Guidelines to minimize *Phytophthora* species pathogens in restoration nurseries: rationale and review

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Outline

1. Big picture rationale

    -Why the concern?

2. Overview major points of the Phytophthora Working Group doc.:

    “Guidelines to Minimize Phytophthora Pathogens in Restoration Nurseries”
    brief reasoning behind each

3. A closer look at specific aspects of guidelines:

    -Table height (Section 6.3. Benches and growing areas)

    -Buy-ins (Section 8. Special note)

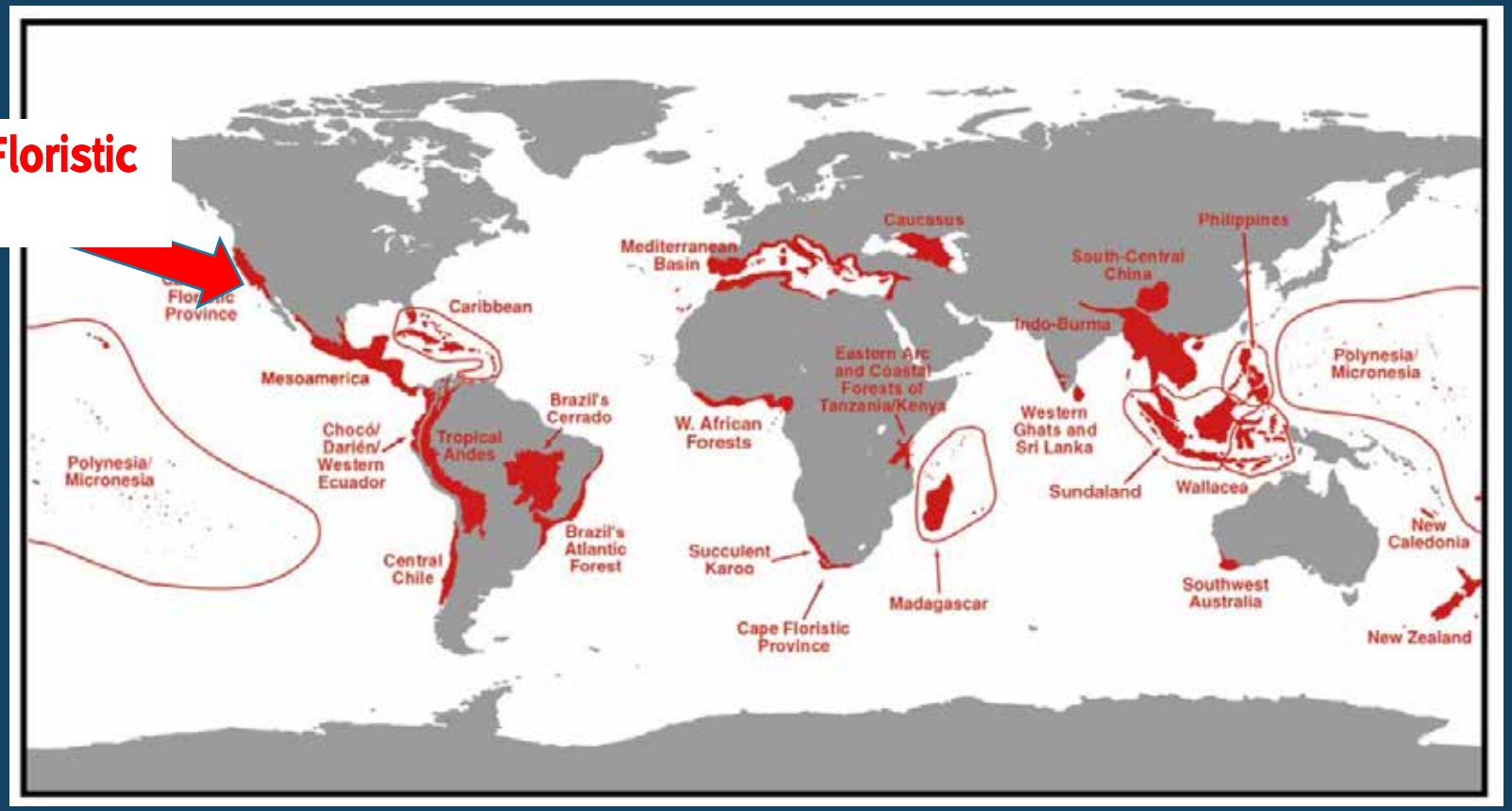
    -Soil pasteurization (Section 4, and 10.4.)

4. Example of how well Phytophthora does *without* Best Practices as a rationale for use:

    -Real examples from two California nurseries that provides restoration nursery stock

1. Big Picture Rational: Importance of Maintaining CA Biodiversity

California Floristic Province



Threats to biodiversity loss

1. Habitat loss from urbanization and overexploitation
2. Climate Change
3. Invasive species (includes *Phytophthora*)

Myers et al. 2012, Nature

Mediterranean biomes are considered particularly at risk to biodiversity loss especially in area with high human activity (2000, Sala et al. 2000, Science)
Threats from forest pathogens in particular Garbelotto & Pautasso (2012 Eur. Journal Plant Path.)

Phytophthora pathogens increasingly in production
Greater species richness & diversity in nurseries sources than wildlands sources

Nurseries vs. Wildlands


Forest Pathology
For. Path. 46 (2016) 134–163
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doi: 10.1111/efp.12239

Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases

T. Jung[1,2,30], L. Orlikowski[3], B. Henricot[4], P. Abad-Campos[5], A. G. Aday[6], O. Aguin Casal[7], J. Bakonyi[8], S. O. Cacciola[9], T. Cech[10], D. Chavarriaga[11], T. Corcobado[12], A. Cravador[1], T. Decourcelle[13], G. Denton[4], S. Diamandis[14], H. T. Doğmuş-Lehtijärvi[15], A. Franceschini[16], B. Ginetti[16], S. Green[17], M. Glavendekic[18], J. Hantula[19], G. Hartmann[20], M. Herrero[21], D. Ivic[22], M. Horta Jung[2], A. Lilja[19], N. Keca[23], V. Kramarets[23], A. Lyubenova[24], H. Machado[25], G. Magnano di San Lio[26], P. J. Mansilla Vazquez[27], B. Marçais[27], I. Matsiakh[23], I. Milenkovic[28], S. Moricca[16], Z. A. Nagy[8], J. Nechwatal[29], C. Olsson[19], T. Ostadjo[30], A. Pane[9], E. J. Paplomatas[31], C. Pinto Varela[1], S. Prospero[32], C. Rial Martinez[1], D. Rigling[33], C. Robin[13], A. Rytkönen[19], M. E. Sanchez[21], A. V. Sanz Ros[34], B. Scanu[35], A. Schlenzig[36], J. Schumacher[29], S. Slavov[24], A. Solla[12], E. Sousa[37], J. Stenlid[19], V. Talgø[15], Z. Tomic[22], P. Tsopelas[31], A. Vannini[16], A. M. Vettraino[16], M. Wenneker[38], S. Woodward[17] and A. Perez-Sierra[17]

Jung et al. 2016, Forest Pathology

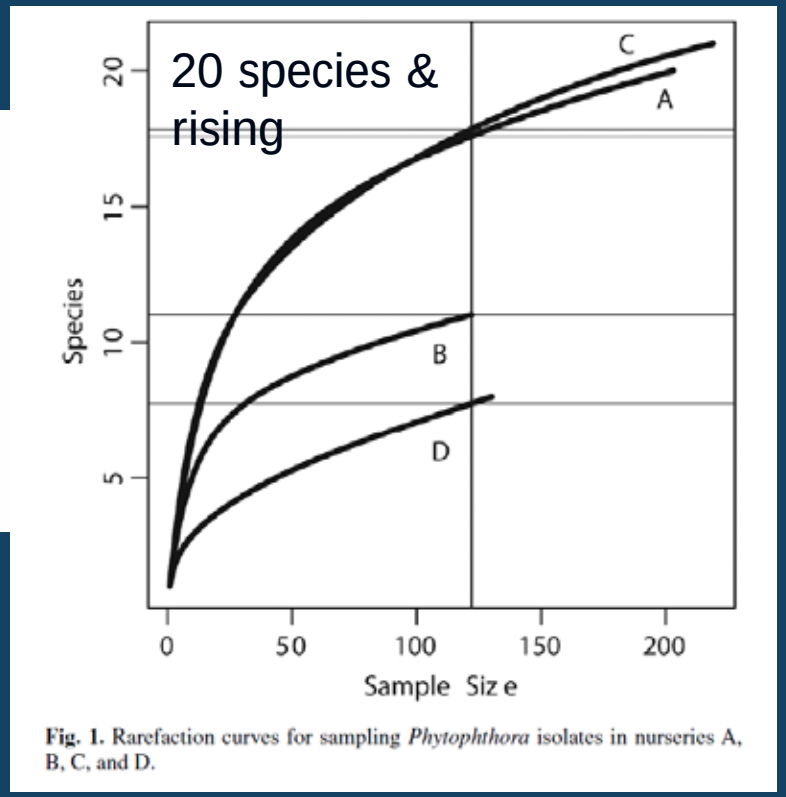
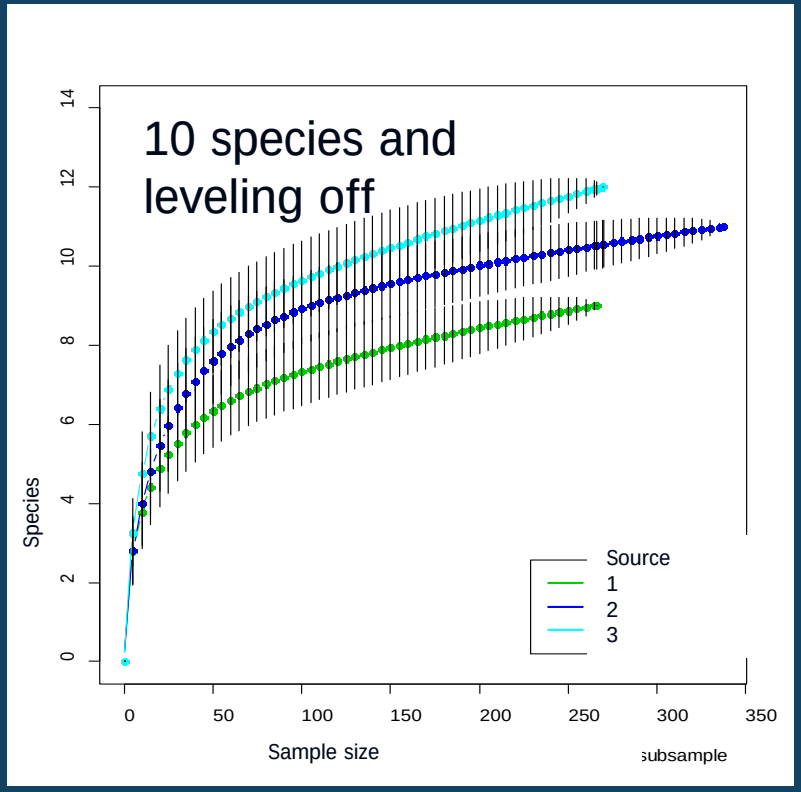


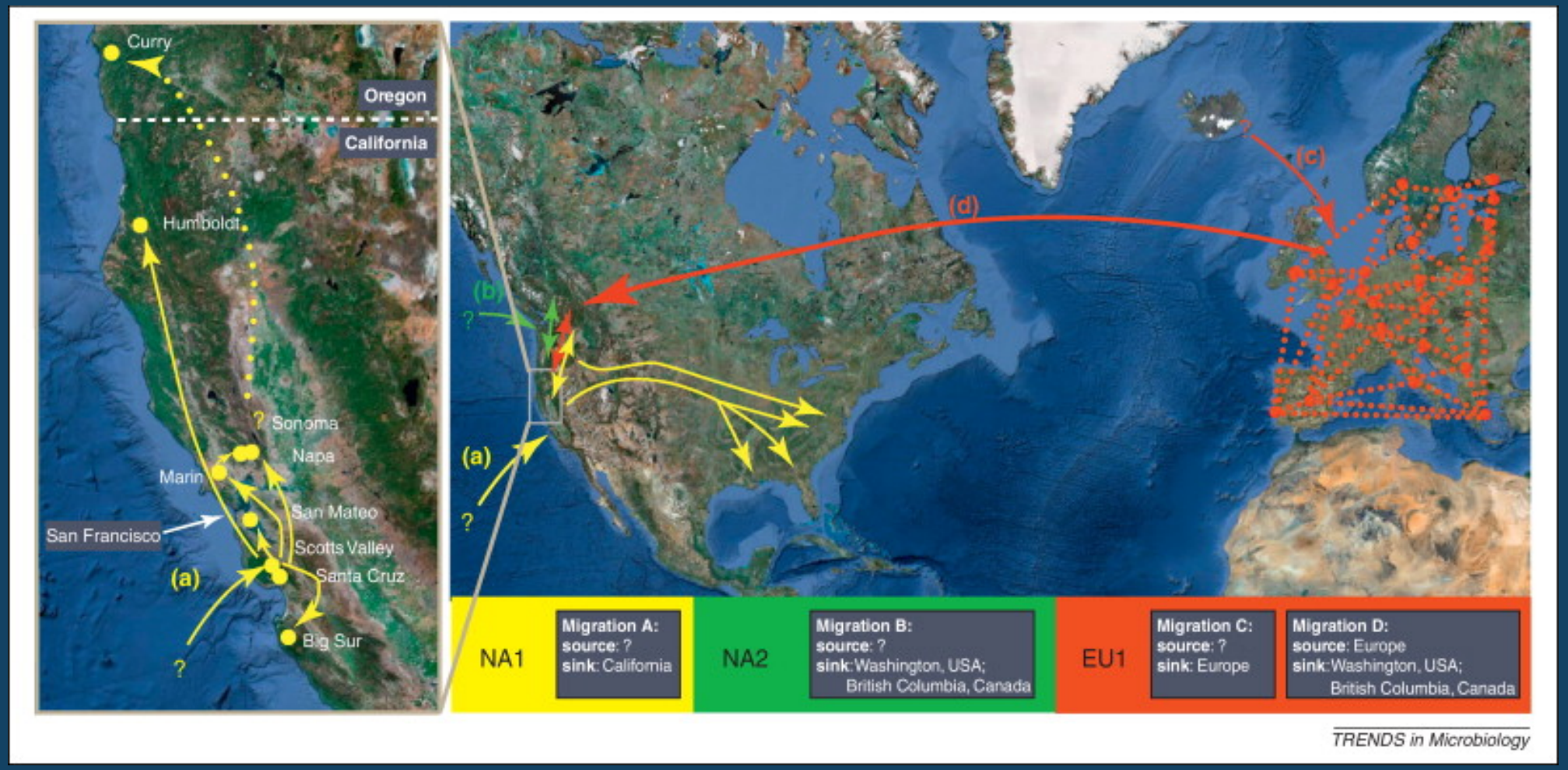
Fig. 1. Rarefaction curves for sampling *Phytophthora* isolates in nurseries A, B, C, and D.

Parke et al. 2014, Phytopathology



Sims et al. 2015, Mycologia

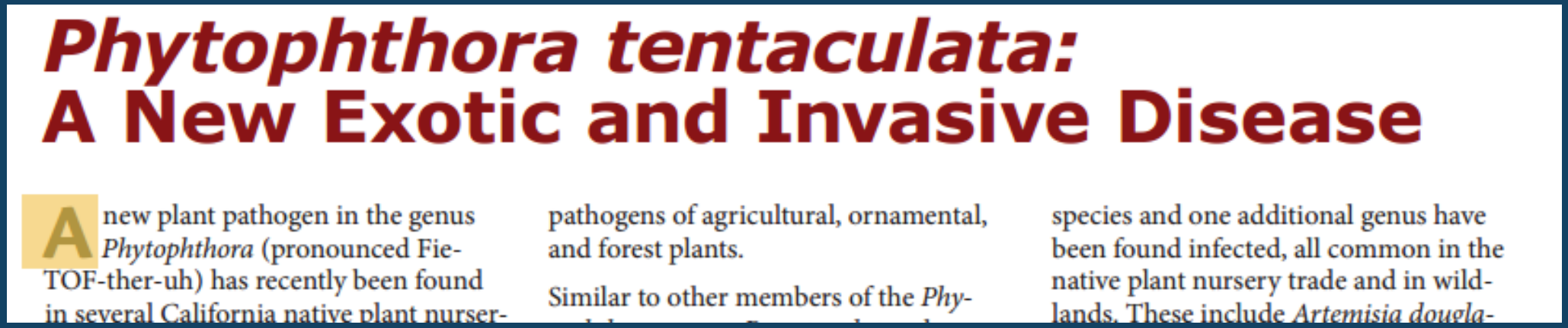
How Phytophthora gets around **Example**- Industry related movement of *Phytophthora ramorum*



Grünwald & Garbelotto et al. 2012

Costs to manage once they are in wildlands are very high

- In just one 19,000 hectare watershed
- Estimated to cost $45 million to clean up and start over
- And we are planting them in wildlands with nursery stock!



Phytophthora tentaculata: A New Exotic and Invasive Disease

A new plant pathogen in the genus *Phytophthora* (pronounced Fie-TOF-ther-uh) has recently been found in several California native plant nurser-

pathogens of agricultural, ornamental, and forest plants.

Similar to other members of the *Phy-*

species and one additional genus have been found infected, all common in the native plant nursery trade and in wild-lands. These include *Artemisia dougla-*

Phytophthora- Microscopic fungal-like organism that produces spores and hyphae (unrelated to true Fungi)

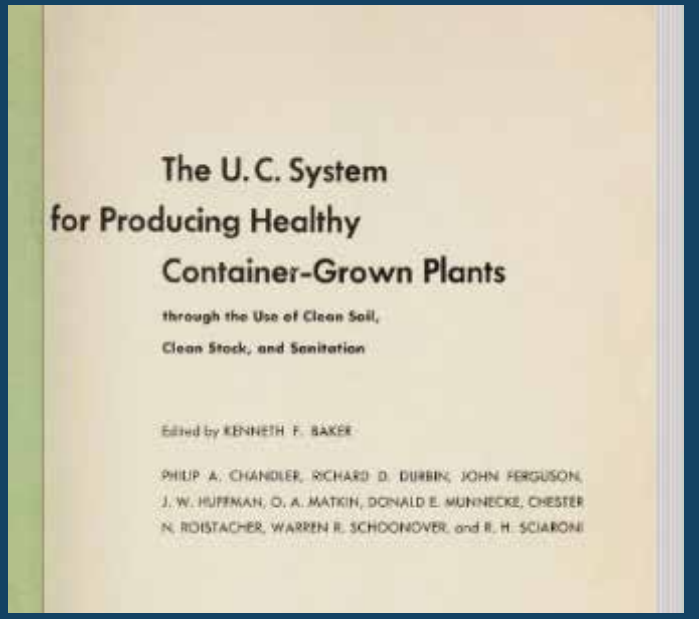
Zoospore
image:Judelson 2005

All others : Laura Sims

(2) Document Overview- *"Guidelines to Minimize Phytophthora Pathogens in Restoration Nurseries"*

- The goal of these guidelines is to help you design and maintain a nursery system that excludes *Phytophthora* and other pathogens and corrects problems if they are found
- From beginning to end- start clean and stay clean

  - *CalPhytos.org*


The U. C. System
for Producing Healthy
Container-Grown Plants
through the Use of Clean Soil,
Clean Stock, and Sanitation
Edited by KENNETH F. BAKER
PHILIP A. CHANDLER, RICHARD D. DURBIN, JOHN FERGUSON, J. W. HUFFMAN, O. A. MATKIN, DONALD E. MUNNECKE, CHESTER N. ROISTACHER, WARREN R. SCHOONOVER, and R. H. SCIARONI

Clean planting materials (Section 2)

Objective: Start with propagative material that is free from infection or external contamination by *Phytophthora* species as well as other pathogens.

Don’t bring in pathogens to the sites

- Once they are there difficult or impossible to remove

Make sure propagation collection site material is healthy and free from debris

- Diseased material brought from the field will allow pathogens to thrive in your nursery if propagated in the nursery

-Increase chance of crop success

-Decrease chance of growing contaminated crop from the start

-Keep source sites healthy

Clean containers (Section 3)

Objective: Use only clean containers

Why? Eliminate this as a source of pathogens

- Dirty containers can have pathogen ‘seed’ source (spores)
- Dirty containers can start epidemics in nurseries if used to grow plants in
  - Spores remaining in containers act as a disease source

Clean potting media (Section 4)

Objective: All potting media must be pathogen free and be handled and stored in a manner that precludes contamination

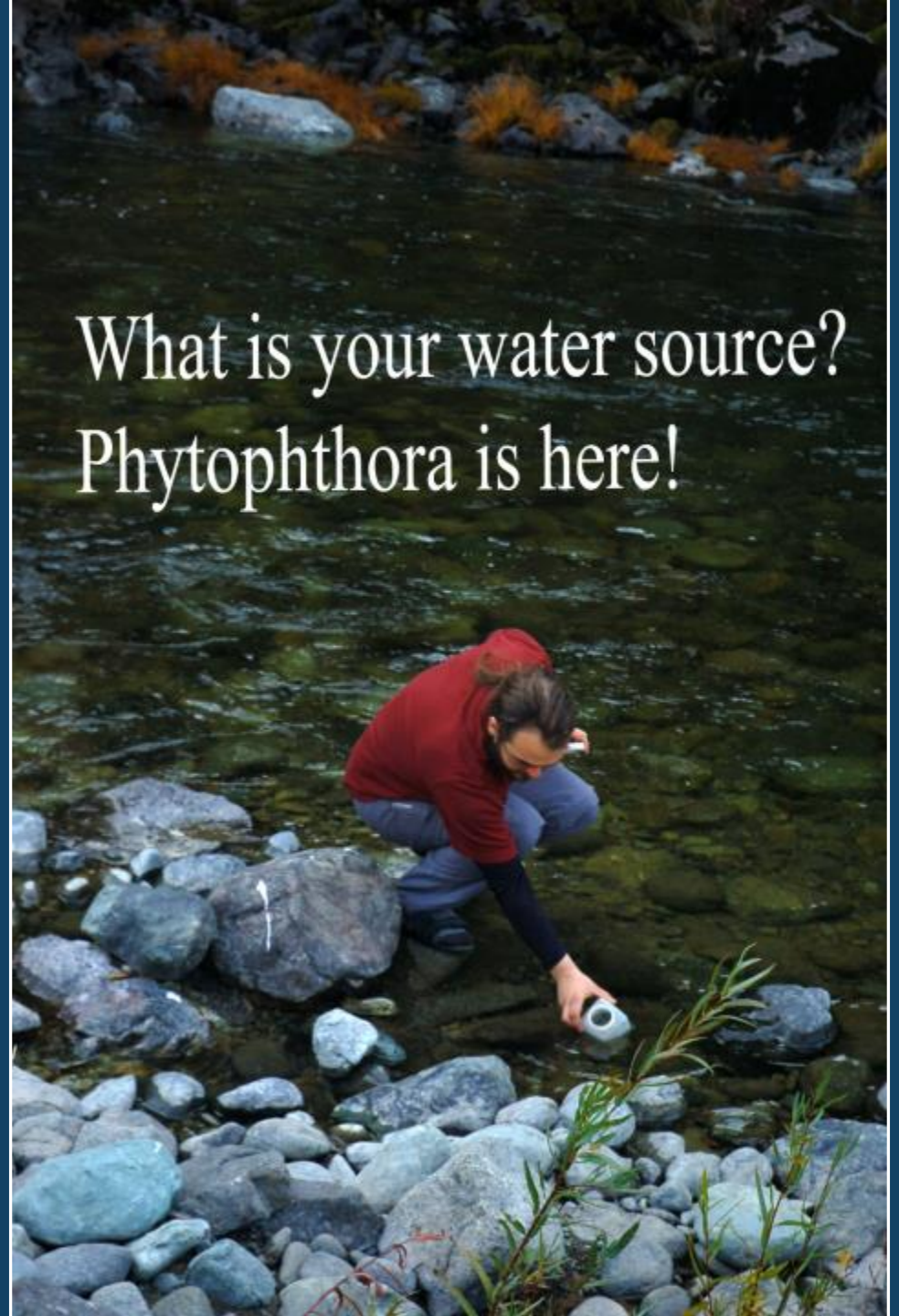
- Pathogens do **not** spontaneously generate
- Resting spores of survive years in soil / debris making it extremely important to remove before use, or to be from a source that does not contain pathogens

Boiler used to generated steam

Clean water (Section 5)

Objective: Use only uncontaminated, appropriately treated water for irrigation

Untreated water may contain *Phytophthora* sporangia and swimming zoospores
Know your water source: Watering plants with *Phytophthora* zoospores and sporangia can lead to infestation problems



Irrigation practices and management (Section 6.5)

- How much water are you using?
  - Excessive water provides a favorable environment for *Phytophthora* to grow and reproduce by zoospores
  - Overwatering can help to spread Phytophthora by creating more opportunites by splash contact and runoff
  - Underwatering stresses plants making them susceptible to even weak pathogens
  - Grouping plants on benches based on watering needs can help to reduce waste, improve plant growth and discourage *Phytophthora*
  - Water moving from plant to plant can move *Phytophthora* and *Pythium* too
  - Over wet conditions support other plant pathogens like *Fusarium* and support an enviroment that is good for fungus gnats which grow and move fungi including plant pahtogenic ones like *Phoma*, *Fusarium* and *Pythium*

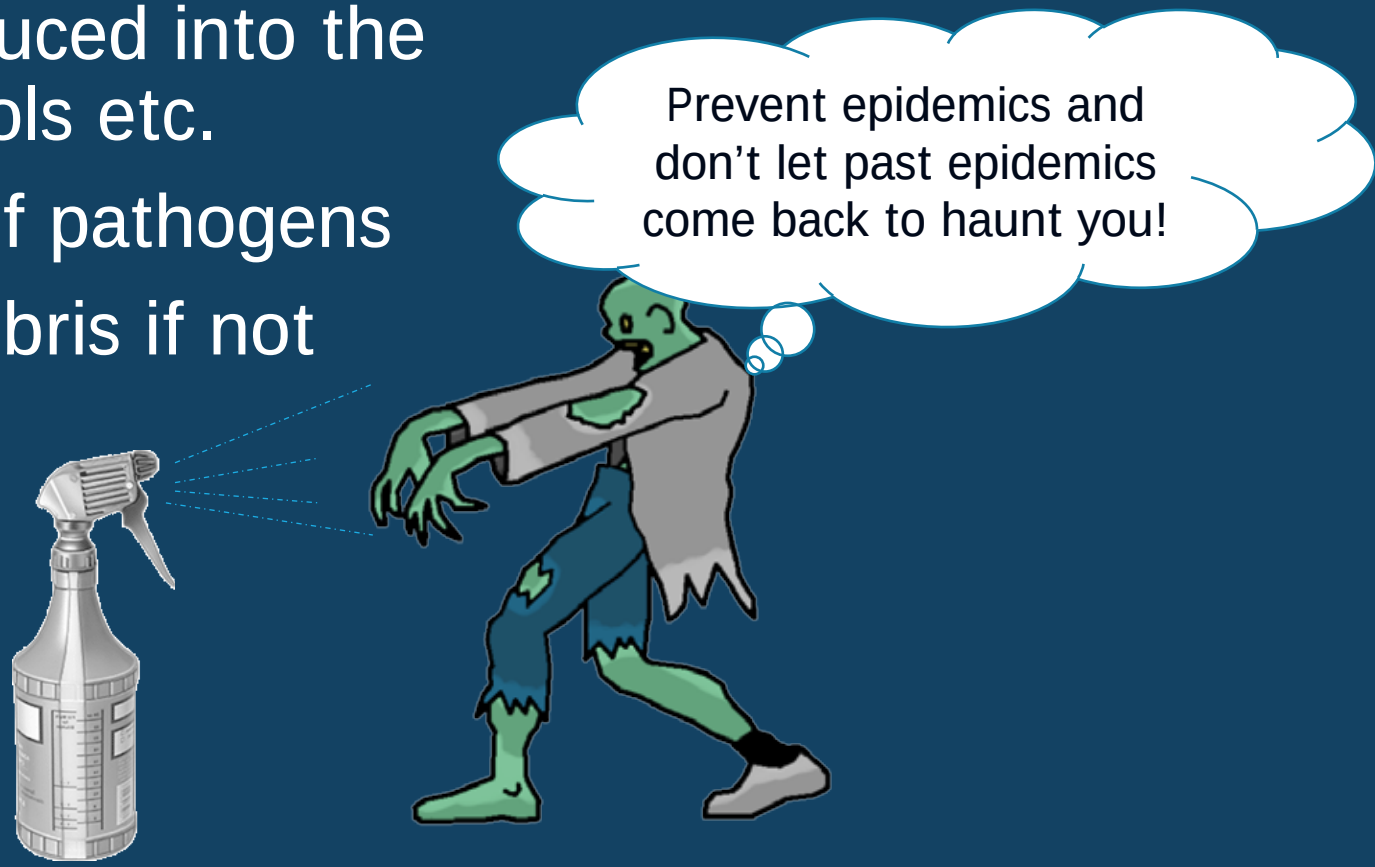
Nursery design layout and workflow (Section 6.2)

- Layout is really important, think about beginning, end and movement through the nursery
- Water from outside nursery should not flow into your nursery
  - water can carry pathogen propagules like zoospores
- Outside plants should not overhang nursery building
  - overhanging material may contain vectors or the pathogen itself spreading it into the nursery –or vice versa a contaminated nursery can move disease outside of the nursery this way
- Propagation and growing areas need to be away from cull pile and discard areas
  - if there was any pathogen in your plant material they may grow in the dead and dying material and then produce resting spores. This material can help to start epidemics in your nursery
- Growing materials need to be seperated from discard materials
  - discarded materials that come into contact with growing materials combine with that material and can bring pathogens with them
- Dirty containers should be away and seperated from new materials
  - assume dirty containers have the pathogen and mixing may accidentally lead to the use of dirty instead of clean containers in nursery production

Sanitation (Sect. 10.3., pg. 25)

Objective: Keep it clean- benches, work areas, tools, surfaces, and the nursery environment

- Past infestation can come back to ‘life’ by presence of spores if re-introduced into the system from dirty benches, tools etc.
- debris can be a great source of pathogens
- surfaces and tools can hold debris if not cleaned and sanitized



Delivery of finished plant material (Section 8., pg. 19)

The process is not over until it is over

Plants need to be delivered clean

Where are they stored before delivery

How are they delivered

What happens while they are being loaded

What is the condition of the truck bed

Is the water source used in transport Phytophthora free?

(3) A closer look at specific guidelines

A. Table height (Section 6.3. Benches and growing areas; pg. 13)
B. Buy-ins (Section 8. Special Note; pg. 19)
C. Soil pasteurization (Section 4. & Section 10.4 Heat treatment of potting media; pg. 25)

Why is it a problem that plants are grown on open ground? Why should benches be three feet high?

Practical Advantages:

Good working height reduces worker strain and accidents

Less likely to drop or damage materials if working at a good height

How it works. Principle of Plant Health Management called Protection:

Establishing **a barrier between** the **pathogen** and the **host plant** or the susceptible part of the host plant

Usually a chemical barrier, e.g., a fungicide, bactericide or nematicide –but this not reccomended for restoration

Protection also physical, **spatial**, or temporal barrier- **spatial** barrier probably most practical for restoration plants grown in nurseries

The specific strategies employed **assume that pathogens are present and** that **infection will occur without the intervention** of protective measures. Assume the ground is where the pathogen is and the distance acts (three feet modest and sufficient) as a **spatial barrier** between the pathogen and the host.

In general 1 foot may suffice and even eight inches is better than zero – how ever in cases of driving rain, storms irrigation breaks, heavy irrigation by fatigued/untrained staff risk of spread is still strong

Three feet still possible but very unlikely

Splash is the most important factor in the spread of epidemics of many plant pathogens.

Splash height is highly dependent on the kinetic energy of impacting drops. The lower the height the less predictable splash height becomes and (generally more frequent) this may be due to the effects of secodary splash

Splash can move and spread soil particles, pathogens and water

When and where would three foot high benches not provide any added protection

- If there is no splash or if splash does not contain pathogens, if the ground does not have pathogens and the plants do not have pathogens
- If the splash height were reduced
    - Perhaps develop a screen system to prevent splash- soft surface absorbs and does not puddle evaluated for this purpose
    - Examples- Splash guards, silicone surfaces, absorbent ground covers, thirsty concrete

In what circumstance are three foot high benches not practical

- Large plant stock

Are there options?

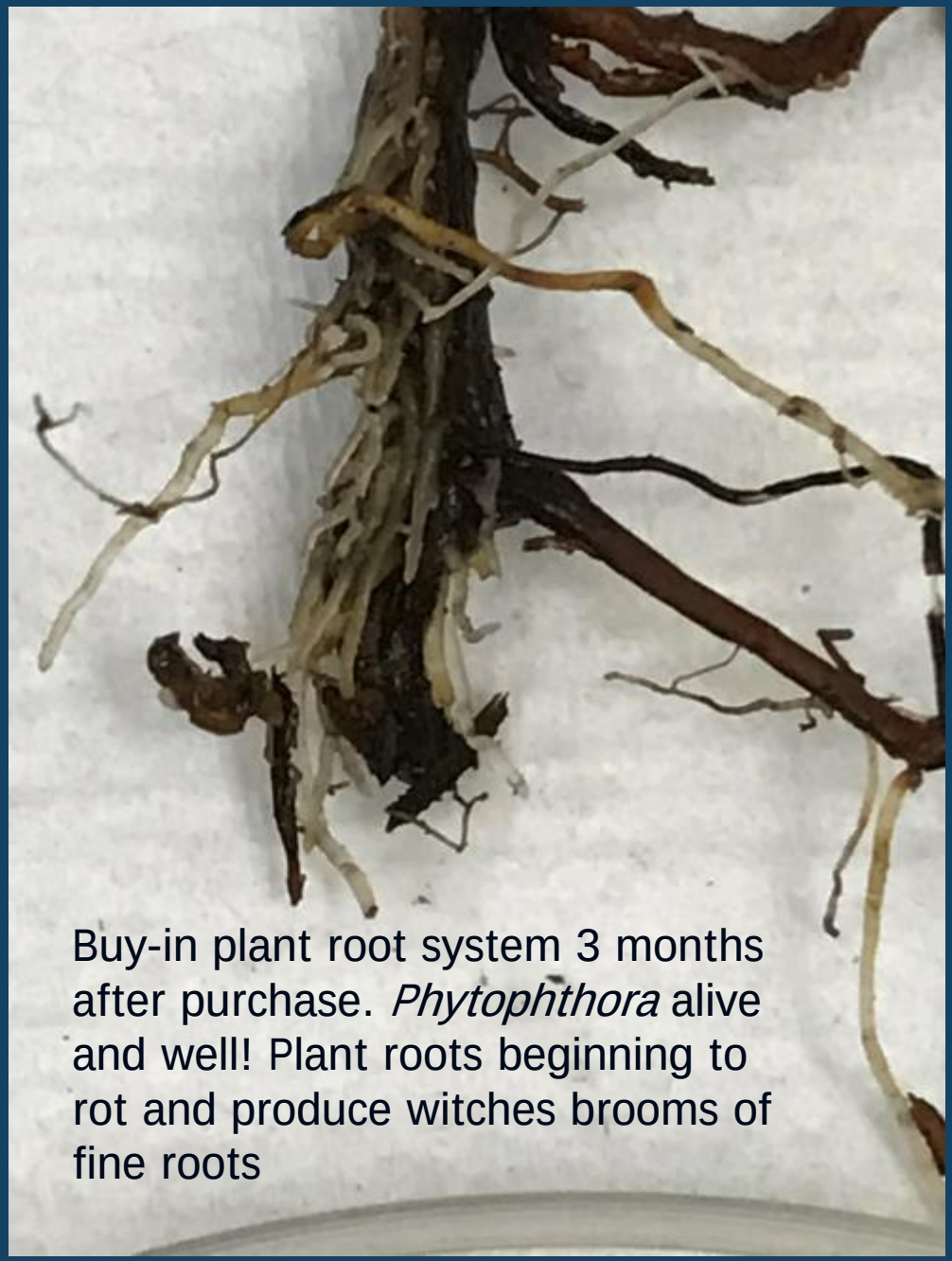
- Greatly reduce the chance the floor has the pathogen – excluding from the nursery as best as possible
- Then decreasing barrier distance is possible and will offer as much protection as a three foor distance
- reduce the chance of splash by protecting from extremes and careful irrigation
  - It is possible that ground cloth and a much lower surface plastic or steel pallet stacked could work better than high tables esp. for large plant stock
  - Double sided plastic pallets provide an environmental alternative to wood pallets or skids.
  - One-piece, high pressure injection molded plastic is used for food processing, meat, poultry and pharmaceutical applications.
  - Could be used for nursery plants
  - 100% recyclable pallets offer a unique rib reinforced design to minimize dirt and dust collection.
  - Easy to clean and washable pallets do not hold or trap water for fast drying.
  - Stackable pallets high load capacities.

48” x 40” and 6” height

Special note: Buy-ins

commercial nurseries use fungicides to reduce damage caused by pathogens in nursery production, does not ususally kill the pathogens can act as a pathway into your nursery

Are you sharing pathogens?



Buy-in plant root system 3 months after purchase. *Phytophthora* alive and well! Plant roots beginning to rot and produce witches brooms of fine roots

Soil pasteurization (Section 10.4 Heat treatment of potting media; page 25)

- Without heat treatment spores that are present can survive years
- Biocontrol usually only works as long as it is continually added NOT a replacement
  - used to suppress not eliminate
- Heat treatment if done correctly kills Phytophthora and other pathogens
- What is killed depends on the temperature that is reached

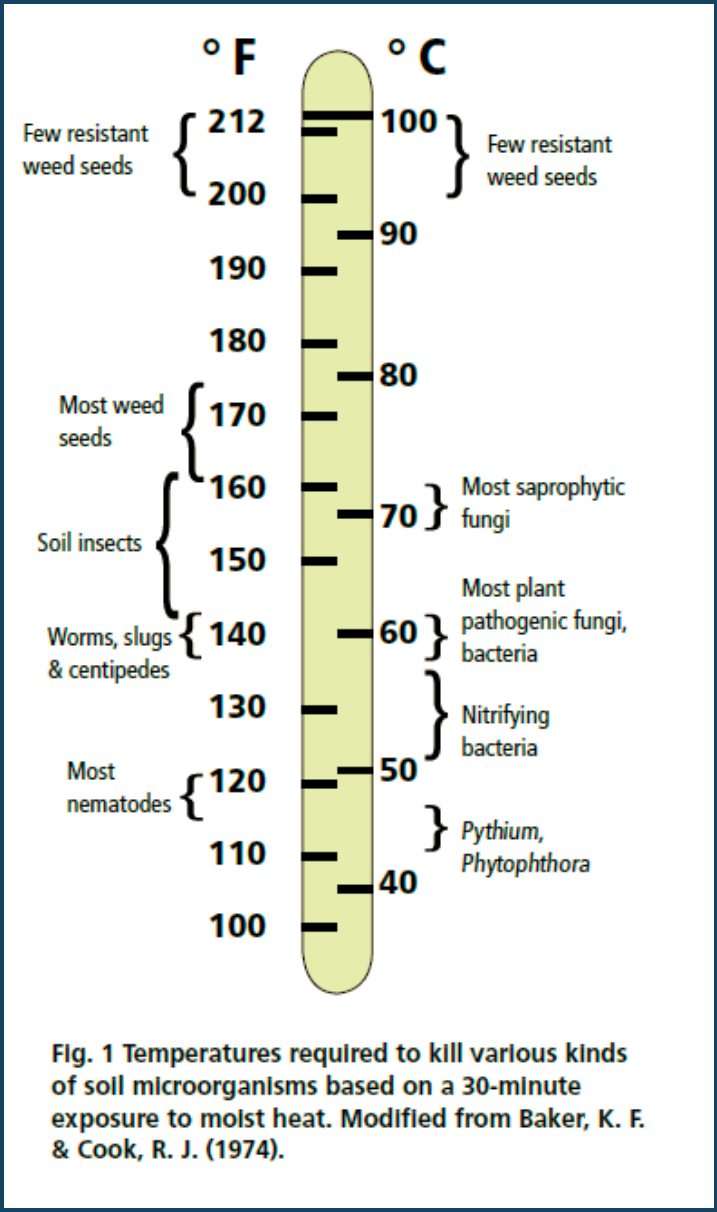


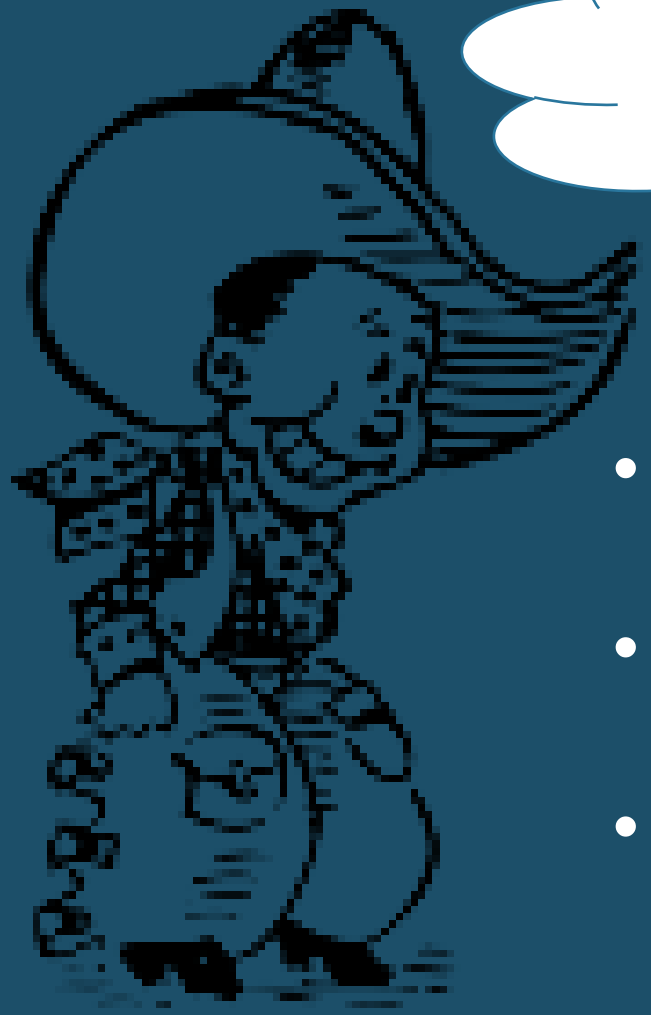
Fig. 1 Temperatures required to kill various kinds of soil microorganisms based on a 30-minute exposure to moist heat. Modified from Baker, K. F. & Cook, R. J. (1974).

Where are there short falls/complications with heat treatment

- Are you heating uneven soil? If yes then heat distribution is uneven as well
- Are you using dry heat then the temperature must be higher and heated for longer may cause increased phytotoxic effects
- Are you heating solid material like old root pieces. No I hope that you are not or else you have a specific protocol for that
- Solarization is tricky if you continually get just below the right temperature may promote the organism that you are trying to get rid of
- Are you monitoring the temperature. Temperature is key

(4) Example of how well Phytophthora does without Best Practices as a rationale for use: real examples from two California nurseries that provides restoration nursery stock

Wild West of Restoration Nursery Management



Yee haw Let's look at some poor management!

- Help to understand how poor practices support Phytophthora
- Rationale for why best practices are so important
- Answer question: Does making small changes make a difference?

Compare two nurseries that supply restoration plant stock in California

- We we call them the red and yellow nursery
- Similarities –both have Phytophthora problems, but one less so than the other

Red Nursery = Stop! Lots of *Phytophthora*!

Yellow Nursery = Watch it! *Phytophthora* still doing well!

Major drivers of contamination problems evidenced at both nursery

- At least some plants grown on open ground
- Improper watering observed during evaluation
- Potting mix contains potential contamination source
- Potting mix stored in the open near dirty containers, cull pile
- Planting in dirty container

Problems specific to each nursery

Red Nursery

1. Most plant production on open ground
2. Excessive weeds and mud on site
3. Improper watering observed during evaulation –mid day (overhead) in already very wet condition
4. Broken irrigation with flooding
5. Contaminated species placed all around nursery grounds near other plant species (mixed with)
6. Mixing potting waste with new potting material and ‘new ‘ mix stored adj. to dirt road and not seperated from potting material
7. No tracking of propagation dates/information

Yellow Nursery

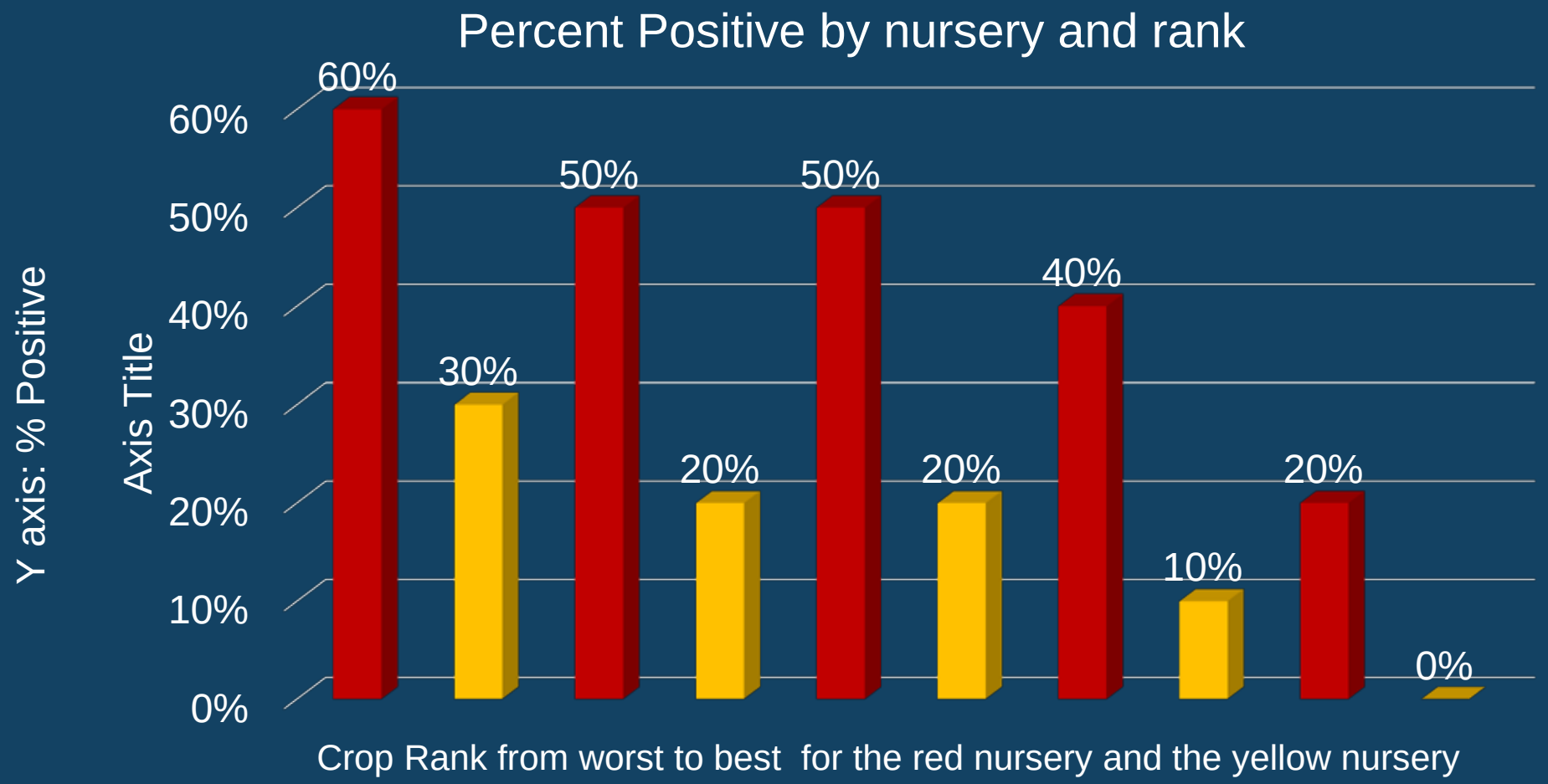
1. At-least some production on open ground
2. Overwatering during propagation
3. Older potting mix placed with new
4. Various piles of mix many of questionable quality
5. Main mix obtained from unverified mix of sources because it was free or cheap, and not treated before use

Yellow nursery drivers toward plant health

- Some areas at-least (focus on restoration project plants) ground cover used to protect plants from splash
- Effort to train staff about pathogens and manage problems as they arise, willingness to learn and adapting to improve practices
- General good management and horticultural knowledge
- Access to at-least some Phytophthora “free” propagation materials
- Working on getting plants up on benches, had moved some plants at-least a few inches off the ground

Nursery red and yellow comparison of diseased crops worst to best

Conclusion: The yellow nursery has less of a problem than the red nursery
Both have far to go



X axis : Worst Crop → Best Crop

Isolate totals: 47 *Phytophthora* species isolates
Taxa totals: 10 identified taxa based on consenus ITS
ITS clade totals: 1 clade 1 isolate from 1 sample, 1 taxa; 13 Clade 2 isolates from 6 samples, 3 taxa; 4

ITS Clade	Phytophthora species (taxon)	host$_1$	host$_2$	# of isolates	# of plants n=10
1	*Phytophthora cactorum*	*Acer circinatum*	-	1	1
2	*P. multivora*	*Acer circinatum*	*Frangula californica*	9	3
2	*P.occultans*	*Acer circinatum*	-	3	3
2	*P* . pini/citricola clade	*Arctostaphlos uva-ursi*	-	1	1
6	*P. crassamura*	*Diplacus aurantiacus*	-	1	1
6	*P.* "taxon raspberry"	*Arctostaphlos uva-ursi*	-	3	3
8	*P.* cryptogea/pseudocryptogea clade	*Diplacus aurantiacus* "trish" type	-	18	5
8	*P* . "taxon kelmania"	*Diplacus aurantiacus*	-	5	1
8	*P* . "taxon kelmania-close"	*Diplacus aurantiacus*	-	2	1
8	*P. pseudocryptogea*	Diplacus aurantiacus "trish" type	*Arctostaphlos uva-ursi*	2	2
8	Unidentified clade 8 species	*Diplacus aurantiacus*		2	2

Red Nursery: 44% of plants were *Phytophthora* species positive

ITS Clade	ITS Sequence Species Results	Host$_1$	Host$_2$	No. of isolates	No. of plants, n=10 per plant species
1	*P. cactorum*	*Heteromeles arbutifolia*		4	3
7	close "*P. niederhauserii*"	*Ceanothus thyrsiflorus*	*Aesculus californica*	5	2
8	*P. cryptogea*	*Frangula californica*	*Aesculus californica*	2	2
2	*P. multivora*	*Frangula californica*		1	1

Yellow Nursery: 15% of plants were *Phytophthora* species positive

Species List: From each nursery 50 plants were evaluated: 10 plants from each of 5 known susceptible crops

Answer question: Does making small changes make a difference?

- Yes. Small changes do make a difference in the amount of crop plants that are infested
- And makes a difference regaring the number and type of Phytophthora species found
- Small changes are not enough –just a beginning moving in the right direction
- Evaluating the population provides evidence of effort

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Thank you! Questions?

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