Population diversity and Disease aggressiveness in isolates

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Characterization of Sclerotinia minor Populations in Texas (139)

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Introduction

Texas is the second largest producer of peanuts in the U.S. with more than 8.6 million pounds produced annually. The plant pathogen, *Sclerotinia minor,* was introduced into a small area in the western region of the state about ten years ago. An unexpected increase in the incidence of *S. minor* in commercial peanut fields in the High Plains of Texas during the 2004 growing season raised concern that the disease increase was due a more aggressive and/or a fungicide insensitive isolate. To test this hypothesis, commercial peanut fields throughout Texas were sampled to obtain isolate of *S. minor* from expanding disease foci. The isolates were characterized for their ability to attack peanut leaves, sensitivity to commonly applied fungicides and genetic variation using microsatellites.

Current Research Activities: Identifying Genotypic Variation

Microsatellites are simple sequence repeats of the nucleotides AT and GC that are located throughout the genome of the target organism. Polymerase chain reaction is employed to amplify these repeats through the use of specific primers. Microsatellites are molecular markers within the genome. These markers can be used to identify major differences among our collected isolates. Fourteen microsatellite primer sets were developed previously by Dr. Linda Kohn (University of Toronto) that amplify regions in *Sclerotinia minor* isolates from North Carolina. The primers were tested on our isolates, and we found that only eight of the primer sets were able to amplify microsatellite regions in our isolates (Table 1). We are currently genotypically characterizing each isolate using these 8 appropriate microsatellite primer sets.

Using codominant markers, such as microsatellites, will allow us to determine if the *Sclerotinia minor* isolates collected throughout Texas are clonal (arising from the same parent fungus) or unique pathogens. These markers can be used to determine if the isolates from Erath County are similar or different from the isolates from Gaines County as well as identifying if there is any difference in the pathogens within one field.

We will also use the microsatellite data to determine if there is any correlation between aggressiveness and genotypic characterization. We are attempting to determine if the most aggressive pathogens have a unique genotype. This is essential to identify because fungal population structures can rapidly shift; therefore, a pathogen in small numbers one year could be found in high numbers the next year.

We will also use these primers to determine if they will amplify regions within the late season pathogen, *Botrytis cinerea*. If these primers can not amplify *Botrytis cinerea*, these markers can be used to differentiate isolates of these pathogens. If the primers do amplify *Botrytis cinerea* genome, we will look at the overall genotypic conservation to determine if there are significant differences between the pathogens.

We have also completed internal transcribed sequence analysis, which demonstrated that *S. minor* is most closely related to *S. sclerotiorum* and *S. trifoliorum* and more distantly related to other sclerotial forming fungi, such as *Botrytis cinerea* and *Phymatotrichopsis omnivora.*

Table 1. Microsatellite Sequences

Locus	Repeat Motif	Size Range	No. of alleles
AF377900	$(GT)_8$	318-325	3
AF377911	$(TTA)_9$	345-390	5
AF377912	$(GT)_7GG(GT)_5$	268-278	4
AF377913	$(TG)_{10}$	384-388	3
AF377914	$CA_6(CGCA)_2CAT_2$	415-429	2
AF377923	$(AGAT)_{14}(AAGC)_4$	351-391	8
AF377924	$(TAC)_6C(TAC)_3$	476-488	2
AF377926	$(GTAA)_2(GCAA)(GTAA)_3$	402-422	2

Materials and Methods for Completed Studies

Isolates of *S. minor* were obtained from symptomatic peanut plants in eight production fields in Gaines (designated A, B, H, E, W, and J) Comanche (designated D), Erath (designated F, L, P, Ter1, Ter2), and Atascosa (designated U) counties during the fall of 2004 and stored as sclerotia at 4°C. A detached leaflet lesion assay was conducted to test the aggressiveness of the isolates. The second leaflets of 3-week-old plants (cultivar Tamrun96) were excised, placed in a sterile glass petri dish, and inoculated with mycelial culture of the isolates. After 48 hours incubation at 20oC, the area of each leaflet and lesion were measured using ImageJ and compiled into a database (Rasban et. al 2005).

The fungicide sensitivity of the isolates to iprodione (Rovral, Rhone-Poulenc Ag Company), thiophanate-methyl (Topsin M, Cerexagri, Inc), dichloran (Botran 75W, Gowan Company), boscalid (Endura, BASF), Fluazinam (Omega 500F, Syngenta) was determined by the spiral gradient dilution method (Forster et. al 2004). The fungicides were applied onto plates using the Autoplate 4000 (Spiral Biotech, Inc). Sterile filter paper strips colonized by different isolates were then applied to each fungicide plate. The radial distance corresponding to 50% growth inhibition (as compared to the controls) was measured and used to calculate the EC50 (effective fungicide concentration at 50% inhibition). In addition to these in vitro tests, field trials were conducted in Gaines County to assay fungicide efficacy during the summer of 2005.

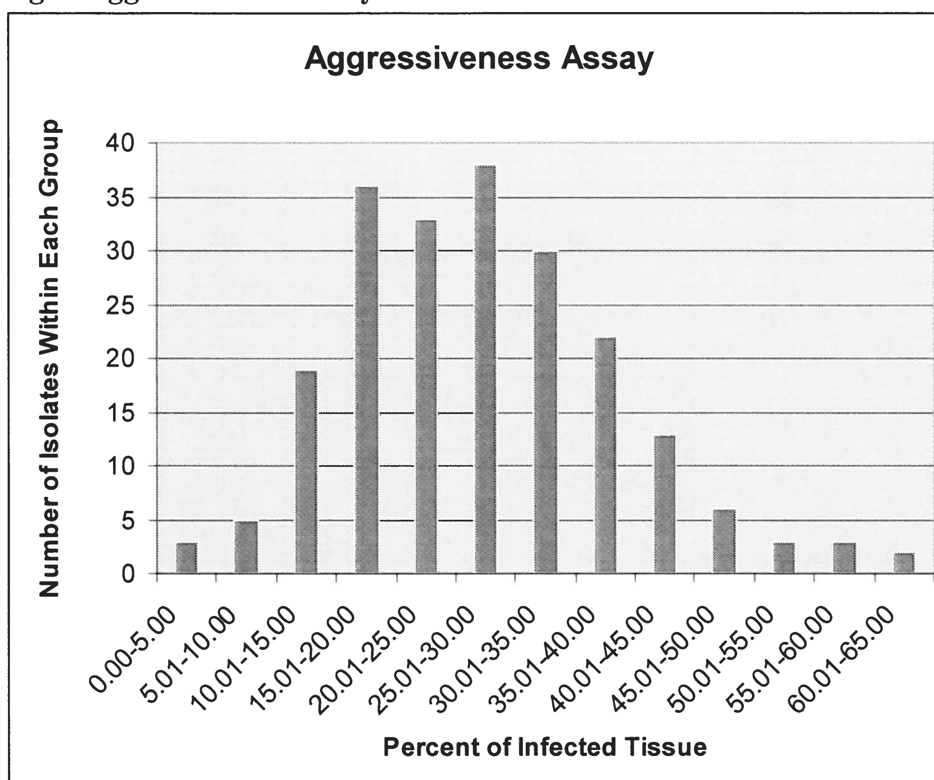
Results and Discussion of Detached Leaflet Assay and Fungicide Sensativity Assay

Detached Leaflet Assay.

Significant differences in aggressiveness based on lesion sized were detected among the isolates in the detached leaflet assay The majority of the isolates produced lesions that encompassed between 20-50% of the leaflet (Fig. 1). The least aggressive isolate was E1690, infecting only 2.7% of the leaflet, while the most aggressive pathogen was W125, infecting than more than 60% of the leaflet; both of these isolates were collected from Gaines County. Isolates found to produce large lesions on the detached leaflet assay were only a small portion of the total isolates recovered; therefore, this does not support the hypothesis that there was an widespread increase in a more aggressive pathogen. However, population structures have the ability to rapidly shift. Therefore, it

would be very informative to re-sample the same fields this growing season to determine if the population structures have shifted or remained static.

Fig 1. Aggressiveness Assay



At this date, the aggressiveness assay has been performed on 217 isolates.

Fungicide Sensitivity

The fungicides reduced the growth of the pathogen at the levels tested; however, there was a significant difference in pathogen sensitivity (average EC50) among the fungicides. Also, there were differences in EC50s and growth patterns among isolates (Table 2). Thiophanate-methyl and dichloran are inhibitory, but they require much higher concentrations of active ingredient than fluazinam, iprodione, and boscalid to limit growth of the pathogen. Fluazinam had a lower tail ending concentration (TEC), the concentration where the isolate no longer can grow from the inoculum, than boscalid. We found that the average EC50 for fluazinam for our isolates of *S. minor* is nine times higher than the fluazinam concentration that effectively suppressing 82 to 84% of the mycelial growth of *S. minor* from Arizona (Materon et. al 2004). However, the average EC50 for boscalid was similar for both sets of isolates (Materon et. al 2004). The in vitro tests correlated to the field trial data (Table 3); both data sets found that boscalid was the most effective and topsin m was the least effective.

Table 2. In vitro Average EC50s

Fungicides	Average EC50	Range of EC50s
Thiophanate methyl	**2.59**	**0.15-8.54 µg/mL**
Dichloran	**1.12**	**0.36-2.52 µg/mL**
Iprodione	**.117**	**0.030-0.250 µg/mL**
Fluazinam	**.102**	**0.045-0.310 µg/mL**
Boscalid	**.079**	**0.020-0.177 µg/mL**

Boscalid was the most effective fungicide while Thiophanate methyl was the least effective in the in vitro and field trials.

Table 3. Field Data for the 2005 Fungicide Trials

Fungicide	**Calendar-Based Application**		**Symptom-Based Application**	
	Profit/acre ($)	*Average disease*	*Profit/acre ($)*	*Average disease*
Boscalid	724.38	11	724.25	26
Fluazinam	594.17	11	703.96	24
Thiophanate Methyl	592.26	39	519.06	40
No fungicide application	556.08	51	503.38	47

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