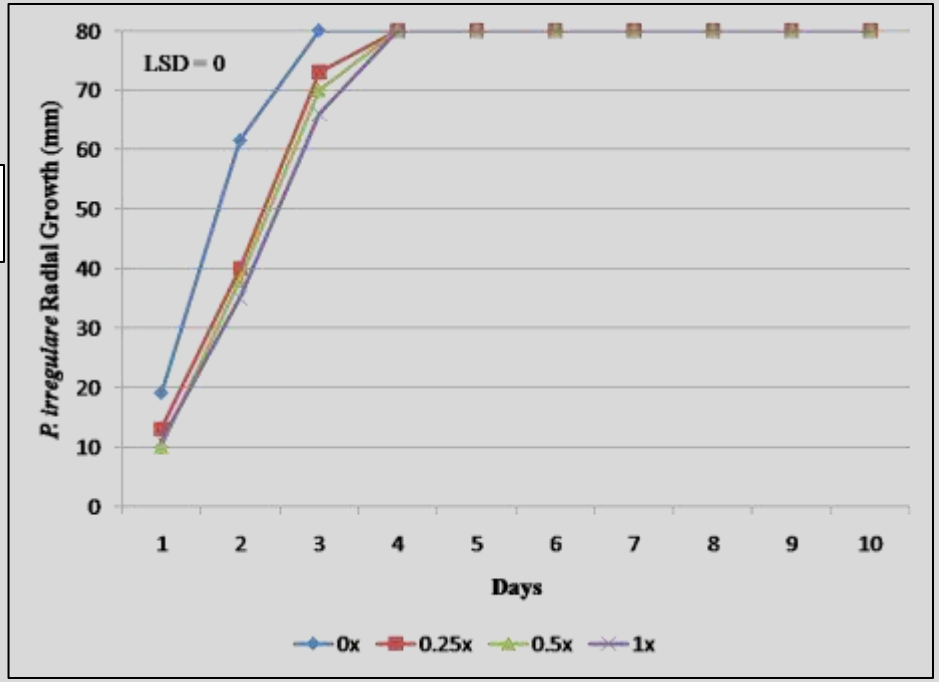
C. ½ label rate (2.5 fl oz /ac)

D. full label rate (5 fl oz/ac)

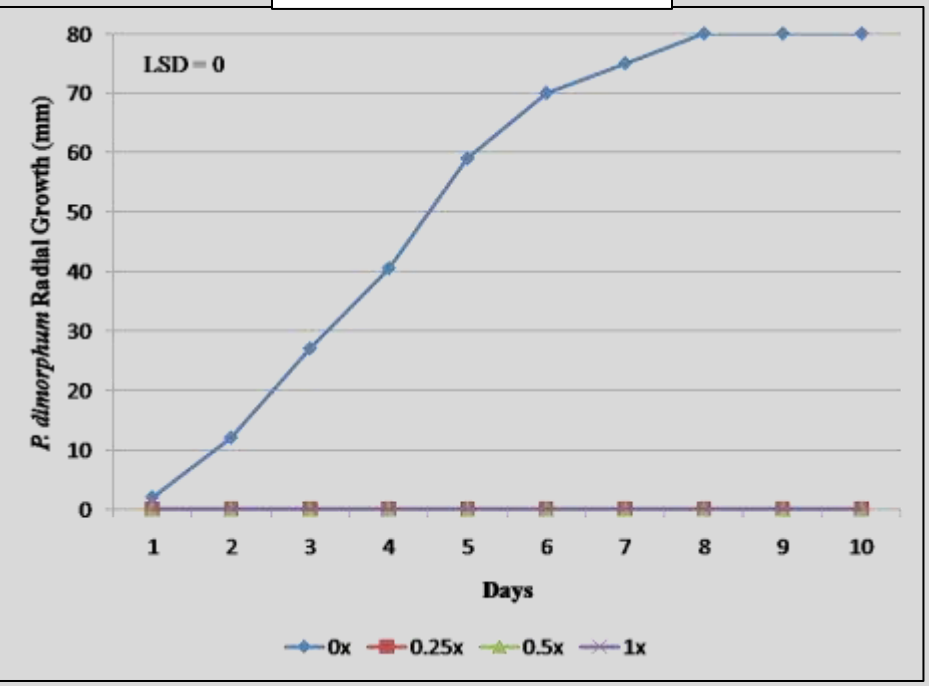
- all rates based on 30 g water/ac
- 13 replicates/rate/fungus

Fungal Growth (mm)

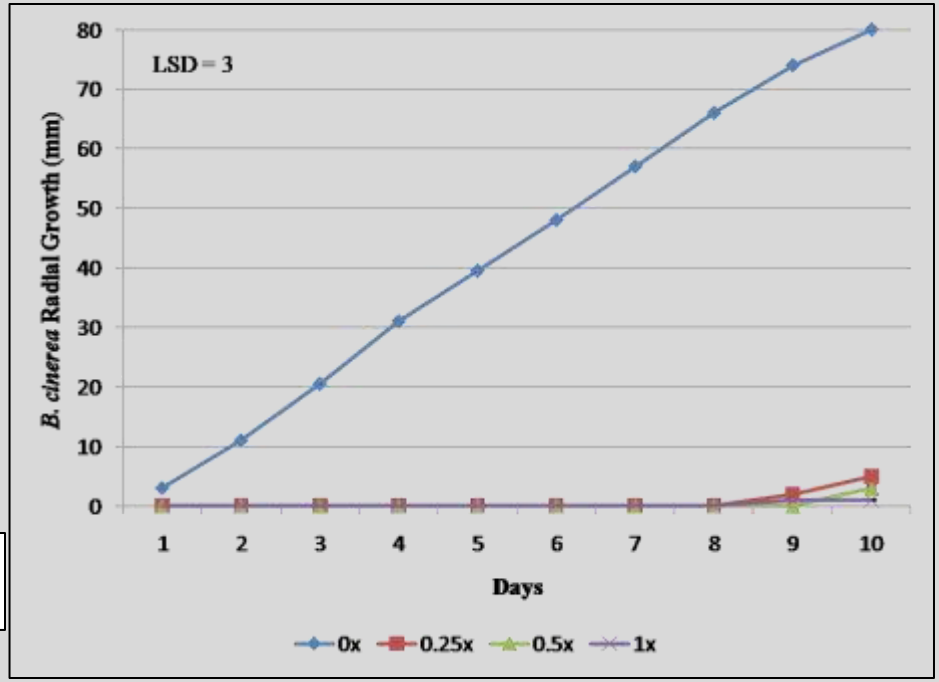
P. irregulare



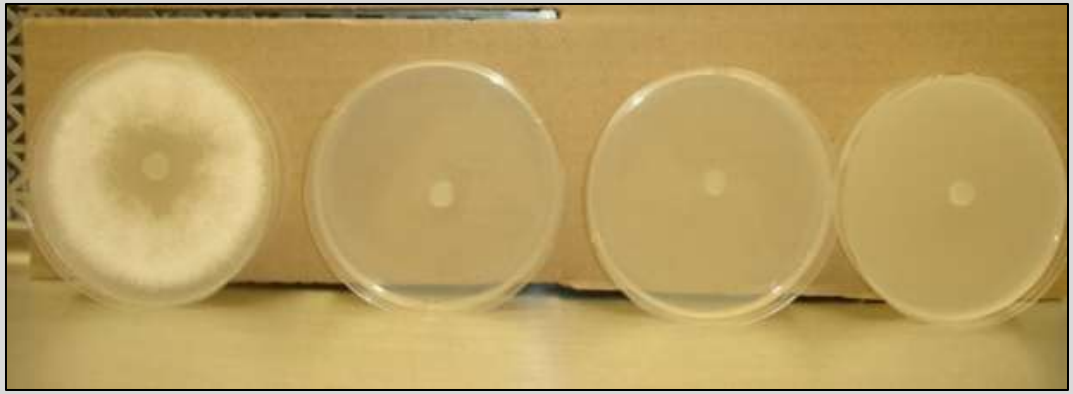
P. dimorphum



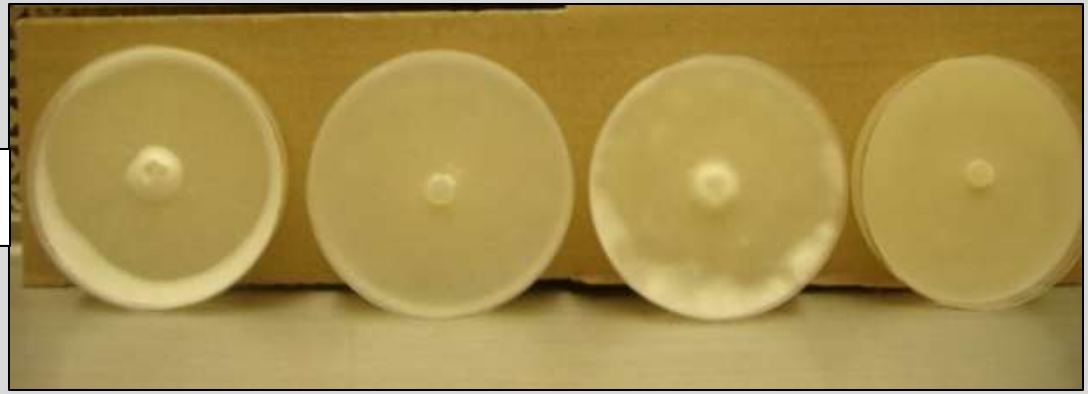
B. cinerea



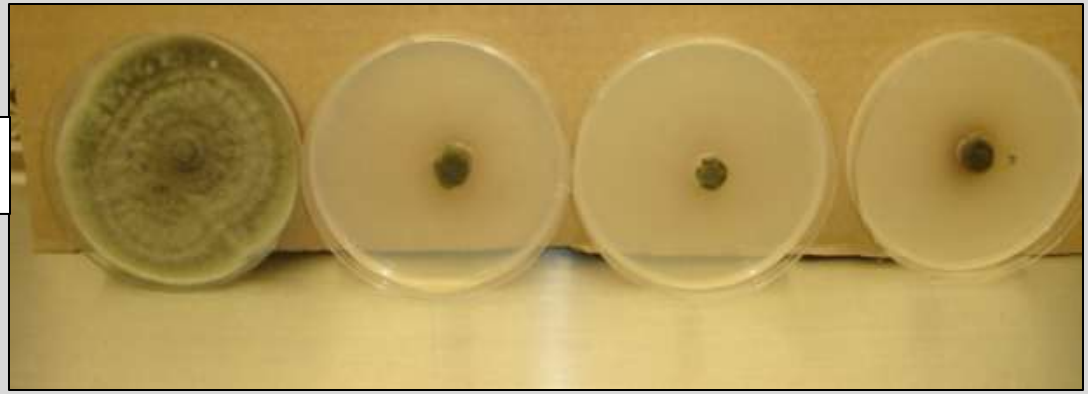
P. dimorphum



P. irregulare

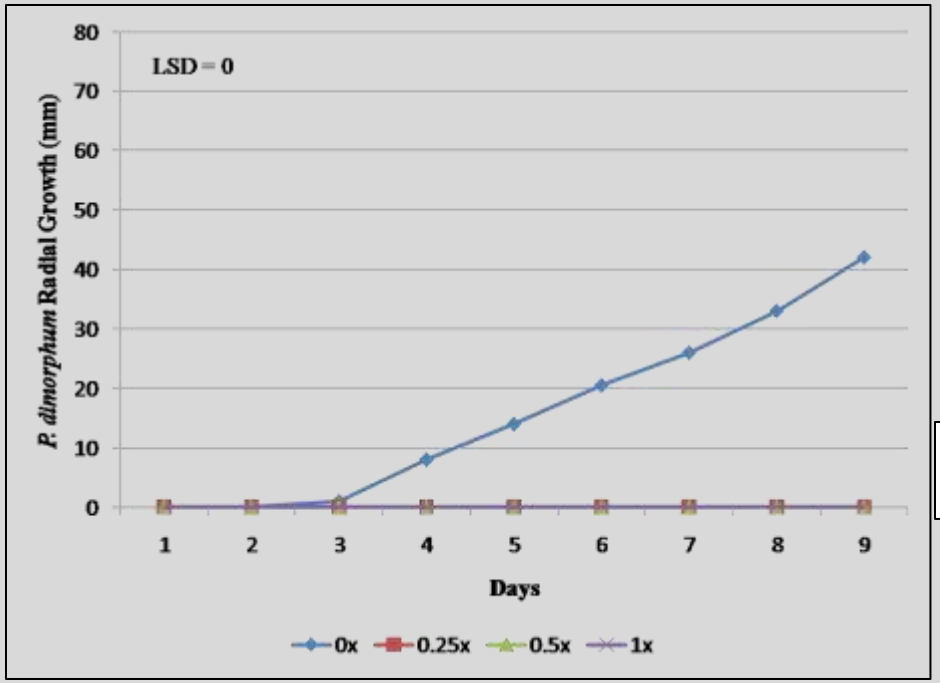


B. cinerea

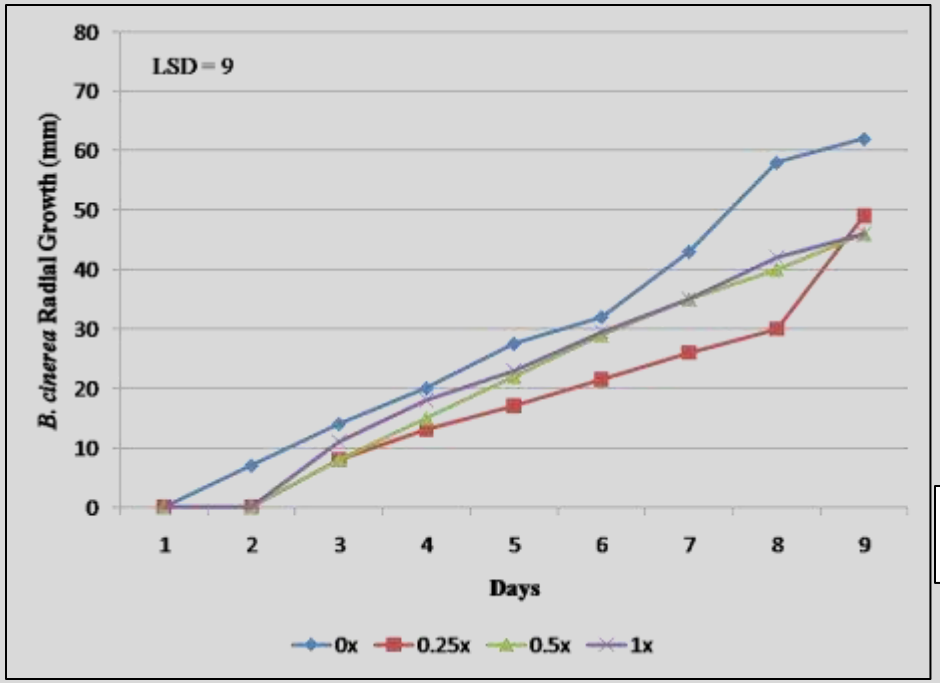


0X 0.25X 0.5X 1X

Fungal Re-growth (mm)



P. dimorphum



B. cinerea

Proline®-Amended Agar

- Study Objective:

  To determine if Proline-amended agar has an effect on the growth and survival of *Pythium dimorphum*, *Pythium irregulare*, and *Botrytis cinerea*.

- Proline® did not affect *Pythium irregulare*.
- Proline® was fungicidal to *Pythium dimorphum*.
- Proline® was fungistatic to *Botrytis cinerea*.

Research Summary

(1) Bareroot & Container Seedling Survival:

- *Pythium* reduced bareroot longleaf pine survival.

(2) Bareroot & Container Seedling Survival in Peat Moss:

- The peat-mix did not provide an antagonistic effect against *Pythium*.
- *P. irregulare* reduced survival of container longleaf in peat and shortleaf in perlite.

(3) Loblolly & Slash Pine Root Growth Potential:

- *Pythium* reduced loblolly pine RGP but did not affect survival.
- *Pythium* reduced slash pine RGP and survival.

Research Summary (cont.)

(4) *Pythium* Soil Surveys:

- Time of year did not affect *Pythium* populations.
- *Pythium* numbers varied between seasons.

(5) Proline-Amended Agar:

- Proline's ability to control *Pythium* was species specific.

Future Research?

- Would fungicide applications to seedlings before lifting improve survival after storage?

- Would fungicide applications in storage improve survival of *Pythium*–inoculated seedlings?

- Could mycorrhizae play a role in the protection of container-grown seedling roots?

- Could *Pythium* affect survival differently in a 100% peat moss media or with peat moss at different levels of decomposition?

Thank You

- Southern Forest Nursery Management Cooperative
- Dept. of Horticulture and School of Forestry and Wildlife Sciences
- Forest Health Dynamics Laboratory

- Committee Members:
  - Dr. Scott Enebak (Co-chair)
  - Dr. David South
  - Dr. Charles Gilliam (Co-chair)
  - Dr. Joseph Eakes

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  - David Dyson
  - Ben Whitaker
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Questions?