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Research Article

Influence of *Verticillium dahliae* Infested Peanut Residue on Wilt Development in Subsequent Cotton

Shilpi Chawla,[1] Jason E. Woodward,[1, 2] and Terry A. Wheeler[3]

[1] *Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409, USA*
[2] *Texas AgriLIFE Extension Service, Texas A&M System, Lubbock, TX 79403, USA*
[3] *Texas AgriLIFE Research, Texas A&M System, Lubbock, TX 79403, USA*

Correspondence should be addressed to Jason E. Woodward, jewoodward@ag.tamu.edu

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Texas ranks first in cotton production in the United States and accounts for approximately 40% of the total production. Most of the cotton production is concentrated in the Texas High Plains where cotton and peanut are commonly grown in rotation. With peanut being a legume crop, farmers routinely leave residue on the soil surface to improve soil fertility; however, *V. dahliae* can survive in the crop residue contributing inoculum to the soil. A microplot study was conducted to investigate the impact of peanut residue infested with *V. dahliae* on subsequent microsclerotia density in soil and Verticillium wilt development in cotton. The effects of infested peanut residue rate on percent germination of cotton seeds and on wilt incidence were monitored in 2008 and 2009. In both years microplots were planted with a susceptible cotton cultivar, Stoneville (ST) 4554B2RF. Increasing infested peanut residue rate was positively correlated with wilt incidence in cotton and negatively correlated with germination of cotton seeds. Density of microsclerotia in the soil increased significantly with increasing rates of infested peanut residue over time. Results indicate infested peanut residue serve as a source of *V. dahliae* inoculum, and removing infested residue can reduce disease development in subsequent cotton crops.

1. Introduction

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb., is an economically important disease of cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.). The pathogen has a broad host range of more than 400 plant species including field crops and most vegetables [1]. Several factors, including cultivar selection, pathogen aggressiveness, inoculum density, and environmental conditions, influence disease development [2, 3]. Cotton and peanut plants affected by Verticillium wilt show stunting and epinasty [4]. Their leaves exhibit interveinal chlorosis, necrosis, curling, and die back from the margins inward. Plants develop characteristic mosaic patterns on foliage, starting from the base of the plant and progressing towards the top [5]. Ramification of the fungus in the xylem vessels leads to a tan-to-brown discoloration, a decrease in hydraulic conductance, wilting, and eventually death [6]. In infested cotton, plants can appear stunted, defoliate prematurely, and have fewer fruiting positions; bolls may abscise or not open [7], whereas in peanut, pegs are formed in less numbers and have fewer seeds [8].

The fungus is capable of infecting plant roots directly or through wounds throughout the growing season between temperatures 21 and 27°C whereas temperatures between 24 and 27°C are best suited for *V. dahliae* growth and survival in the plant [6, 9]. According to Huisman [10], *V. dahliae* primarily colonizes the rhizoplane of the host plant, penetrates roots early in the growing season, and then infects the vascular system and grows systemically throughout the plant. Microsclerotia, the survival structures of *V. dahliae,* are produced once the plant dies. Microsclerotia are composed of masses of melanized hyphae and are considered the principal source of inoculum for Verticillium wilt development. Microsclerotia can survive for more than 20 years in the soil [11], and root exudates stimulate germination initiating infection [6, 12]. Microsclerotia are formed depending on temperature and moisture availability with the decay of plant tissues [13]. Microsclerotia are dispersed in the soil, and only a single cycle of inoculum is produced during a growing season. Inoculum density in soil at planting has been shown to play a critical role in disease development [14]. Production of *V. dahliae* microsclerotia on infected plant parts of many field crops has been found [15, 16]. Formation of microsclerotia on dying host debris in the soil can cause an increase in inoculum density in the following year, especially if the increase is greater than the reduction in microsclerotia due to mortality [15, 16].

Cotton is economically the most important crop in Texas High Plains. Cotton is commonly grown in rotation with peanut in this region. Farmers in this region routinely utilize peanut residue on the soil surface to improve soil fertility. Typical rates of peanut residue left in the field after harvest are approximately 3500 kg/ha (Woodward, *unpublished data*). This practice can help reduce soil erosion, conserve energy, maintain soil moisture, improve organic matter content and soil fertility [17]. However, *V. dahliae* can survive in the crop residue, and disease problems may be more severe by protecting the residue from microbial degradation and lowering soil temperature.

Both peanut and cotton are suitable hosts for *V. dahliae* allowing for a continued increase in microsclerotia production. Currently, there is no quantitative data available regarding the influence of *V. dahliae* infested peanut residue on wilt development in cotton. The objective of this study was to determine the effect of *V. dahliae* infested peanut residue rate on release of microsclerotia in the soil and its implications for Verticillium wilt development in cotton over time. The hypothesis was that peanut residue infested with *V. dahliae* will increase microsclerotia density in soil and Verticillium wilt development in cotton.

2. Materials and Methods

2.1. Microplot Experiment. A microplot experiment was conducted in 2008 and 2009 to examine the effect of increasing rate of peanut residue infested with *V. dahliae* on microsclerotia production and disease development in cotton over time. Microplots were constructed out of cylindrical galvanized aluminum rings (90 cm diameter and 60 cm height) and buried at the depth of 50 cm. Treatments (0, 370, 925, 1850, 2775, 3700, 18,495, and 37,000 kg/ha infested peanut residue) were arranged in a randomized complete block design with nine replications. Peanut residue was collected from a field that was infested with *V. dahliae* and had experienced severe Verticillium wilt for several growing seasons. In 2008, two months prior to planting infested residues were incorporated in microplots at the rate of assigned treatments by mixing the residues with top soil by hand tilling to mimic the peanut residue naturally left on the field. Microplots were planted with a susceptible cotton cultivar, Stoneville (ST) 4554B2RF at the rate of 25 seeds per microplot in a circular pattern. Irrigation, fertilizer, and weeding practices were conducted as needed, according to local extension recommendations for both seasons. In 2008 and 2009, cotton plants were hand harvested leaving about six inch stems above ground, and remaining cotton residues were removed.

2.2. Soil Sampling and Data Collection. All microplots were sampled in February 2008, prior to the assigning of residue treatments, to determine baseline populations of *V. dahliae* within the soil. Subsequent soil samples were taken in April and November 2009 and April 2010. A 2.5 cm diameter auger to a depth of 20 cm was used in taking soil samples from each microplot. Each sample consisted of four cores and had a total soil weight of approximately 250 g. The samples were air-dried at room temperature for 14 days. A soil dilution plating technique [18], utilizing Sorensen's NP-10 semiselective medium [19] amended with 0.025 N NaOH as suggested by Kabir et al. [20], was used for enumeration of microsclerotia in soil. Air-dried soil was ground with a roller pin; a 20 cm^3 soil sample was combined with 80 ml of deionized water and stirred using a magnetic stir plate. A 1 ml aliquot of the soil solution was distributed on each Petri dish (10 replications) containing Sorensen's NP-10 semiselective medium and was spread with a glass rod. After 14 days of incubation at room temperature in the dark, the soil was rinsed from the Petri dishes by gentle rubbing and then air-dried for 2 hours prior to counting. The numbers of colonies of *V. dahliae* were counted under a stereo dissecting microscope and expressed as the number of microsclerotia (ms)/cm^3 of dry soil. Percent germination of cotton seed was recorded in June, and disease incidence was assessed in September as percent symptomatic plants in each microplot for both seasons.

2.3. Statistical Analysis. Data for percent germination of cotton seeds, percent disease incidence and inoculum density of *V. dahliae* in soil (ms/cm^3) were analyzed using Proc MIXED (SAS Institute Inc., 2008, Ver. 9.2, Cary, NC, USA). Infested peanut residue rate (treatments) were log transformed. Data were analyzed as a split-plot in time where the sub-plots were four sampling dates as described in Steel and Torrie [21]. The method used to adjust the degrees of freedom (df) to match adjustments in the sums of square was the Satterthwaite option in the LSMEANS statement in Proc MIXED. Standard error were determined from the PDIFF (probability of difference of two means) option. Regression analysis was carried out for percent germination, percent disease incidence and inoculum density of *V. dahliae* in soil (ms/cm^3) with $\log_{10}$ transformed infested peanut residue rates using Proc MIXED. Linear regression ($f = y^0 + ax + bx^2$) with quadratic terms and the slopes were analyzed.

3. Results

Soil samples collected in February 2008 were void of *V. dahliae* inoculum in all the microplots prior to artificial incorporation of infested peanut residue (Figure 1). There was a positive correlation between increasing infested peanut residue rates and microsclerotia densities in soil (Figure 1). Linear regression with a quadratic term and slope represented the overall effect as we go from low to high values

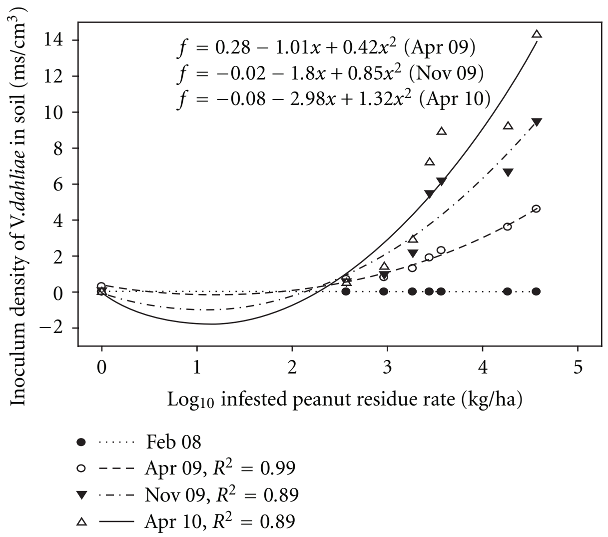

Figure 1: Effect of infested peanut residue rates on inoculum density of *Verticillium dahliae* in soil (microsclerotia/cm^3). Infested peanut residue rates 0, 370, 925, 1850, 2775, 3700, 18495, and 37000 kg/ha were $\log_{10}$ transformed and were expressed as 0, 2.6, 3.0, 3.3, 3.4, 3.6, 4.3, and 4.6 on X-axis. Soil samples collected in February 2008 were void of *V. dahliae* inoculum prior to artificial incorporation of infested peanut residue. Linear quadratic equation $f = y^0 + ax + bx^2$ was used to calculate intercept (y^0), slope (a) and curvature (b).

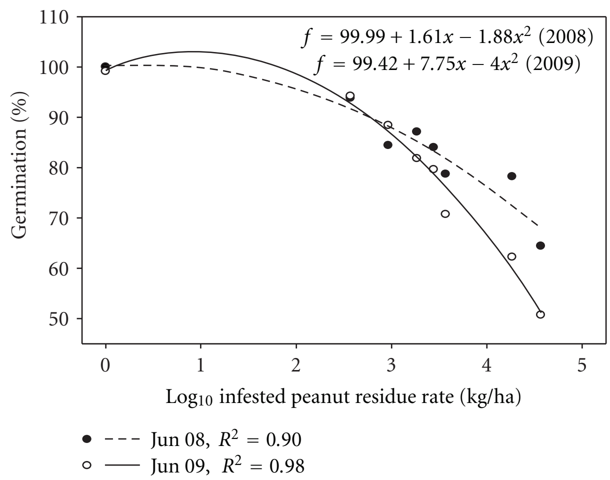

Figure 2: Effect of infested peanut residue rates on germination of cotton seeds (%). Infested peanut residue rates 0, 370, 925, 1850, 2775, 3700, 18495, and 37000 kg/ha were $\log_{10}$ transformed and were expressed as 0, 2.6, 3.0, 3.3, 3.4, 3.6, 4.3, and 4.6 on X-axis. Linear quadratic equation $f = y^0 + ax + bx^2$ was used to calculate intercept (y^0), slope (a) and curvature (b).

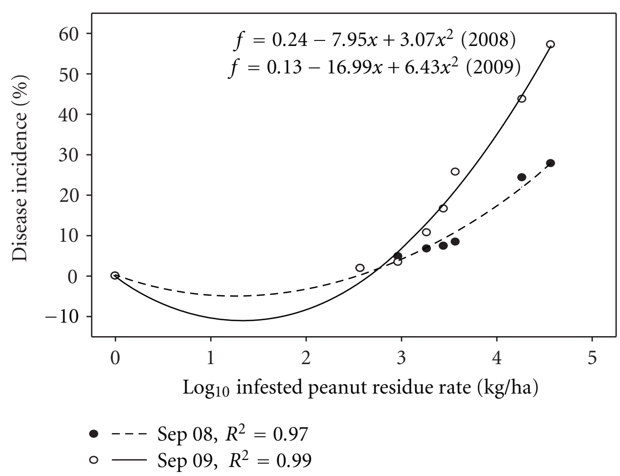

Figure 3: Effect of infested peanut residue rates on Verticillium wilt incidence (%). Infested peanut residue rates 0, 370, 925, 1850, 2775, 3700, 18495, and 37000 kg/ha were $\log_{10}$ transformed and were expressed as 0, 2.6, 3.0, 3.3, 3.4, 3.6, 4.3, and 4.6 on X-axis. Linear quadratic equation $f = y^0 + ax + bx^2$ was used to calculate intercept (y^0), slope (a) and curvature (b).

of the inoculum density. The slope of the quadratic term increased from 0.42 ($R^2 = 0.99$) in April 2009 to 0.85 ($R^2 = 0.89$) in November 2009 to 1.32 ($R^2 = 0.89$) in April 2010. Overall, microsclerotia densities were found to increase with higher rates of *V. dahliae* infested peanut residue and with time (Figure 1).

Increasing peanut residue rates had a negative effect on percent germination of cotton seeds (Figure 2). Cotton germination in microplots amended with the lowest rate of residue averaged 93.8 and 94.2% for 2008 and 2009, respectively, whereas microplots amended with the highest rate of infested peanut residue resulted in 64.4 and 50.7% over the two years (Figure 2). Germination of cotton seeds in the nonamended controls averaged 99.6%, whereas germination for all other treatments was intermediate (Figure 2). Linear regression with a quadratic term and slope represented the overall effect as we go from high to low values of the germination of cotton seeds. The reduction in germination per unit peanut residue increased from 2008 to 2009 (quadratic curvature of -1.88 ($R^2 = 0.90$) in June 2008 and -4.00 ($R^2 = 0.98$) in June 2009).

A positive correlation occurred between peanut residue rates and Verticillium wilt incidence on cotton (Figure 3). Linear regression with a quadratic term and slope represented the overall effect as we go from low to high values of the disease incidence. The increase in wilt incidence per unit of peanut residue rate was higher in 2009 than in 2008 (slope of 3.07 ($R^2 = 0.97$) in September 2008 and 6.43 ($R^2 = 0.99$) in September 2009). Disease incidence increased from 1.9% to 27.8% when comparing the lowest and highest residue rates, respectively, in 2008 (Figure 3). A similar trend was observed for the 2009 growing season. Disease incidence increased substantially between years in microplots amended with 1850 kg/ha residue rate or greater (Figure 3).

4. Discussion

Microsclerotia of *V. dahliae* are produced in large numbers on senescing parts of host plants and may remain viable in the soil for many years [12]. Davis et al. [22] found a strong correlation between microsclerotia population in the soil and the number of microsclerotia in stem tissue, which can further contribute to soil inoculum for the next season. Peanut is commonly used as a rotation crop with cotton in parts of the United States, and both crops are suitable hosts for *V. dahliae*. This study demonstrates that microsclerotia can survive in infested peanut residue and then can infect susceptible cotton in the next season. Root exudates stimulate germination of microsclerotia leading to a decline in the population of viable propagules in soil until the incorporation of new inoculum from infected tissues at the end of the crop [16, 23]. Inoculum potential is related to the density and distribution of infested residues as well as to the susceptibility of the host crop [2]. Being a monocyclic disease, disease incidence in Verticillium wilt is positively related to the concentration of primary inoculum; thus understanding the relationship between microsclerotia density in soil at planting and wilt development is essential for developing a disease risk assessment based on preplant soil assays and also for disease management [14].

The beneficial effects of leaving crop residue as a nutrient source can be offset by the negative effects of providing shelter for survival, growth, and reproduction of plant pathogens and raise concerns over the role of crop residue in epidemics caused by soilborne pathogens. Crop residue at the soil surface is a principal source of inoculum. Infested peanut residue increased soil inoculum density and disease incidence on subsequent cotton in this study and similar results were found by Adee et al. [24] for other pathosystems. Tjamos [25] and Zilberstein et al. [26] found that the degree of pathogenicity of *V. dahliae* was related to the plant species from which the isolate was obtained and was also dependent on the previous cropping history. Continual uses of susceptible host cultivars and cultural practices that leave abundant infested residue on the soil surface have been observed to increase the damage caused by other soilborne pathogens in subsequent crops [27].

Microsclerotia mainly occur in the aerial parts of the crop [12], and, therefore, removal of the aerial crop debris from the field is potentially an effective measure to prevent the accumulation of microsclerotia in the soil. In the present study, lower rates of infested peanut residue had lower ms/cm^3 of soil, which agrees with results of Hoekstra [28], who found that many microsclerotia can be produced in plant residue of field bean, and removing the debris of field bean resulted in a lower microsclerotia population in the next spring. The apparent increase in microsclerotia population, a year after incorporation of infested peanut residue, may be explained by the disintegration of plant debris containing microsclerotia or reproduction on the susceptible cotton cultivar in 2008. Higher numbers of *V. dahliae* microsclerotia were found on cauliflower roots eight weeks after harvest [29]. Huisman and Ashworth [15] and Joaquim et al. [30] found a sharp increase in microsclerotia population in the second year after growth of a susceptible crop, despite host susceptibility.

Peanut residue is composed of vines and leaves remaining after harvest. These materials provide a good source of nutrition [31] and are often used as a feed supplement. According to Darrell [32], the cost of feed represents the largest single cost item in most beef operations. This is an additional way to add economic benefits to removing peanut residue from the field. Balkcom et al. [17] found that peanut residue does not contribute significant amounts of nitrogen to the subsequent cotton crop; however, these interactions need to be investigated further under the arid conditions. While removing residue may remove nitrogen from the soil, the potential benefits are twofold reducing soil populations of *V. dahliae* and sale of hay for feedstock.

Results from the present study suggest the importance of removing the peanut residue infested with *V. dahliae*, for managing the Verticillium wilt in the subsequent cotton crop. Due to the lack of effective fungicides and truly resistant cultivars, disease management will likely rely on an integrated management program using a number of management options. Reducing inoculum of *V. dahliae* in host debris and other reservoirs may be a key to Verticillium wilt management. Cropping systems have a changing dynamics of disease and soilborne pathogens that are influenced by cultural practices such as residue management. The importance of primary inoculum in Verticillium wilt development, justifies the use of cultural management practices, including the use of partially resistant cotton cultivars and destruction of infested peanut residues for effective disease management.

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