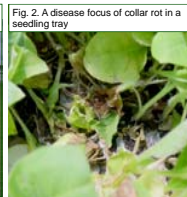


Temporal and spatial distribution of *Sclerotinia sclerotiorum* ascospore dispersal around tobacco-seedling greenhouses

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ABSTRACT

Ascospores of the soilborne pathogen *Sclerotinia sclerotiorum* serve as the primary inoculum for the collar rot disease of greenhouse-produced tobacco seedlings. The objectives of this study were to determine the temporal and spatial distribution of ascospores around greenhouses and use somatic compatibility to determine the level of pathogen diversity among isolates of *S. sclerotiorum* present around a greenhouse. Quantification of ascospores was completed by placing Petri dishes of a semi-selective agar medium at predetermined locations around each greenhouse. Temporal distribution of ascospores was monitored over a 3 year period and somatic compatibility studies followed this period for an additional 2 years. Ascospores were trapped from late February to late April with peak release from early March to early April in each of the three years of this study. There were few or no ascospores detected other times of the year. Spatial distribution of ascospores around greenhouses indicated a steep dispersal gradient, with numbers varying greatly between contiguous trapping dishes placed at 9 m intervals around the outside of greenhouses. In general, ascospore numbers remained either high or low in each of these areas over the 3-yr duration of the study, indicating that the pathogen may be cycling on weed hosts or as sclerotia for long periods. Ascospore numbers were not consistently correlated with temperature or rainfall data. All compatibility tests were conducted on modified Patterson's medium (MPM). Each colony was transferred to MPM and grown for seven days at 24°C. Colonized agar plugs were then transferred to fresh plates of MPM and grown for 14 days at 24°C in the dark. All isolates from a greenhouse were paired in all combinations and compatibility was determined after seven and fourteen days. Isolates collected around two of the greenhouses were very diverse while isolates around the third house were much less diverse. Data indicate that *S. sclerotiorum* was probably present on native plants prior to the introduction of tobacco greenhouses and continues to reproduce on these plants.

The Disease

Environmental conditions required for optimal seedling production (Fig. 1) also favor development of the collar rot disease caused by *Sclerotinia sclerotiorum* (Fig. 2). With no chemical controls labeled for the disease, emphasis has been on inoculum and environmental management. Ascospores of the pathogen enter greenhouses through open side vents (Fig. 3), and disease develops once a dense canopy is formed (Fig. 4).



Fig. 3. Inoculum movement from apothecia into a greenhouse through open side vents



Fig. 4. Symptoms of collar rot on seedlings. Note cottony mycelium, sclerotia, soft rot, and girdling of stems of seedlings.

S. sclerotiorum ascospores, which are the primary inoculum for the disease, can be trapped and quantified with a semi-selective agar medium (1,2). Trapping between 9 a.m. and noon, the peak ascospore deposition times around greenhouses (2,4), can help identify inoculum sources and trends in ascospore activity.

Objectives

- Determine the temporal and spatial distribution of ascospores of tobacco isolates of *S. sclerotiorum*
- Identify environmental factors associated with ascospore production and release
- Propose inoculum management strategies for tobacco seedling production in greenhouses
- Investigate the genetic variability among isolates from around greenhouses

Materials and Methods

Four greenhouses in central North Carolina were chosen to monitor ascospore populations of *S. sclerotiorum* over a five year period. In each of the first 3 years trapping was performed three times per week during the spring transplant production period (February-April) and three to four times monthly the rest of the year. Petri dishes containing the semi-selective agar medium were arranged around the periphery of each house (Fig. 5) and opened for one hour between 9:30 and 11:30 a.m. Weather data loggers (HOBO series, Spectrum Technologies) were used at two of the greenhouses (Fig. 5), and hourly measurements of rainfall, temperature, and relative humidity were recorded.

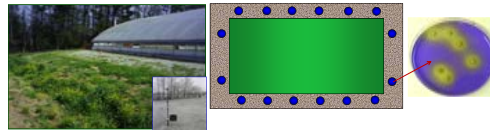


Fig. 5. Pattern of dishes around periphery of a greenhouse and colonies of *S. sclerotiorum* on the selective medium. Data logger outside of greenhouse.

Ascospores collected on a semi-selective medium around three of the greenhouses over the final 2 years of the study were included in somatic compatibility tests. All compatibility tests were conducted on modified Patterson's medium (MPM). All isolates from a greenhouse were paired in all combinations and compatibility was determined after seven and fourteen days. Incompatible interactions (below left and center) indicated isolates were not clonal, and compatible (self) reactions (below right) indicated the isolates were clonal (Fig. 6).



Fig. 6. Somatic compatibility tests on a modified Patterson's medium after the method of Kohn et al.

Results

Ascospores were first trapped the last week of February each of the three years, with peak ascospore release in March (Fig. 6). No ascospores were trapped from May to January. Localized areas of inoculum were detected around greenhouses, and this pattern was maintained over years (Fig. 8).

Fig. 7. Dates ascospores were trapped and average numbers.

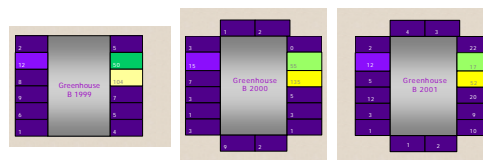
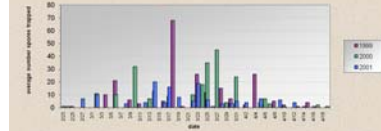


Fig. 8. Number of ascospores trapped around one of the tobacco greenhouses over a three year period. Note similar numbers at certain locations. Disease foci often developed in areas adjacent to highest concentrations of inoculum.

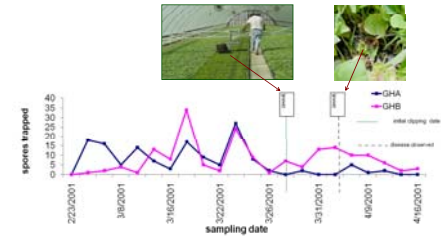


Fig. 9. Timing of clipping and spore showers in relation to when disease foci developed in the greenhouse.

Disease was closely associated with clipping of plants, which left abundant leaf material on plants that served as a food source for the pathogen (Fig. 9). The level of diversity was high around 2 of the 3 greenhouses, with numerous mycelial compatibility groups (MCGs) found (Fig. 10). Several MCGs were recovered across years indicating that isolates may be reproducing on plants near the greenhouse. Isolates around one greenhouse were represented by few MCGs.

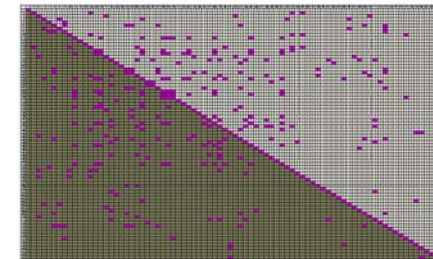


Fig. 10. Pairing matrix for isolates of *S. sclerotiorum* obtained around a single greenhouse in central NC. Note high degree of incompatibility among isolates, but that some isolates were compatible with multiple isolates.

Conclusions

- Year round trapping studies demonstrated that tobacco isolates of *S. sclerotiorum* produce ascospores only in the spring and show discrete predictable periods of activity that correspond to known stages of host susceptibility.
- The reoccurrence of similar spore deposition over multiple years suggests the fungus is cycling on nearby alternative hosts or that sclerotia previously formed are surviving for at least 5 years.
- Spatial distribution of ascospores indicated a steep dispersal gradient
- Plant clipping is a good indicator of when plants are most susceptible to collar rot
- Removal of clipping debris at least 100 meters from the greenhouse has resulted in a very sharp decline in the disease
- The pathogen is highly diverse around each greenhouse sampled in this study, indicating the pathogen is an ancient resident of the area and not spread between greenhouses

Acknowledgements

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