

# Use of several natural products from tobacco and Nicotiana species to prevent black shank disease in tobacco

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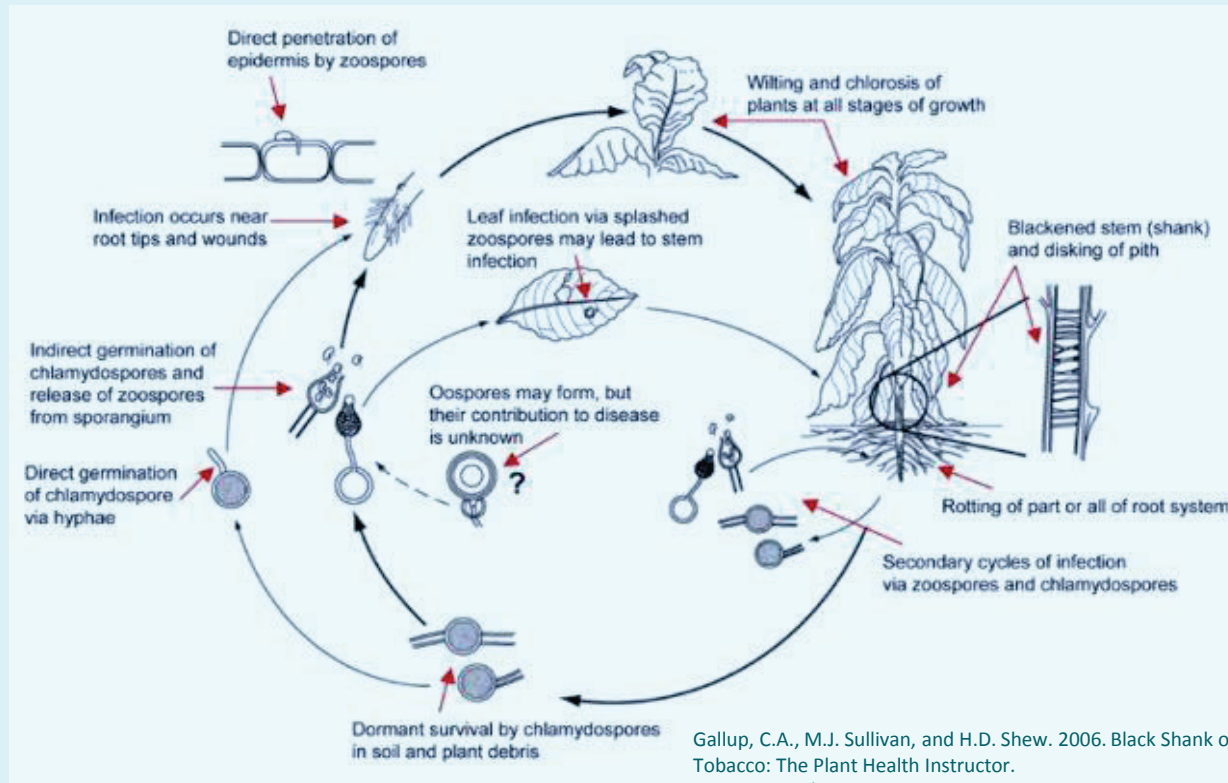
KTRDC, Lexington, KY, USA

# Introduction

- Black shank is the largest annual disease threat to Kentucky tobacco, surpassing blue mold in importance, if we look at annual revenue losses.
- The disease is caused by the soil fungus *Phytophthora parasitica*, var. *nicotianae* that attacks plant's roots and stems and leads to dark-brown to black lesions, eventually killing the plant.
- Currently, *P. parasitica* is found in the majority of tobacco fields in the southern United States, causing damage in 2 to 5.5% of them, but in some fields losses can be 100%.

# *P. parasitica* life cycle

- There are two races of this fungus on the territory of Kentucky: race 0 and race 1



- Infection occurs through the roots and is favored by moisture and warm temperatures

# Approaches to fight the disease:

- Breeding of resistant varieties (e.g. burley lines 209 LC and 206 LC, MS KY14xL8 or MS Burley 21xL8)
  - L8 hybrids are resistant only to Race 0.
  - Resistance is conditional.
- Fungicide application (mefenoxam-containing fungicides)
  - Long-term negative effect: acquired resistance to them, collateral toxicity and environmental pollution.
- Crop rotation
- Biological approach - still experimental (*Rhizoctonia fung*; *Paenibacillus polymyxa*).

# Continue...

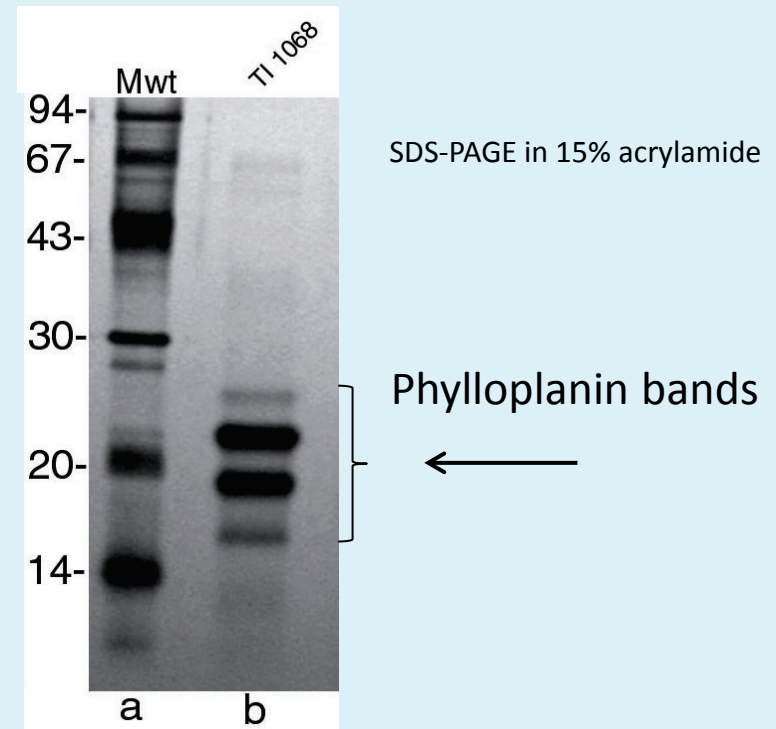
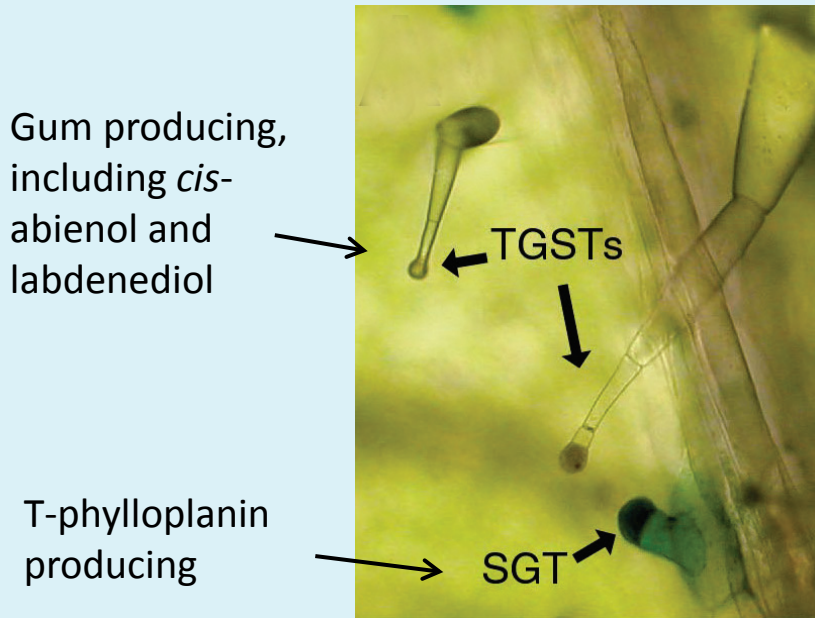
- Because of the long-term negative effect of the fungicides, there is a need for development of alternative ones that are sustainable and eco-friendly.
- Some plant-made products are shown to have antibacterial, antifungal and insecticidal properties.
- Some essential oils and tobacco root constituents have been found effective against black shank disease.

# Hypothesis:

- Our laboratory is specialized in the study of surface chemistry of tobacco leaves.
  - Recently, it was discovered that a tobacco trichome-produces glycoprotein (T- phylloplanin) is active against blue mold spores.
  - T-phylloplanin and trichome-produced diterpene *cis*-abienol showed a potential for inhibit black shank in laboratory tests.
- We hypothesized that application of these natural products, and also of terpenoids sclareol and labdenediol, in potted plants in the green house can inhibit *P. parasitica* zoospore penetration into the roots and can prevent/reduce disease symptoms.

# Sites of synthesis of *cis*-abienol, T-phylloplanin and labdenediol

*N. tabacum* T.I.1068 is known to produce both - T-phylloplanin and also terpenoids, such as *cis*-abienol or labdenediol in quantity.

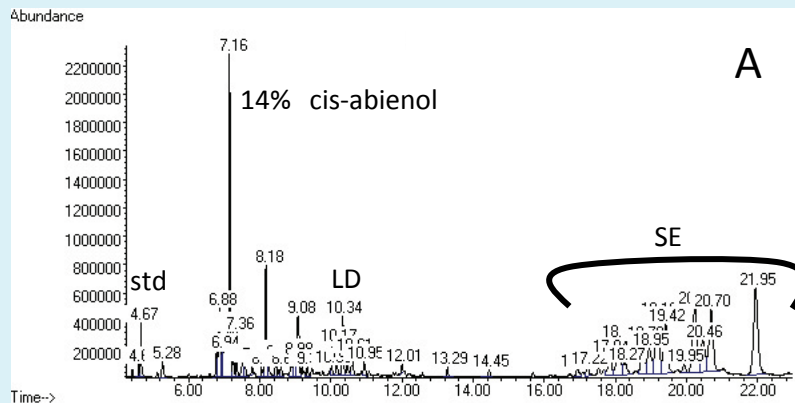


# Natural products collection

- **T-Phylloplanin:** T.I. 1068 leaf water wash, followed by concentration via freeze-drying.
- ***cis*-Abienol:** We used a line of T.I. 1068, created in our lab, that produces increased (at least 3 times) amount of *cis*-abienol. The leaf exudate was acetonitrile washed, concentrated, and further purified by partitioning between hexane and 80% methanol.
- **Labdenediol:** We used a line of T.I. 1068, created in our lab, producing labdenediol in high abundance (90% of all terpenoids). The compound was collected the same way as *cis*-abienol, but was not purified additionally.
- **Sclareol:** Obtained commercially.



# *cis*-Abienol purification



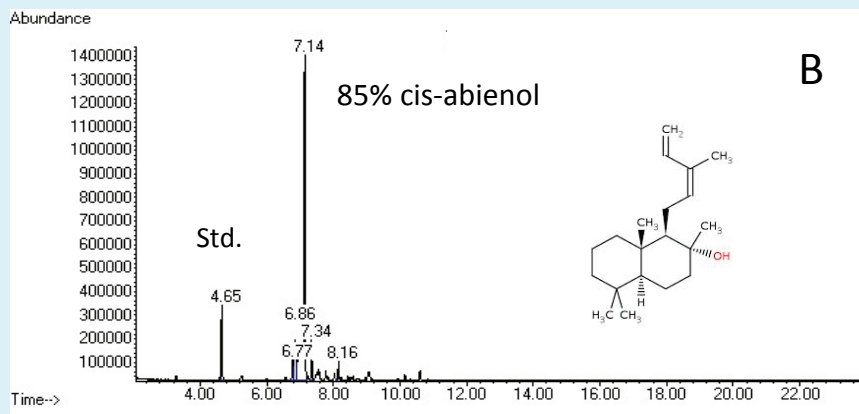
## A. Crude acetonitrile wash

7.16 min – *cis*-abienol

17-23 min – Sugar esters

10.34 min – Labdenediol

4.67 min - standard



## B. Purified *cis*-abienol and structure

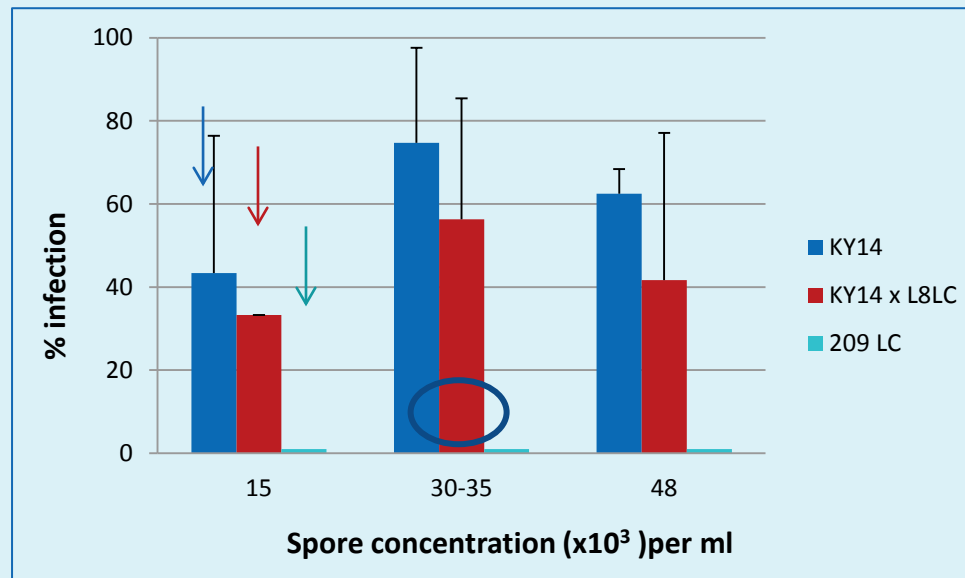
7.14 min – *cis*-abienol

4.65 min - standard

# A customized method for *P. parasitica* spores preparation

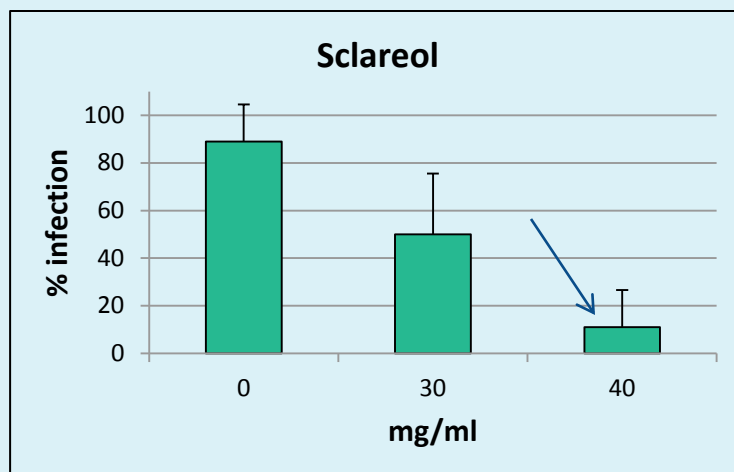
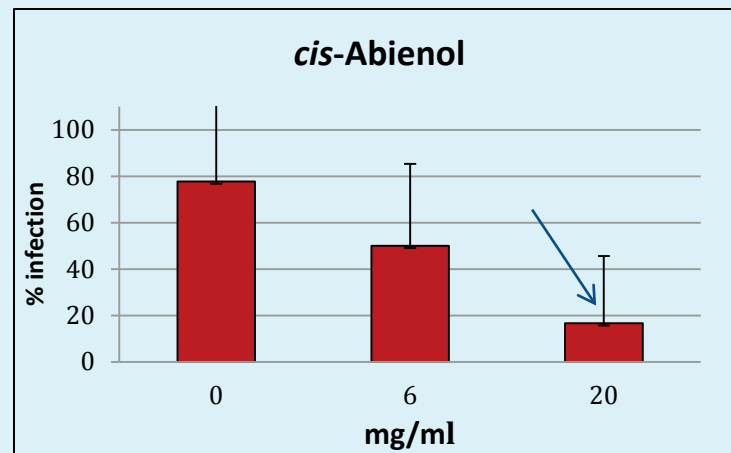
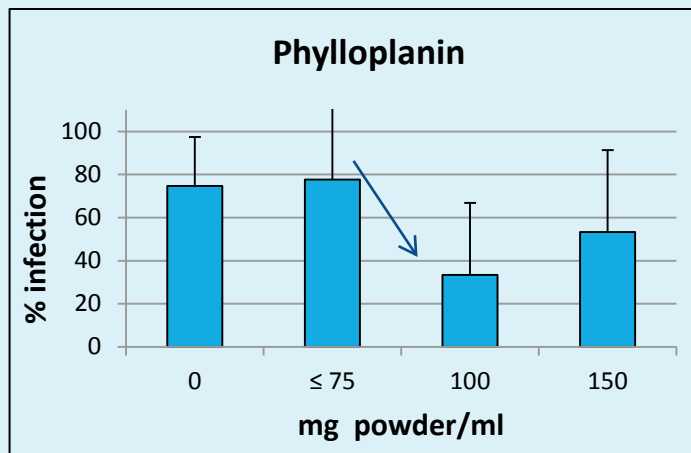
- One plug (1<sup>3</sup>cm) of agar-mycelium from a 3-7 d old culture is placed in a plate with V8 medium. The plate is kept in the dark for 4-6 days.
- 5 plugs (1<sup>3</sup>cm) of agar-mycelium from the edge of the mycelial mat are placed in a Petri dish with 20 ml sterile tap water. The plate is kept in the dark, at room temperature for 7 days.
- Hyphal mat is monitored under scope for sporangia formation.
- Upon formation, sporangia are induced to release spores by temperature stress: 4°C for 30 min, 37°C for 8 min, and 22° for 20 min .
- Moving spores are monitored under microscope.
- Spores are immobilized and counted with hemocytometer.

# Establishing the working concentrations of spores

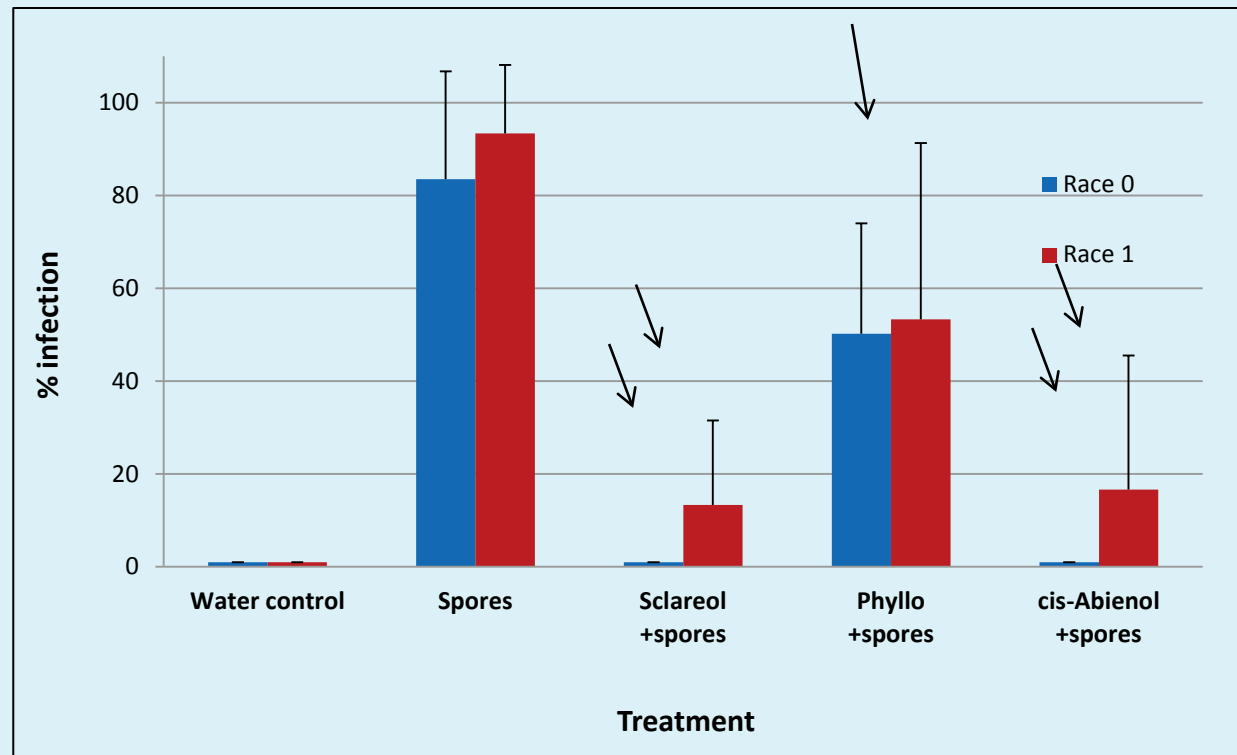


*P. parasitica* race 1 spores were diluted with water to the desired concentration and 1 ml of each concentration was applied to the base of the stems of 5 week-old seedlings.

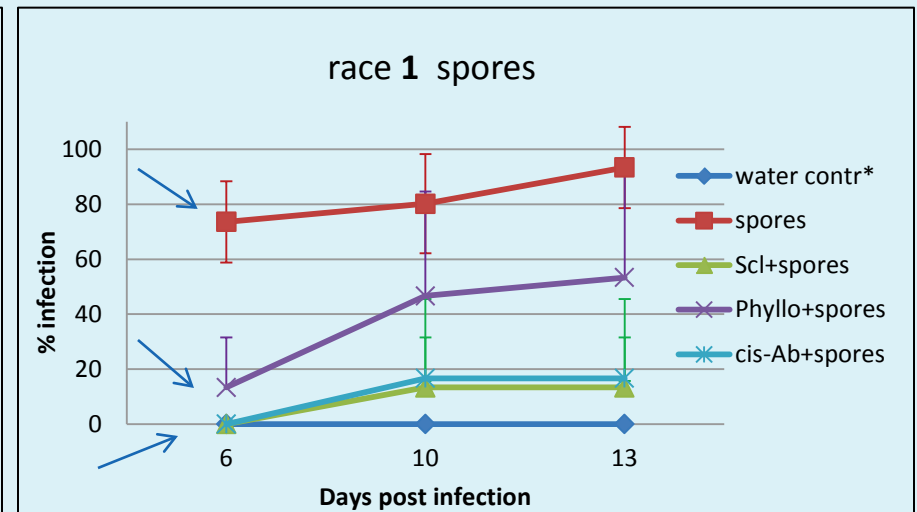
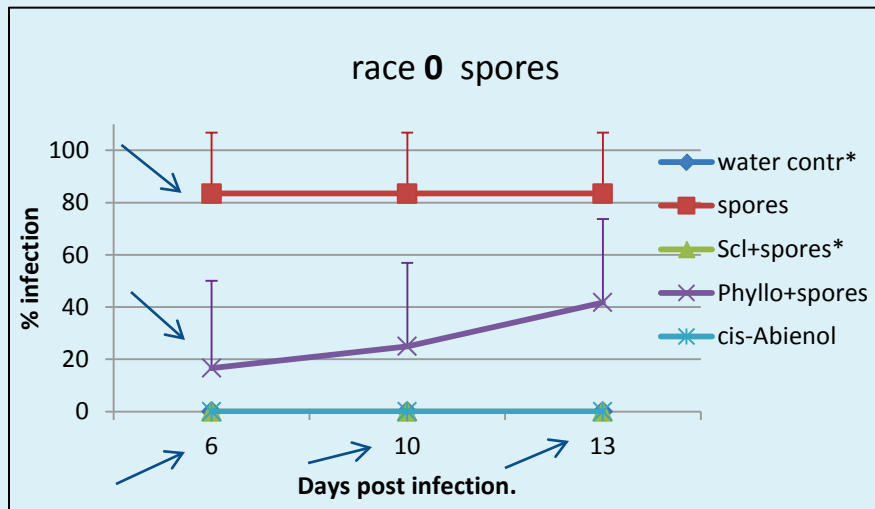
# Establishing the working concentrations of natural products



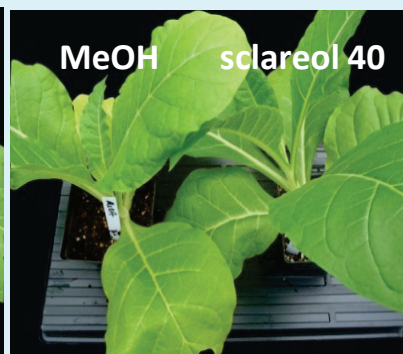
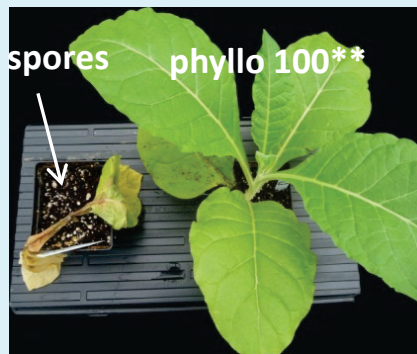
# Effect of sclareol, phylloplanin and *cis*-abienol on suppression of black shank disease in 7 week-old KY14 plants



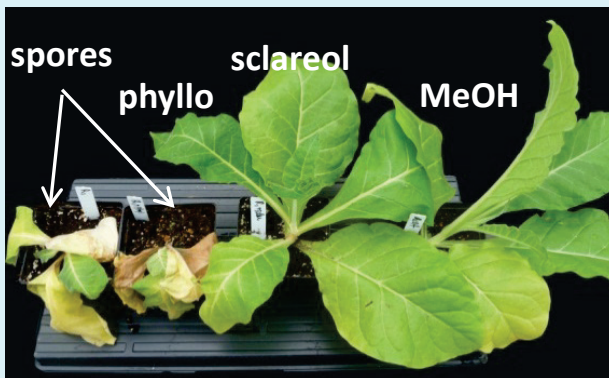
# The natural products delayed/suppressed black shank symptoms in 7 week-old KY14



# Observation of the disease symptoms in KY14 seedlings



Race 0

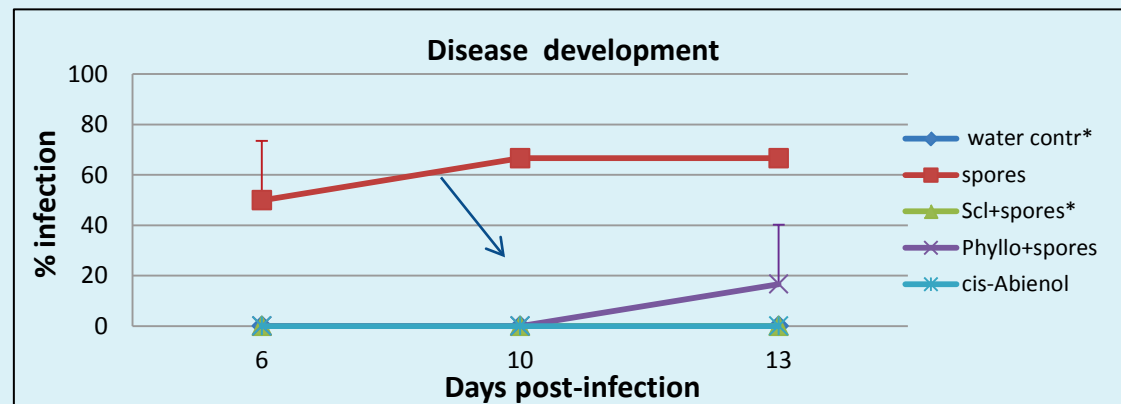
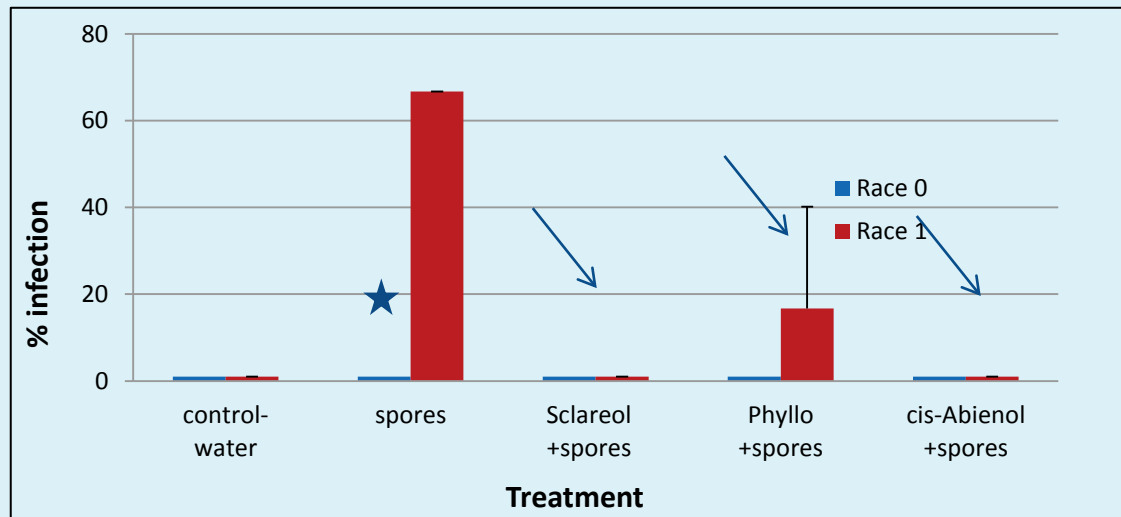


Race 1

\*Pictures were taken on **the 10<sup>th</sup>** day post-infection

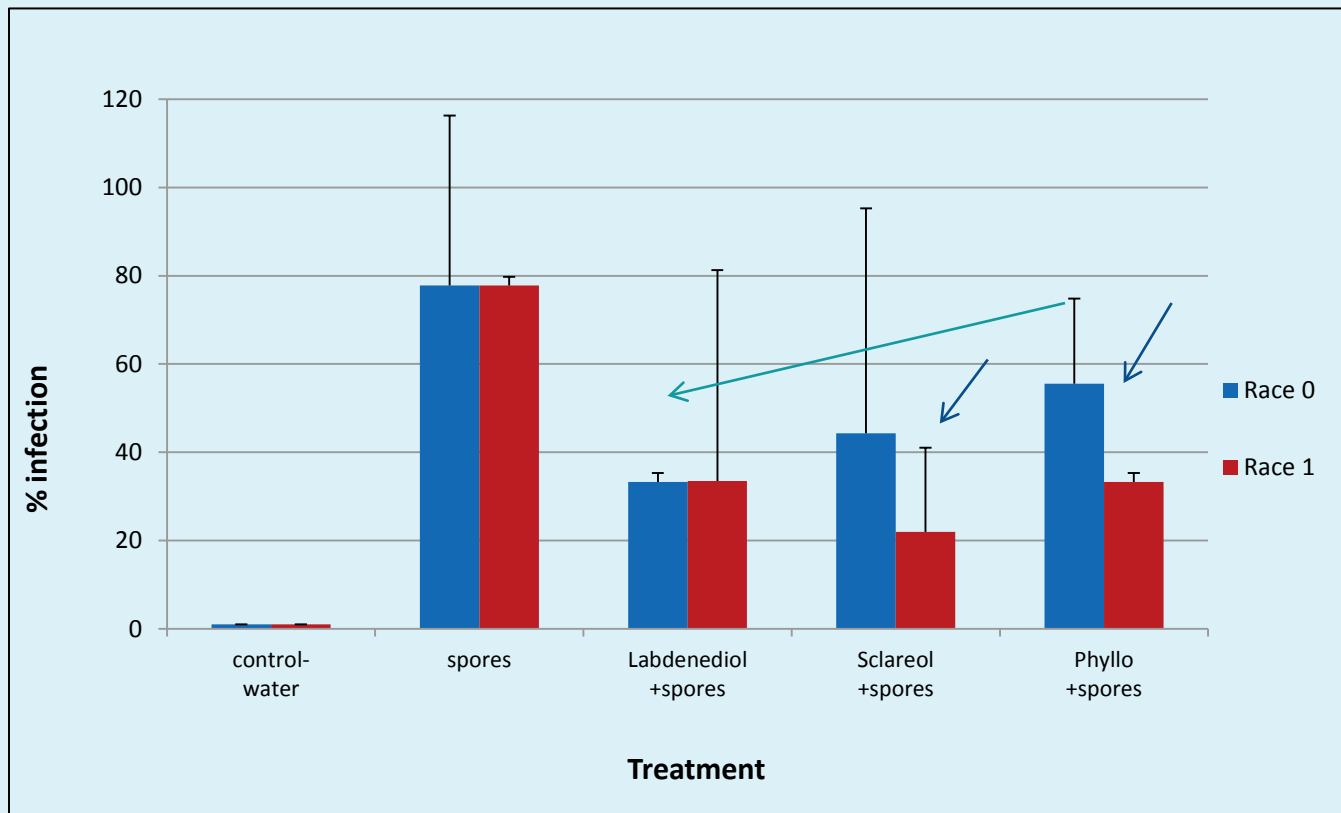
\*\*Numbers represent mg/ml

# Effect of sclareol, phylloplanin and cis-abienol on suppression of race 1-caused disease in 7 week-old KY14xL8\*

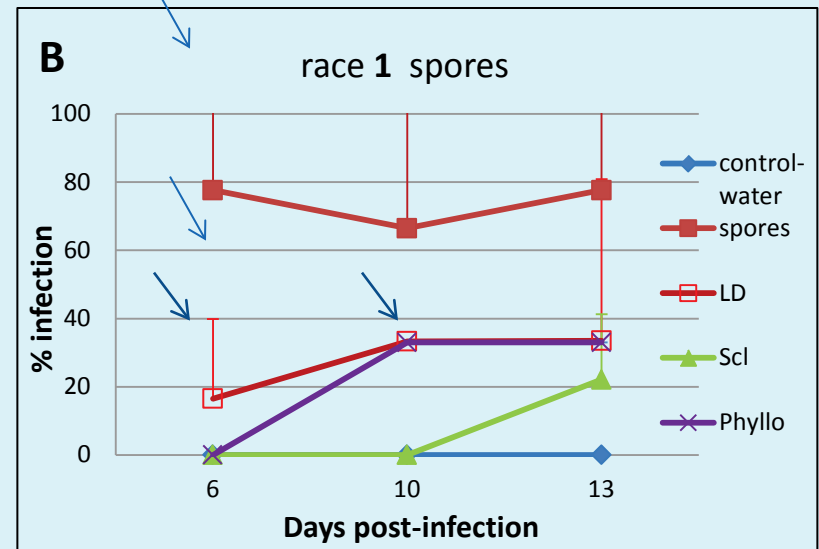
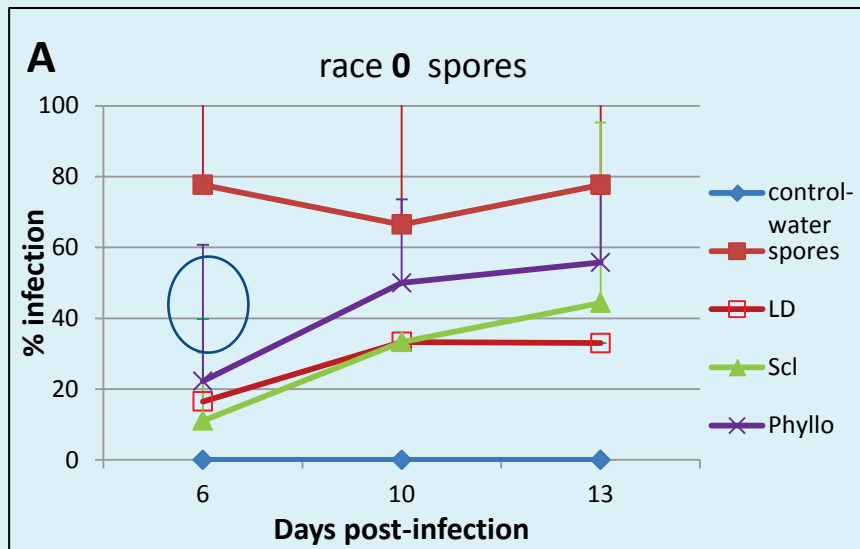




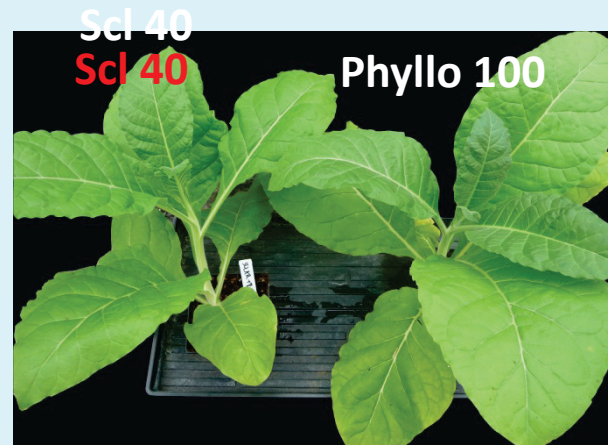
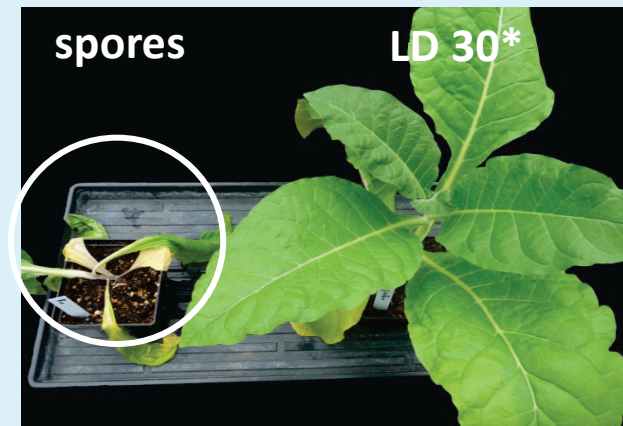
# Effect of sclareol, phylloplanin and labdenediol on suppression of black shank disease in 8 week-old KY14



# Delay of black shank symptoms in 8 week-old KY14

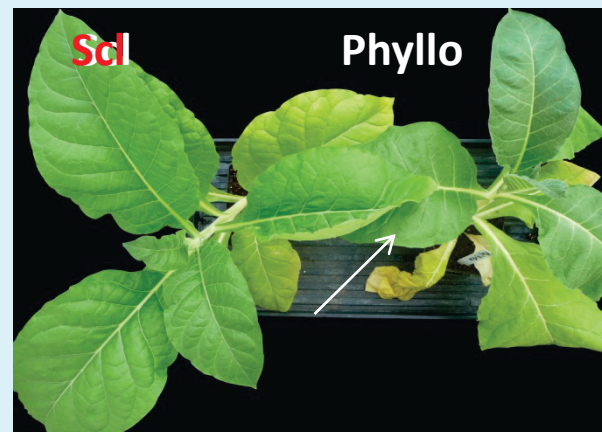
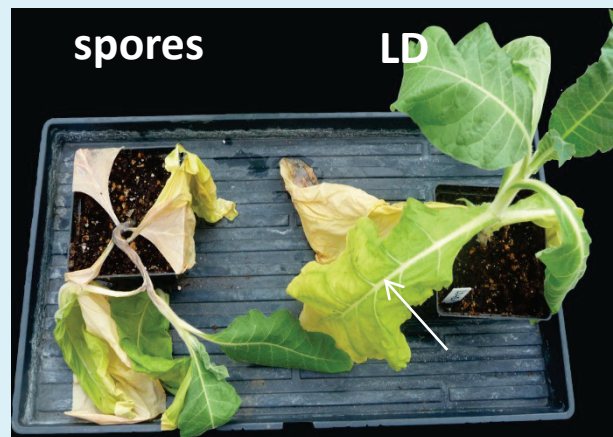


# Delay of race 0-caused disease by labdenediol and phylloplanin, and disease prevention by sclareol



\*mg/ml

6<sup>th</sup> day PI



13<sup>th</sup> day PI

# Conclusions:

- Sclareol appeared to be the best candidate for future study on a large scale. Sclareol strongly suppressed the disease from both races in younger plants from both lines. It is commercially produced from clary sage (*Salvia sclarea*) at a price of ~\$70-100/kg. One kg could be used to treat 25,000 plants.
- This work is the first to show that sclareol suppresses black shank disease in tobacco.
- *cis*-Abienol , despite its good antifungal activity, is not feasible for large scale use due to the production and stability limitations.

# Conclusions (continued):

- Labdenediol worked well in suppressing the disease in older KY14 plants. Further studies are needed, however, with well-purified substance.
- Phylloplanin is not as efficient black shank suppressor as the other natural products.

# Acknowledgments:

- Council of Burley Tobacco, Kentucky, USA
- KTRDC and BAT
  
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GRACIAS  
ARIGATO  
SHUKURIA  
JUSPAYAR  
DANKSCHEEN  
TASHAKKUR ATU  
WAQHRAYELA  
ISUKSAMA  
MAKKE  
GRAZIE  
MEHRBANI  
GOSAHASRIYA  
DI-CHANGSTO  
KENDUJANDEA  
BIYAN  
SHUKRIA  
THANK  
YOU  
BOLZIN  
MERCII

