Systemic Movement of *Raffaelea lauricola* Through The Rhizomes In Pondberry (*Lindera melissifolia*)

Susan Best

BACKGROUND

Grantham, N.C.
Population: 3,959 (all rural)

Small farming community

Crops:
Tobacco
Sweet potatoes
Cucumbers
Strawberries
Corn
Soybeans

Livestock:
Hogs
Turkeys
Chickens

U.S. Air Force

Ssgt G.S. Best
Fuel Specialist
1980 - 1992

Lackland, AFB
San Antonio, Texas

Basic Training

Little Rock AFB
Jacksonville, Arkansas

C-130 Cargo Planes

Spangdahlem, AB Germany

F-16 Fighter Planes

Persian Gulf War - Operation Desert Storm

Diego Garcia Military Base

Loring AFB
Caribou, Maine

B-52 Bombers

Post-Secondary Education

Pensacola Junior College

Pensacola, FL

- Natural Resource Conservation, A.A.
- Forest Technology/Management, A.S.

University of West Florida
Pensacola, FL

- Environmental Studies / Policy, B.S.
- Minor in Geography

Athens, GA

U.S. Forest Service

Insects, Diseases, and Invasive Plants

Plant Pathologist

We provide biological and ecological knowledge for management and control of native and non-native insect pests, disease pathogens and invasive plants.

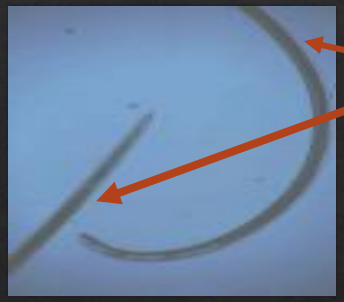
Current Research

Laurel Wilt Disease

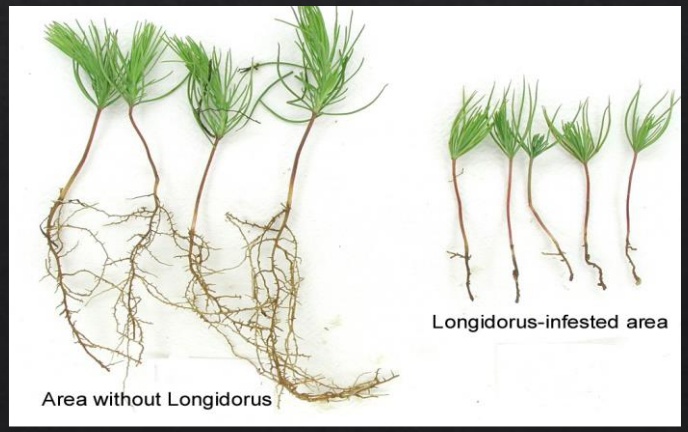
Redbay ambrosia beetle (*Xyleborus glabratus*)

Thousand Canker Disease

Pest Management and Seedling Quality In Tree Nurseries



Longidorius Nematode



Hobbies

Smoking / Grilling

Gardening

Building Projects

Motorcycles

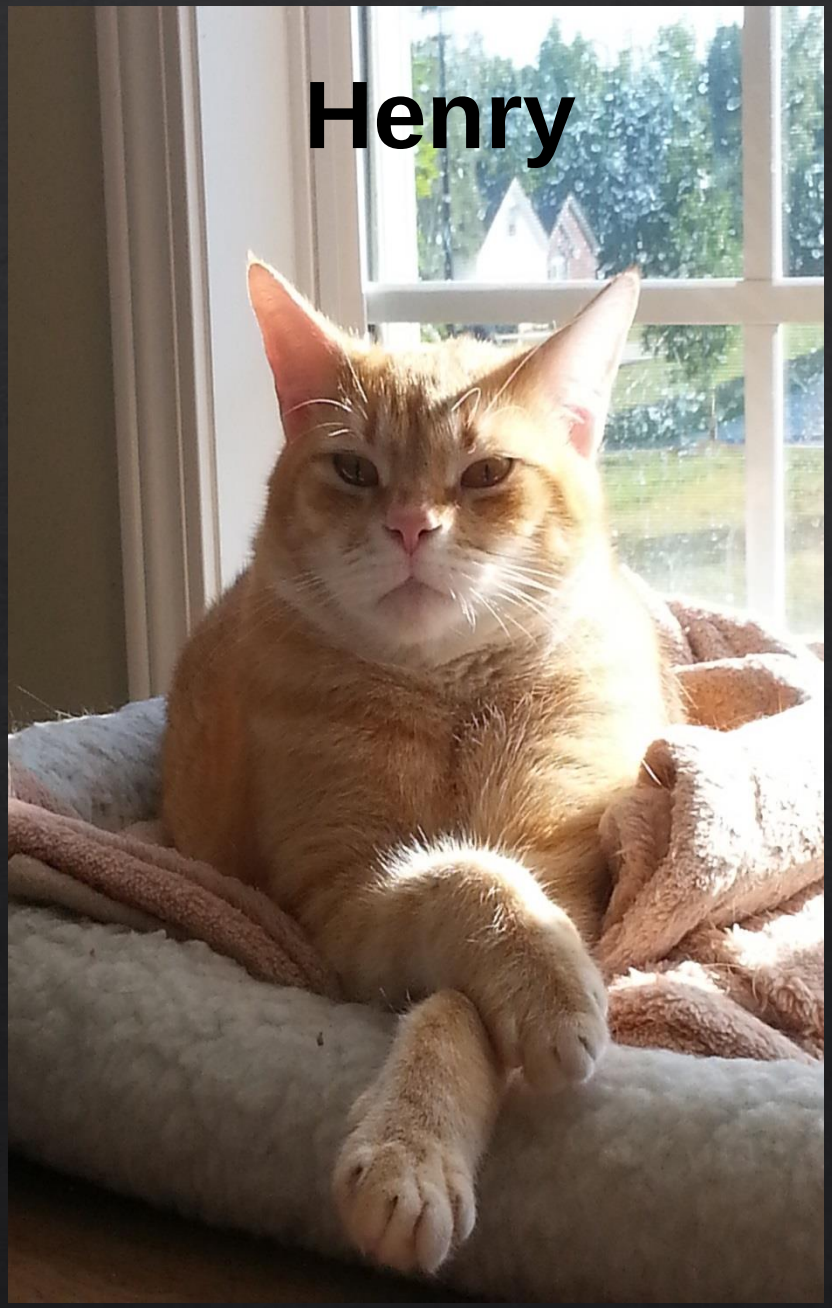
Geocaching

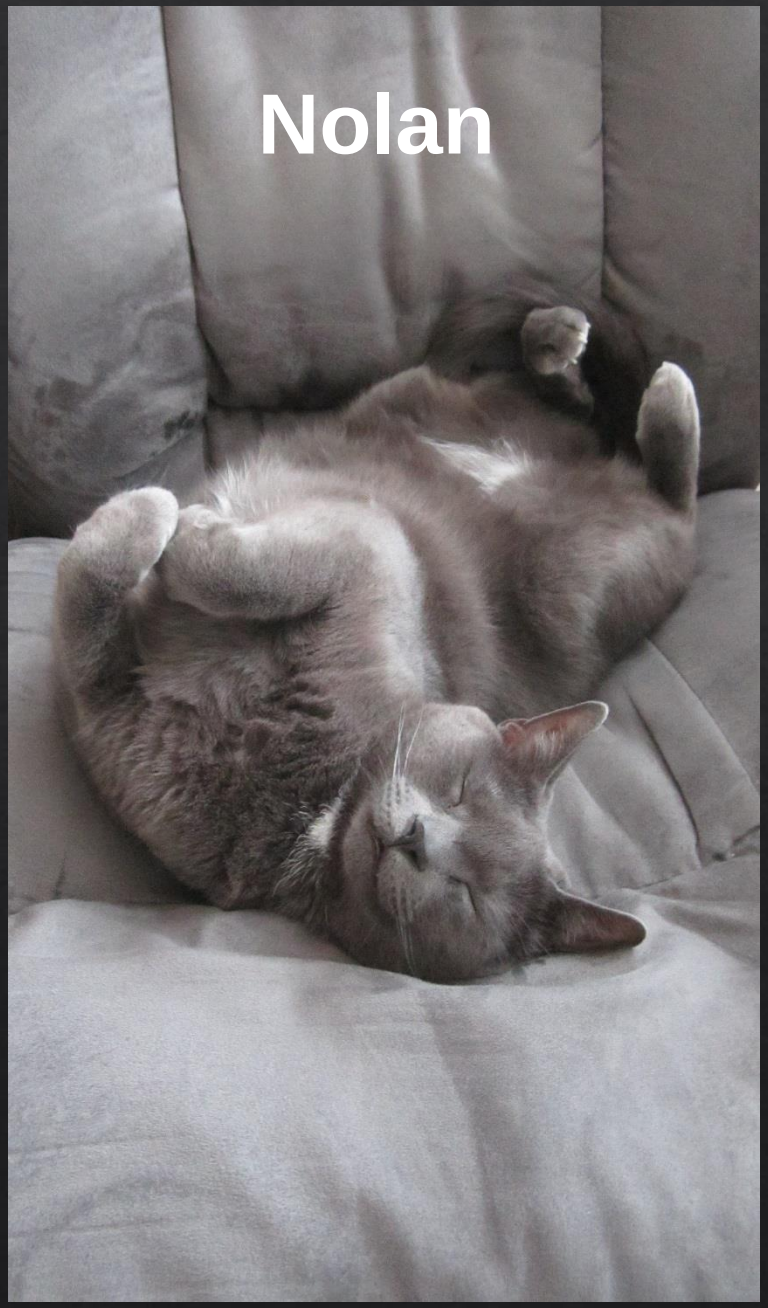
Kayaking

Camping

Hiking

Golfing


Henry


Nolan

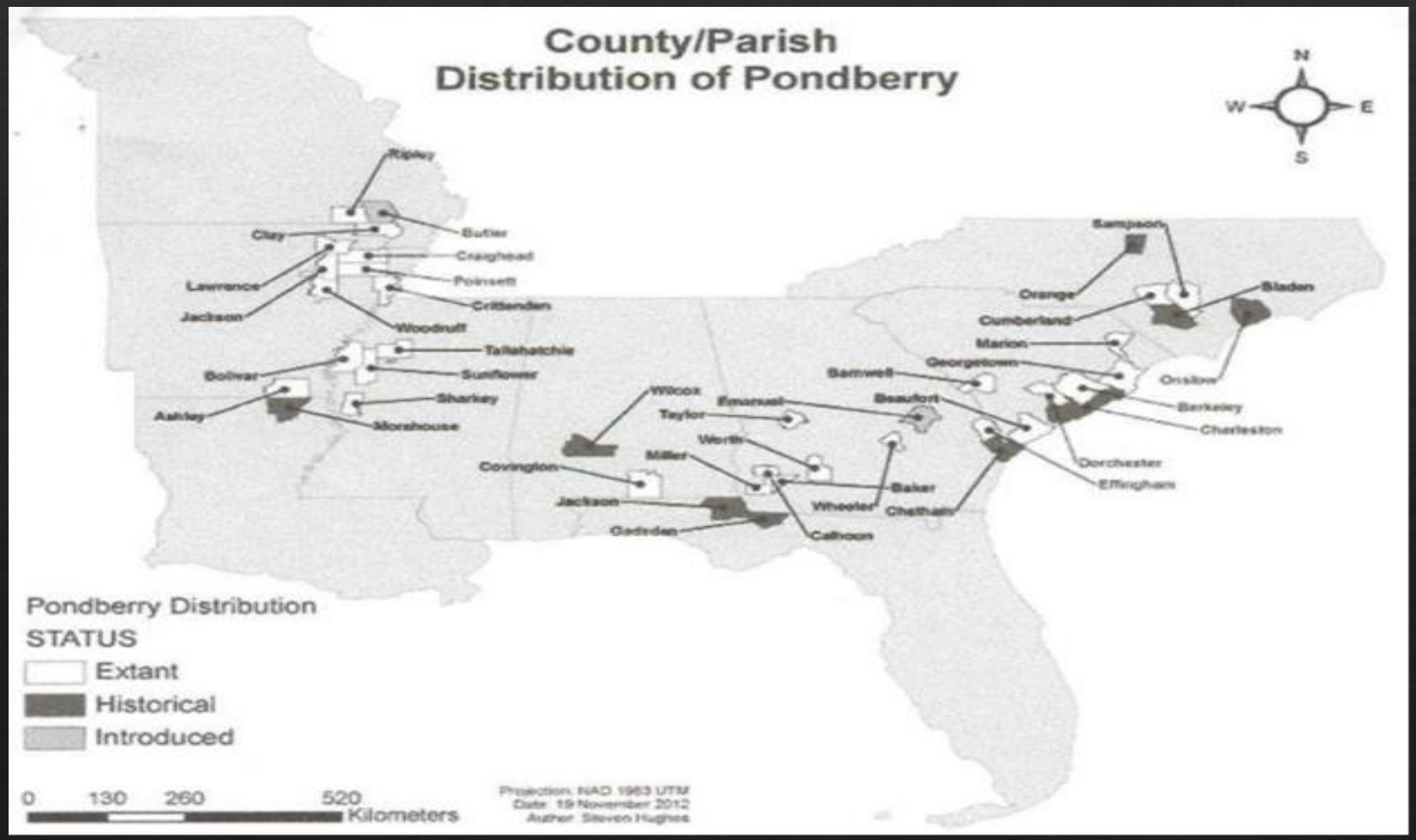
INTRODUCTION

Pondberry
(*Lindera melissifolia)*

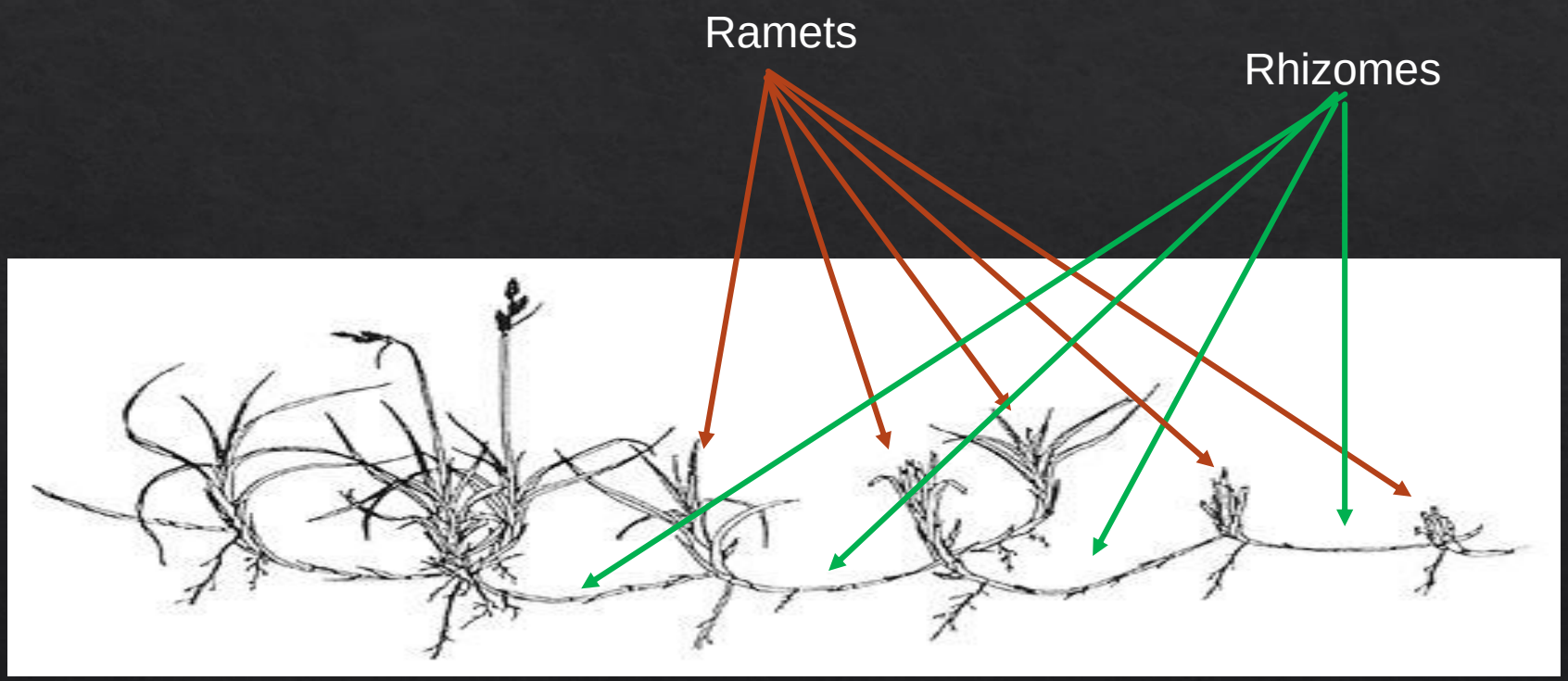
Pondberry in Clyo, GA

- History
- Endangered Species
- Understory Shrub
- Highly Clonal
- Lauraceae Family

Distribution map of pondberry in the southeastern U.S.



Ramets are interconnected and clones of the same plant.

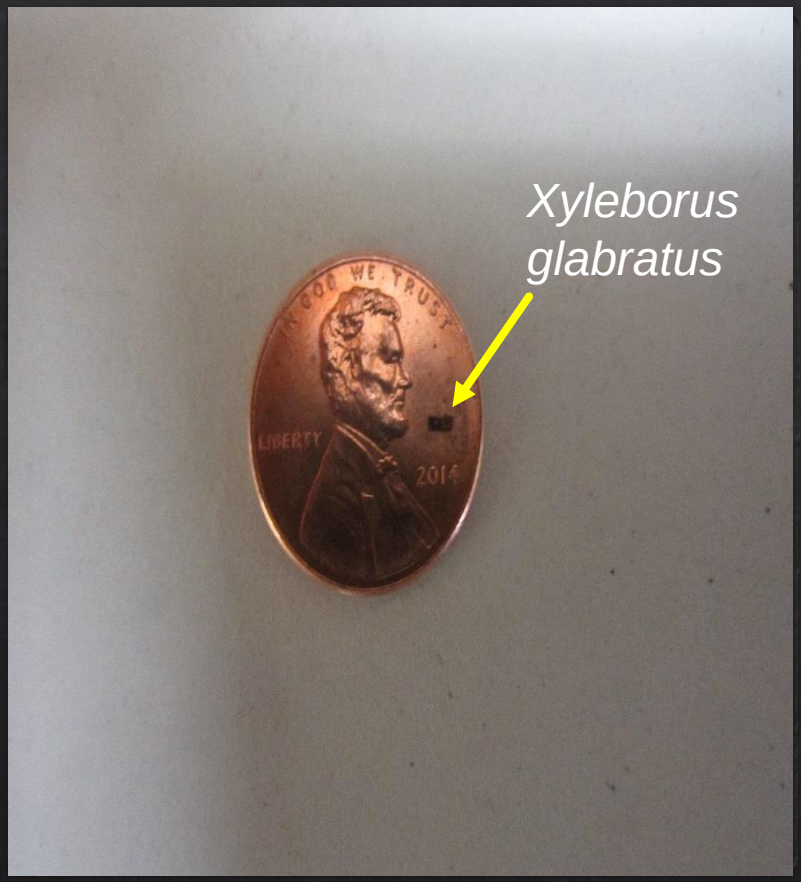


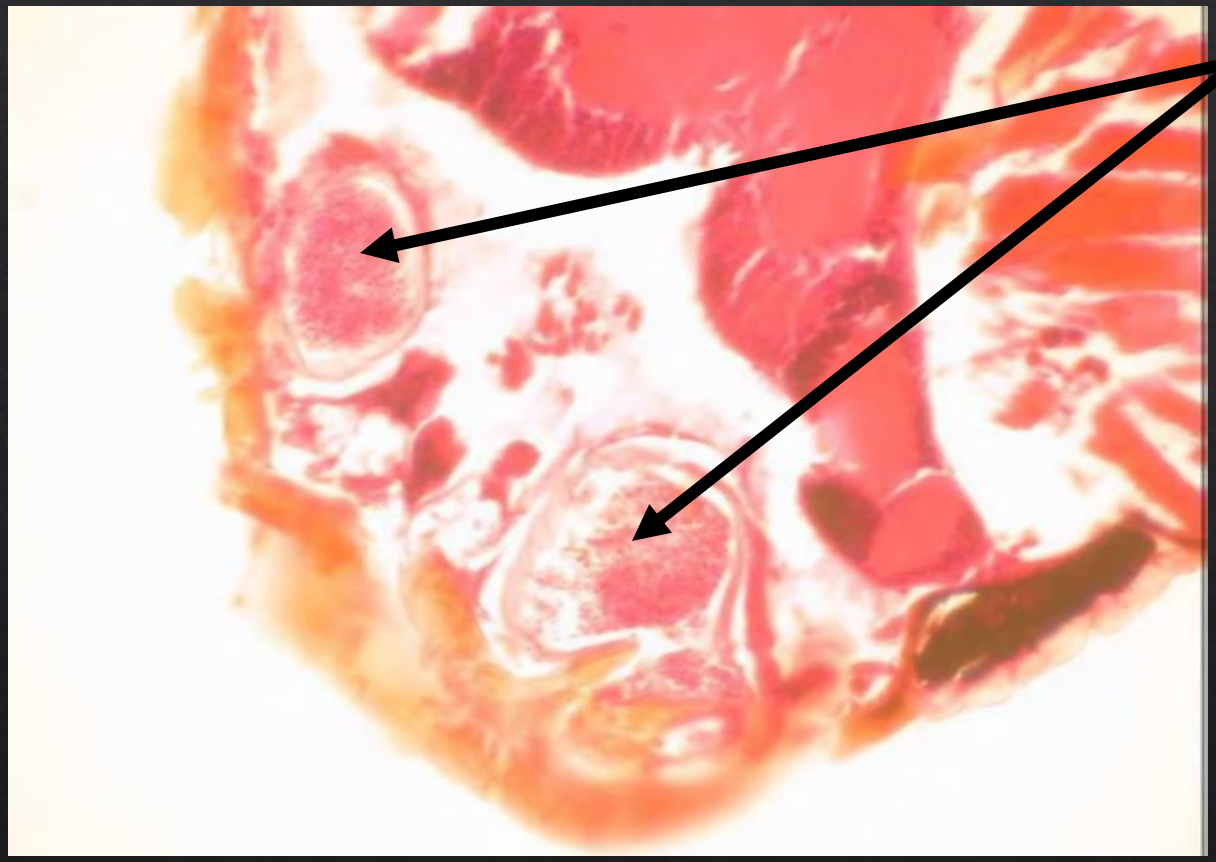
Fruit of Pondberry (*Lindera melissifolia*).

Shaded Pondberry (*Lindera melissifolia)*

Asian native female *Xyleborus glabratus.*

Female *Xyleborus glabratus* on a penny.



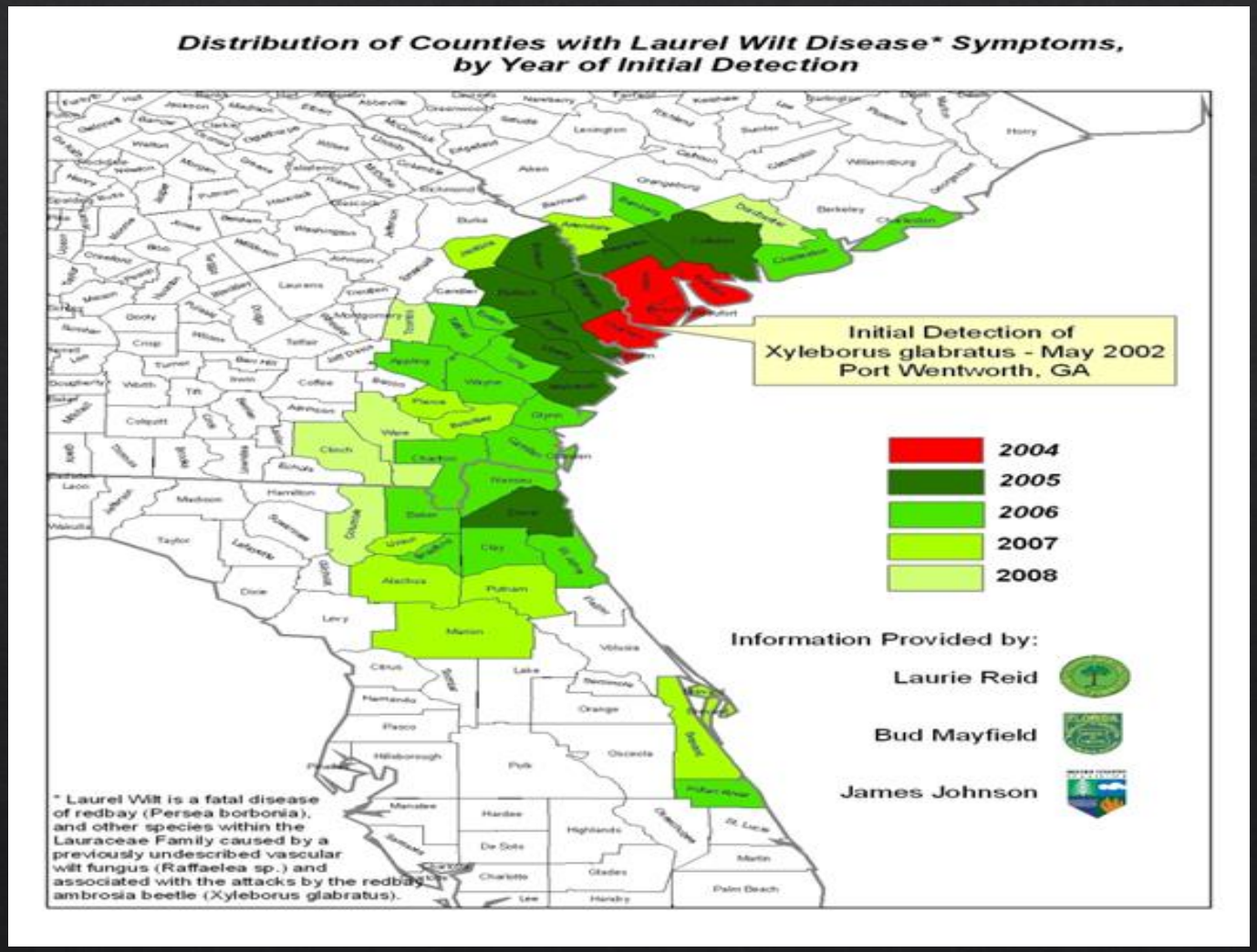


Mandibular mycangia of female *X. glabratus*

Raffaelea lauricola, fungus that causes the disease, laurel wilt.

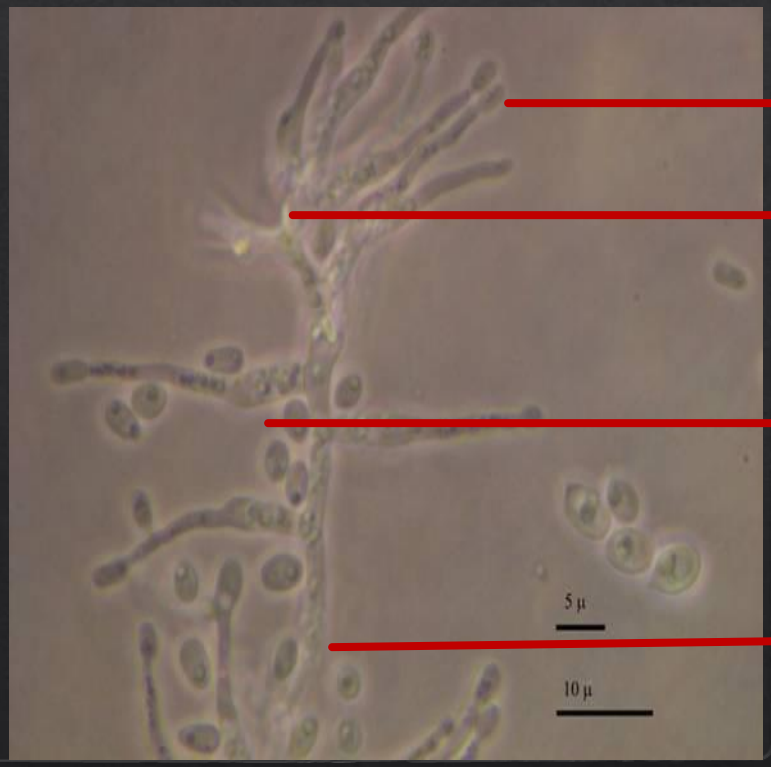
Developmental stages for *Xyleborus glabratus* larvae and adults.

Initial detection of *Xyleborus glabratus* in Port Wentworth, GA.

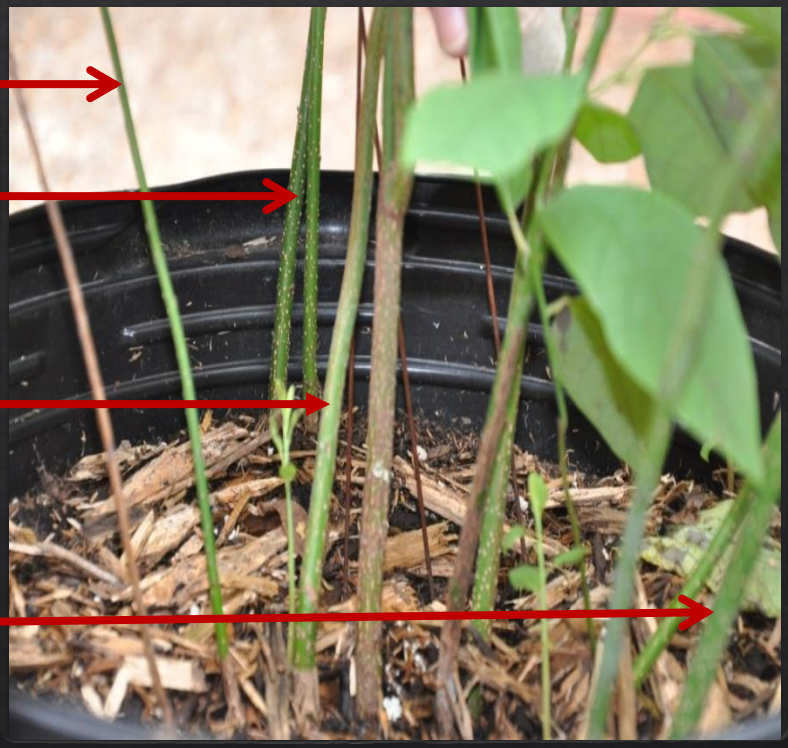


The objective of this study was to determine if the fungus, *Raffaelea lauricola,* could move systemically through rhizomes from an infected plant to other clonally reproduced ramets, within the same population.

Raffaelea lauricola



Pondberry ramets



MATERIALS AND METHODS

Two inoculation experiments were conducted to determine if *Raffaelea lauricola* could move systemically through rhizomes from an infected plant to other ramets.

Pondberry Fruit

- Originated from Mississippi
- De-pulped
- Stratified in moist coarse sand for 30 days at 5 degrees C

- One pondberry plant was transplanted into a 15 gallon plastic blow-molded nursery pot
- 1:4 soil media ratio of coarse sand and peat

- Placed in a courtyard, receiving up to four hours of direct morning sunlight
- Grown for three years

Growth / Sprouting of Pondberry

Early spring / Year 2

Mid-summer / Year 3

The stem of the original planted pondberry in each of four pots was wounded by drilling a hole one-half the diameter of the main stem, using 2.25 mm drill bit.

To prevent cross contamination, the 2.25 mm drill bit was flamed and sterilized between each inoculation.

Pondberry plants, day of inoculation

Wounding pondberries using a cordless drill and 2.25 mm drill bit

Plants were inoculated with 0.1 ml drops of conidial suspensions (5.6 x 10^6 spores/ml) from isolates of the *R. lauricola.*

R. lauricola isolates were obtained from wilted redbay trees on Hilton Head Island, S.C. and were used for both inoculations.

- Inoculation points on all seedlings were wrapped with Parafilm M (Pechiney Plastic Packaging, Menasha, WI)
- Plants were watered as needed
- Observations of disease progression were noted daily

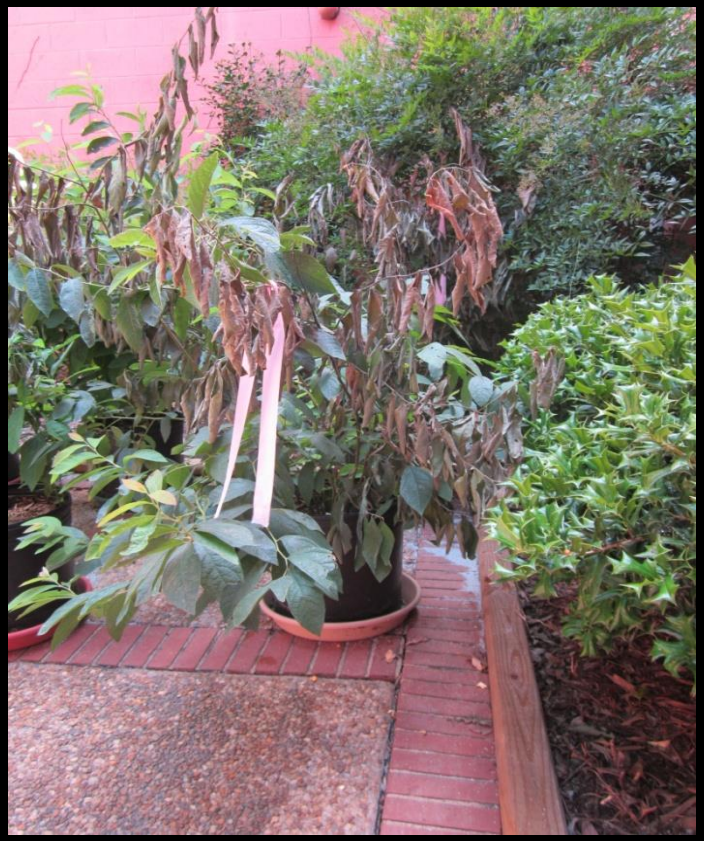
Symptom Development
14 days after inoculation

Leaves showing signs of wilting in inoculated stem and adjacent ramets

Symptom Development
24 days after inoculation

Wilting and dieback continues to spread among ramets

Symptom Development
45 days after inoculation



Many ramets are dead and dying.

Symptom Development
72 days after inoculation

10 weeks after inoculations, the plants were taken out of the pots and soil removed from the roots, revealing all rhizome connections within each individual pot

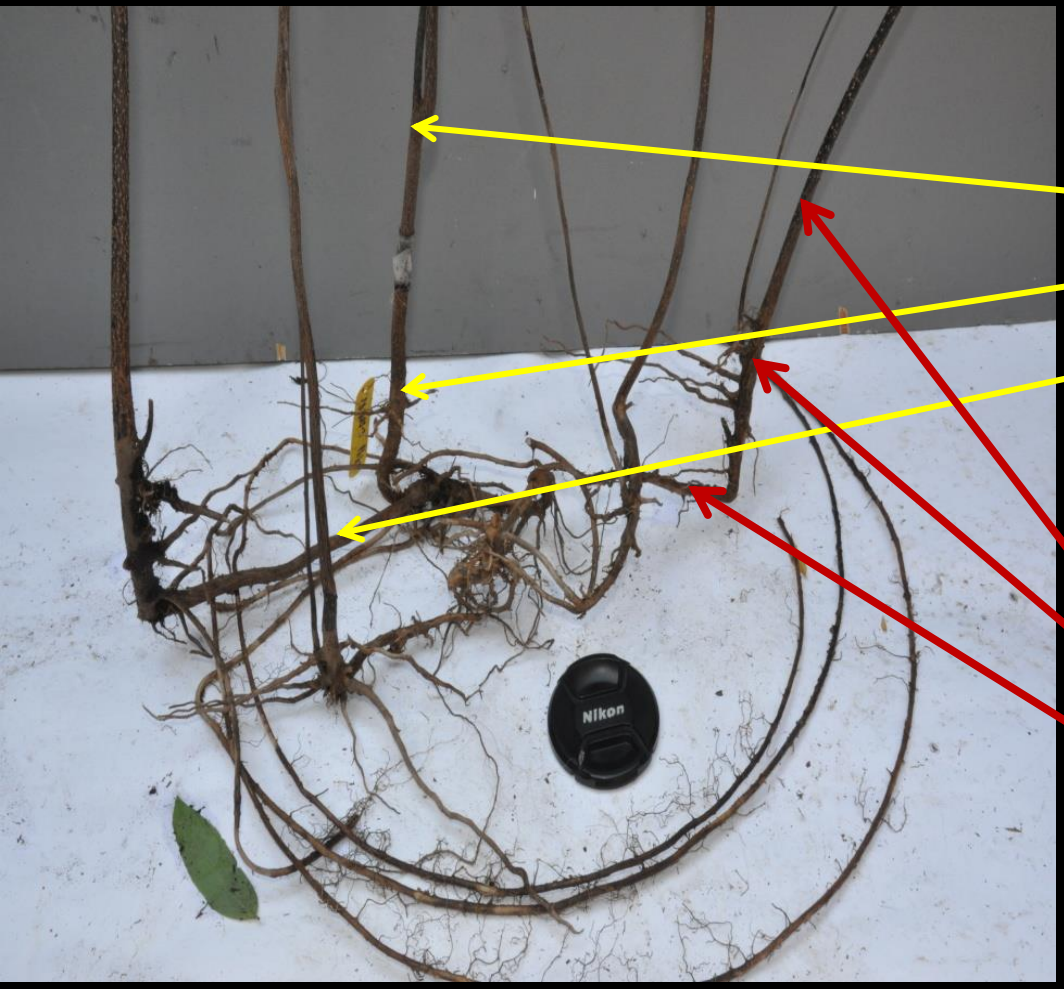
Pondberry rhizomes and root systems

Original inoculated stem

Rhizomes that connected the original inoculated stem, with discolored, and dark brown stain.

Examination of the xylem of rhizomes that connected the original inoculated stems to adjacent wilted ramets, were consistently discolored, with a dark brown stain.

Sampling for *R. lauricola*



Inoculated Main Stem

Stem

Root collar

Root connections with ramets

Ramets

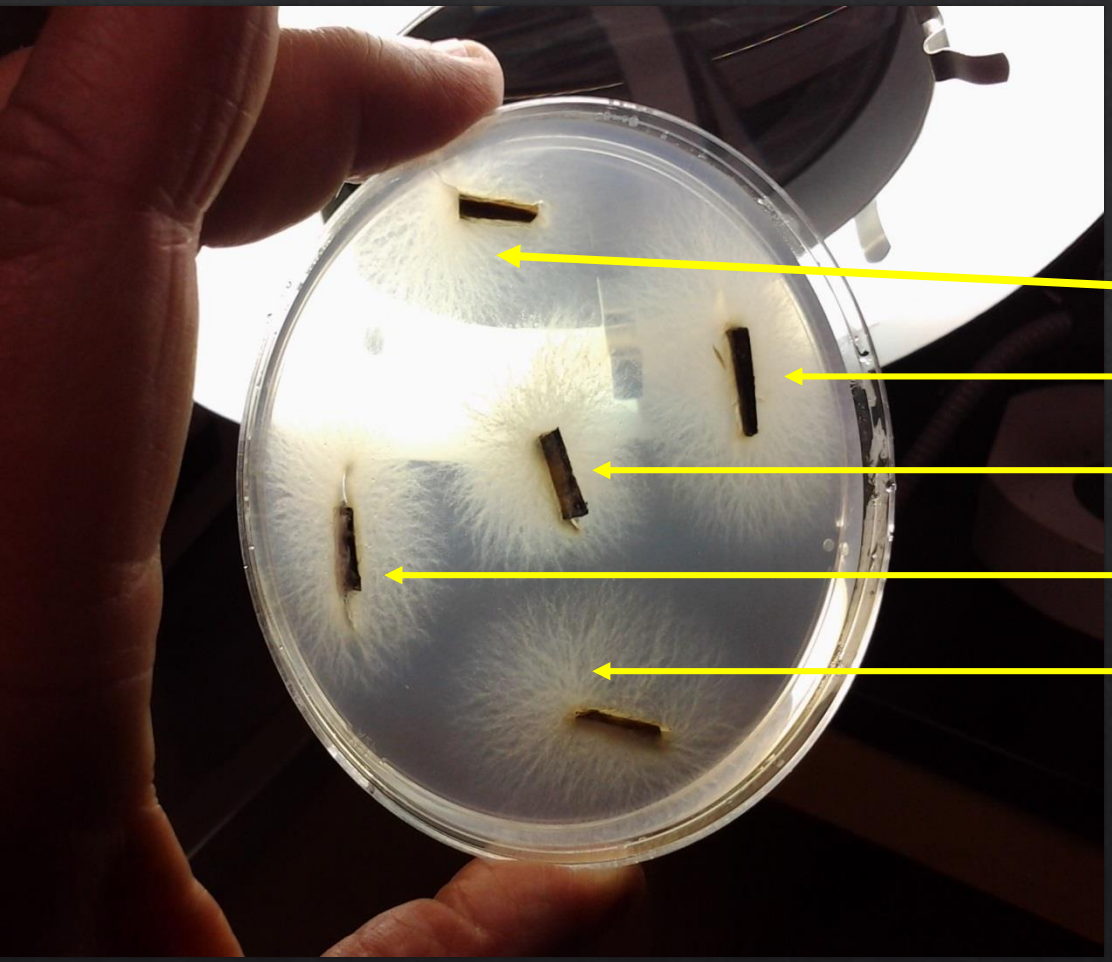
Stem

Root collar

Root connections with other ramets

Plated discolored xylem, from inoculated pondberry rhizomes, onto cycloheximide-streptomycin malt agar (CSMA).

Isolation of *R. lauricola*



Samples were surface sterilized and plated on Cycloheximide-Streptomycin Malt Agar (CSMA) CSMA

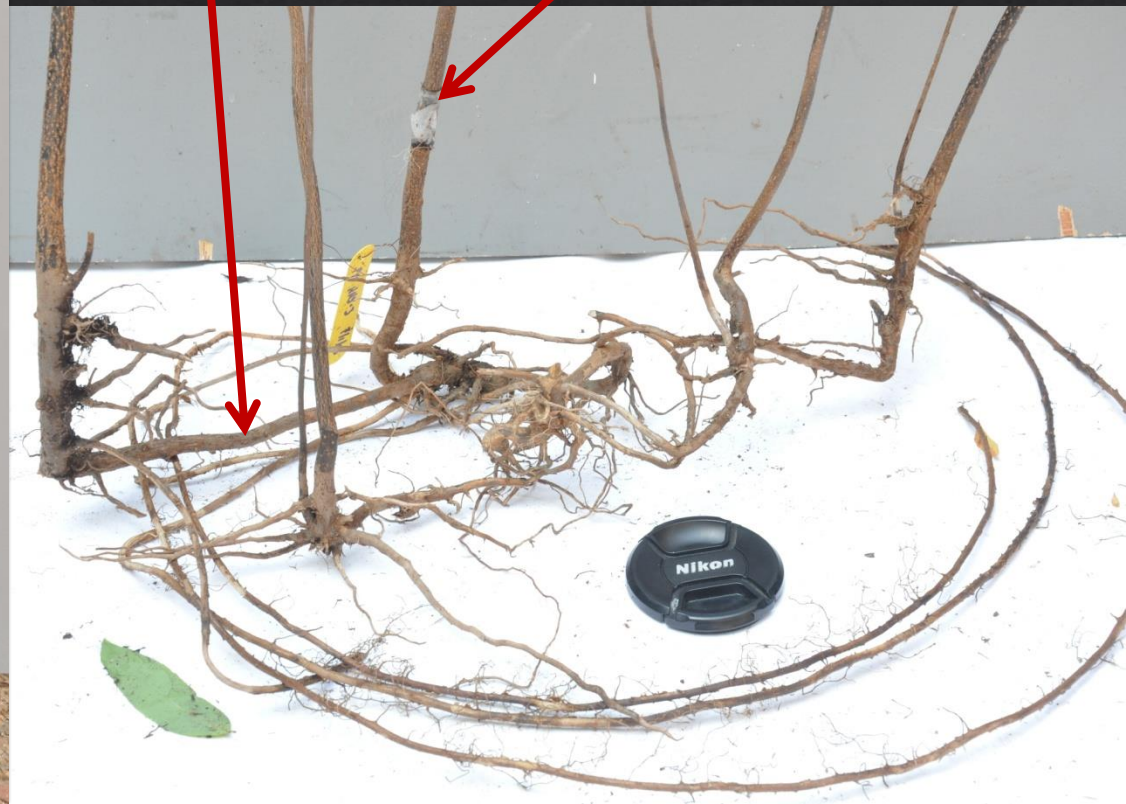
After 5-8 days, *R. lauricola* emerged on cycloheximide-streptomycin malt agar (CSMA) petri dish plates, that were stored in an incubator at 24°C.

RESULTS AND DISCUSSION

Table 1. Results of two inoculation experiments to examine the movement of *Raffaelea lauricola* through the rhizomes of pondberry and subsequent spread of laurel wilt to adjacent ramets.

Date	Treatment	Pots	Mean number (range) of ramets/pot	Mean percentage (range) of wilted ramets/pot	Mean percentage of wilted ramets with R. lauricola	Mean distance (range) from inoculated plants to outermost wilted ramets (cm)
Jul-13	*R. lauricola*	4	20(10-26)	76(39-90)	100	21(17-26)
	Control	2	14(9-18)	0	N/A	N/A
Aug-13	*R. lauricola*	4	14(9-26)	56(35-78)	95	18(15-22)
	Control	2	20(7-32)	0	N/A	N/A

R. lauricola **was consistently recovered from the stem, root collar, and root connections of dead and dying ramets that were connected to the main inoculated stem.**



CONCLUSION

This study confirms that the fungal pathogen that causes laurel wilt, *Raffaelea lauricola,* can spread rapidly through rhizomes from infected pondberry plants to adjacent ramets in a population.

Each experiment was conducted for only a 10-week duration, in relatively small containers, but during this time *R. lauricola* moved rapidly through many rhizomes that were connected to the original inoculated plant and to other ramets that became infected.

These two experiments documented that when pondberry plants are infected with *R. lauricola,* other members of the population are at risk, resulting in mortality.

Consequently, the infection of a single plant by this fungal pathogen could have detrimental effects to the pondberry plant population to which the infected individual belongs.

Additional studies are currently underway in experimental beds to develop a better understanding of the distance and rate at which *R. lauricola* can move through populations of pondberry .

Strategies for control of the disease are also being tested.

Pondberry test beds at the Whitehall Experimental Forest in Athens, GA.

Questions