Verticillium Wilt Disease Management in Organic Production

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Understanding Verticillium wilt

Verticillum dahliae

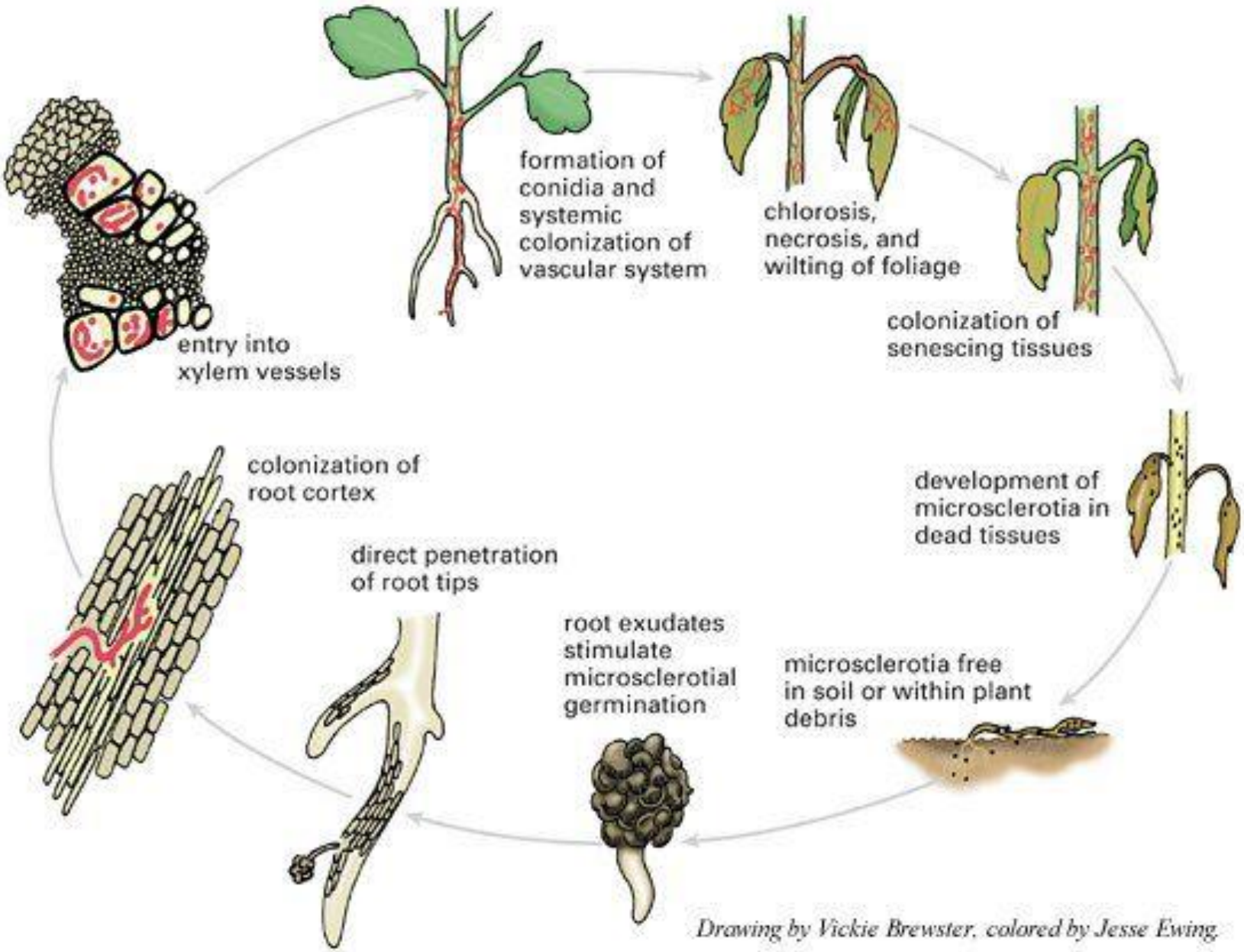
Growth, infection, propagation and survival

MANAGEMENT IN ORGANIC PRODUCTION



Take away message:

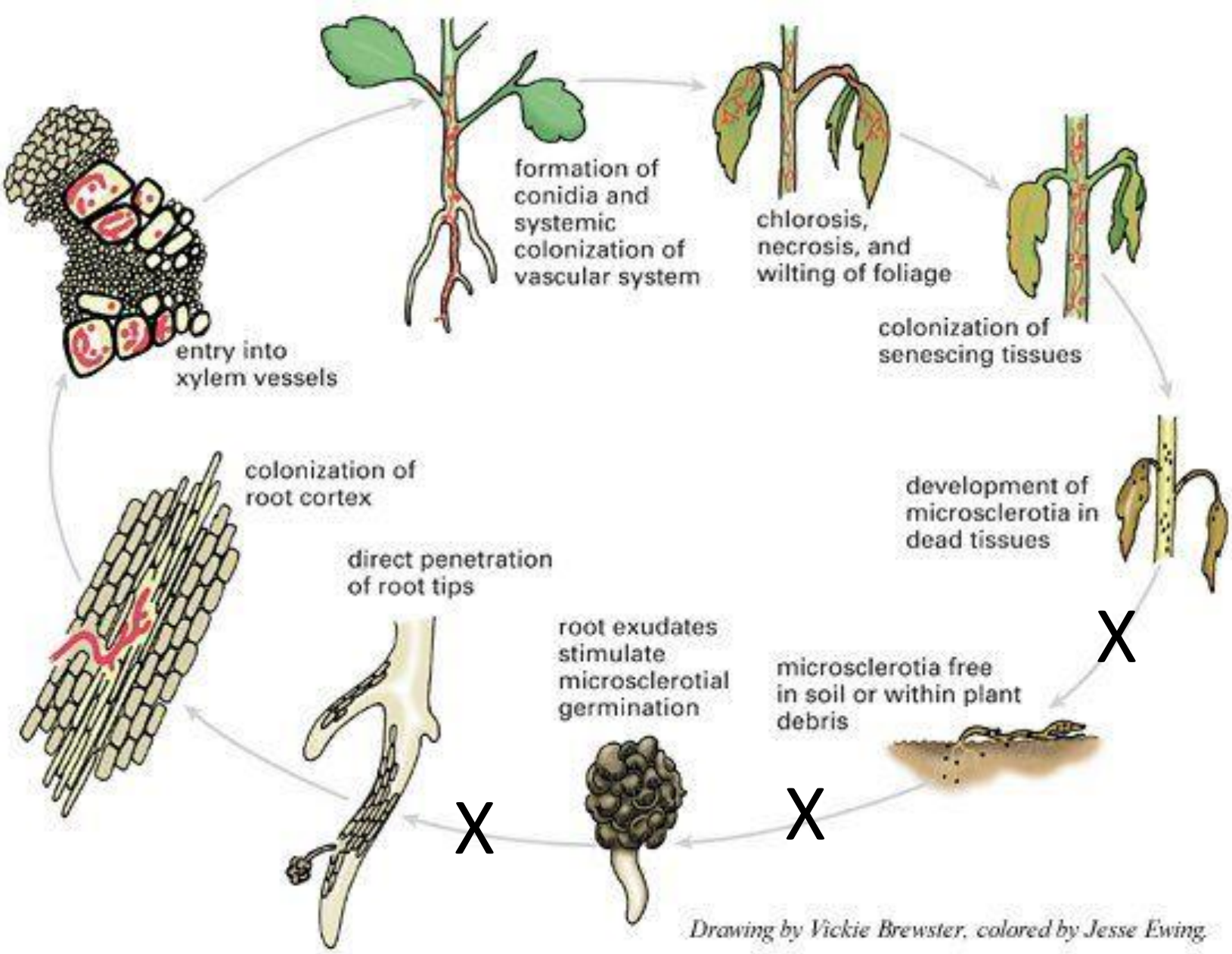
Keep inoculum levels low


entry into
xylem vessels
formation of
conidia and
systemic
colonization of
vascular system
chlorosis,
necrosis, and
wilting of foliage
colonization of
senescing tissues
development of
microsclerotia in
dead tissues
microsclerotia free
in soil or within plant
debris
root exudates
stimulate
microsclerotial
germination
direct penetration
of root tips
colonization of
root cortex
Drawing by Vickie Brewster, colored by Jesse Ewing

V. dahliae **microsclerotia emerging from plant tissue**

V. dahliae
Microsclerotia in plant tissue

Opportunities to interrupt the cycle



Verticillium dahliae

- Ubiquitous soil inhabitants
- Non-motile
- Dormant state in soil
- Root-inhabiting, major pathogens
- Monocyclic, no plant-to-plant spread during a season
- Spreads by contaminated soil via equipment, air or water; infested plant material

Verticillium Wilt Symptoms

- Leaves wilt on sunny days and recover at night
- Symptoms are most noticeable during later stages of plant development when fruit begin to size.
  - Often, no symptoms are seen until the plant is bearing or during dry periods
- Often plants are stunted

Light vascular discoloration and is confined to lower areas of the plant (4” from soil)

Bottom leaves become pale, then tips and edges die and leaves drop off

V-shaped lesions

One-sided symptoms

Impact

Sun-related fruit damage is increased because of the loss of foliage.

Verticillium wilt seldom kills tomato plants but reduces their vigor and yield.

Photo: Gene Miyao

Sampling and Diagnosis

- Challenging to differentiate Fusarium from Verticillium wilt
- Lab confirmation is important since management options can be quite different

- Take stem samples. Look for vascular discoloration.
  - It's rarely in the petioles/leaflets

  So take a lower section of the main stem

Fusarium oxysporum

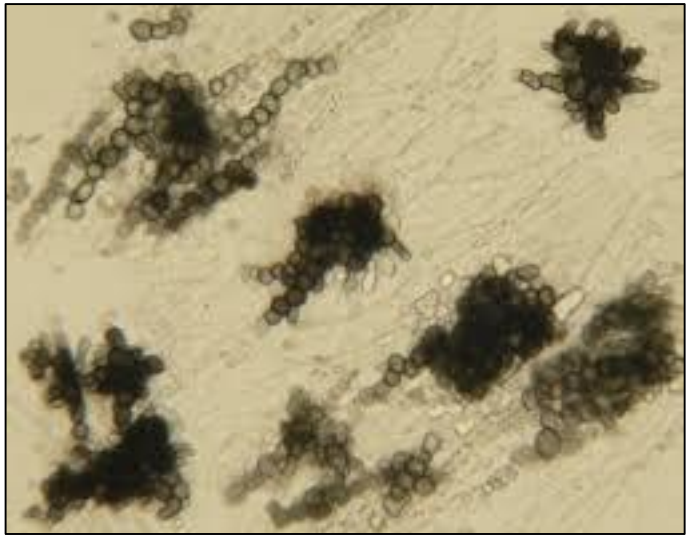
- Host-specific
  - Over 100 different formae speciales described
- Shorter lived survival structures, 3-5 years (chlamydospores)
- Many *Fusarium* spp. are reported to be seedborne.
- Optimal soil temp 86°F+

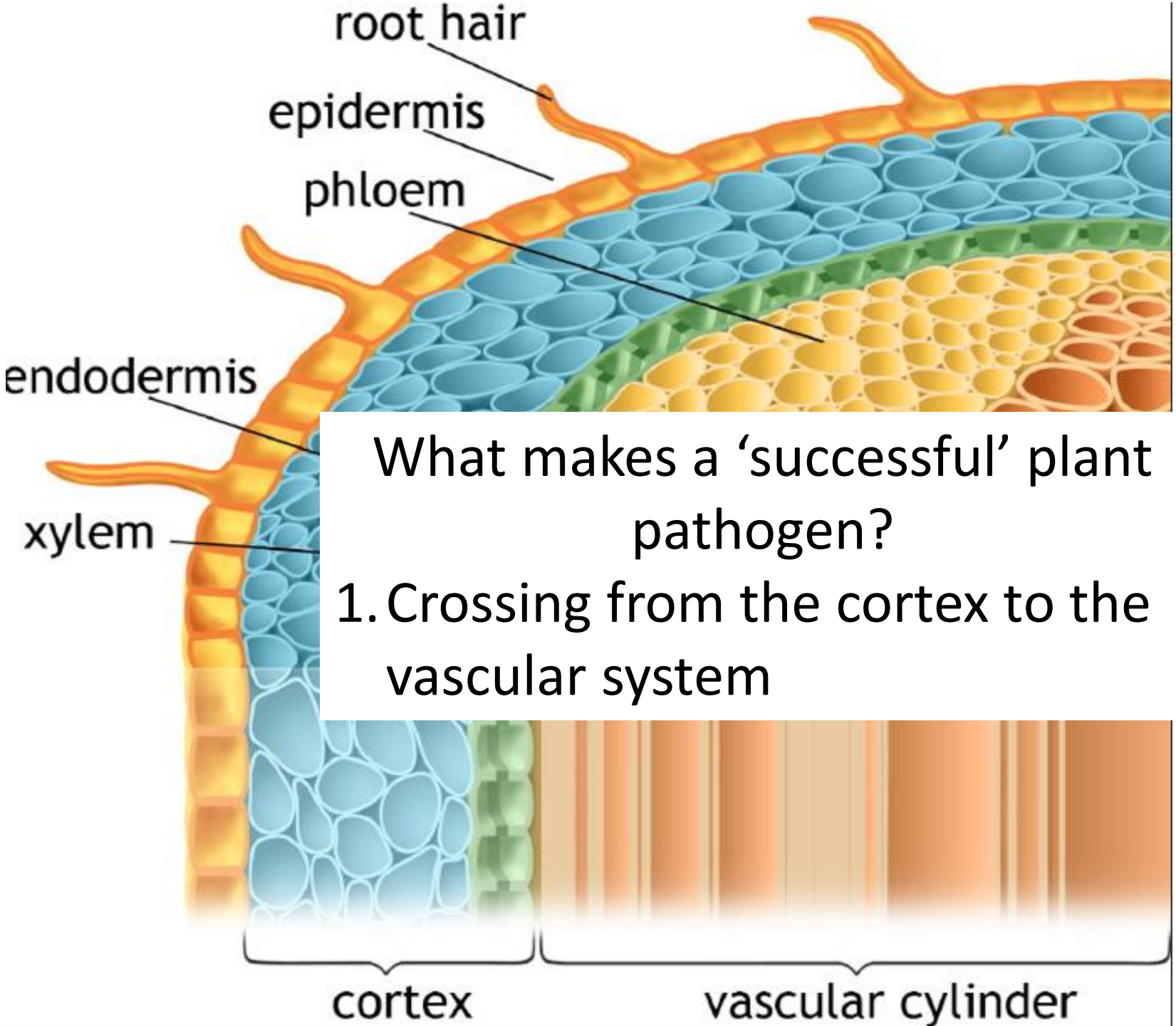
Chlamydospores

Verticillium dahliae

- Wide host range
- Long lived survival structures, 10+ years (microsclerotia)
- Occasionally seedborne
- Optimal soil temp 70-80°F

Microsclerotia

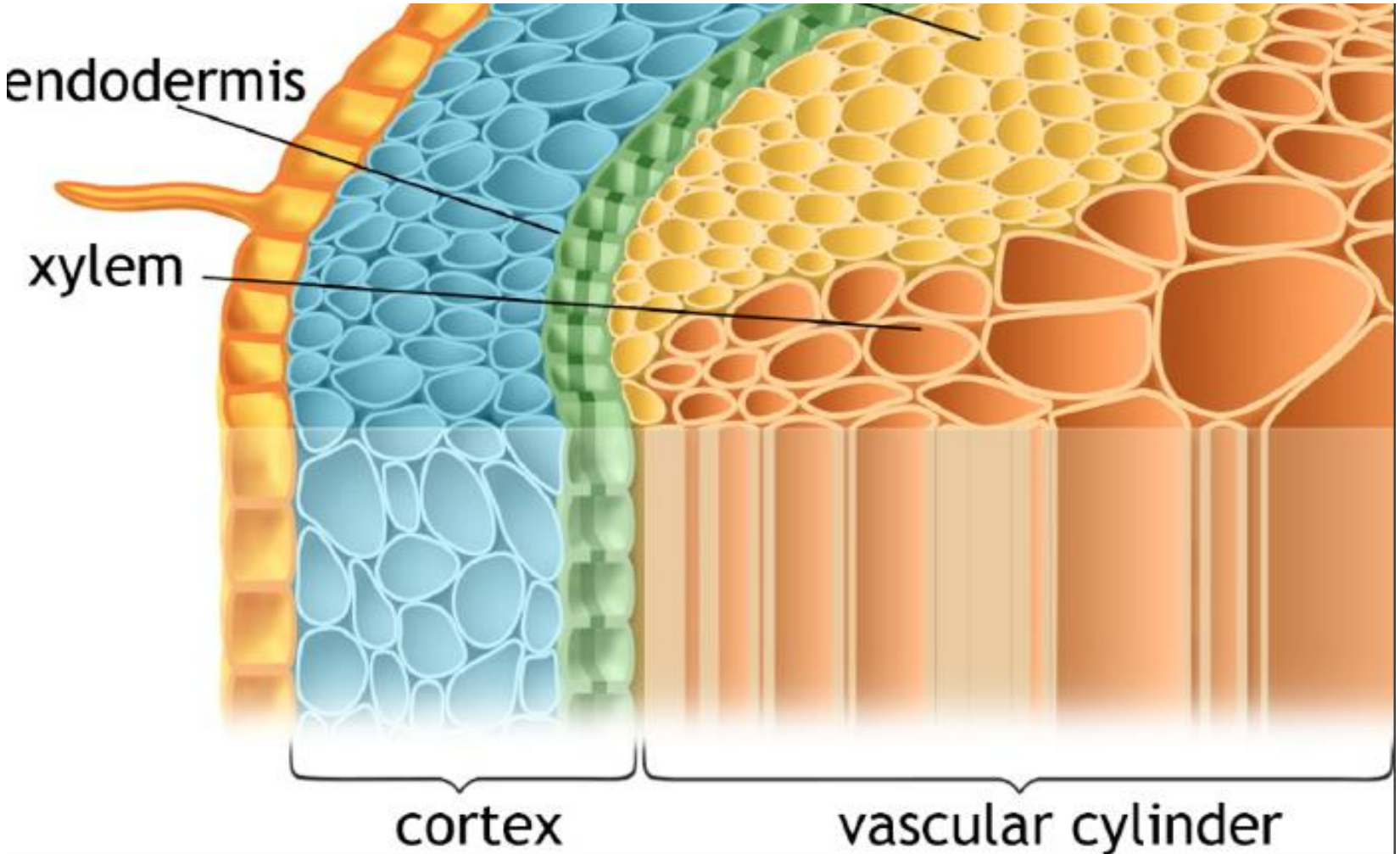




What makes a ‘successful’ plant pathogen?

1. Crossing from the cortex to the vascular system

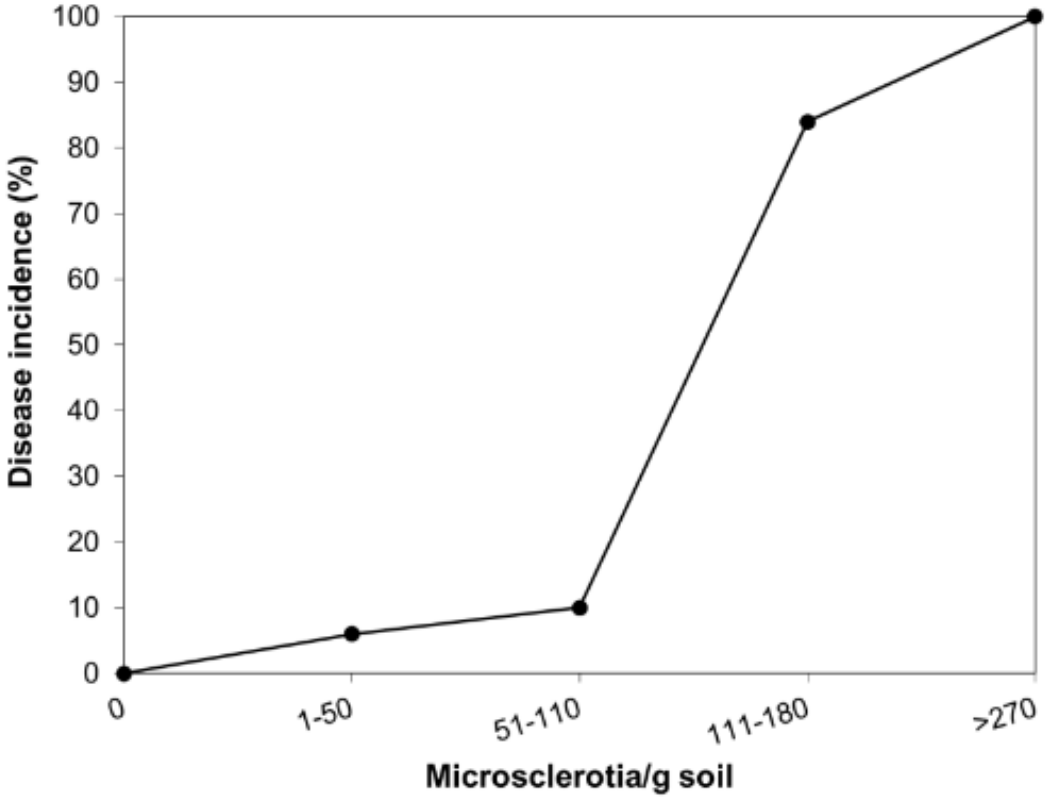
Huisman and Gerik observed that only 1 in 5,000 cortical invasions of cotton roots by *V. dahliae* results in successful vascular infection.



Soil Inoculum Thresholds

- Threshold depends on the crop and variety, even the operation

- Sampling?
  - Not very realistic as a check in an unknown field
  - More useful for monitoring an area with known infection
- Mark and track infested areas
  - GPS coordinates



Relationship of disease incidence and microsclerotia density in lettuce

Soil inoculum density and disease incidence

- *Vd*: Most crops suffer near-total losses at densities of <50 microsclerotia/g soil.

Crop	*V. dahliae* (cfu/g soil)	% loss
Strawberry	3-5	50%
Lettuce (iceberg)	>100-150	
Artichoke	5-6	50%
Tomato	2-6	
Cauliflower	1	5%

Root Entry:
Consider the location of the root tip and inoculum

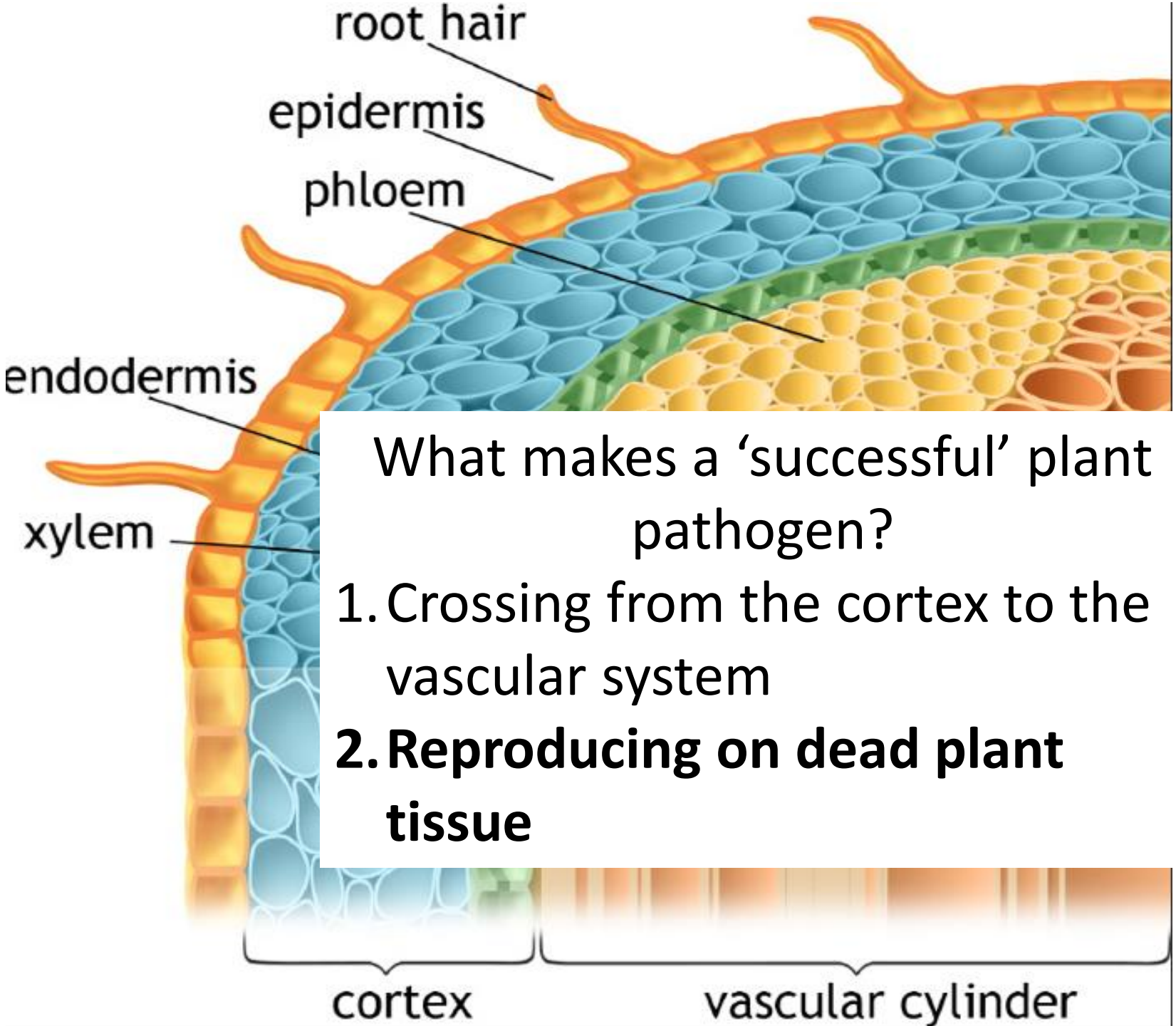
BIOLOGY:

- Pathogens are non-motile, the roots must encounter the pathogen
- Root tip is the hot spot: “Activity Zone” or “Infection Zone”
- Only infects in the root tip
- *V. dahliae* propagules occur in highest concentration in the top 12 inches of the soil profile

Root Entry:
Consider the location of the root tip and inoculum

MANAGEMENT STRATEGY:

- Planting deep if inoculum is higher in the soil
- Delaying infection by keeping the pathogen deeper in the soil
- Use irrigation to direct roots (shallow or deep-rooted)
- Plant into infested areas during less optimal temperatures for the pathogen **Optimal temp. for Vd: 70-81° F**
- Keep inoculum levels low



What makes a 'successful' plant pathogen?

1. Crossing from the cortex to the vascular system
2. **Reproducing on dead plant tissue**

What could be the source of a new infection?

Verticillium is endemic in California

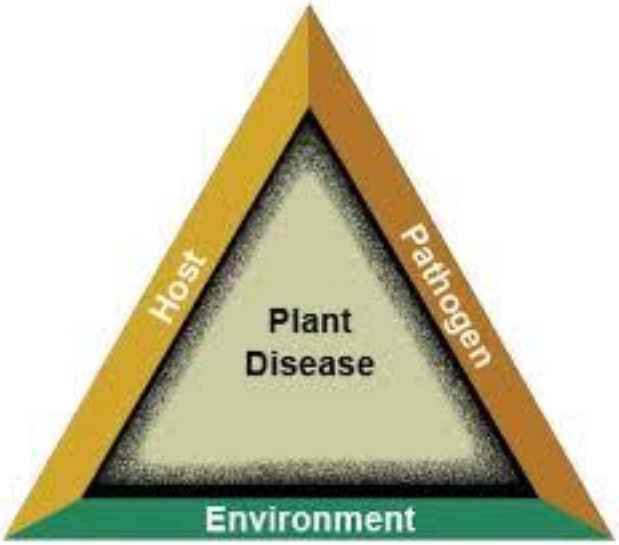
V. dahliae is present in higher amounts in most ground, now or previously cropped to potatoes, peppermint and cotton

- Deep tillage
- 'Virgin land'- previous cropping history
- Increased inoculum to damaging threshold

- Plant material
  - infected planting stock or seed can move the pathogen long distances
- Equipment movement
  - *Main method for spread of established infection

Principles of Soilborne Disease Management

1. Keep inoculum levels low
2. Maximize plant health
3. Create a less favorable environment



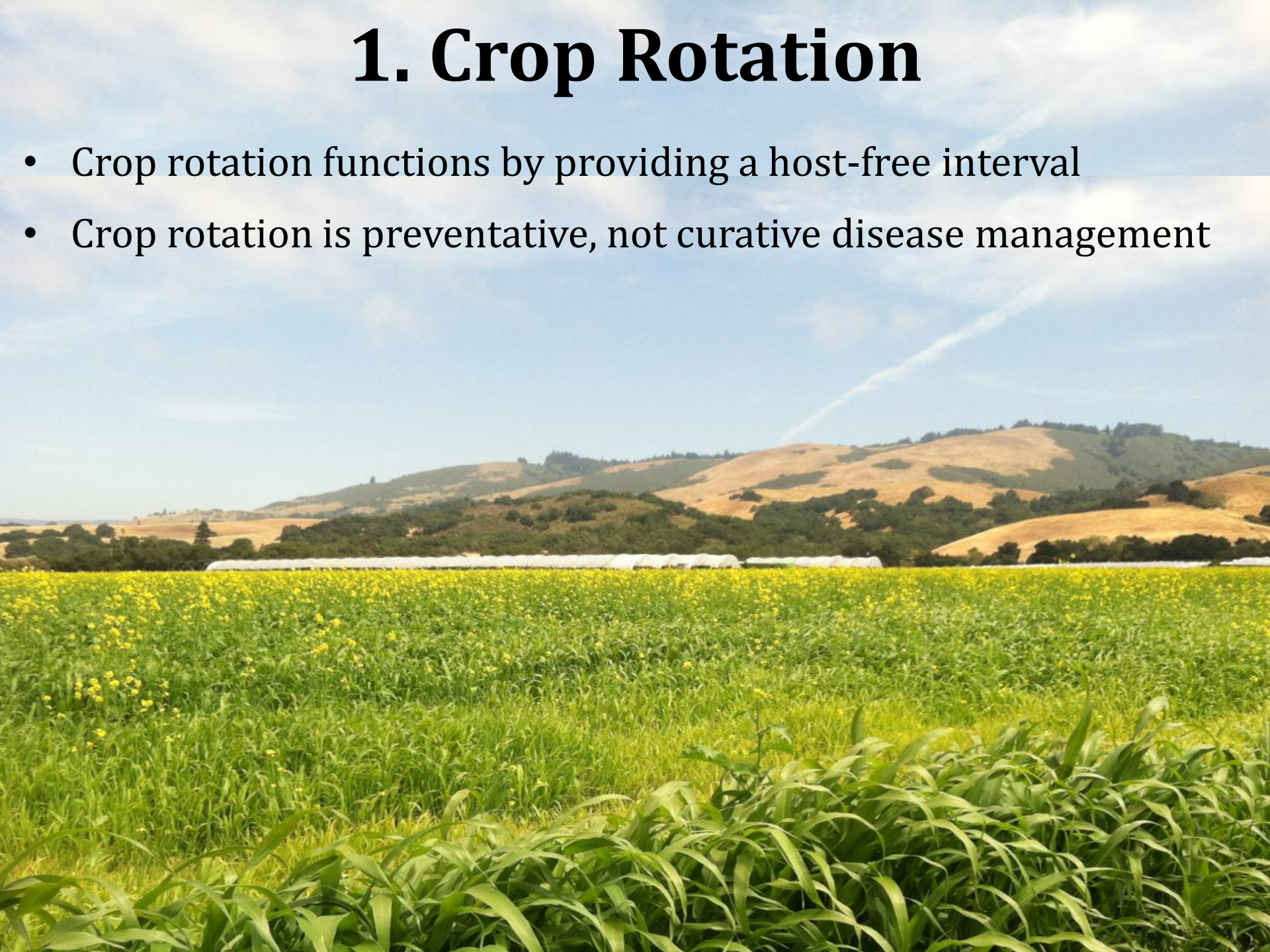
You are managing the fungus and the disease

Integrated Disease Management in Organic Production

1. Crop rotation
2. Genetic resistance and grafting
3. Pre-plant soil treatments
4. Avoid new introductions
5. Amendment application
6. Destruction of infected crop debris
7. In-season management

1. Crop Rotation

- Crop rotation functions by providing a host-free interval
- Crop rotation is preventative, not curative disease management



Effective Crop Rotation

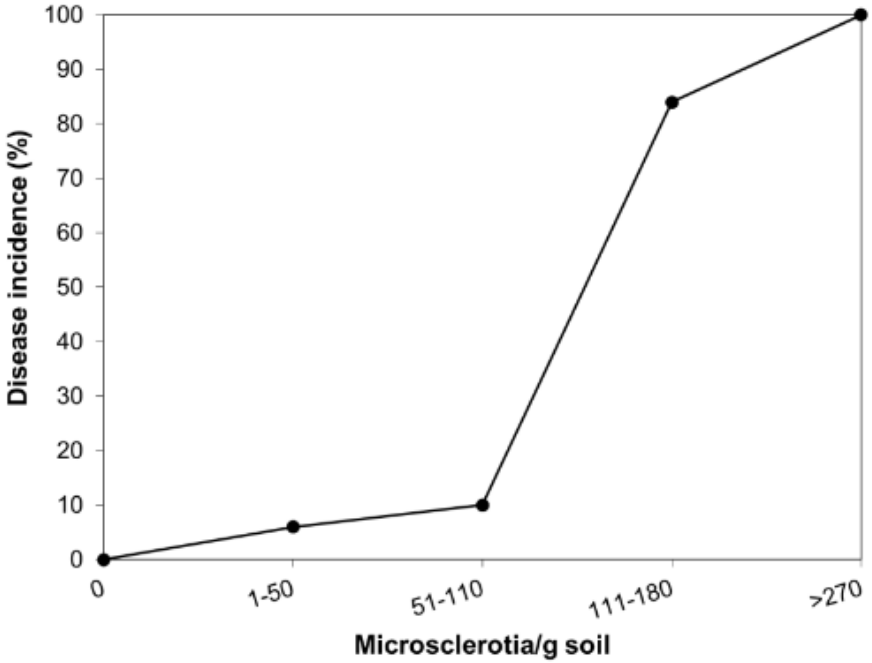
V. dahliae

200+ hosts

- Unexpected hosts: lettuce, legumes
- Crops that suppress *V. dahliae*
- Good options for rotation

Lettuce

- **No symptoms until high disease pressure** and later in growth
- **Roots produce abundant microsclerotia**
  - 2,500-100,000 cfu of microsclerotia per gram of dry taproot tissue
- **Seed is not a major concern**
  - 18% of the 250 seed lots tested positive for *V. dahliae*, and disease incidence from was less than 5%



Subbarao,Krishna. Biology and Epidemiology of Verticillium wilt of Lettuce. California Lettuce Research Program. 2010-2011.

Cryptic infection of legumes

- No difference in plant height
- No typical symptoms like wilt or v-shaped lesions

Most suitable host for systemic *V. dahliae* colonization and reproduction

Legume	***Selection for managing V. dahliae***
Common vetch (*Vicia sativa* subsp. *sativa*)	**Poor choice** (Good host for *V. dahliae* colonization and reproduction)
Field pea (*Pisum arvense*) **Purple vetch** (*Vicia benghalensis*) **Fava Windsor bean** (*Vicia faba,* large seed) **Lana Woolypod vetch** (*Vicia dasycarpa*)	**Intermediate choice** (Intermediate host for *V. dahliae* colonization and reproduction)
Hairy vetch (*Vicia sativa* subsp. *sativa*) **Bell bean** (*Vicia faba,* small seed)	**Best choice** (Less good host for *V. dahliae* colonization and reproduction

Broccoli and Brussels Sprout Residue

- Broccoli did not develop Verticillium wilt and microsclerotia were not observed on broccoli roots (cauliflower is a host)

Broccoli Residue
(for *V. dahliae*)

- Reduces microsclerotia density in the soil
- May have an inhibitory effect on the root-colonizing potential of surviving microsclerotia.

Implementation:

- Plastic tarps are not necessary
  - Residue effects were unaffected by tarp presence/absence
- Most effective with soil temps >77°F
- Maximum microsclerotia reductions occurred within 15 days, regardless of the temperature
- Fresh broccoli provided significantly greater reductions

Mechanism of Broccoli Residue

Antifungal compounds: Glucosinolates

- Glucosinolates: converted into toxic fungal compounds
- But are volatile

Microbial community shifts

- Greatly increased competition among root colonizers
- Increased lignin specialists

Lignin-degrading fungi

- Enzymes involved in lignin biodegradation can also degrade fungal melanin (ex. Microsclerotia)

Shetty, K. G., Subbarao, K. V., Huisman, O. C., and Hubbard, J. C. 2000. Mechanism of broccoli-mediated Verticillium wilt reduction in cauliflower. Phytopathology 90:305-310

Good rotation crops

- Grasses: sudan grass, corn, wheat, rye
- Broccoli
- Mustard seed meals have been shown to inhibit *V. dahliae*

2. Resistant Cultivars and Grafting

- Resistant cultivars may exhibit symptoms of Verticillium wilt under high inoculum pressure

Grafting susceptible varieties onto resistant tomato rootstocks

Demonstrated control for Verticillium wilt of :

- cucumber
- melon
- watermelon
- tomato
- eggplant

3. Preplant Soil Treatments: Soil Solarization

- Lower populations in soil sufficiently to delay onset and reduce disease incidence

4. Avoid New Introductions

The prevention of biological invasions is the most cost-effective strategy of invasive species control

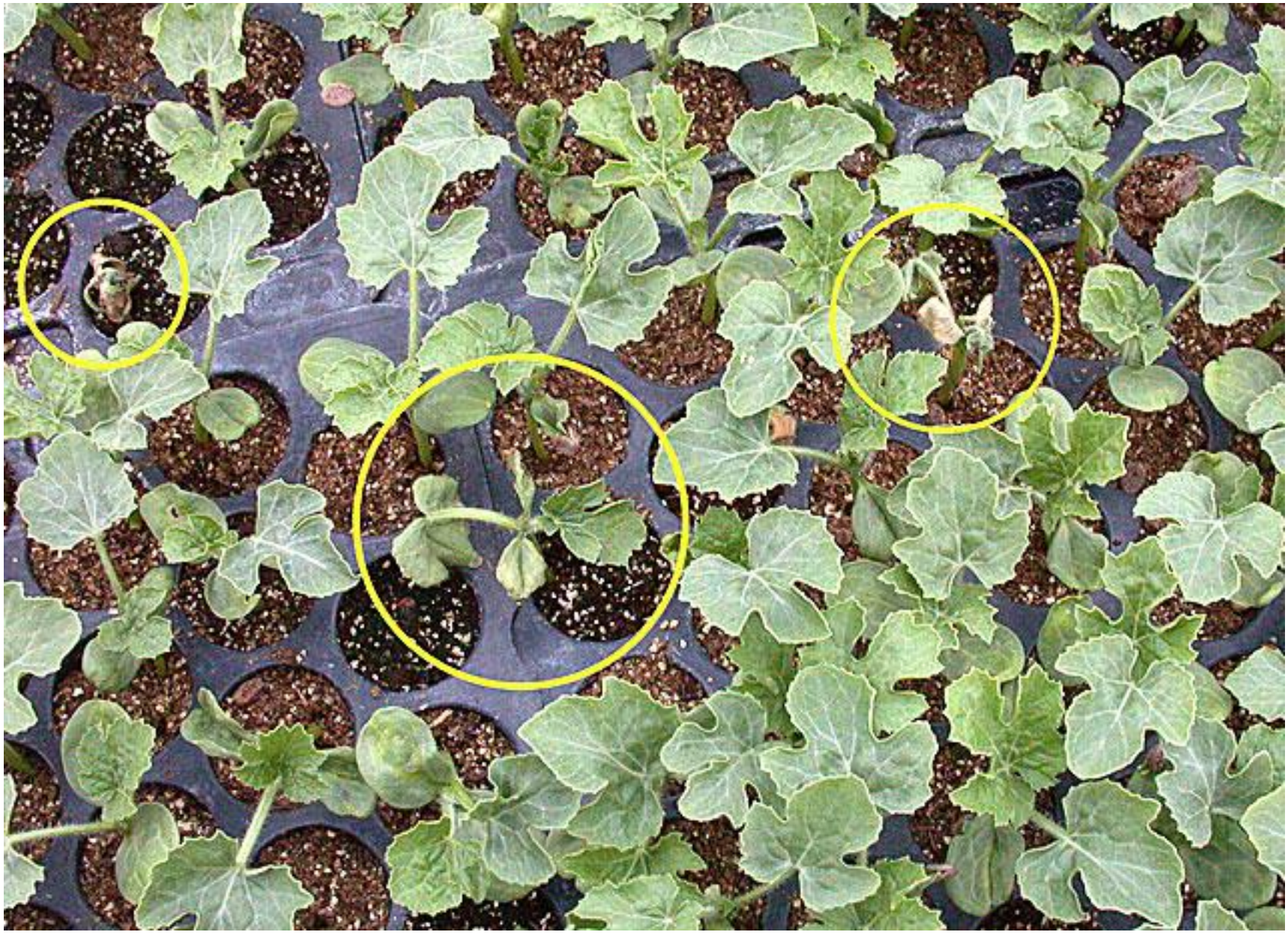
Transplants

Seeds

Planting stock

Plant Material:
Disease-free transplants

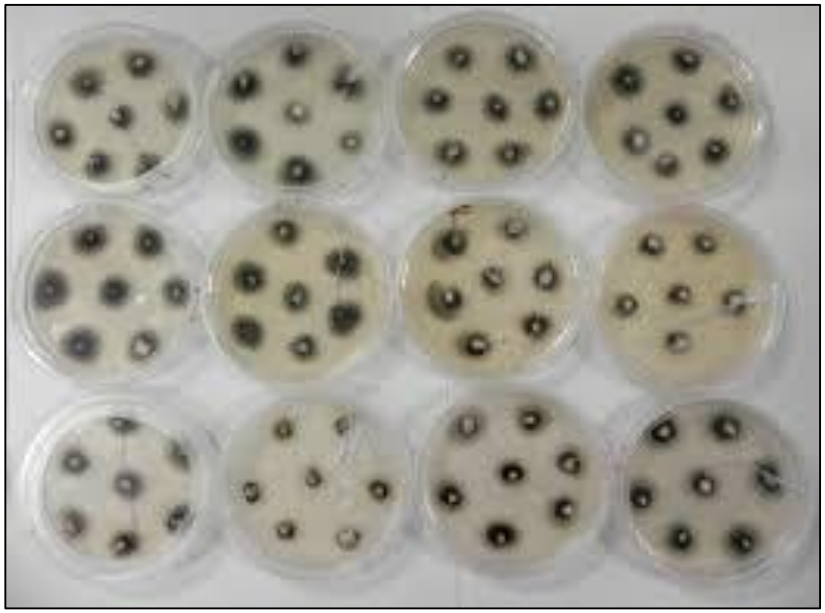
- Vegetatively propagated plants
  - Non-symptomatic, but infected, planting stock
  - such as ornamentals, peppermint, potato seed tubers, strawberries
- Screen transplants and young plants
  - Stunting and/or wilting symptoms can be observed in transplants and should be eliminated



(Courtesy D.S. Egel)

Seedborne

- *V. dahliae*
  - Safflower
  - Spinach
  - Pumpkin



KLISIEWICZ, J. M. 1974. Assay of Verticillium in safflower seed. Phytopathology 52:998-1000. Plant Dis. Rep. 44:901-903.

Short, D. P. G., Gurung, S., Koike, S.T., Klosterman, S. J., and Subbarao, K. V. 2015. Frequency of Verticillium species in commercial spinach fields and transmission of V.dahliae from spinach to subsequent lettuce crops. Phytopathology 105:80-90.

Spinach Seed:
V. dahliae introduced via spinach seed

- 80% of seed comes from US (Pacific NW) where *V. dahliae* is endemic
- Race 1 and 2 have been identified on spinach seed
- Incidence of the fungus was >75% in many seed lots
- Current reports restricted to the Pajaro and Salinas Valley

Short, D. P. G., Gurung, S., Koike, S.T., Klosterman, S. J., and Subbarao, K. V. 2015. Frequency of Verticillium species in commercial spinach fields and transmission of V.dahliae from spinach to subsequent lettuce crops. Phytopathology 105:80-90.

Pumpkin Seed

- *Verticillium dahliae* in pumpkin seed was found to be external and transmissible to plants.
- 21.0% of seeds were infected
- **25% infection of 6-week-old plants** grown from untreated seeds.
- **Surface sterilization**
  - Fungus was present **in the seed coat** but not in the embryo or cotyledons.

Seed Treatments

Hot Water

- 55 °C for 30 min
- 60° C for 10 min

Sodium hypochlorite (bleech)

- 1.2% solution, 30 minutes
- 0.6% solution, 20 min eradicated *V. dahliae* from infected pumpkin seeds without affecting germinability.

5. Amendment Applications: Biological Controls

- Soil environment is conducive to abundant and diverse microbes
- Effective control under field conditions is hard to demonstrate
- Dose-response relationship

Mechanism:

1. Use of antibiotic-producing soil fungi and bacteria i.e., *Gliocladium* spp., *Trichoderma* spp. *Pseudomonas* spp.
2. Natural or induced soil suppressiveness
3. Chelating siderophores
4. Fungistasis (nutrient deprivation)

Shishido, M., Miwa, C., Usami, T., Amemiya, Y., and Johnson, K. B. 2005. Biological control efficiency of Fusarium wilt of tomato by nonpathogenic Fusarium oxysporum Fo-B2 in different environments. Phytopathology 95:1072-1080.

6. Crop Residue Destruction

- **Propane flaming**
  - High temperatures are lethal to Verticillium propagules.
- **Removal**

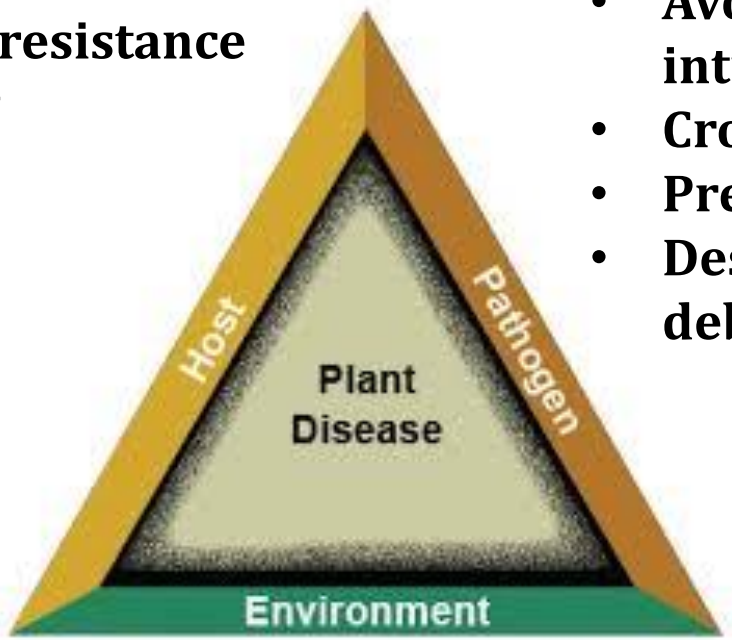
7. In-season Management

- **Fertilization**
  - Optimal rates of nitrogen and phosphorus reduces the severity of Verticillium wilt

- **Water management**
  - Limiting the amount of water applied to the field can reduce severity of the disease in some plant species, such as potato.
  - Overwatering, especially early in the growing season, results in increased infection and increased disease severity.

HOST
- **Genetic resistance**
- **Grafting**

PATHOGEN
- **Avoidance of new introductions**
- **Crop rotation**
- **Pre-plant soil treatments**
- **Destruction of infected crop debris**



ENVIRONMENT
- **Amendment application**
- **In-season management**

Conclusion

- Diagnose the pathogen
- Multiple tactics
- Keep inoculum levels low!
  - Rogue and destroy infected plant residue
- Use biological controls to minimize plant infection
- Remember the biology
  - Roots must encounter the pathogen
  - Fungus reproduces on dead tissue

Thank you for your attention

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QUESTIONS

- Races in the area
- Diagnosing f.sp. How easy?
- Picture of clamydospores (gordon lab)
- What is the local availability of grafted plants? The local history?

Definition of Soilborne Pathogens

1) Soil invaders vs soil inhabitants
2) Inoculum potential, including the stimulation of plant pathogens by root exudates and other sources of nutrients
3) Environmental stress can predispose hosts to infection and disease
4) Soil microorganisms influence pathogen survival and development

- Soil fungi that are ubiquitous (inhabitants) and those found only locally (invaders)

Soil Inhabiting

- Primitive, unspecialized organisms
  - Infect seedlings and juvenile root tissues
  - Parasitism was incidental to a saprophytic existence
- Transitory existence in soil in the absence of a host
- Either have pathogenic specialization or have competitive saprophytic ability

Either have pathogenic specialization or have competitive saprophytic ability

- Pathogenic specialization, “major pathogens”:
  - *Fusarium* spp.
  - Fusarium can colonize weed hosts without causing apparent symptoms

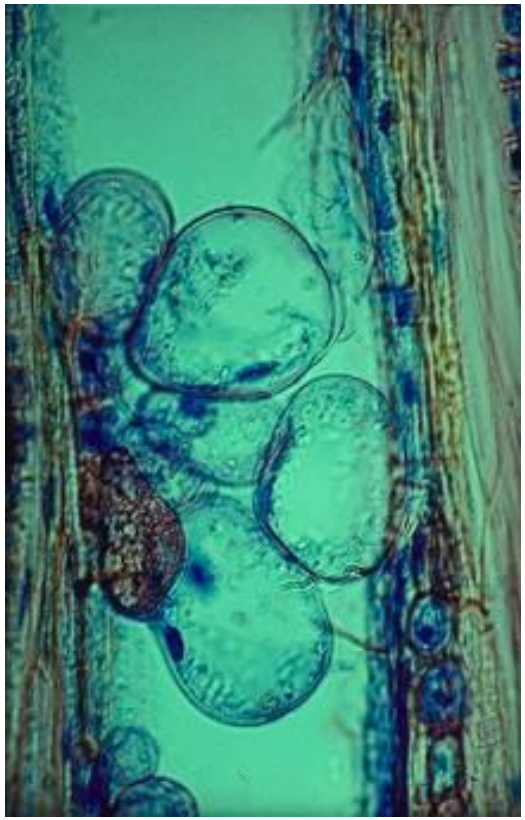
- Unspecialized, soil inhabiting fungi, “minor pathogens”
  - *Pythium* spp. : nibble root tips

Soil microorganisms influence pathogen survival and development

- The differences between pathogen-suppressive and pathogen-conducive soils are at least in part microbial

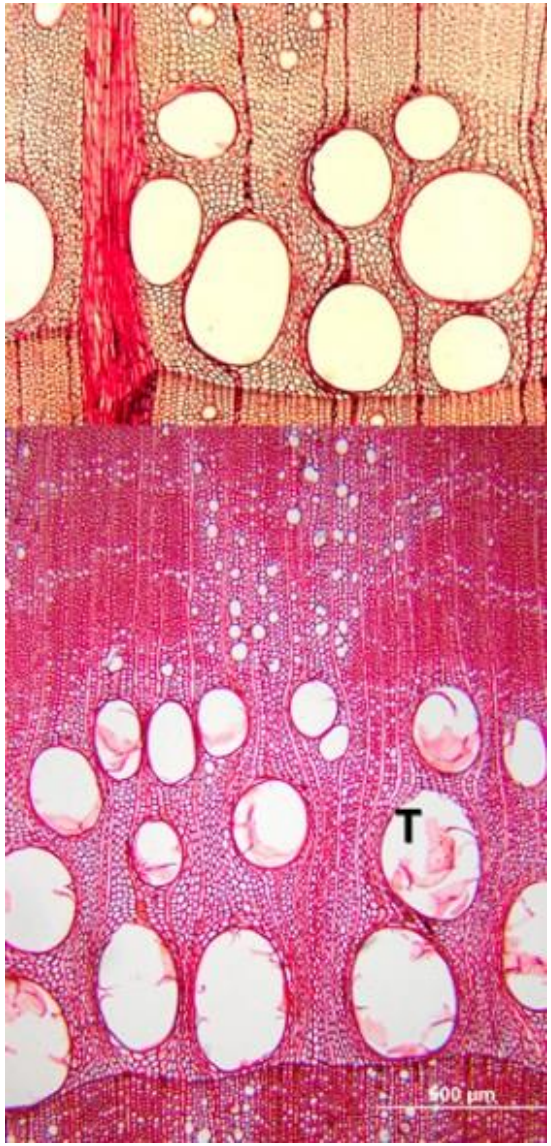
Tyloses in the xylem contribute to wilt symptoms

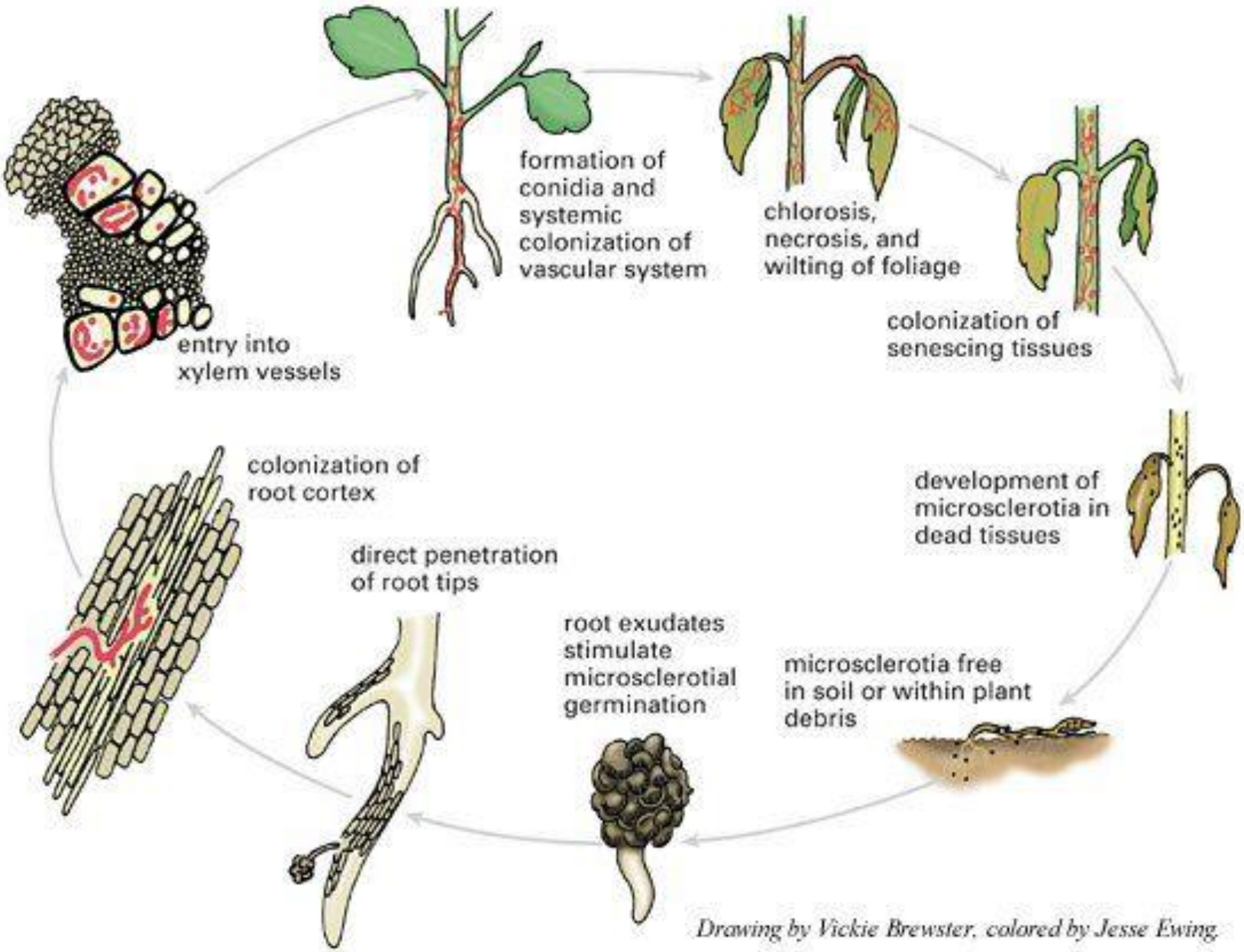
- Leads to one-sided wilt symptoms



Tyloses in xylem

Healthy xylem

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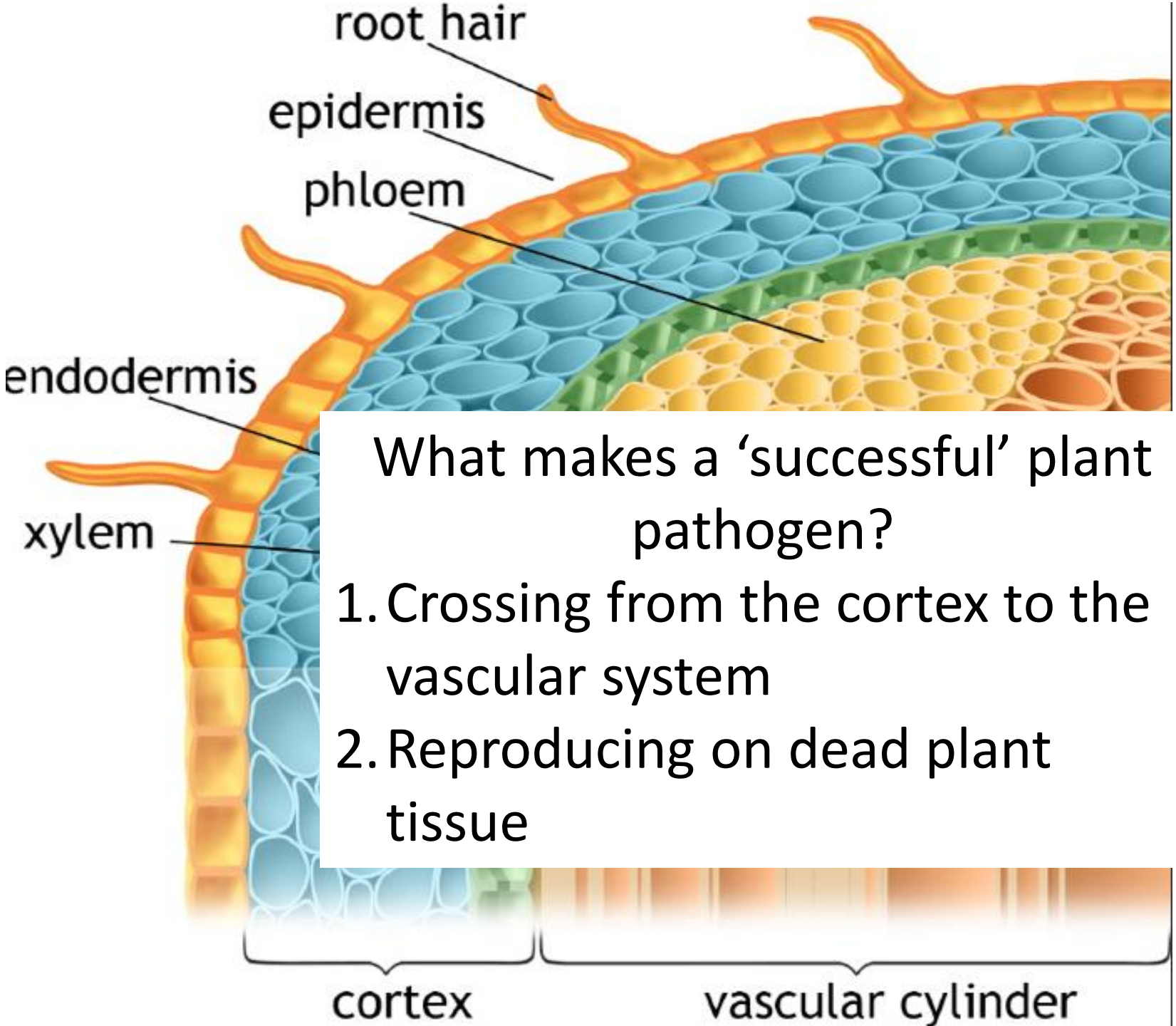

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1. Crossing from the cortex to the vascular system
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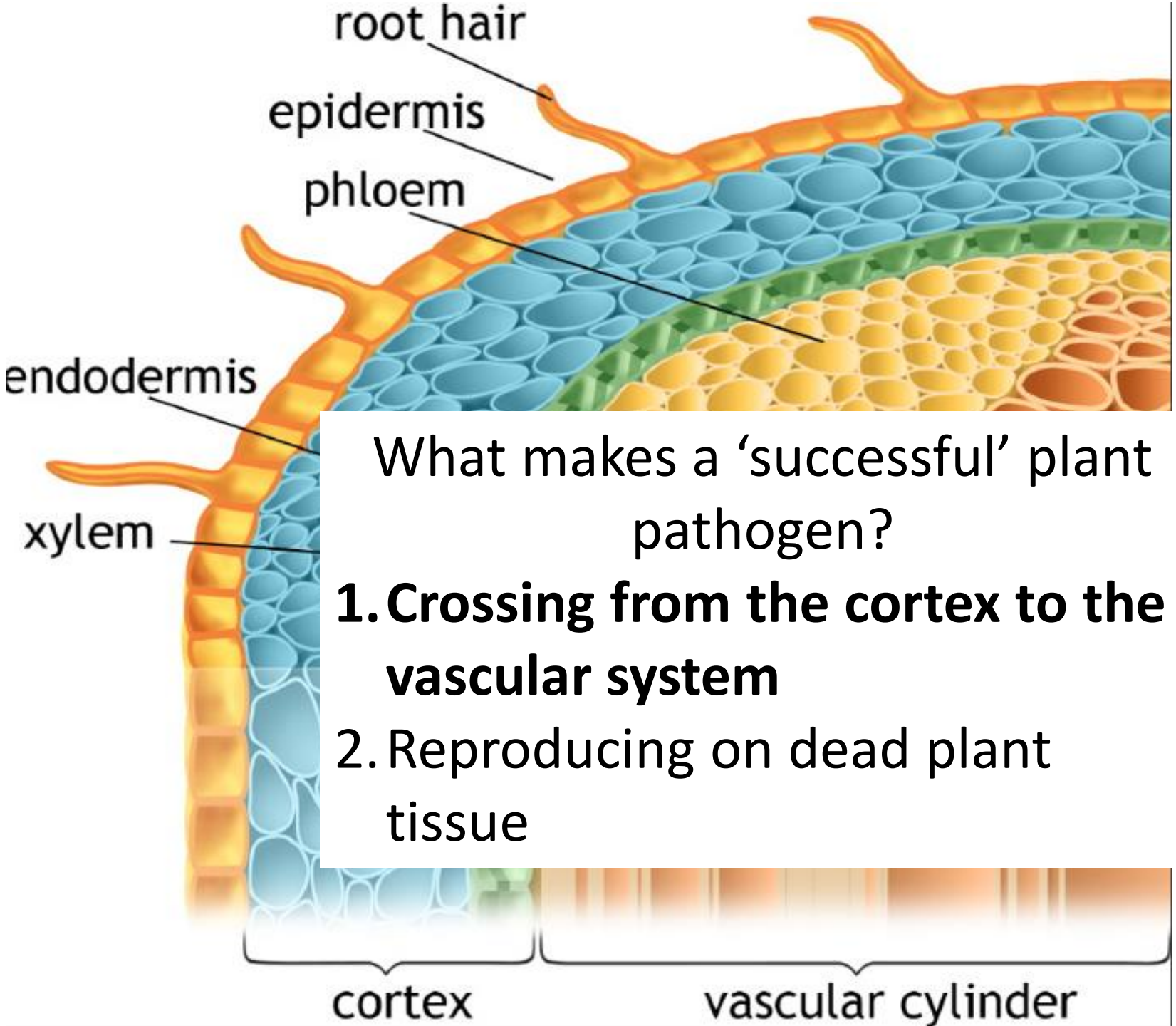

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Races

- Fusarium wilt of tomato: The fungus only infects tomato but exists as three races. Race 1 is widespread; Race 2 is common in the Sacramento Valley and in the northern San Joaquin Valley; and Race 3 is in the Sacramento Valley and spreading into the San Joaquin Valley

Root exudates

- Large amounts of photosynthate is lost from plants via root exudation
  - As much as 25% in some instances

- Root exudates stimulate activity of pathogens
  - Support germination of dormant propagules
  - Provide energy to aid in infection

Root exudates

- Rhizosphere as a pathogen site
- Pathogens can utilize exudates to maintain themselves or increase their inoculum
  - *F. solani* f. sp. *phaseoli* can colonize and produce chlamydospoes in rhizospheres and spermospheres in numerous nonhosts
  - *F. oxysporum* can colonize the cortex of many nonhosts