Project Description: Sensitivity, cross-resistance and genetic mechanisms of resistance to DMI fungicides in the peanut leaf spot pathogens
Principle Investigator: Katherine L. Stevenson
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Executive Summary

Since 1994, the fungicide tebuconazole has been widely used in Georgia to control early leaf spot of peanut, caused by *Cercospora arachidicola*. Tebuconazole is one of a large group of demethylation-inhibiting (DMI) fungicides that inhibit fungal growth by interfering with biosynthesis of essential sterols in fungi. In the early 2000s, a significant decline in performance of tebuconazole for leaf spot control was reported in Georgia and neighboring states. This decline in performance was associated with a significant decrease in tebuconazole sensitivity based on laboratory assays of isolates collected from field sites. Underlying genetic and physiological mechanisms of DMI resistance reported in other fungi include mutations in the *CYP51* gene that encodes the target demethylase enzyme, over-production of the target enzyme, and an active efflux mechanism that removes toxins, including fungicides, from the fungal cell. However, mechanisms of resistance to DMIs in *C. arachidicola* have not been previously studied. The general objectives of this study were to develop a rapid assay to detect tebuconazole resistance in field populations of *C. arachidicola* and investigate the mechanisms of DMI resistance in *C. arachidicola*.

A new procedure was developed to rapidly detect DMI resistance in *C. arachidicola*. Fungal spores were transferred directly from leaf lesions to tebuconazole-amended medium and sensitivity was based on diameters of 3-day-old fungal colonies observed under the microscope. The main advantage of the new assay is that it can be completed in 3 days, compared to 2-3 months for the standard microtiter plate assay. Fungal isolates were collected in 2008 and 2009 from peanut fields with or without a history of DMI use and tested for sensitivity to tebuconazole using both the new method and the standard mycelial growth assay in microtiter plates. There was good correlation between the results of the two assay methods, but sensitivity values were consistently higher for the new assay compared to the microtiter plate assay.

Mechanisms of resistance to the DMI fungicides in the early leaf spot pathogen, *Cercospora arachidicola*, were investigated. Sequencing of the CYP51 gene from DMI-resistant and DMI-sensitive field isolates of *C. arachidicola* revealed alterations at codons 453 or 461 in 4 of the 10 DMI-resistant isolates. This is the first report of mutations in the *CYP51* gene associated with DMI resistance in *C. arachidicola*. The fact that mutations were not detected in all resistant isolates suggested that other mechanisms must be involved. However DMI resistance was not associated with over-production of the demethylase enzyme of *CYP51* or activity of ABC transporters in the isolates tested. The mutations in the *CYP51* gene that were found to be associated with DMI resistance in some *C. arachidicola* isolates can be used to develop PCR-based assays for detection of DMI resistance in this pathogen. The results of this research provide important new information about the mechanisms of DMI resistance in this important peanut pathogen that will lead to more efficient fungicide sensitivity monitoring and improved peanut leaf spot management.

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INSTITUTION: University of Georgia Research Foundation

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NPB CONTACT: Marie Fenn or M Mehok
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FINAL REPORT:

More than 100 single-spore isolates of the early leaf spot pathogen, *C. arachidicola*, and 46 isolates of the late leaf spot pathogen, *C. personatum*, were obtained from infected peanut leaves and assayed for sensitivity to the demethylation-inhibiting (DMI) fungicides tebuconazole and prothioconazole based on mycelial growth in fungicide-amended medium in microtiter plates. For *C. arachidicola*, EC_{50} values for tebuconazole ranged from 0.018 µg/ml to 2.706 µg/ml, with a median value of 0.558 µg/ml. EC_{50} values for prothioconazole ranged from 0.004 µg/ml to 1.827 µg/ml, with a median value of 0.240 µg/ml. For *C. personatum*, EC_{50} values for tebuconazole ranged from 0.052 µg/ml to 2.884 µg/ml, with a median value of 0.701 µg/ml. EC_{50} values for prothioconazole ranged from 0.021 µg/ml to 1.425 µg/ml, with a median value of 0.228 µg/ml. There was a significant correlation between EC_{50} values for tebuconazole and prothioconazole for 71 isolates of *C. arachidicola* ($r=0.642$, $p0<0.0001$) and for 37 isolates of *C. personatum* ($r=0.413$, $p=0.0110$), indicating a significant potential for cross resistance in these pathogens to these two DMI fungicides. Based on the median EC_{50} values of the isolates tested, the sensitivity of both pathogens to prothioconazole was similar, but both pathogens were slightly less sensitive to tebuconazole than prothioconazole. Based on the correlation analysis, there is evidence of significant potential for cross resistance between tebuconazole and prothioconazole in both leaf spot pathogens.

A subset of isolates of *C. arachidicola* was selected for molecular genetic analysis to determine if mutations in *CYP51*, the gene that encodes for the target binding site of the DMI fungicide, are responsible for DMI resistance in *C. arachidicola*, DNA was extracted from each isolate and one pair of degenerate primers and six pairs of specific primers were designed to amplify the region containing the *CYP51* gene using PCR. The gene from each isolate was sequenced and sequences were compared to detect specific base pair alterations corresponding to amino acid substitutions in the target protein in isolates with reduced sensitivity to DMIs compared to more sensitive isolates. No differences in the sequence of the *CYP51* gene were detected among the isolates, regardless of DMI sensitivity, suggesting that mutations in the *CYP51* gene are not responsible for DMI resistance in these isolates. However, the sequences will be compared to known *CYP51* sequences in other fungi to verify that the sequences are consistent with those of other DMI-sensitive phenotypes. Other possible mechanisms of DMI resistance, including ATP-binding cassette (ABC) transporters and overexpression of *CYP51* are currently being investigated in DMI-resistant isolates of *C. arachidicola*.