CONTRIBUTIONS TO MANAGEMENT OF DISEASES OF PEANUT (*ARACHIS HYPOGAEA*) THROUGH BOLIVIAN-DERIVED HOST RESISTANCE, INTEGRATED DISEASE MANAGEMENT AND KNOWLEDGE OF PATHOGEN VARIABILITY

by

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(Under the Direction of Albert K. Culbreath)

ABSTRACT

Applied and basic research experiments were conducted to improve current management of the fungal pathogens *Cercospora arachidicola*, cause of early leaf spot, *Cercosporidium personatum*, cause of late leaf spot, *Puccinia arachidis*, cause of peanut rust, and Tomato spotted wilt (TSW), caused by *Tomato spotted wilt virus*. A series of breeding lines and the Bolivian cultivar, Bayo Grande (BG), were evaluated for resistance to these diseases compared to Georgia Green (GG), a cultivar with high susceptibility to early and late leaf spot and moderate resistance to TSW. When grown in the U.S., BG and the breeding lines showed improved leaf spot resistance compared to GG. When grown in Bolivia, no improved leaf spot resistance was observed among any genotypes tested. No improved rust resistance was observed among genotypes in any experiment. When evaluated as part of an integrated disease management (IDM) system, the improved resistance of BG and the breeding lines coupled with zero to four reduced fungicide sprays reduced leaf spot to levels comparable to those seen under a full season, six to eight spray fungicide regimes. The addition of the cultural practice of strip tillage negated

the need for fungicides in most genotypes in one year, when compared to those genotypes grown under conventional tillage. However, in the following year, strip tillage did not contribute to spray reduction. To predict the potential for development of resistance in populations of *C. arachidicola*, genetic diversity was measured in populations from the U.S. and Bolivia by comparing sequences of the β-tubulin and calmodulin genes and by comparing spore length. Genetic and phenotypic results indicate that populations of *C. arachidicola* have low diversity.

INDEX WORDS: leaf spot, *Cercospora arachidicola*, *Passalora*, *Cercosporidium personatum*, tomato spotted wilt, peanut rust, *Puccinia arachis*, tillage, fungicide, host resistance, breeding

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DEDICATION

This dissertation is dedicated to Dr. Ralph H. Gremillion.

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CHAPTER 1

INTRODUCTION

With a world population that is increasing by one billion people nearly every decade (1) maintaining a stable food supply is a crucial goal for mankind. Grown around the world, peanut (*Arachis hypogaea* L.) is a crop capable of contributing to a global diet. Peanut plants produce edible seed that contain 25% protein, unsaturated fats and various vitamins and minerals (37). In addition to the nutritional benefits of peanut, production of this legume is a profitable industry, resulting in approximately $1 billion annually in the United States alone (35).

While peanuts offer both health and economic benefits, production issues threaten the crop every year. One of the most important challenges facing peanut growers is management of destructive diseases. A variety of plant pathogens including bacteria, fungi, nematodes and viruses, as well as abiotic factors cause physiological damage in peanut, resulting in poor plant health, reduced yields and economic returns. Among the most devastating fungal diseases of peanut yields are the leaf spots, early leaf spot (*Cercospora arachidicola* S. Hori) and late leaf spot (*Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton), and peanut rust (*Puccinia arachidis* Speg.) (40). Tomato spotted wilt (TSW), caused by the tomato spotted wilt tospovirus (TSWV), is a worldwide viral disease with the most severe impact on yield occurring in the U.S.

Many fungicides are available for management of diseases caused by *C. arachidicola*, *C. personatum* and *P. arachidis*, but insecticides, with one exception, have not been useful in suppressing TSW spread even when they show good performance for reducing feeding damage by thrips vectors (12). To control fungal pathogens, multiple fungicide applications are required throughout the season, resulting in a time- and resource-consuming burden to growers, and

concerns have arisen about the negative effect that fungicides may have on surrounding natural environments (8, 23, 31, 32). The efficacy of fungicides has also been compromised with development of pathogen resistance as documented in the leaf spot pathogens with regards to benzamidizoles (10, 25, 38) and triazole fungicides (39).

In response to the issues surrounding fungicide use, major research efforts have been made to develop and implement alternative disease management options to lessen reliance on these chemicals. Planting resistant cultivars is perhaps the easiest, least expensive and most effective method of disease control, and several U.S. runner-type peanut cultivars with partial resistance to multiple diseases are currently available (15-17). Another management option includes the use of conservation tillage, which can suppress TSW (2, 5, 9, 22, 28, 29), and early leaf spot (5, 6, 28, 29, 36). By combining host resistance and conservation tillage into an integrated disease management (IDM) system, fewer fungicide applications are required. Studies combining moderate to enhanced host resistance and strip tillage, a form of conservation tillage, indicated that increasing the interval between fungicide applications resulted in less overall sprays without a loss in the control of *C. arachidicola* and *C. personatum* (7, 28, 29).

The success of the aforementioned IDM system consisting of resistant cultivars, strip tillage and reduced fungicides is well-supported and likely to continue in future attempts at control of the leaf spot pathogens. To guarantee continued effectiveness, the IDM system will rely heavily upon the durability of each component. Disease management tactics exert selection pressure on pathogen populations that may result in pathogen resistance and an erosion of the durability of control (3). Recent reports indicate that the high genetic uniformity of U.S. peanut cultivars classifies the national crop as vulnerable to disease (21, 41). This characterization is due to the few sources of parent germplasm used to produce U.S. cultivars (21) as well as the

continued monoculture of cultivars like Georgia Green (GG), which is planted to 80% of the southeastern U.S. in recent years (41). There is a need for a diversification of peanut germplasm to support breeding programs and the future of host resistance. The South American country of Bolivia where the domesticated peanut is believed to have originated (24), has been recognized as an under-utilized source of genotype diversity for wild *Arachis* species (22, 42), and is noted by Holbrook and Stalker (2003) as a center for both early and late leaf spot resistance in cultivated peanut (19).

As part of a United States Agency for International Development's Peanut Collaborative Research and Support Program (USAID Peanut CRSP) to utilize Bolivia's diverse peanut germplasm and disease resistance, a series of 'CRSP' breeding lines was developed from crosses of the U.S. cultivar Florida MDR-98 and the Bolivian land-race cultivar Bayo Grande (BG). Florida MDR-98 was selected as a parent cultivar due to greater resistance to leaf spot and equal to better TSW resistance when compared to GG (7, 11, 28, 29), Bayo Grande was selected due to moderate to greater resistance to *C. arachidicola* and *C. personatum* compared to GG in preliminary field evaluations (J.W. Todd, *unpublished data*). The contributions of the parent germplasms has not yet been evaluated in the CRSP breeding line progeny, but the potential for improved multi-disease resistance is great. The eventual release of a cultivar from these lines would also contribute to U.S. peanut crop diversity.

Efforts like the diversification of peanut germplasm will help prevent the potentially serious threat of host resistance breakdown in pathogen populations. It has been suggested that predicting the potential for resistance in a population of pathogens is possible and has been described as a function of genetic variation (26, 27), with populations of high genetic diversity having a greater evolutionary potential to overcome host resistance (26). Population genetic

studies aimed at the quantification of diversity are common within the genus *Cercospora* and its teleomorph, *Mycosphaerella* (13, 20, 30, 33, 34), but little work has described the genetic variability of *C. arachidicola* or *Cercosporidium personatum*. In turn, knowledge of the genetic diversity of these pathogens will aid in predicting management durability and support the selection of long-term management strategies (4, 14, 18, 26, 27).

The goal of this research is to improve current management of peanut diseases through newly-incorporated Bolivian host resistance, integrated disease management and knowledge of pathogen variability. The specific objectives of this work are: i) to evaluate early and late leaf spot and rust resistance in the CRSP breeding lines at multi-continent locations, and determine the components of resistance to early leaf spot; ii) to determine the potential for fungicide reduction with an integrated disease management system composed of strip tillage and the CRSP lines and; iii) to assess genetic variation among populations of *Cercospora arachidicola* in the U.S. and Bolivia.

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CHAPTER 2

LITERATURE REVIEW

The peanut plant. The cultivated peanut (*Arachis hypogaea* L.) is grown on approximately 19 million hectares in over 20 countries around the world (96). The genus *Arachis,* a member of the Fabaceae family, is native to South America with the origins of *A. hypogaea* believed to be in Bolivia and northwestern Argentina (59, 97). Within *A. hypogaea*, subspecies and varieties have been identified based on variations in structures, growth habits, maturity, and seed characteristics. Subspecies produced in the U.S. include Virginia (subsp. *hypogaea* var. *hypogaea*), runner (subsp. *hypogaea* var. *hypogaea*), Valencia (subsp. *fastigiata* var. *fastigiata*) and Spanish (subsp. *fastigiata* var. *vulgaris*) (96). Peanut plants grow 15-60 cm in height and produce zygomorphic, yellow inflorescences 28-42 days after seedling germination. Four to six hours after pollination, flowers senesce and the fertilized ovaries develop into carpophores, or "pegs." Pegs grow gravitrophically into soil, and the tips form pods (1.0-3.5 x 0.5-1.5 cm) containing one to five seeds between 0.2 to 2.0 g in weight (85).

Peanut seeds are consumed whole, processed to make peanut butter and candy, or crushed to extract oil for cooking. With contents of 25% protein, unsaturated fats and various vitamins and minerals (96), peanuts are a healthy legume, and the wide area of production indicates that its popularity as a component of many global diets.

Peanut leaf spot and rust. The major fungal disease threats to peanut yields are leaf spot and peanut rust (102, 111). Leaf spot is a collective term for two peanut diseases; early leaf spot, caused by *Cercospora arachidicola* S. Hori (*Mycosphaerella*

arachidis Deighton), and late leaf spot, caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) (*Mycosphaerella berkeleyii* Jenk.) (95). The appearance of one or both diseases varies by year and location and is influenced by environmental factors (78). Leaf spot symptoms include irregular, necrotic lesions, or spots, on leaves, petioles, stems and pegs. In response to infection, plants prematurely drop leaves, and if untreated, leaf spot can defoliate plants completely. The defoliation caused by both pathogens causes a reduction in photosynthesis in peanuts which can negatively affect pod fill. The necrosis of lesions on pegs can causes breakage during harvest, leaving pods in the soil, also contributing to yield loss. The direct damage and resulting crop loss caused by leaf spot and the fungicide cost to manage both diseases totaled $42 million in GA in 2005 (53).

Lesions caused by *Cercospora arachidicola* are approximately 1-10 mm in diameter and are light to medium brown in color, sometimes with the presence of a chlorotic halo (95). Sporulation occurs within lesions, typically on the adaxial leaf surface. Conidiophores range in size from 15-45 × 3-6 μm and form in clusters. Conidia (35-110 × 3-6 μm) are subhyaline, elongated and can have up to 12 septa. *Cercosporidium personatum* causes dark brown to black lesions typically without halos. Sporulation occurs on the abaxial surface of lesions, often in concentric rings. Conidiophores (10.0-100.0 × 3.0-6.5 μm) give rise to conidia (20.0-70.0 × 4.0-9.0 μm) that are cylindrical and slightly curved with up to nine septations.

The disease cycles of *C. arachidicola* and *C. personatum* are very similar. Both pathogens overwinter in infected crop debris. When temperature and moisture increase in the spring, both fungi infect new peanut crops via asexual conidia, sexual ascospores, or mycelial fragments. Dispersal of these forms of initial inoculum include rain-splash, wind or insect (78, 95). When temperatures reach 25-31 C and leaf wetness is abundant due to rainfall or heavy dew,

spores germinate and penetrate the host through the natural openings like stomata or through direct penetration of plant tissue (95). Infection by *C. arachidicola* is favored by temperatures greater than 19 C and relative humidity higher than 95% humidity. Infection by *C. personatum* is favored by temperatures greater than 20 C and relative humidity higher than 93% for 12 h or 10 or more hr of leaf wetness. Within the host, *C. personatum* grows intercellularly and forms haustoria to obtain nutrients . *C. arachidicola* also grows intercellularly, but lacks haustoria and absorbs plant cell materials directly . Late leaf spot lesions can appear 10-14 d after infection and early leaf spot 11-17 d (78, 96). Asexual conidia are formed within the necrotic tissue of lesions, and secondary infections result from dispersal of fungal propagules to new host tissue (85).

Peanut rust, caused by *Puccinia arachidis* Spegazzini, is a reoccurring pathogen in North, Central and South America, as well as Asia, Australasia, Oceania and Africa (42, 85, 102, 104). In the U.S., peanut rust has caused notable economic loss in Texas (102), but also can occur and impact yields in the southeastern U.S. (7). Symptoms of rust include leaf necrosis without defoliation (78), and infection can result in nonviable seed, pod detachment at digging, and, ultimately, low yields and oil content (42, 78). Disease signs of *P. arachidis* include orange uredinia pustules typically found on the abaxial side of leaves and occasionally on stems and developing pods. Each uredinium ranges in diameter from 0.5-1.4 mm and may contain hundreds of asexual urediniospores. The urediniospores are obovoid and 23-29 ×16-22 µm in size with a thick, brown wall often with diagnostic surface echinulations (102). This uredinial stage is the most prevalent, while the sexual, telial stage has been rarely observed (42, 45). Unlike urediniospores, teliospores are brown, two-celled survival spores (38-24 ×14-16 µm) which form on the abaxial leaf surface. Most *Puccinia* species require two hosts to complete a complex life

cycle, yet no alternative host has been identified for *P. arachidis* which appears to be restricted solely to peanut (106).

In locations where peanuts are produced year-round, *P. arachidis* urediniospores can survive on volunteer peanuts, while in areas with annual production, rust primary inoculum is believed to be blown in on wind currents from areas of year-round production (78). It has been suggested that the southern U.S. receives rust inoculum from Central America and the Caribbean islands (7, 45).The overwintering of *P. arachidis* is unlikely because urediniospores can survive on crop debris for no more than four weeks (105). Urediniospores are disseminated by wind, water or insect to susceptible host tissue, and germination is favored by minimal light, humidity above 87% and temperatures of 20-25 C. Symptoms typically occur 7-20 d after infection (102, 105).

Tomato spotted wilt of peanut. The *Tomato spotted wilt virus* (TSWV), causal agent of tomato spotted wilt (TSW), is a tospovirus that infects a wide host range including peanut, tomato, tobacco and numerous weed species. Tomato spotted wilt on peanut has been reported in Africa, Australia, South America and India and causes major yield losses in North America production (21). In the state of Georgia in 2005, TSW was responsible for $37.1 million in damage and reduced crop value (53).

Aboveground symptoms of TSW infection include severe stunting of plants, and leaves typically show unique patterns of chlorotic rings and puckering. Underground, pegs, pods and seeds may deform in size, color and seed coats may split (21, 23, 24, 41, 93). Roots can also become necrotic, and, in severe epidemics, whole plants can die (22). In some instances, infected plants show no visible symptoms (28), and reports have indicated that the physiology of asymptomatic plants are negatively affected by the presence of the virus (90).

Three insects are recognized as vectors of this virus; *Thrips tabaci* Lindeman (onion thrips), *Frankliniell occidentalis* Pergande (western flower thrips) and *F. fusca* Hinds (tobacco thrips) (91, 92), with the two latter species being the most predominant TSWV vectors in U.S. peanuts (21). While thrips adults and larvae are capable of acquiring the TSWV, only adults that obtained the virus as larvae may infect new plants. Because of its wide host range, TSWV can survive in alternative hosts between peanut seasons and initial inoculum can be derived from numerous sources.

Disease management with chemicals. Chemical pesticides are perhaps the most commonly used and most effective disease management strategy. Many fungicides are available for the effective control of *C. arachidicola*, *C. personatum* and *P. arachidis*, but insecticides, with one exception, have not been useful in suppressing TSW, even when they reduce feeding damage by thrips vectors (21). Recommendations for management of leaf spot and rust in Georgia include a variety of fungicides with different modes of action such as organochlorines (e.g. chlorothalonil), triazoles (e.g. tebuconazole and propiconazole), and strobilurins (e.g. trifloxystrobin and pyraclostrobin) (54). The currently recommended spray schedule for leaf spot in Georgia begins 30 days after planting (DAP) and continues at a 14-day interval until harvest. To manage rust, peanuts with three or more weeks until maturity at the time of rust onset should be sprayed weekly until harvest. As a result, the number of fungicide applications recommended for leaf spot and rust management can be very high during a season. These multiple fungicide applications can significantly increase the time, resource and cost of peanut production. The impact of fungicides on surrounding natural environments is also a concern associated with chemical use. Numerous studies on the effects of triazole fungicides on water quality (12) as well on non-target organisms such as fish (57), birds (75) and mice (76) have been conducted. Lastly,

fungicide resistance has become a major concern in fungal pathogen control. The efficacy of fungicides has been compromised with development of pathogen resistance as documented in the leaf spot pathogens with regards to benzamidizole and triazole fungicides (17, 62, 98, 101). The many negative issues surrounding fungicide use have caused a shift towards alternative disease management strategies that lessen reliance on these chemicals.

Disease management with host resistance. The use of cultivars with improved disease resistance is one of the most effective options for leaf spot and rust management (67, 81, 84) and is one of the most promising single factors for suppressing epidemics of TSW in peanut (21). Sources of genetic resistance to these pathogens have been identified, yet the mode of inheritance of resistance is complex and often unclear (2, 14, 21, 38, 51, 55, 58). While immunity or very high resistance to leaf spot and rust has been identified in wild *Arachis* species (1, 99, 100, 109, 110), a majority of cultivated peanuts demonstrate only partial resistance to these pathogens (13, 14, 105). The TSW resistance of currently available cultivars is also considered to be moderate, or partial (21). Partial resistance is often the result of the additive effects of numerous components of resistance which affect epidemics by reducing the rate of disease progress (82).

The components of resistance to *C. arachidicola* and *C. personatum* used for genotype screening include incubation period, or the time from inoculation to appearance of lesion, infection frequency, lesion size, necrotic area, latent period, or the time from infection to sporulation, amount of spore production, amount of defoliation, and time until defoliation. The components that are most often associated with a reduction in rate of early leaf spot epidemics are a longer latent period (32) and an increase in the maximum percentage of sporulating lesions

(89). Reduced lesion size and sporulation, as well as a longer latent period have been associated with reduced rates of late leaf spot epidemics (3, 13, 15).

Components of resistance to peanut rust include incubation period, infection frequency, pustule size, percent diseased leaf area, and spore production and germinability. Increased incubation period, decreased infection frequency, decreased percent diseased leaf area, reduced pustule size, spore production and spore germinability contributed to rust resistance and correlated to field resistance (81, 107, 108). Ontogenic resistance to *P. arachidis* has also been identified. An increase in the age of leaves was found to be negatively correlated with disease levels (18). A decrease in leaf wettability observed with age was identified as the reason for loss of urediniospore retention on the leaf surface, a step required for uredinial formation. Similar results were observed in another study in which susceptibility decreased with increasing whole plant age (103). Breeding lines may differ in rates of decreasing leaf wettability with age; therefore, leaf and overall plant age should be considered when screening for rust resistance.

Components of resistance to TSW are not well understood (21). Evaluations of factors associated with the thrips vector such as reproduction, host plant appeal and feeding damage were evaluated and no correlations were found with TSW resistance observed in the field (20, 22, 23, 25, 26, 29). Also, artificial inoculations of the virus rarely resulted in the same resistance found in naturally occurring epidemics in the field (46, 64, 83).

Currently, several runner-type peanut cultivars with partial disease resistance are available in the U.S. Georgia Green (GG), the most predominantly grown cultivar in the southeastern U.S., was released with moderate resistance to TSW (5), but low resistance to leaf spot. The later release of Florida MDR-98 in 1998 and C-99R in 1999 provided the U.S. with cultivars with better resistance to early leaf spot (11, 71, 72) and late leaf spot (11, 36, 37), and

equal or better resistance to TSW (20, 25, 29, 30, 34, 115) compared to GG. Field resistance to *P. arachidis* has not been well documented in GG, C-99R or MDR-98. The potential for greater rust resistance exists within C-99R and MDR-98, as the parental lineage of both cultivars contains UF81206, a breeding line with documented rust resistance (36, 37). More recently, cultivars like DP-1 and Hull have been released with improved leaf spot and TSW resistance (19, 35).

In addition to naturally occurring genetic resistance, transgenic, or genetically engineered peanuts have the potential to successfully enhance disease management. Progeny of peanuts that were genetically modified by inserting the viral gene for nucleocapsid protein showed improved TSW resistance in the field (61, 63, 117). However, concerns have arisen about the probable breakdown of this single-gene resistance (47, 87). Although peanuts with genetically enhanced fungal disease resistance are not currently available, potential genes for fungal resistance have been identified and show promise for future resistance toward fungi like *C. arachidicola*, *C. personatum* and *P. arachidis* (68).

Disease management with tillage practices. In addition to genetic and chemical control, cultural practices have proven reliable methods of disease suppression. Examples of effective cultural practices include manipulation of planting date, crop rotation with a non-host crop and more recently, conservation tillage. Peanuts have traditionally been planted using conventional tillage in which soil is turned with a switch plow and bedded with a disk bedder in order to bury weed seed and previous season crop debris. More recently peanuts have been planted using conservation tillage to prevent soil erosion, conserve water, and consume less time and labor (43, 86). This form of reduced tillage consists of maintaining a cover crop in the soil while peanuts are planted in 20- to 25- cm strips of soil using a subsoil shank. In two studies, researchers found reduced tillage lowered incidence of TSW compared to peanuts planted under

conventional tillage (27, 52). Although the underlying mechanism is unknown, this disease management practice continues to be successful and has led to an increase in acreage of peanuts planted under conservation tillage in Georgia.

Because TSW typically occurs in areas where leaf spot is also prevalent, interest arose in the effects of conservation tillage on leaf spot. When compared to conventionally tilled soils, strip tillage, a form of conservation tillage, resulted in less defoliation due to *C. arachidicola* (86). Early leaf spot epidemics within strip tilled soils were less severe than those in conventionally tilled soils under both natural and chemically-influenced disease environments (9, 71, 72). The mechanism underlying early leaf spot suppression under strip tillage has been suggested to be a result of a physical disruption of initial inoculum dispersal (10). No information has been published on the effects of conservation tillage on rust epidemics.

Integrated disease management. To reduce complete reliance on fungicides for leaf spot and rust, host resistance and strip tillage can be combined into an integrated disease management (IDM) system. Monfort et al. (2004) were the first to explore the use of partially resistant cultivars coupled with strip tillage for management of leaf spot (71). Results indicated that peanuts grown under strip tillage and treated with an effective fungicide on a 21- to 28-d schedule had levels that were comparable to those in conventionally-tilled peanuts on a 14-d spray regime. This combination of control tactics provided effective management of early leaf spot with fewer fungicide applications. Similar results with a combination of improved host resistance and strip tillage were found (11). Results demonstrated that planting resistant peanut cultivars in strip-tilled soils can reduce early leaf spot severity and fungicide inputs. However, inconsistencies in economic net returns across years compared to net return under conventional

tillage emphasizes the need for more work in this area before recommendations can be made to growers.

Protecting host resistance through genetic diversification. While many cultivars currently available in the U.S. have partial resistance to one or more pathogens, recent evaluations of the peanut crop as a whole indicated an overall lack in genetic diversity and a classification of 'vulnerable to diseases' (111). This is due to the limited sources of parent germplasm used to produce U.S. cultivars (49) as well as the continued monoculture of cultivars like GG, which is planted to 80% of the southeastern U.S. every year (111). Cultivars, like fungicides and other management tactics, exert selection on pathogen populations that may result in pathogen resistance and the erosion of the durability of control (4). The narrow genetic base of germplasm offers a restricted source for disease resistance, increasing the chance of the development of resistance in pathogen populations. To avoid future resistance breakdown, more diverse germplam must be incorporated into future breeding programs. The South American country of Bolivia where the peanut is believed to have originated (59), has been recognized as an under-utilized source of genotype diversity for wild *Arachis* species (50, 116), and is noted by Holbrook and Stalker (2003) as a center for both early and late leaf spot resistance in cultivated peanut (47).

As part of a United States Agency for International Development's Peanut Collaborative Research and Support Program (USAID Peanut CRSP) to utilize Bolivia's diverse peanut germplasm and disease resistance, a series of 'CRSP' breeding lines was developed from crosses of the U.S. cultivar MDR-98 and the Bolivian land-race cultivar Bayo Grande (BG). MDR-98 was selected as a parent cultivar due to greater resistance to leaf spot and equal to better TSW resistance when compared to GG (11, 20, 71, 72). Bayo Grande was selected due to moderate to

greater resistance to *C. arachidicola* and *C. personatum* compared to GG in preliminary field evaluations (J.W. Todd, *unpublished data*). The contributions of the parent germplasms has not yet been evaluated in the CRSP breeding line progeny, but the potential for improved multi-disease resistance is great. The eventual release of a cultivar from these lines would also contribute to U.S. peanut crop diversity.

For disease and yield evaluations, the CRSP breeding lines must be assessed at locations within the U.S. and Bolivia if they are to be utilized in either or both locations. Multiple location assessments are necessary because 'genotype x environment' interactions are common when screening breeding lines for resistance to rust (105), early leaf spot (56, 77, 112, 113), late leaf spot (16), TSW (20, 21) as well as screening for yield potential (6). Pathogen virulence has been shown to differ among locations (88, 94). This variability is a likely contributor to the interactions in genotype evaluations.

Protecting disease management through knowledge of pathogen variability. In natural ecosystems, the genetic structure of pathogen populations is in a constant state of change in response to processes like mutations (changes in the DNA of individuals), gene flow (the exchange of alleles, or genes, among geographically isolated populations), genetic drift (random processes that change alleles in a population) and mating (asexually, sexually or both). In agricultural settings, human-implemented disease control methods, such as fungicides, crop rotation and host genotype, cause an artificial change in pathogen populations by targeting individuals that are susceptible to the specific control measure(s) used, while leaving behind resistant individuals. Continued selection of this nature leaves behind resistant individuals, which, over time can increase in number and render control strategies ineffective. Predicting the potential for resistance in a population of pathogens has been described as a function of genetic

variation (65, 70), with populations of high genetic diversity having a greater evolutionary potential to overcome resistance (65). An understanding of genetic variation of pathogen populations and forces that contribute to it, such as sexual reproduction, can lead to more informed selections of control strategies and overall stronger durability of disease management programs (8, 33, 40, 70).

Many population genetic studies of plant pathogens have been conducted on fungi (60, 66, 69), and only within the last decade, have expanded in number with reference to the genus *Cercospora* and its teleomorph, *Mycosphaerella* (31, 48, 73, 79, 80). Using neutral molecular markers like DNA fingerprinting and DNA sequences, many studies have not only described the amount and geographic distribution of genetic diversity, but have also used this information to make inferences about the origin and spread of pathogens.

Genetic diversity within populations of *C. beticola* was found to be high within and between four locations in Greece, indicating substantial movement of the pathogen throughout the sugarbeet production region (73). A study of ten isolates from within a single lesion revealed high genetic diversity and varying reactions to fungicides, offering proof that *C. beticola*, a pathogen believed to predominantly reproduce asexually, may be undergoing sexual reproduction (74).

Mycosphaerella fijiensis populations on banana from the Australasian-Pacific region had moderate genetic diversity within populations in most locations, and moderate to high diversity as populations became more geographically separated (44). All populations were characterized as being in gametic equilibrium, indicating that recombination, or sexual reproduction was occurring and likely contributing to diversity. The country of Papua New Guinea had the most

diverse population structure and was suggested to be the center of origin of *Mycosphaerella fijiensis*.

In a global study of 14 populations of *M. graminicola* from four continents, high genetic diversity within populations was correlated with increased age, according to the spread of wheat with spreading civilizations; the most diversity was seen in populations from the Middle East, the oldest known area of civilization and wheat cultivation. Moderate diversity was noted in Europe, and the lowest diversity in the North and South America and Australia, areas considered younger or "New World" civilizations.

In *C. zeae-maydis*, a study of U.S. populations described the presence of two genetically distinct sibling species within the country, group I and II (114). Later studies revealed that the genetic structure of group II populations was similar to African populations, suggesting that an African isolate migrated to the U.S. and established the group II (31, 79). The appearance of two sibling species suggests that speciation is occurring within global populations of *C. zeae-maydis*, a process that will affect future breeding and disease management.

From South American and Asian collections of *C. kikuchii*, Imazaki et al. (2006), found three genetically distinct population lineages from Brazil and Argentina and two unique to Japan (48), while two additional lineages were shared among both locations, suggesting that the pathogen may have been introduced to South America from Japan where soybean originated. Fungicide sensitive isolates were also documented within lineages to identify populations in which fungicide selection will have the most impact.

The presence and distribution of the mating types of various *Cercospora* species from around the world have been studied by amplifying the loci, *MAT1-1* and *MAT1-2* in individual isolates using PCR (39). Populations of *C. apii, C. apiicola*, *C. beticola*, *C. zeae-maydis*, and *C.*

zeina were found to have both mating types, indicating the potential for sexual reproduction, a process that can lead to increased genetic diversity. Knowledge of mating types within a population may also indicate differences in pathogenicity, as seen among global isolates *Mycosphaerella graminicola* (118).

Despite the breadth of population genetic work with the aforementioned *Cercospora* and *Mycosphaerella* species, no work has explored population structures of *C. arachidicola* or *C. personatum*. In recent years, *C. arachidicola* has been the prominent pathogen in fields in GA. Knowledge of the genetic variability and frequency of mating types of this pathogen may give insight into the origin and spread of *C. arachidicola* as well as reveal evolutionary forces acting upon these populations. In addition to genetic evaluation, assessments of fungal morphology such as spore size, may provide evidence of genetic changes in populations of *C. arachidicola*. Both genotypic and phenotypic information will lead to the support of more informed selections of control strategies and overall stronger durability of management programs (8, 33, 40, 70).

The goal of this research is to improve current management of peanut diseases through newly-incorporated Bolivian host resistance, integrated disease management and knowledge of pathogen variability. The specific objectives of this work are: i) to evaluate early and late leaf spot and rust resistance in the CRSP breeding lines at multi-continent locations, and determine the components of resistance to early leaf spot; ii) to determine the potential for fungicide reduction with an integrated disease management system composed of strip tillage and the CRSP lines and; iii) to assess genetic variation among populations of *Cercospora arachidicola* in the U.S. and Bolivia.

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CHAPTER 3

INCORPORATION OF BOLIVIAN RESISTANCE TO FUNGAL DISEASES OF PEANUT (*ARACHIS HYPOGAEA*) [1]

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Field, greenhouse and growth chamber trials were conducted over a four-year period on two continents to assess resistance of newly-incorporated Bolivian peanut germplasm to the yield-reducing fungal pathogens *Cercospora arachidicola*, cause of early leaf spot, *Cercosporidium personatum*, cause of late leaf spot, and *Puccinia arachidis*, cause of peanut rust. A series of breeding lines and the Bolivian cultivar, Bayo Grande (BG), were compared with the U.S. cultivars Florida MDR-98, C-99R and Georgia Green (GG), a highly susceptible cultivar to both early and late leaf spot. Field resistance of the breeding lines and BG to the leaf spot was apparent when disease incidence and defoliation were compared among genotypes. The components of resistance to *C. arachidicola* most associated with field resistance were lower maximum percent sporulating lesions and lesion size, but not all breeding lines were significantly different from the susceptible cultivar GG based on these components, and the overall results were not consistent. Compared to the Bolivian standard BG, no improved leaf spot resistance was observed among the other genotypes in Bolivian trials. No improved rust resistance was observed in genotypes. Overall, rust resistance did increase as plants matured in age, but these results were not repeatable. Yield in the U.S. was improved in BG and the breeding lines compared to the other genotypes in one year but not significantly better in the following season. In Bolivia, where leaf spot intensity was low, GG out-yielded BG and the breeding lines, but when disease levels increased, yields were comparable to MDR-98 and C-99R and better yields than GG. The application of two tebuconazole sprays significantly suppressed leaf spot when utilized in Bolivia compared to the U.S., possibly indicating a higher level of fungicide resistance in the U.S.

Keywords: *Cercospora arachidicola*, *Cercosporidium personatum*, *Puccinia arachidis*

Introduction

Peanut (*Arachis hypogaea* L.) is a legume that offers both health and financial benefits to the areas of the world where it is grown. However, crop production and economic returns are threatened yearly by destructive diseases. Among the most devastating fungal diseases of peanut are the leaf spots, early leaf spot (*Cercospora arachidicola* S. Hori) and late leaf spot (*Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton), and peanut rust (*Puccinia arachidis* Speg.) (44, 48). Rust infection results in leaf death, and leaf spot triggers defoliation. Both leaf spot and rust affect the integrity of pegs, which causes pod detachment at harvest and, ultimately, reduced yields.

The use of cultivars with improved disease resistance is an effective means of leaf spot and rust management (27, 31, 32). While many cultivars currently available in the U.S. have partial resistance to one or more of pathogens (3, 16-18), recent evaluations of the peanut crop as a whole indicated an overall lack in genetic diversity and a classification of 'vulnerable to diseases' (48). This is due, largely, to the few sources of parent germplasm used to produce U.S. cultivars (23). The narrow genetic base of germplasm offers a restricted source for disease resistance, increasing the chance of the formation of resistance in pathogen populations. To avoid future resistance breakdown, more diverse germplams should be incorporated into breeding programs.

The South American country of Bolivia where peanut is believed to have originated (26), has been recognized as an under-utilized source of genotype diversity for wild *Arachis* species (24, 51), and is noted by Holbrook and Stalker (2003) as a center for both early and late leaf spot resistance in cultivated peanut (20, 21). With one of its main objectives to utilize Bolivia's diverse germplasm and potential disease resistance, the UFL-16 project of the United States

Agency for International Development's Peanut Collaborative Research and Support Program (USAID Peanut CRSP) has developed a series of breeding lines from crosses of the U.S. cultivar, Florida MDR-98, and the Bolivian land-race cultivar Bayo Grande (BG). Florida MDR-98 has improved leaf spot and TSW resistance equal to or better than Georgia Green (GG) (8, 14, 18, 28), and BG has shown moderate to improved resistance to *C. arachidicola* and *C. personatum* compared to GG in preliminary field evaluations (J.W. Todd, *unpublished data*). Little is known about the levels of rust resistance in BG or MDR-98. The potential for resistance exists within MDR-98, as its parental lineage contains UF81206, a breeding line with documented rust resistance (17, 18).

Preliminary field evaluations of the new germplasm and has narrowed selections to a group with the potential for cultivar release in the U.S. and Bolivia. However, more information on the disease and yield response of the CRSP breeding lines in multiple locations is needed to support final selection, as 'genotype x environment' interactions are common when screening for resistance to rust (40), early leaf spot (25, 30, 49, 50), late leaf spot (11) and yield potential (4). To determine the mechanism behind the disease resistance found in the CRSP lines, genetic resistance can be assessed by measuring components of resistance. With leaf spot, components that typically indicate resistance are lower lesion density, lesion diameter and maximum percentage of sporulating lesions, higher disease severity, and a longer latent period (1, 6, 9, 10, 15, 34). For rust, a longer incubation period, lower uredinia density, lower percent diseased leaf area, smaller pustule size, and reduced spore production and germinability are most associated with resistance (31, 43, 46). Physiological resistance associated with disease suppression is also a possibility in regards to rust. Ontogenetic resistance, or the change in resistance as plant tissue ages, is a common characteristic of many agronomic crops infected by various *Puccinia* species

(22) and has been documented in peanut (13, 39). A more complete understanding of genetic and physiological components of resistance that describe field resistance should aid breeding line selection.

The objectives of this study were to i) evaluate disease resistance and yield potential in BG and a series of CRSP breeding lines in field experiments in multiple locations in the U.S. and Bolivia, over multiple years, and to ii) describe genetic resistance to leaf spot and ontogenic resistance to rust in BG and the CRSP lines.

Materials and Methods

Field evaluations. Genotype evaluations for leaf spot and rust resistance and yield potential were conducted at three U.S. locations including the University of Georgia (UGA) Attapulgus Research and Education Center in Attapulgus, GA (AP), the UGA Southwestern Research Station in Plains, GA (PL), and the UGA Coastal Plain Experiment Station, Lang Farm in Tifton, GA (TF) in 2004-2005. Field experiments were also conducted in Bolivia, South America at San Pedro (SP) in 2005 during the dry, winter growing season (June-December), and Saavedra (SV) and Veintiséis de Agosto (VA) in 2005-2006 during the wet, summer growing season (December-May).

A split-plot design with three replications was used for all experiments. Fungicide regimes represented the whole-plot treatments and included: i) non-treated control (0 sprays); and ii) tebuconazole (Folicur 3.6F in U.S. or Folicur 250 FC in Bolivia, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 days later (2 sprays). The two-spray fungicide regime was used as a preventative measure to help separate host genotype resistance in case of severe epidemics that can overcome host defenses.

The fungicide rate was selected to mimic practices of Bolivian farmers who typically spray at reduced rates to lower production costs. Sub-plot treatments included MDR-98, Bayo Grande (BG) and the CRSP breeding lines CRSP-01, CRSP-08 and CRSP-14. All breeding lines were in the F7 generation in 2004 and are considered late-maturing genotypes. Georgia Green (GG), a common medium maturing cultivar grown in the southeastern U.S. that is susceptible to *C. arachidicola* and *C. personatum*, was also included to serve as a reference point for leaf spot resistance evaluations. The late-maturing cultivar C-99R, with a moderate level of resistance to leaf spot (8, 17, 28, 29) was included as well.

In the U.S., peanuts were planted on 26 May 2004 and 26 May 2005 in AP, 24 May 2004 and 25 May 2005 in PL, and 20 May 2004 and 24 May 2005 in TF. All plots were planted at the rate of 16.4 seed/m in 1.829 m × 6.096 m plots. Plots were irrigated throughout the season as needed. In Bolivia, SA peanuts were planted 22 December 2004 and 7 December 2005, VA on 15 December 2004 and 30 December 2005, and SP on 23 June 2005. All Bolivian plots consisted of two rows 6 m in length with 0.07 m between the row and 0.07 m between plots. In accordance to typical Bolivian peanut production practices, plots did not receive any irrigation in addition to natural rain events.

In 2004, leaf spot at AP was evaluated 23, 27, 33, 47, 54, 61, 68, 80, 96, 101 and 108 DAP for all genotypes and a final assessment was made 101 DAP for all genotypes except GG, which was previously harvested. The following year, leaf spot intensity was evaluated 60, 74, 82, 92, 97, 111, 125 and 132 DAP for all genotypes, and a final assessment was made 141 DAP for all genotypes except GG. In 2004 at PL, leaf spot was evaluated 28, 48, 55, 62, 69, 78, 83, 103 DAP for all genotypes and a final assessment was made 109 DAP for all genotypes except GG. In 2005 disease was assessed 61, 75, 83, 93, 107 and 126 DAP for all genotypes and the final

assessment was made 138 DAP for all genotypes except GG. In 2004 at TF, leaf spot intensity was evaluated 22, 38, 52, 62, 68, 81, 90, 104, and 108 DAP for all genotypes and 116 DAP for all genotypes except GG. Georgia Green plots were inverted 131 DAP at AT and PL and 143 DAP in TF in 2004. Remaining genotypes were inverted 142 DAP in AT, 139 DAP in PL, and 146 DAP in TF. Peanuts were harvested 7-10 days after digging at each location.

In Bolivia at SA, leaf spot intensity was evaluated 92, 104 and 124 DAP in 2005 and 74, 91, 102, and 115 DAP in 2006. Peanut rust was assessed 124 DAP in 2005. Leaf spot intensity was assessed 83 and 114 DAP in 2005 and 72, 100, 114, 121 and 132 DAP in 2006 at VA. Peanut rust was evaluated 114 DAP in 2005. At the SP location, leaf spot intensity was assessed 91, 134 and 160 DAP, and peanut rust intensity was evaluated 160 DAP. Plots at SA were inverted 141 DAP in 2005 and 124 DAP in 2006. Plots at VA were inverted 132 DAP in 2005 and 136 DAP in 2006. Plots at SP were inverted 170 DAP in 2005.

Leaf spot intensity was assessed using the Florida 1-10 scale where 1=no disease (0% defoliation), 2=very few lesions, more on upper canopy (0% defoliation), 3=few lesions, very few on upper canopy (0% defoliation), 4=some lesions with more on upper canopy, noticeable defoliation (5% defoliation), 5=lesions noticeable even on upper canopy, noticeable defoliation (20% defoliation), 6=lesions numerous and very evident on upper canopy, significant defoliation (50% defoliation), 7=lesion numerous on upper canopy with much defoliation (75% defoliation), 8=upper canopy covered with lesions with high defoliation (90% defoliation), 9=very few leaves remain and those covered with lesions, some plants completely defoliated (98% defoliation) and 10=dead plants (100% defoliation), completely defoliated and killed by leaf spot (10). Area under the disease progress curve (AUDPC) based on visual estimates of % defoliation was

calculated for each replication of genotype and fungicide treatment (35) and standardized by dividing AUDPC by the number of days between the first and final leaf spot rating (5).

Simple models of temporal disease progress were fit to leaf spot intensity data to compare the effect of genotype on rate. Percent defoliation was converted to proportion [proportion of disease = ((% defoliation)/100))]. Defoliation ratings of "0%" were assigned a proportion value of "0.001" to avoid dividing by zero. The Gompertz [-ln(-ln y)], logistic [ln(y/1-y)] and monomolecular [ln(1/1-y)] models were fit to linearly regression transformed disease proportion on time (DAP). The best fit model was determined by evaluations of residual plots and back-transformed, recalculated R^2 values. Data that did not fit any of the aforementioned models were excluded from further analyses. The slope, or rate (r), parameter estimate was used to compare genotypes and treatments. If different models within the Richard's family of models (monomolecular, logistic and Gompertz) were fit within a location, the variable weighted mean absolute rate (ρ) was calculated with the following formula: $\rho = rK/(2m + 2)$ where r represents the rate parameter of the Richard's family disease progress curve and m is the shape parameter which is given a value of '0' if data fits the monomolecular model, '1' if data fits the logistic model, and '2' if data fits the Gompertz model. The theoretical maximum level of disease, K, was set to 1.0 (100% severity) in all evaluations.

Peanut rust severity was measured using a modified 1-9 scale where 1= 0%, 2=1-5%, 3=6-10%, 4=11-20%, 5=21-30%, 6=31-40%, 7=41-60%, 8=61-80% and 9=81-100% severity (38). Yields for individual plots were recorded at harvest as kilograms per hectare (kg/ha) for in-shell peanuts in the U.S. and shelled peanuts in Bolivia.

The effects of fungicide and genotype on AUDPC, r, rust severity and yield were analyzed using SAS Proc MIXED (SAS v 9.1, SAS Institute, Inc. Cary, NC). The "Satterth"

option was used for determining degrees of freedom. Significant interactions were included in the model as a random effect. Non-significant interactions ($P \leq 0.05$) were removed from the model. If non-significant interactions contributed substantially to variation (F-value > 1.00), they were included as random effects. Differences among genotypes were determined based on a "pdiff" option included in each main effect and significant interaction LSMEAN statement. Significant differences treatments for AUDPC, r, rust and yield were determined based on Fisher's LSD ($P \leq 0.05$). Significance levels reported in the text are $P \leq 0.05$ unless otherwise indicated.

Pre-defoliation early leaf spot evaluations. Early leaf spot epidemics are often assessed by percentage of whole plant defoliation over time. This evaluation, however, does not describe the spots that appear at the beginning of epidemics, previous to the defoliation. To describe entire epidemics, pre-defoliation early leaf spot evaluations were taken. Data were collected in 2002 and 2003 from field trials with a split-split plot experimental design. Conventional and strip tillage composed of the main plots, a series of full and reduced fungicide regimes as well as a non-sprayed control made up the split plots and genotype representing the split-split plots including the cultivars GG, BG, C-99R and MDR-98 and the progeny lines CRSP-01, CRSP-08, CRSP-14 and CRSP-20. One lateral branch from 10 random plants from each plot was removed from non-sprayed, conventional tillage plots at 87, 95, 101 and 109 DAP in 2002 and 56, 63, 70, and 77, 81 and 88 DAP in 2003. Disease was assessed on the first 9 leaves starting from the node closest to the main stem. Disease incidence was recorded as the percentage of leaves with one or more leaf spots or defoliation and averaged across the 10 lateral branches. Then AUDPC was calculated for each replication. The effect of genotype on AUDPC was determined using Proc MIXED. The "Satterth" option was selected for determining degrees

of freedom. Significant differences among genotypes for AUDPC was defined by Fisher's LSD ($P \leq 0.05$). Significance level reported in the text is $P \leq 0.05$ unless otherwise indicated.

Components of early leaf spot resistance. Because *C. arachidicola* was more prevalent than *C. personatum* during field experiments in North and South America, components of resistance in the CRSP breeding lines to early leaf spot was investigated in an experiment using a modified detached leaf inoculation technique (7). To produce spores for inoculations, *C. arachidicola* isolates from Tifton, GA were grown on PDA.. Three tissue samples of approximately 1.0 cm^2 of were homogenized separately in 1.0 ml of deionized water (dH_2O) for 10 sec using a TissueMiser (Fisher Scientific, Pittsburgh, PA). The homogenate was spread across the surface of separate plates of V8 media and allowed to dry under sterile conditions until surface water evaporated. Plates were sealed with Parafilm and placed in a light box at room temperature (approximately 24 C) under continuous light. After 7 days, spores were collected by 10.0 ml washes with 0.005% Tween 20 solution. The spore concentration was determined using a hemacytometer (Fisher Scientific) and adjusted to final inoculation concentration of 1.0×10^4 spores/ml.

The genotypes included in this study were GG, BG, CRSP-01, CRSP-08, CRSP-14, CRSP-20 and MDR-98. Leaves were excised from approximately five 50-day-old greenhouse plants in the first trial and 52-day-old plants in the second trial, and petioles were dipped in napthaleneacetamide and thiram (Rootone, Security Products Co., Atlanta, GA) and placed in 100-ml beakers filled with damp sterilized sand. The spore suspension was sprayed onto individual leaves using compressed air (CleanSafe, Houston, TX) for 1 sec. Leaves of GG sprayed with 0.005% Tween 20 solution served as controls. A randomized, complete block design with four or five replications was used. A moist chamber, $58 \times 46 \times 56$ cm, was

constructed with PVC pipe, covered in transparent plastic, and placed in a growth chamber set at 24C, 90 % RH, and a 12-hr photoperiod. A humidifier and the tray of leaves were placed inside the moist chamber. The humidifier was scheduled to turn on and off every 30 min. After 48 hr, the tray and humidifier were moved to an enclosed light box with a 12-hr photoperiod, where leaves were maintained at room temperature (24 C). The humidifier was scheduled to turn on for 90 min and off for 30 min during the light period and off continually for the dark period. Sand was remoistened with dH_2O as needed.

Components of resistance to early leaf spot that were evaluated included % severity, or the percent area of leaf tissue with leaf spot symptoms at 30 days after inoculation (DAI), lesion size, or the average area (cm^2) of three randomly selected lesions, lesion density, or the number of lesions per leaf area (cm^2) at 30 DAI, latent period, or the DAI until one spot produced spores, and % maximum percent sporulating lesions (MPSL), or the percentage of spots with visible sporulation at 30 DAI. Lesions were counted daily and examined for sporulation starting 15 DAI and ending 30 DAI. Lesions that did not produce spores were not included in the study. Digital photographs of leaves taken 30 DAI were used to measure total leaflet area using the ASSESS Image Analysis Software for plant disease quantification (APS Press, St. Paul, Minnesota). Wilted or dead leaves were excluded from analyses. Up to three sporulating lesions were excised and placed in 0.5 ml of 0.005% Tween 20 solution for spore quantifications using a hemacytometer. The experiment was repeated twice. Air temperature and relative humidity (RH) were measured every 30 min in the growth chamber using HOBO dataloggers (H8 Pro Series, Onset Computer Corporation, Bourne, MA).

The effect of genotype on all components of resistance was determined using Proc MIXED. The “Satterth” option was selected for determining degrees of freedom. Significant

differences among genotypes were determined based on Fisher's LSD ($P \leq 0.05$). If data were unbalanced and standard errors were similar, the largest standard error was used to calculate LSDs for means comparisons. Significance level reported in the text is $P \leq 0.05$ unless otherwise indicated.

Greenhouse rust evaluations. An experiment was conducted in the greenhouse to evaluate genetic and physiological resistance to *P. arachidis*. Eight genotypes including the four cultivars GG, BG, C-99R and MDR-98 and the breeding lines CRSP-01, CRSP-08, CRSP-14 and CRSP-20 were included in the study. Three seed of each genotype were planted in each of eight plastic, 30.48 cm pots containing commercial potting soil (Miracle Gro Moisture Control Potting Mix with Miracle Gro Continuous Release, Scotts Company, Marysville, OH). After one week, the most vigorous seedling was selected and the remaining seedlings were removed from pots. *Rhizobium sp.* was added to the soil as Rhizo-Stick (Becker Underwood, Inc. Ames, Iowa) according to the manufacture's directions. Plants were watered as needed.

To test for ontogenic resistance, plants of two age groups were established so that overall resistance in genotypes could be evaluated in plants of different ages. The age of the first group was around flowering (FL), or the R1 growth stage (2), approximately 28-42 days after planting, and the second group after flowering, or post-flowering (PF), approximately 43 days or older. At the time of inoculation, the FL plants were 28 days old in 2002 and 40 days old in 2003, and the PF plants were 56 days old in 2002 and 71 days old in 2003. Plants were transported to the University of Florida Green Acres farm about 20 km west of Gainesville, FL on August 6, 2002 and August 5, 2003 to be exposed to natural rust inoculum present in the area. Seven replications of 5-7 plants in randomized complete block design were placed among rows of unsprayed control peanut plots. The plants remained in the field for 48 hr and received either one rain or

irrigation event. Plants were then transported to a greenhouse, misted with dH_2O, and covered in clear plastic bags to establish a humid environment conducive for infection. Plastic bags were removed after 48 hr. Twenty randomly selected leaves selected 17 days after exposure (DAE) in 2002 and 21 DAE in 2003 were dried and pressed. Leaflets were detached and petioles were discarded. Dead leaves were discarded. All leaflets collected were scanned, and images were used to measure total leaflet area using the ASSESS Image Analysis Software. Because of the relatively small size of the rust uredinia, or pustule, the area of infected tissue could not be accurately estimated, so counts were made by eye. Disease incidence was calculated as the number of leaflets with 1 or more uredinia divided by the total number of leaflets for each genotype and age group (FL and PF) and expressed as a percentage. Uredinia density was calculated as the average number of uredinia per leaflet divided by the average area of leaflet (cm^2). Whole plant disease severity was measured in 2003 using a modified 1-9 scale for rust as previously described (47).

The effect of genotype on disease incidence, uredinia density and severity was determined using Proc MIXED. The "Satterth" option was selected for determining degrees of freedom. Significance among genotypes for each disease measurement was defined by Fisher's LSD ($P \leq 0.05$). Significance level reported in the text is $P \leq 0.05$ unless otherwise indicated.

Results

Field evaluations. The severity of leaf spot epidemics varied across continents, domestic locations and years. Early and late leaf spot were present at all U.S. locations in 2004, but early leaf spot was predominant in 2005. Across years, final defoliation ranged from 90-100% in the most susceptible genotype (GG) (Fig 3.1). Early and late leaf spot were present at all Bolivian locations in both years, except SA in 2005 where early leaf spot was the predominant disease. In

Bolivia, final defoliation observed was dramatically lower in 2005 compared to 2006 (Fig. 3.2). In 2005, defoliation was greatest (22.5%) in plots of C-99R at SA and defoliation of all genotypes at SP and VA was less than 1.5%. In 2006, final defoliation ranged from 55-60% in the most susceptible genotype (GG) at SA and VA.

At all U.S. locations in both years, BG and the CRSP breeding lines suppressed leaf spot better than GG, C-99R and MDR-98, except at PL in 2005, where leaf spot levels were similar among the CRSP lines and C-99R and MDR-98 (Table 3.1). Two applications of tebuconazole at a reduced rate significantly lowered leaf spot at PL and TF in 2004 and AP in 2005. Although not significant, means at AP in 2004 and PL in 2005 numerically followed the same trend. The fungicide by genotype interaction was significant for AUDPC in Tifton in 2005 (Table 3.2). Under no fungicide sprays, GG had the greatest AUDPC value and BG and CRSP-08 had the lowest. The remaining genotypes were intermediate. An improvement in leaf spot intensity was seen in all genotypes with the addition of two fungicide applications, and AUDPC values of all genotypes were significantly similar. Partial resistance of the breeding lines reduced the rate of leaf spot epidemics at AT and TF in 2004, but not in 2005 at these locations (Table 3.1). The rate of disease development was lower in Bayo Grande and the CRSP lines than in GG and less than or equal to that of MDR-98 and C-99R at both locations in 2004. Fungicide did not significantly affect rate at any location in either year. None of the models provided an adequate fit to disease progress curves at PL in both years; therefore, epidemic rates were not compared among genotypes.

In Bolivia, neither genotype nor fungicides had any impact on AUDPC at SA or SP in 2005 (Table 3.3). The reduced fungicide regime significantly suppressed leaf spot compared to the non-sprayed control only in 2006 at SA, but trends were numerically similar at SA and SP in

2005. The fungicide by genotype interaction was significant at VA in both years (Table 3.4). Under very low leaf spot pressure in 2005, a majority of the genotypes resulted in similar AUDPC levels with no fungicide applications. The addition of fungicides further suppressed leaf spot only in C-99R compared to the non-sprayed plots. With an increase in disease pressure in 2006, BG, MDR-98 and the CRSP lines had the lowest AUDPC levels without fungicide applications. Georgia Green resulted in the greatest AUDPC levels, and C-99R was intermediate. All genotypes responded to fungicide applications with lower AUDPC levels, and when compared, AUDPC values for all genotypes similar. No models were fit to Bolivian data due to the low number of disease evaluations; therefore, epidemic rates could not be compared among genotypes at the SA, SP and VA locations.

Overall rust severity was moderate in Bolivia (<40%), yet no differences among genotype were observed (Table 3.5). At SA in 2005 and VA in 2006, locations where disease levels approached 20-30%, the application of fungicides significantly suppressed rust. Although not significant, the same trend was seen at other locations.

Yields of in-shell peanuts varied greatly across U.S. locations and years. Mean yields across genotypes were 1036.29, 3755.80 and 2840.23 kg/ha at AT, PL and TF, respectively, in 2004. The pre-plant herbicide Atrazine (Shell Chemical Company, Houston, TX) was inadvertently applied to soils before peanuts were planted at AP. Yields were unusually low at this location. In 2005, yields averaged 3303.67, 1475.58 and 2452.67 kg/ha at AT, PL and TF, respectively.

In 2004, yields of BG and the CRSP breeding lines were significantly higher than those of GG at PL and TF. The reduced fungicide treatment significantly increased yields by approximately 600 kg/ha compared to the non-sprayed control at PL (Table 3.6). The same trend

was observed in TF, though not significant. A significant fungicide by genotype interaction occurred at AT in the same year (Table 3.7). Under no fungicides, CRSP-08 had the highest yields and CRSP-20 the lowest. The remaining genotypes were intermediate. Yields of GG and MDR-98 were improved with the addition of two fungicide sprays compared to no fungicide application. The yields of the remaining genotypes were numerically higher under the two-spray regime, but not significantly different from those of the unsprayed controls. With the addition of fungicide applications, GG was the top-yielding genotype. Bayo Grande had the lowest yields and the remaining genotypes were intermediate. In 2005, neither genotype nor fungicide significantly affected yield, and no consistent trends were observed (Table 3.8).

In Bolivia, mean yields of shelled peanuts were 2472.31, 729.27, and 2652.94 kg/ha in SA, SP and VA in 2005, respectively, and 1524.05 and 3011.05 kg/ha in SA and VA in 2006, respectively. In 2005 at SA, yields of GG and C-99R were significantly higher than those of the other genotypes tested (Table 3.9). Yields of Florida MDR-98, BG and CRSP-01 were lowest, and those of CRSP-08 and CRSP-14 were intermediate. In the same year at SP, grown during the dry season, GG out-yielded all other genotypes, followed by CRSP-14 and C-99R. Bayo Grande, MDR-98 and the remaining CRSP lines had the lowest yields. At VA, yields of all genotypes were similar except that of CRSP-14, which was significantly lower than the others. Fungicides significantly improved yields at SA and numerically at VA and SP in 2005. In 2006, VA yields of all genotypes were higher than that of GG and were similar to C-99R and MDR-98, except CRSP-08 which had higher yields than the latter two genotypes (Table 3.10). The fungicide applications significantly increased yields across all genotypes. The fungicide by genotype interaction was significant at SA in 2006 (Table 3.11). Under no fungicide applications, C-99R yielded the highest and GG the lowest with the remaining genotypes yielding intermediately.

With the application of two fungicides, GG and MDR-98 had the highest yields and BG and the CRSP lines were significantly lower. Within each genotype, applications of fungicides resulted in significantly higher yields, except with BG and CRSP-08 in which yields under the control and fungicide regime were not significantly different.

Pre-defoliation early leaf spot evaluations. Assessments of early leaf spot incidence began after epidemics were already underway in both years (Fig. 3.3). Disease incidence approached 100% by the final assessments in 2002 and was approximately 80% in 2003. AUDPC was significantly different among genotypes in both years (Table 3.12). The AUDPC of BG, CRSP-01, CRSP-08, CRSP-14 and C-99R were lowest in 2002, MDR-98 and CRSP-20 were intermediate, and GG was the highest. In 2003, GG had the highest AUDPC value and CRSP-01 and CRSP-20 were not significantly different. The remaining genotypes had significantly lower AUDPC values than GG.

Components of early leaf spot resistance. For the two repeated trials of the *C. arachidicola* components of resistance study, the mean relative humidity (RH) during the day was 80% and 59% at night. Mean temperature was 23.6 C during the day and 20.9 C at night. Early leaf spot symptoms were visible in both trials, but not all experimental leaves showed visible symptoms of infection. No disease symptoms were observed on control leaves. Sporulation was observed in both trials but could not be quantified accurately due to loss of spores during the physical handling of the leaves during the lesion excision process. A significant difference in resistance components among genotypes was observed only in the second trial (Table 3.13). The genotype CRSP-14 had the largest lesion area and BG, CRSP-01, and MDR-98 resulted in the smallest. Georgia Green, CRSP-08 and CRSP-20 were intermediate in size. Although not significant, a similar trend was seen with disease severity; CRSP-14

resulted in the most severe disease. Latent period was significantly longer for BG, CRSP-01, CRSP-08 and MDR-98 compared to CRSP-14 which had the shortest latent period. The latent period for GG and CRSP-20 were intermediate. The MPSL of all genotypes, except CRSP-20, was significantly lower than that of GG. Lesion density and % severity were not significantly different among genotypes in the second trial.

Greenhouse rust evaluations. In 2002, no images were taken for the FL group; therefore, uredinia density means for genotype are reflective of the PF group only. The decline of a majority of CRSP-01 plants in 2003 resulted in a lack of disease assessments for the genotype in that year. Whole plant disease severity was assessed only in 2003. Rust was more severe in 2003 than in 2002 and incidence was near 100% in most pots (Table 3.14). No differences in rust incidence, uredinia density or whole plant severity among genotypes were observed in either year. Plant age at inoculation did influence resistance, but results were not consistent across years. Disease incidence was lower in the younger FL plants than in the older PF plants in 2002. Uredinia density and whole plant severity assessments in 2003 indicated an increased resistance in the older PF plants compared to younger FL plants. Although not significant, means of % incidence in 2003 followed the trend seen in uredinia density and severity.

Discussion

Assessments of leaf spot, rust and yield provided information on the relative resistance and production capabilities of Bolivian-incorporated peanut germplasm in the U.S. and Bolivia. Improved leaf spot resistance in BG and the CRSP lines was apparent at all U.S. locations as measured by lower percent defoliation when compared to the U.S. standard cultivar, GG. Pre-defoliation evaluations of early leaf spot corroborated these results; in 2002, BG and the CRSP

line resulted in improved early leaf spot resistance compared to GG. In 2003, under less disease pressure, only BG, CRSP-08 and CRSP-14 were more resistant than GG.

Previous reports indicated that reduced rates of early leaf spot development were due to a longer latent period (6, 15), smaller lesion size (6) and a decrease in the MPSL (34). In the current study, a lower MPSL indicated improved early leaf spot resistance in BG and most CRSP lines, except CRSP-20, when compared to the GG. Reduced lesions size was also observed in BG and CRSP-01 but not in the other, more susceptible breeding lines. It should be noted that results were only significant in one of two trials, and CRSP-14, a line with field resistance to *C. arachidicola*, showed larger lesions and a shorter latent period than the other CRSP lines and BG. More consistent data is needed to describe true genetic resistance in these genotypes.

Overall, the leaf spot resistance observed in Bolivian-incorporated germplasm in U.S. field trials supports the suggestion by Holbrook et al. that peanuts from this region possess qualities resistance to the leaf spot pathogens (19)**.** However, when these genotypes were evaluated in the region of origin, improved resistance was only observed at VA in 2006 under no fungicide applications. This differential response of genotypes is common when screening for resistance to *C. arachidicola* (25, 30, 49, 50) and *C. personatum* (11) and may be due to differences in favorability of environmental conditions to disease development or variation in virulence of *C. arachidicola* and *C. personatum* isolates within populations among locations (33, 36).

Bolivian-incorporated resistance did not suppress rust to the extent observed with leaf spot. Under moderate rust pressure in Bolivian fields (> 40% severity) and in greenhouse trials in the U.S., there was no indication of resistance to *P. arachidis*, compared to the U.S. standard, GG. Ontogenic resistance was observed, and was not exclusive to BG and the CRSP lines. In two of

three evaluations, rust resistance was found to increase with plant age, specifically when plants matured from flowering stage to the early stages of maturity. This evidence of ontogenic resistance corroborates results of other studies (13, 39, 41), and is suggested to be a result of the decrease in leaf wettabilty as plants age, a trait necessary for urediniospore retention and establishment of disease (12). However, a lack of resistance correlation of % incidence in 2002 is unclear. Because it was found in previous greenhouse screening studies that a decrease in uredinia density and severity in genotypes indicates rust suppression and correlates with field resistance (40, 43, 46), the uredinia density and severity recorded in 2003 in the current study is likely to be the best indicator of actual rust resistance in the field. Further investigations of components of rust resistance such as incubation period, pustule size, spore production and spore germinability (42, 45) should be conducted and results correlated with genotype field assessments under heavy rust pressure for a more accurate evaluation of resistance.

Yields of BG and the CRSP lines were better than GG and better than or equal to C-99R and MDR-98 in two locations in the U.S. in 2004. Yields were unusually low at the third location, AP, possibly due to negative effects caused by an accidental application of the pre-plant herbicide Atrazine. The growing season in 2005 had ideal environmental conditions and yields at two locations were high for all genotypes. The only exception was at PL where Cylindrocladium black rot (CBR), caused by the soilborne fungus *Cylindrocladium parasiticum*, likely reduced yields with 6.4 plants per row showing CBR symptoms (data not shown).

In Bolivia in 2005, GG had the best yields at SP, and both GG and C-99R had the superior yields at SA. All genotypes yielded similarly at VA. The high relative yields of GG at all locations are likely due to a lack of heavy leaf spot pressure in 2005. It should also be noted that the yields of GG at SP were double that of other genotypes despite the dry conditions during

the June-December growing season. These results indicate that in the absence of leaf spot and rainfall and/or irrigation, GG has promise of attaining high yields. In 2006, BG and the CRSP lines had similar or higher yields compared to C-99R and MDR-98 and higher yields than GG at one location.

The half-rate, two spray fungicide program, based on cost-saving practices of Bolivian farmers, was effective in reducing leaf spot and rust levels and aiding in the increase of yields at locations in Bolivia where leaf spot and rust pressure was moderate to high. Similar results were not observed in the U.S. Leaf spot was moderate to severe at all locations across years in the U.S., yet lower leaf spot intensity and higher yields in response to fungicide treatments were not consistently seen. These results may be indicative of partial pathogen resistance to fungicides in the U.S. (37). Because fungicides are rarely used in Bolivian peanut production due to either a lack of affordability or availability to growers, *C. arachdicola* and *C. personatum* populations in that area have little exposure to fungicides and are less likely to be resistant, explaining the strong response of leaf spot and yields to fungicides.

A major objective of this overall project was to develop cultivars from the CRSP breeding for use in Bolivia and the U.S. Overall, no single breeding line was dominant for leaf spot resistance or yield potential, indicating that any would be appropriate for cultivar selection. During this study, BG was observed to have considerable variability in growth habit, pod size and seed size and shape, making it unacceptable as a cultivar for commercial use in the U.S (Gremillion, unpublished data). However, the apparent heterogeneity indicated by that variability may provide opportunity for selection from within BG itself for traits that would be desirable for use in either the U.S. or Bolivia. The CRSP genotypes were more uniform than BG

in growth habit and pod and seed characteristics, and two lines, CRSP-08 and CRSP-14 are being considered for release as a cultivar.

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Figure 3.1. Peanut leaf spot disease progress curves at multiple locations in the U.S. in 2004-05. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot. Genotypes included Bolivian Bayo Grande (BG), MDR-98, C-99R and the breeding lines CRSP-01, CRSP-08 andCRSP-14. Georgia Green (GG) served as the standard due to its high susceptibility to *C. arachidicola* and *C. personatum*. Plots of GG, a medium maturing cultivar, were harvested 7-10 days before the remaining genotypes. Percent (%) defoliation was determined based on corresponding ratings of the Florida 1-10 Intensity scale (Chiteka et al., 1988b). Disease progress curves are represented across all fungicide regimes.

Figure 3.1

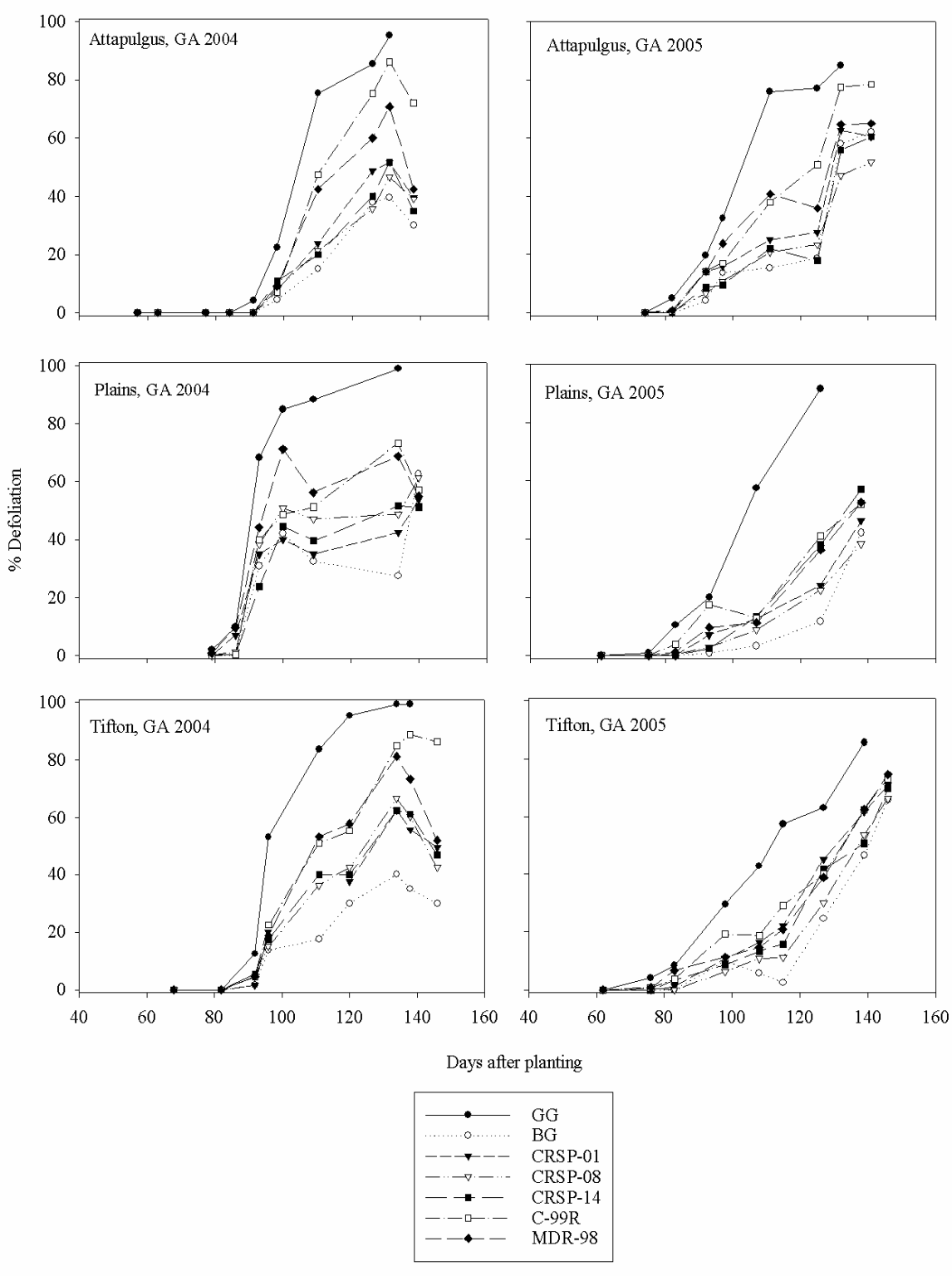
Attapulgus, GA 2004
Attapulgus, GA 2005
Plains, GA 2004
Plains, GA 2005
Tifton, GA 2004
Tifton, GA 2005
% Defoliation
Days after planting
GG
BG
CRSP-01
CRSP-08
CRSP-14
C-99R
MDR-98

Figure 3.2. Peanut leaf spot disease progress curves at multiple locations in Bolivia in 2005-06. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot. Genotypes included Bolivian Bayo Grande (BG), MDR-98, C-99R and the breeding lines CRSP-01, CRSP-08 andCRSP-14. Georgia Green (GG) served as the standard due to its high susceptibility to *C. arachidicola* and *C. personatum*. Percent (%) defoliation was determined based on corresponding ratings of the Florida 1-10 Intensity scale (Chiteka et al., 1988b). Disease progress curves are represented across all fungicide regimes.

Figure 3.2.

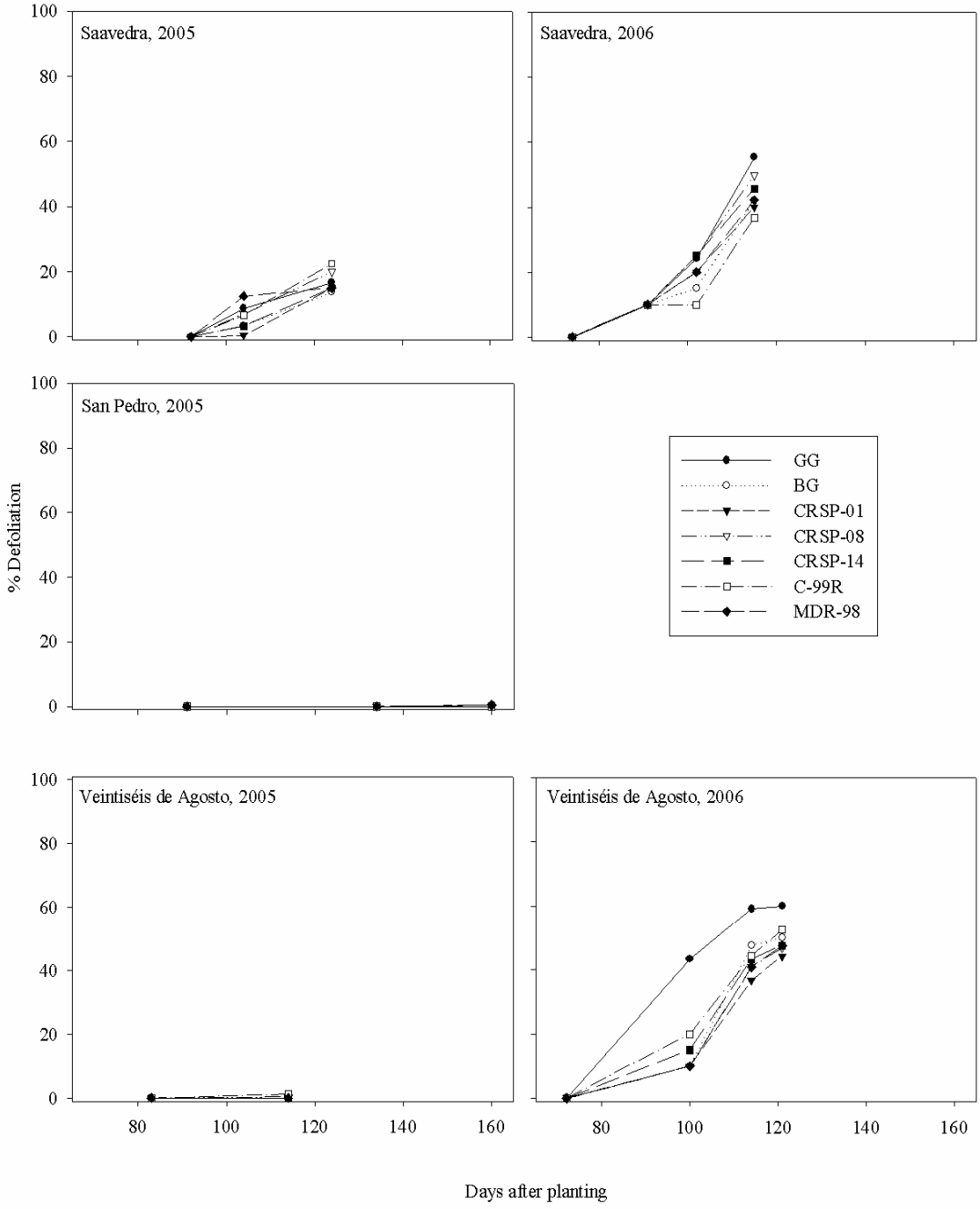
Saavedra, 2005
Saavedra, 2006
San Pedro, 2005
Veintiséis de Agosto, 2005
Veintiséis de Agosto, 2006
% Defoliation
Days after planting
GG
BG
CRSP-01
CRSP-08
CRSP-14
C-99R
MDR-98

Figure 3.3. Disease progress curves of pre-defoliation early leaf spot (*Cercospora arachidicola*) of peanut (*Arachis hypogaea*) in 2002-03. Genotypes included Bolivian Bayo Grande (BG), MDR-98, C-99R and the breeding lines CRSP-01, CRSP-08, CRSP-14 and CRSP-20. Georgia Green (GG) served as the standard due to its high susceptibility to *C. arachidicola*. Disease incidence was recorded as the percentage of leaves with one or more leaf spots or defoliation and averaged across the 10 lateral branches. Assessments were recorded for the first 9 leaves starting from the node closest to the main stem. Branches were taken from plots that did not receive fungicide sprays.

Figure 3.3.

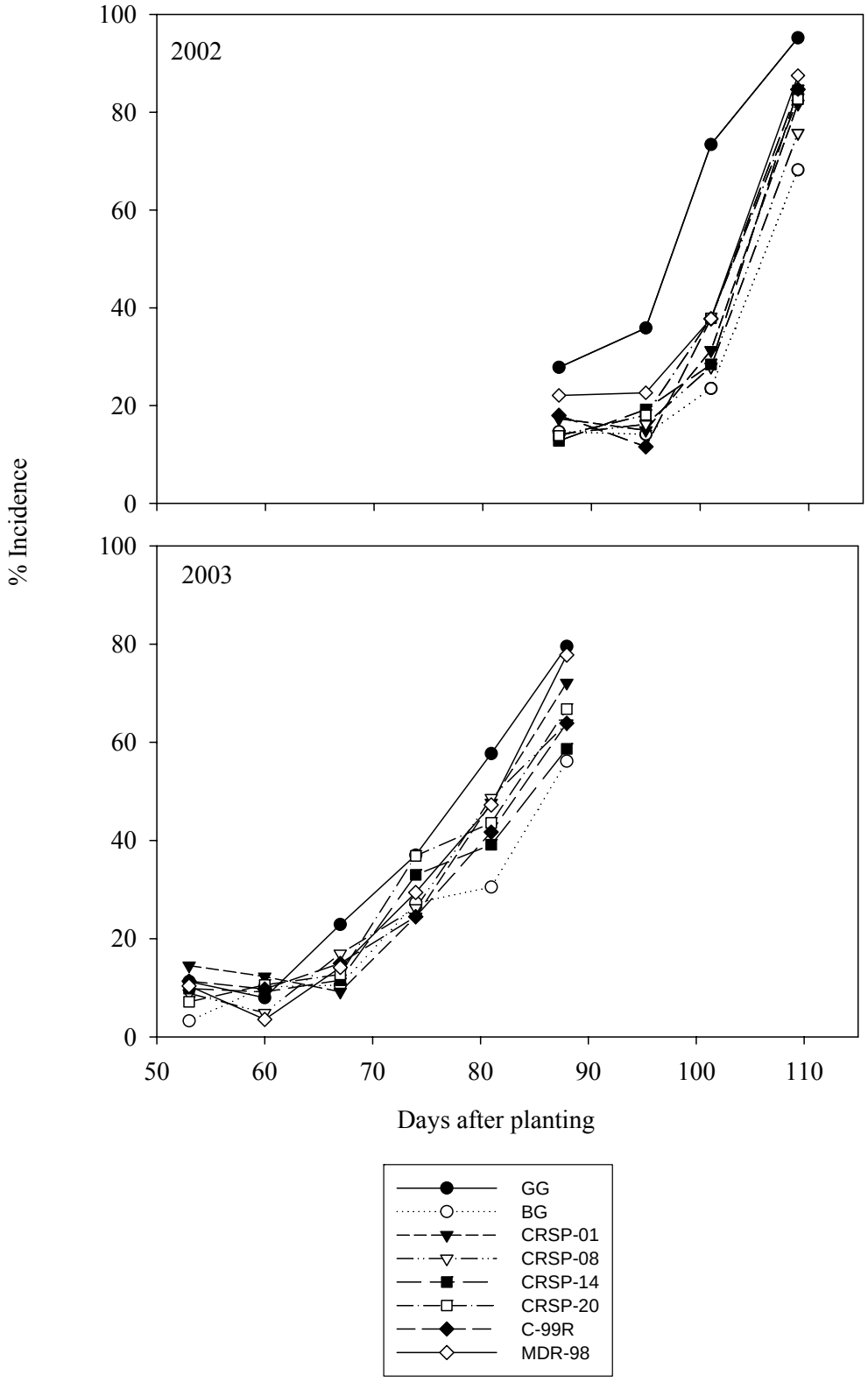
2002
2003
% Incidence
Days after planting
GG
BG
CRSP-01
CRSP-08
CRSP-14
CRSP-20
C-99R
MDR-98

Table 3.1. The effect of peanut genotype and fungicide on the rate of leaf spot disease progress and AUDPC at multiple U.S. locations, 2004-05[ab]

[a]Least square means from Proc MIXED of area under the disease progress curve (AUDPC) based on percent (%) defoliation due to leaf spot. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

[c]Gompertz models were fit to data for Attapulgus, GA in 2004 and resulting rates were used. Different models within the Richard's family of models were fit to data from other locations; therefore rho (ρ) was used for rate observations. Rho was calculated with the formula "$\rho = rK/(2m + 2)$" where r represents the rate parameter of the Richard's family disease progress curve and m is the shape parameter which is given a value of '0' if data fits the monomolecular model, '1' if data fits the logistic model, and '2' if data fits the Gompertz model. The theoretical maximum level of disease, K, was set to 1.0 (100% severity) in all evaluations.

Dash (--) indicates that models tested did not fit data; therefore, no rates were calculated

[d] indicates a significant fungicide by genotype interaction occurred in Tifton in 2005 and data is shown in Table 3.2.

NS= not significant.

Table. 3.1

Location Genotype Fungicide	2004				2005			
	AUDPC		Rate[c]		AUDPC		Rate[c]	
Attapulgus, US								
Georgia Green	32.95	a	0.081	a	32.95	a	0.020	
Bayo Grande	11.73	d	0.037	c	15.14	c	0.024	
CRSP-01	16.10	d	0.042	c	19.16	c	0.020	
CRSP-08	13.77	d	0.040	c	14.97	c	0.017	
CRSP-14	14.53	d	0.041	c	15.78	c	0.024	
C-99R	26.99	b	0.060	ab	26.54	b	0.025	
MDR-98	22.35	c	0.048	bc	24.10	b	--	
LSD, df	*4.55*	*34*	*0.012*	*30*	*4.231*	*30*	*NS*	
No fungicide	20.80		0.050		25.98	a	0.017	
Fungicide	18.75		0.051		16.49	b	0.025	
LSD, df	*NS*		*NS*		*7.98*	*2*	*NS*	
Plains, US								
Georgia Green	70.98	a	--		32.25	a	--	
Bayo Grande	25.57	d	--		6.15	d	--	
CRSP-01	31.63	cd	--		11.65	bcd	--	
CRSP-08	37.63	c	--		9.41	cd	--	
CRSP-14	33.57	c	--		14.58	bc	--	
C-99R	45.54	b	--		17.36	b	--	
MDR-98	49.70	b	--		14.68	bc	--	
LSD, df	*6.21*	*6*			*6.27*	*30*		
No fungicide	46.52	a	--		19.96		--	
Fungicide	37.70	b	--		10.35		--	
LSD, df	*3.32*	*6*			*NS*			
Tifton, US								
Georgia Green	53.07	a	0.054	a	[d]		0.021	
Bayo Grande	16.73	d	0.005	c			0.021	
CRSP-01	23.79	c	0.010	bc			0.017	
CRSP-08	26.82	c	0.010	bc			0.017	
CRSP-14	27.17	c	0.010	bc			0.017	
C-99R	37.73	b	0.022	b			0.016	
MDR-98	34.72	b	0.013	bc			0.018	
LSD, df	*6.11*	*6*	*0.012*	*32*			*NS*	
No fungicide	33.70	a	0.017				0.017	
Fungicide	29.16	b	0.018				0.019	
LSD, df	*4.05*	*3.78*	*NS*				*NS*	

Table 3.2. The effect of peanut genotype and fungicide sprays on AUDPC at Tifton, GA U.S. in 2005[ab]

[a]Least square means from Proc MIXED of area under the disease progress curve (AUDPC) based on % defoliation due to leaf spot. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Different letters indicate a significant difference between genotype treatment within the same fungicide treatment, LSD=5.57, df =28 ($P \leq 0.05$).

*Indicates a significant difference between fungicide treatment within the same genotype treatment, LSD=5.57, df =28 ($P \leq 0.05$).

	AUDPC	
Genotype	**No fungicide**	**Fungicide**
Georgia Green	37.95 a	6.42 ab*
Bayo Grande	7.91 d	0.93 b*
CRSP-01	19.44 bc	3.07 ab*
CRSP-08	9.65 d	3.82 ab*
CRSP-14	15.55 c	3.14 ab*
C-99R	23.59 b	4.41 ab*
MDR-98	15.48 c	6.51 a*

Table 3.3. The effect of genotype and fungicide on AUDPC of leaf spot of peanut at Saavedra and San Pedro, Bolivia in 2005-06[ab]

[a]Least square means of area under the disease progress curve (AUDPC) based on percent (%) defoliation due to leaf spot from Proc MIXED. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Dash (--) indicates that no experiments were conducted at San Pedro, Bolivia in 2006.

NS= not significant.

Location	**2005**	**2006**	
Genotype Fungicide	**AUDPC**	**AUDPC**	
Saavedra			
Georgia Green	6.26	7.20	
Bayo Grande	3.90	5.39	
CRSP-01	3.20	5.89	
CRSP-08	6.40	7.01	
CRSP-14	4.15	6.79	
C-99R	6.77	4.50	
MDR-98	7.14	6.00	
LSD, df	*NS*	*NS*	
No fungicide	6.99	11.62	a
Fungicide	3.81	0.60	b
LSD, df	*NS*	*2.72*	*3.19*
San Pedro			
Georgia Green	0.07	--	
Bayo Grande	8.73×10^{-04}	--	
CRSP-01	8.73×10^{-04}	--	
CRSP-08	8.73×10^{-04}	--	
CRSP-14	8.73×10^{-04}	--	
C-99R	8.73×10^{-04}	--	
MDR-98	0.07	--	
LSD, df	*NS*		
No fungicide	4.00×10^{-02}	--	
Fungicide	8.73×10^{-04}	--	
LSD, df	*NS*		

Table 3.4. The effect of a fungicide by genotype interaction on AUDPC at Veintiséis de Agosto in Bolivia in 2005-6[ab]

[a]Least square means of area under the disease progress curve (AUDPC) based on % defoliation due to leaf spot from Proc MIXED. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Different letters indicate a significant difference between genotype treatment within the same fungicide treatment, LSD=0.46, df =28 in 2005, LSD=2.25, df =28 in 2006 ($P \leq 0.05$).

Asterisk (*) indicates a significant difference between fungicide treatment within the same genotype treatment, LSD=0.46, df =28 in 2005, LSD=2.25, df =28 in 2006 ($P \leq 0.05$).

Year	**2005**		**2006**	
Genotype	**No fungicide**	**Fungicide**	**No fungicide**	**Fungicide**
Georgia Green	0.001 b	0.001 a	27.27 a	4.50 a*
Bayo Grande	0.418 b	0.001 a	15.50 c	3.05 ab*
CRSP-01	0.001 b	0.001 a	14.06 c	1.56 b*
CRSP-08	0.418 b	0.001 a	15.50 c	1.81 b*
CRSP-14	0.001 b	0.001 a	15.46 c	3.31 ab*
C-99R	1.251 a	0.001 a*	18.60 b	2.79 ab*
MDR-98	0.001 b	0.001 a	14.93 c	2.02 b*

Table 3.5 The effect of peanut genotype on rust at multiple locations in Bolivia in 2005-06.
[a] Least square means of final season evaluations of rust (*Puccinia arachidis*) based on a modified 9-point scale where '1'= 0%, '2'=1-5%, '3'=6-10%, '4'=11-20%, '5'=21-30%, '6'=31-40%, '7'=41-60%, '8'=61-80% and '9'=81-100% rust severity (Subrahmanyam, 1995b) from Proc MIXED.
[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).
[c]No rust was observed at this location in 2006.
[d]No experiment was conducted at this location in 2006.
NS= not significant.

	Severity [a]		
Location	**Saavedra**	**San Pedro**	**Veintiséis de Agosto**
Year			
2005			
Genotype			
Georgia Green	5.67	1.67	2.50
Bayo Grande	5.00	1.00	2.50
CRSP-01	5.67	1.33	2.17
CRSP-08	5.50	1.17	2.50
CRSP-14	5.67	1.00	2.33
C-99R	5.67	1.00	2.33
MDR-98	5.83	1.33	2.50
LSD, df	*NS*	*NS*	*NS*
Fungicide program			
No fungicide	6.29 a	1.29	2.67
Fungicide	4.86 b	1.14	2.14
LSD, df	*0.53 6*	*NS*	*NS*
2006			
Genotype			
Georgia Green	--[c]	--[d]	3.50
Bayo Grande	--	--	3.50
CRSP-01	--	--	3.67
CRSP-08	--	--	3.67
CRSP-14	--	--	3.83
C-99R	--	--	3.83
MDR-98	--	--	4.00
LSD, df			*NS*
Fungicide program			
No fungicide	--	--	4.42 a
Fungicide	--	--	3.00 b
LSD, df			*1.23 2*

Table 3.6. The effect of peanut genotype and fungicide on yield at Plains and Tifton, GA, U.S. in 2004[ab]
[a] Least square means from Proc MIXED of yields of in-shell peanuts.
[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).
NS= not significant.

	Yield (kg/ha)			
Location	**Plains**		**Tifton**	
Genotype				
Georgia Green	2377.11	c	1132.60	d
Bayo Grande	3920.02	ab	3563.97	a
CRSP-01	3970.89	ab	3285.90	ab
CRSP-08	4123.49	ab	3635.18	a
CRSP-14	4215.04	a	3353.72	ab
C-99R	4031.93	ab	2763.69	b
MDR-98	3652.13	b	2146.52	c
LSD, df	*487.25*	*28*	*785.76*	*6*
Fungicide program				
No fungicide	3443.34	b	2647.91	
Fungicide	4068.26	a	3032.55	
LSD, df	*260.45*	*28*	*NS*	

Table 3.7. The effect of a fungicide by genotype interaction in yield at Attapulgus, GA, U.S. in 2004[a]

[a]Least square means from Proc MIXED of yield (kg/ha) of in-shell peanuts.
Different letters indicate a significant difference between genotype treatment within the same fungicide, LSD =195.76, df =24 ($P \leq 0.05$).
Asterick (*) indicates a significant difference between fungicide treatments within the same genotype treatment, LSD=246.27, df =16.6 ($P \leq 0.05$).

	Yield (kg/ha)			
Genotype	**No fungicide**		**Fungicide**	
Georgia Green	956.27	bc	1329.28	a*
Bayo Grande	859.96	cd	895.23	c
CRSP-01	849.11	cd	1014.59	bc
CRSP-08	1228.91	a	1120.39	b
CRSP-14	1104.12	ab	1144.81	b
CRSP-20	743.31	d	1047.15	bc
MDR-98	1087.84	ab	1127.18	b*

Table 3.8. The effect of peanut genotype and fungicide on yield at multiple locations in the U.S. in 2005 [ab]

[a] Least square means from Proc MIXED of yields of in-shell peanuts.

[b] Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

NS= not significant.

	Yield (kg/ha)		
Treatment	**Attapulgus**	**Plains**	**Tifton**
Genotype			
Georgia Green	3238	1682	2170
Bayo Grande	3344	1397	2347
CRSP-01	2804	1417	2397
CRSP-08	3166	1523	2353
CRSP-14	3530	1668	2825
C-99R	3547	1451	2808
MDR-98	3312	1187	2269
LSD, df	*NS*	*NS*	*NS*
Fungicide program			
No fungicide	3144	1269	2320
Fungicide	3463	1682	2585
LSD, df	*NS*	*NS*	*NS*

Table 3.9. The effect of peanut genotype and fungicide on yield at multiple locations in Bolivia in 2005[ab]
[a]Least square means from Proc MIXED of yield of shelled peanuts.
[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).
NS= not significant.

	Yield (kg/ha)		
Treatment	**Saavedra**	**San Pedro**	**Veintiséis de Agosto**
Genotype			
Georgia Green	29234 a	1251 a	2845 a
Bayo Grande	2148 c	579 c	2920 a
CRSP-01	2160 c	537 c	2684 a
CRSP-08	2384 cb	552 c	2515 ab
CRSP-14	2509 b	729 bc	2189 b
C-99R	3093 a	856 b	2908 a
MDR-98	2087 c	601 c	2510 ab
LSD, df	*336 30*	*207 30*	*415 30*
Fungicide program			
No fungicide	2093 b	566	2445
Fungicide	2852 a	893	2861
LSD, df	*539 4*	*NS*	*NS*

Table 3.10. The effect of peanut genotype and fungicide on yield at Veintiséis de Agosto, Bolivia in 2006[ab]

[a]Least square means from Proc MIXED of yield of shelled peanuts.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

	Yield (kg/ha)	
Genotype		
Georgia Green	1473	c
Bayo Grande	3600	ab
CRSP-01	3195	ab
CRSP-08	3741	a
CRSP-14	3287.	ab
C-99R	2860	b
MDR-98	2920	b
LSD, df	*794*	*26*
Fungicide program		
No fungicide	2393	b
Fungicide	3629	a
LSD, df	*425*	*26*

Table 3.11. The effect of a fungicide by genotype interaction on yield at Saavedra, Bolivia in 2006[ab]

[a]Least square means from Proc MIXED of yield of shelled peanuts.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Different letters indicate a significant difference between genotype within the same fungicide, LSD=323, df =24 ($P \leq 0.05$).

Asterisk (*) indicates a significant difference between fungicide treatments within the same genotype treatment, LSD=325, df=24.1 ($P \leq 0.05$).

Genotype	Yield (kg/ha)	
	No Fungicide	**Fungicide**
Georgia Green	1050 b	2326 a*
Bayo Grande	1331 ab	1626 c
CRSP-01	1302 ab	1651 c*
CRSP-08	1291 ab	1503 c
CRSP-14	1203 ab	1591 c*
C-99R	1374 a	1731 bc*
MDR-98	1318 ab	2041 ab*

Table 3.12. The effect of genotype on AUDPC of early leaf spot incidence taken during pre-defoliation stage of epidemics, 2002-03[a]

[a]Least square means from Proc MIXED of area under the disease progress curve (AUDPC) based on percentage of leaves with 1 or more early leaf spots or defoliation.

Different letters in a column indicate a significant difference between treatments within the same year ($P \leq 0.05$).

Genotype	AUDPC, 2002		AUDPC, 2003	
Georgia Green	57.13	a	34.20	a
Bayo Grande	27.03	d	21.64	c
CRSP-01	32.72	bcd	29.06	ab
CRSP-08	30.37	cd	26.61	bc
CRSP-14	32.83	bcd	25.42	bc
CRSP-20	35.33	bc	28.14	ab
C-99R	34.35	bcd	25.71	bc
MDR-98	39.13	b	27.69	bc
LSD, df	*7.93*	*14*	*6.37*	*14*

Table 3.13. Components of early leaf spot resistance of detached peanut leaves inoculated with *Cercospora arachidicola*.
[a]Least square means from Proc MIXED of the percent area of leaf tissue (cm^2) with leaf spot symptoms at 30 days after inoculation (DAI).
[b]Least square means from Proc MIXED of the average area (cm^2) of three randomly selected lesions.
[c]Least square means from Proc MIXED of the number of lesions per leaf area (cm^2) at 30 DAI.
[d]Least square means from Proc MIXED of the DAI until one spot produces spores.
[e]Least square means from Proc MIXED of maximum percentage of sporulating lesions (MPSL), the percent of spots with visible conidiophores at 30 DAI.
Different letters within a column indicate a significant difference between genotypes ($P \leq 0.05$).
Dash (--) indicates that latent period could not be calculated due to a lack of sporulation.
NS= not significant.

	Trial 1					**Trial 2**		
	Severity [a]	**Lesion size [b]**	**Lesion frequency[c]**	**Latent period[d]**	**MPSL[e]**	**Severity [a]**	**Lesion size[b]**	
Genotype								
GG	1.42	0.049	50.26	28.50	46.67	3.41	0.036	ab
BG	1.20	0.033	28.57	30.00	28.93	1.55	0.016	c
CRSP-01	1.10	0.035	45.93	29.50	27.86	0.04	0.006	c
CRSP-08	1.25	0.038	13.86	--	62.20	0.51	0.018	bc
CRSP-14	1.65	0.038	34.22	--	36.39	4.14	0.048	a
CRSP-20	0.53	0.037	28.28	--	37.50	1.91	0.019	bc
MDR-98	1.13	0.045	10.24	--	23.33	0.44	0.011	c
LSD, df	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.023*	*20.5*

Table 3.13. (continued)
[a]Least square means from Proc MIXED of the percent area of leaf tissue (cm^2) with leaf spot symptoms at 30 days after inoculation (DAI).
[b]Least square means from Proc MIXED of the average area (cm^2) of three randomly selected lesions.
[c]Least square means from Proc MIXED of the number of lesions per leaf area (cm^2) at 30 DAI.
[d]Least square means from Proc MIXED of the DAI until one spot produces spores.
[e]Least square means from Proc MIXED of maximum percentage of sporulating lesions (MPSL), the percent of spots with visible conidiophores at 30 DAI.
Different letters within a column indicate a significant difference between genotypes ($P \leq 0.05$).
Dash (--) indicates that latent period could not be calculated due to a lack of sporulation.
NS= not significant.
Different letters within a column indicate a significant difference between genotype and physiological age treatments ($P \leq 0.05$).

	Trial 2					
	Lesion frequency[c]	**Latent period[d]**		**MPSL[e]**		
Genotype						
GG	1.18	26.60	ab	42.51	a	
BG	0.52	30.75	a	13.64	b	
CRSP-01	0.04	31.00	a	3.55×10^{-15}	b	
CRSP-08	0.25	30.75	a	0.01	b	
CRSP-14	1.28	23.40	b	15.05	b	
CRSP-20	0.47	28.60	ab	16.00	ab	
MDR-98	0.14	30.75	a	2.78	b	

Table 3.14. The effects of genotype on rust in greenhouse trials in 2002-03[a].
[a]Flowering (FL) plants were 28 days old in 2002 and 40 days old in 2003 while post-flowering plants were 56 days old in 2002 and 71 days old in 2003.
[b]Least square means from Proc MIXED of the percent of leaflets with one or more uredinia.
[c]Least square means from Proc MIXED of the average number of uredinia per average leaflet area (cm^2).
[d]Least square means from Proc MIXED of percent disease severity based on a modified 1-9 scale (Subrahmanyam et al., 1982).
Dash (--) indicates that no data were collected for the treatment.
Asterick (*) indicates means of lesion frequency in 2002 is for post-flowering plants only as no data was collected for flowering plants.
Different letters within a column indicate a significant difference between genotype and physiological age treatments ($P \leq 0.05$).

Year	% Incidence [b]		Uredinia frequency[c*]		Severity[d]	
2002						
Genotype						
Georgia Green	84.92		2.34		--	
Bayo Grande	73.44		2.04		--	
CRSP-01	77.04		2.44		--	
CRSP-08	76.46		2.08		--	
CRSP-14	77.29		2.65		--	
CRSP-20	85.40		3.20		--	
C-99R	84.64		1.96		--	
MDR-98	81.53		4.13		--	
LSD	*NS*		*NS*			
Physiological Age						
Flowering (FL)	70.89	b	--		--	
Post-flowering (PF)	89.63	a	--		--	
LSD, df	*4.55*	*87.8*				
2003						
Genotype						
Georgia Green	100.00		18.87		5.87	
Bayo Grande	97.80		19.05		5.25	
CRSP-01	--		--		--	
CRSP-08	99.95		18.41		5.71	
CRSP-14	100.00		17.63		5.39	
CRSP-20	99.98		17.47		5.87	
C-99R	99.81		19.69		5.32	
MDR-98	99.81		20.11		6.07	
LSD, df	*NS*		*NS*		*NS*	
Physiological Age						
Flowering (FL)	99.97		21.56	a	6.19	a
Post-flowering (PF)	99.28		15.95	b	5.08	b
LSD, df	*NS*		*1.74*	*90*	*0.37*	*97*

APPENDIX FOR CHAPTER 3

Appendix Table 3A. The effect of peanut genotype on AUDPC from leaf spot epidemics at multiple locations in U.S. in 2002-03[a]

[a] Least square means of Proc MIXED of area under the disease progress curve (AUDPC) based on percent (%) defoliation due to early and late leaf spot under no fungicide treatment. Different letters in a column indicate a significant difference between treatments ($P \leq 0.05$). Dash (--) indicates that Georgia Green was not included at this location.

	AUDPC		
Genotype	**Attapulgus, GA, 2002**	**Marianna, FL, 2002**	**Marianna, FL, 2003**
Georgia Green	--	40.04 a	--
Bayo Grande	41.25 ab	16.63 c	16.21 c
CRSP-01	47.59 ab	22.84 bc	37.46 b
CRSP-08	36.48 b	21.07 c	37.04 b
CRSP-14	43.94 ab	22.15 bc	25.13 bc
CRSP-20	32.64 b	20.21 c	27.50 bc
C-99R	57.34 a	29.45 b	76.50 a
MDR-98	44.96 ab	29.02 b	32.29 bc
LSD, df	*18.13 20*	*7.55 22*	*16.16 14*

Appendix Table 3B. The effect of a fungicide by genotype interaction on *Cylindrocladium parasiticum* on peanuts at Plains GA, U.S. in 2005[ab]

[a] Least square means from Proc MIXED of the number of plants per row with symptoms of Cylindrocladium black rot (*Cylindrocladium parasiticum*)

[b]Fungicide regimes include i) no fungicide (0 sprays); and ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Dash (--) indicates that Georgia Green was harvested at the time of disease assessment

Asterisk (*) indicates a significant different between genotypes within the same fungicide treatment, LSD=5.91, df =24 ($P \leq 0.05$).

Different letters denote a significant different between fungicide treatment within the same genotype, LSD=5.91, df =24 ($P \leq 0.05$).

	No fungicide		Fungicide	
Genotype				
Georgia Green	--		--	
Bayo Grande	15.33	a	3.33	a*
CRSP-01	8.33	bc	4.67	a
CRSP-08	3.00	c	7.67	a
CRSP-14	5.67	bc	6.83	a
C-99R	9.33	b	7.33	a
MDR-98	3.33	c	3.00	a

Appendix Table 3C. Parameters for best fit models of leaf spot epidemics at multiple U.S. and Bolivian locations over three years[a]

[a]Disease progress curves based on percent (%) defoliation due to leaf spot of peanut.

[b]R^2 from regression of back-transformed predicted disease on observed disease.

[c]Y-intercept (Y_0) for model fitting.

[d]Data for Attapulgus, GA in 2004 fit to Gompertz models and resulting rates were used; therefore, rho (ρ) was not calculated and is indicated by '.' All other location data fit more than one model within the Richard's family of models; therefore rho was used as rate observations. Rho was calculated with the formula "$\rho = rK/(2m + 2)$" where r represents the rate parameter of the Richard's family disease progress curve and m is the shape parameter which is given a value of '0' if data fits the monomolecular model, '1' if data fits the logistic model, and '2' if data fits the Gompertz model. The theoretical maximum level of disease, K, was set to 1.0 (100% severity) in all evaluations.

Dash (--) indicates a replication plot where no model significantly fit Gompertz, logistic or monomolecular model.

Appendix Table 3C.

Year/Location Genotype/Fungicide	Rep	Model Parameters				
		R^2[b]	Y_0[c]	Slope	Rho (ρ)	Best Fit Model
2004, Attap., U.S.						
GG	1	0.8987	-6.833	0.066	.[d]	Gompertz
Zero-spray	2	0.8544	-8.834	0.091	.	Gompertz
	3	0.9097	-8.629	0.090	.	Gompertz
GG	1	0.8984	-8.152	0.080	.	Gompertz
Two-spray	2	0.9283	-7.982	0.078	.	Gompertz
	3	0.8913	-8.412	0.083	.	Gompertz
BG	1	0.7568	-4.778	0.032	.	Gompertz
Zero-spray	2	0.8913	-8.412	0.083	.	Gompertz
	3	0.9283	-7.982	0.078	.	Gompertz
BG	1	0.7568	-4.778	0.032	.	Gompertz
Two-spray	2	0.5239	-5.254	0.040	.	Gompertz
	3	0.8115	-5.100	0.039	.	Gompertz
CRSP-01	1	0.7857	-5.404	0.043	.	Gompertz
Zero-spray	2	0.8238	-5.293	0.042	.	Gompertz
	3	0.784	-4.763	0.033	.	Gompertz
CRSP-01	1	0.7706	-5.541	0.042	.	Gompertz
Two-spray	2	0.9554	-5.409	0.042	.	Gompertz
	3	0.9278	-6.123	0.051	.	Gompertz
CRSP-08	1	0.8997	-4.999	0.038	.	Gompertz
Zero-spray	2	0.8997	-4.999	0.038	.	Gompertz
	3	0.8258	-5.252	0.040	.	Gompertz
CRSP-08	1	0.7152	-5.014	0.037	.	Gompertz
Two-spray	2	0.8486	-4.881	0.035	.	Gompertz
	3	0.9448	-6.343	0.054	.	Gompertz
CRSP-14	1	0.7221	-4.807	0.035	.	Gompertz
Zero-spray	2	0.916	-5.557	0.044	.	Gompertz
	3	--[e]	--	--	--	--
CRSP-14	1	0.9423	-5.450	0.044	.	Gompertz
Two-spray	2	--	--	--	--	--
	3	0.8837	-5.838	0.048	.	Gompertz
C-99R	1	0.941	-6.657	0.060	.	Gompertz
Zero-spray	2	0.8721	-6.120	0.052	.	Gompertz
	3	0.9482	-7.050	0.065	.	Gompertz
C-99R	1	0.9433	-6.984	0.064	.	Gompertz
Two-spray	2	0.9359	-7.011	0.064	.	Gompertz
	3	0.9097	-6.713	0.057	.	Gompertz

Appendix Table C (continued).

Year/Location	Rep	Model Parameters				
Genotype/Treatment		R^{2b}	$Y_0{}^c$	Slope	Rho (ρ)	Best Fit Model
MDR-98	1	0.8591	-5.819	0.049	.	Gompertz
Zero-spray	2	0.8735	-6.070	0.053	.	Gompertz
	3	0.869	-6.094	0.053	.	Gompertz
MDR-98	1	0.7882	-5.389	0.042	.	Gompertz
Two-spray	2	0.7718	-5.725	0.045	.	Gompertz
	3	0.7629	-5.898	0.047	.	Gompertz
2004, Tifton, U.S.						
GG	1	0.8472	-36.597	0.352	0.059	Logistic
Zero-spray	2	0.9151	-35.774	0.347	0.058	Logistic
	3	0.9248	-10.982	0.118	0.030	Gompertz
GG	1	0.8357	-19.859	0.214	0.054	Gompertz
Two-spray	2	0.9459	-30.889	0.288	0.048	Logistic
	3	0.9386	-31.658	0.293	0.073	Logistic
BG	1	0.937	-5.045	0.036	0.009	Gompertz
Zero-spray	2	0.8564	-4.873	0.038	0.009	Gompertz
	3	0.7498	-0.630	0.009	0.004	Monomolecular
BG	1	0.7326	-0.330	0.004	0.002	Monomolecular
Two-spray	2	0.8675	-0.542	0.007	0.003	Monomolecular
	3	0.7179	-0.373	0.005	0.003	Monomolecular
CRSP-01	1	0.8503	-5.722	0.046	0.012	Gompertz
Zero-spray	2	0.8689	-5.521	0.046	0.011	Gompertz
	3	0.9078	-5.268	0.042	0.010	Gompertz
CRSP-01	1	0.9266	-5.915	0.047	0.012	Gompertz
Two-spray	2	0.8683	-5.545	0.045	0.011	Gompertz
	3	0.8045	-0.575	0.008	0.004	Monomolecular
CRSP-08	1	0.8346	-5.877	0.048	0.012	Gompertz
Zero-spray	2	0.7574	-5.043	0.040	0.010	Gompertz
	3	0.7205	-1.047	0.014	0.007	Monomolecular
CRSP-08	1	0.7727	-5.663	0.043	0.011	Gompertz
Two-spray	2	0.8752	-5.287	0.046	0.011	Gompertz
	3	0.9049	-5.209	0.042	0.010	Gompertz
CRSP-14	1	0.8485	-5.278	0.044	0.011	Gompertz
Zero-spray	2	0.7598	-4.977	0.040	0.010	Gompertz
	3	0.8419	-5.243	0.043	0.011	Gompertz
CRSP-14	1	0.8228	-5.034	0.040	0.010	Gompertz
Two-spray	2	0.9126	-5.681	0.049	0.012	Gompertz
	3	0.732	-0.918	0.012	0.006	Monomolecular
C-99R	1	0.9634	-8.524	0.080	0.020	Gompertz
Zero-spray	2	0.9652	-7.139	0.068	0.017	Gompertz
	3	0.9575	-7.960	0.074	0.019	Gompertz

Appendix Table C (continued).

Year/Location	Rep	Model Parameters				
Genotype/Treatment		R^{2b}	Y_0^c	Slope	Rho (ρ)	Best Fit Model
2004, Tifton, U.S.						
C-99R	1	0.8877	-22.623	0.183	0.031	Logistic
Two-spray	2	0.9182	-22.444	0.180	0.030	Logistic
	3	0.9272	-6.355	0.056	0.014	Gompertz
MDR-98	1	0.8127	-6.722	0.060	0.015	Gompertz
Zero-spray	2	0.7868	-6.166	0.053	0.013	Gompertz
	3	0.7838	-5.752	0.051	0.013	Gompertz
MDR-98	1	0.8701	-5.891	0.050	0.012	Gompertz
Two-spray	2	0.8831	-5.831	0.050	0.013	Gompertz
	3	0.9296	-5.303	0.046	0.011	Gompertz
2005, Attap., U.S.						
GG	1	0.880	-6.876	0.072	0.018	Gompertz
Zero-spray	2	0.955	-7.234	0.075	0.019	Gompertz
	3	--	--	--	--	--
GG	1	0.94	-3.912	0.032	0.008	Gompertz
Two-spray	2	--	--	--	--	--
	3	0.78	-25.008	0.209	0.035	Logistic
BG	1	--	--	--	--	--
Zero-spray	2	0.9628	-5.910	0.052	0.013	Gompertz
	3	0.8929	-22.426	0.176	0.029	Logistic
BG	1	0.9307	-22.670	0.159	0.026	Logistic
Two-spray	2	0.9088	23.381	0.178	0.030	Logistic
	3	0.9721	-24.110	0.150	0.025	Logistic
CRSP-01	1	0.7616	-21.186	0.165	0.027	Logistic
Zero-spray	2	0.7796	-5.763	0.050	0.013	Gompertz
	3	--	--	--	--	--
CRSP-01	1	0.858	-21.183	0.163	0.027	Logistic
Two-spray	2	0.9228	-5.360	0.045	0.011	Gompertz
	3	0.8739	-23.306	0.167	0.028	Logistic
CRSP-08	1	0.947	-5.335	0.043	0.011	Gompertz
Zero-spray	2	0.7897	-5.049	0.041	0.010	Gompertz
	3	0.9538	-5.451	0.045	0.011	Gompertz
CRSP-08	1	0.92	-17.568	0.135	0.023	Logistic
Two-spray	2	0.7918	-20.640	0.151	0.025	Logistic
	3	--	--	--	--	--
CRSP-14	1	0.9117	-22.104	0.173	0.029	Logistic
Zero-spray	2	--	--	--	--	--
	3	0.8458	-5.534	0.043	0.011	Gompertz

Appendix Table C (continued).

Year/Location	Rep	Model Parameters				
Genotype/Treatment		R^{2b}	$Y_0{}^c$	Slope	Rho (ρ)	Best Fit Model
2005, Attap., U.S.						
CRSP-14	1	0.95	-24.390	0.167	0.028	Logistic
Two-spray	2	0.80	-21.308	0.166	0.028	Logistic
	3	--	--	--	--	--
C-99R	1	0.87	-6.770	0.063	0.016	Gompertz
Zero-spray	2	0.86	-5.609	0.049	0.012	Gompertz
	3	0.97	-6.911	0.066	0.017	Gompertz
C-99R	1	0.94	-19.333	0.158	0.026	Logistic
Two-spray	2	0.94	-25.178	0.192	0.032	Logistic
	3	0.92	-20.800	0.157	0.026	Logistic
MDR-98	1	--	--	--	--	--
Zero-spray	2	0.81	-6.299	0.058	0.015	Gompertz
	3	--	--	--	--	--
MDR-98	1	--	--	--	--	--
Two-spray	2	0.89	-5.274	0.043	0.011	Gompertz
	3	--	--	--	--	--
2005, Tifton, U.S.						
GG	1	0.92	-19.031	0.177	0.029	Logistic
Zero-spray	2	0.92	-6.634	0.074	0.019	Gompertz
	3	0.96	-8.115	0.087	0.022	Gompertz
GG	1	0.89	-15.730	0.126	0.021	Logistic
Two-spray	2	0.78	-20.180	0.146	0.024	Logistic
	3	0.86	-5.539	0.046	0.011	Gompertz
BG	1	0.91	-22.657	0.163	0.027	Gompertz
Zero-spray	2	0.86	-5.244	0.041	0.010	Gompertz
	3	0.94	-22.865	0.173	0.029	Logistic
BG	1	0.96	-24.953	0.162	0.027	Logistic
Two-spray	2	0.87	-5.532	0.037	0.009	Gompertz
	3	1.00	-23.545	0.144	0.024	Logistic
CRSP-01	1	0.97	-5.505	0.046	0.011	Gompertz
Zero-spray	2	0.93	-23.922	0.189	0.032	Logistic
	3	0.92	-7.091	0.068	0.017	Gompertz
CRSP-01	1	0.87	-5.628	0.038	0.010	Gompertz
Two-spray	2	0.92	-19.274	0.125	0.021	Logistic
	3	0.91	-5.072	0.038	0.009	Gompertz
CRSP-08	1	0.97	-4.477	0.034	0.009	Gompertz
Zero-spray	2	0.94	-5.889	0.048	0.012	Gompertz
	3	0.89	-5.240	0.041	0.010	Gompertz
CRSP-08	1	0.93	-25.341	0.189	0.031	Logistic
Two-spray	2	0.99	-24.169	0.172	0.029	Logistic
	3	0.88	-5.360	0.035	0.009	Gompertz

Appendix Table C (continued).

Year/Location	Rep	Model Parameters				
Genotype/Treatment		R^{2b}	Y_0^c	Slope	Rho (ρ)	Best Fit Model
2005, Tifton, U.S.						
CRSP-14	1	0.81	-5.537	0.045	0.011	Gompertz
Zero-spray	2	0.89	-4.585	0.038	0.010	Gompertz
	3	0.97	-6.002	0.051	0.013	Gompertz
CRSP-14	1	0.89	-24.096	0.172	0.029	Logistic
Two-spray	2	0.94	-26.964	0.194	0.032	Logistic
	3	0.88	-5.360	0.035	0.009	Gompertz
C-99R	1	0.95	-6.284	0.059	0.015	Gompertz
Zero-spray	2	0.97	-5.364	0.048	0.012	Gompertz
	3	0.85	-5.205	0.046	0.012	Gompertz
C-99R	1	0.88	-5.737	0.039	0.010	Gompertz
Two-spray	2	0.91	-19.041	0.135	0.023	Logistic
	3	0.90	-22.668	0.161	0.027	Logistic
MDR-98	1	0.91	-4.348	0.039	0.010	Gompertz
Zero-spray	2	0.94	-14.510	0.120	0.020	Logistic
	3	0.90	-22.372	0.168	0.028	Logistic
MDR-98	1	0.88	-5.632	0.042	0.010	Gompertz
Two-spray	2	0.92	-24.733	0.172	0.029	Logistic
	3	0.95	-5.432	0.044	0.011	Gompertz

Appendix Table 3D. The effect of genotype and fungicide on yield of in-shell peanuts at multiple locations in Bolivia in 2005[ab]

[a]Least square means from Proc MIXED of yield (kg/ha) of in-shell peanuts.

[b]Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Different letters denote a significant difference between genotype within the same fungicide. NS=not significant.

	Yield (kg/ha)					
Location	**Saavedra**		**San Pedro**		**Veintiséis de Agosto**	
Genotype						
Georgia Green	3303	b	2294		3747	
Bayo Grande	3253	b	1568		4276	
CRSP-01	3208	b	2496		3603	
CRSP-08	3418	ab	1362		3443	
CRSP-14	3260	b	2006		3203	
C-99R	4119	a	2085		3627	
MDR-98	2837	b	2076		3400	
LSD, df	*728*	*24*	*NS*		*NS*	
Fungicide program						
No fungicide	2784	b	1600	b	3281	
Fungicide	3901	a	2368	a	3947	
LSD, df	*878*	*4*	*449*	*6*	*NS*	

Appendix Table 3E. The effect of peanut genotype on %TSW at multiple locations in GA, U.S. in 2004-05[ab]

[a] Least square means from Proc MIXED of percent (%) tomato spotted wilt (TSW) ((# of 0.3 m sections of row with plants severely affected by TSW / total length of row per plot)*100))

[b] Fungicide programs include i) no fungicide (0 sprays); and ii) fungicide: tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at the rate of 0.138 kg a.i./ha (TEB) with an initial application at the first observation of leaf spot symptoms followed by a second application 14 d later (2 sprays).

Dash (--) indicates that TSW was not observed at this location.

NS=not significant.

	%TSW		
Location	**Attapulgus**	**Plains**	**Tifton**
Year			
2004			
Genotype	--	--	
Georgia Green	--	--	9.79
Bayo Grande	--	--	1.25
CRSP-01	--	--	6.04
CRSP-08	--	--	4.79
CRSP-14	--	--	5.21
C-99R	--	--	2.08
MDR-98	--	--	13.96
LSD, df			*NS*
Fungicide program			
No fungicide	--	--	5.48
Fungicide	--	--	6.85
LSD, df			*NS*
2005			
Genotype			
Georgia Green	14.79	7.70	16.25
Bayo Grande	7.29	11.25	18.96
CRSP-01	8.75	8.12	11.88
CRSP-08	6.67	7.70	10.00
CRSP-14	5.63	6.66	8.54
C-99R	7.92	6.45	10.00
MDR-98	7.71	8.95	13.75
LSD, df	*NS*	*NS*	*NS*
Fungicide program			
No fungicide	9.29	6.60	11.73
Fungicide	7.50	9.64	13.81
LSD, df	*NS*	*NS*	*NS*

CHAPTER 4

RESPONSE OF BOLIVIAN-BRED RESISTANCE IN INTEGRATED MANAGEMENT SYSTEMS FOR LEAF SPOT AND TOMATO SPOTTED WILT OF PEANUT (*ARACHIS HYPOGAEA*) [1]

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[1]Gremillion, S., Culbreath, A., Gorbet, D., Kemerait, R. Pittman, R., Stevenson, K., and Todd, J. To be submitted for publication to *Plant Pathology*

Leaf spot, caused by the fungi *Cercospora arachidicola* and *Cercosporidium personatum*, and Tomato spotted wilt (TSW), caused by *Tomato spotted wilt virus*, are major yield-reducing diseases of peanut (*Arachis hypogaea* L.) in the southeastern U.S. While fungicides effectively control leaf spot, high costs, negative environmental effects and selection for pathogen resistance can result from their use. In addition, pesticides are unreliable options for management of TSW. Effective control of these diseases may be reached with integrated disease management (IDM) systems. A Bolivian land-race cultivar, Bayo Grande (BG), and a series of breeding line progeny were evaluated in combination with full and reduced-spray fungicide regimes as well as with conventional and strip tillage in field trials over two years. Bayo Grande and progeny lines had better leaf spot and TSW resistance than the standard southeastern U.S. cultivar, Georgia Green (GG). Bayo Grande and the breeding lines suppressed leaf spot to levels similar to those seen under full-season regimes with the addition of only three or four applications, and in some cases, without any fungicides. Strip tillage negated the need for fungicides in most genotypes in one year, yet did not contribute to spray reduction between conventional and strip tillage in the following year. Results of the effect of strip tillage on TSW were inconsistent. Yields were higher in BG and the breeding lines compared to GG in three of the four locations. No reduced fungicide regime supported yields comparable to those under the full season regime. Yields were negatively impacted by strip tillage in one year and lower in the genotypes BG, CRSP-01 and CRSP-08 in another year. Overall, the use of Bolivian resistance in a reduced fungicide and/or strip tillage IDM system will reduce fungicide use compared to standard production practices but may result in lower yields.

Keywords: *Cercospora arachidicola*, *Cercosporidium personatum*, tillage, host resistance, fungicide, breeding

Introduction

Plant diseases are major yield-limiting factors in production of peanut (*Arachis hypogaea*) around the world. Two of the most devastating diseases in the southeastern United States (U.S.) are leaf spot and tomato spotted wilt (TSW), causing $6.3 and $37.1 million in damage to the 2005 peanut crop in GA alone, respectively (27). Leaf spot is a collective term for two diseases, early leaf spot, caused by *Cercospora arachidicola* S. Hori (teleomorph: *Mycosphaerella arachidis*) and late leaf spot, caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (teleomorph: *Mycosphaerella berkeleyi*) (41). Infection by both *C. arachidicola* and *C. personatum* causes necrotic lesions on leaves, stems, petioles, pegs and pods. Defoliation occurs shortly after the appearance of lesions, and if not controlled, leaf spot can cause complete defoliation. Lesions on pegs compromise the integrity of these structures (29), and the combination of damage to pegs and loss of healthy plant tissue by defoliation results in yield loss.

Tomato spotted wilt is caused by the *Tomato spotted wilt virus*. In the U.S., this tospovirus is vectored by tobacco thrips (*Frankliniella fusca* Hinds) and western flower thrips (*F. occidentalis* Pergande) and has a wide host range including peanut, tomato, tobacco and numerous weed species (14). Symptoms of TSW include whole plant stunting and an array of foliar symptoms including chlorotic rings on leaves (20). Underground, the virus causes pod and seed deformation and root necrosis. In some instances, infected plants show no visible symptoms (17), yet reports have indicated that the physiology of asymptomatic plants is negatively affected by the presence of the virus (39). In cases of severe TSW epidemics, whole plant death and reduced yields are common.

Management of leaf spot and TSW is critical for peanut production in the southeastern U.S. In the last forty years, the control of *C. arachidicola* and *C. personatum* has been heavily reliant upon fungicides. Recommendations for management of leaf spot in

Georgia include fungicide applications 30 days after planting (DAP) and continuing 14-day intervals until harvest (28). As a result, the number of fungicide applications recommended for leaf spot management can reach up to seven sprays during a season, depending upon the duration of epidemics. Multiple fungicide applications can increase time, resources and cost required for peanut production. Also, the development of pathogen resistance to fungicides is a threat that has already been noted in the leaf spot pathogens with regards to benzamidizole and triazole fungicides (12, 31, 43, 44). Lastly, concerns have arisen around the negative effects that fungicides have on water quality (8) and non-target organisms (34, 35). For TSW, with one exception, insecticides have not been effective for controlling viral spread even when they show good performance for reducing feeding damage by thrips vectors (14). In response to these issues surrounding chemical use, the utilization of alternative leaf spot and TSW management options to lessen reliance on pesticides is highly desirable.

Conservation tillage and host resistance are effective options for leaf spot and TSW management. When concerns about soil erosion, water conservation, and production labor caused a shift in peanut production from conventional to conservation tillage (24, 38), a notable suppression of TSW epidemics was observed (2, 5, 7, 9, 26, 32). Although the underlying mechanism is unknown, this disease management practice continues to be successful and has led to an increase in peanut acreage planted under conservation tillage in Georgia (1, 42). Later, a similar suppression was observed in regards to *C. arachidicola*; defoliation was less severe under strip tillage, a form of conservation tillage, when compared to conventional tillage (5, 7, 32, 33, 38). Disease suppression under strip tillage has been suggested to be a result of a physical disruption in initial inoculum dispersion, causing a delay in epidemics (6).

Partial host resistance is available in many cultivars in the U.S. Georgia Green (GG), the most predominantly grown cultivar in the southeastern U.S., was released with moderate

resistance to TSW (3), but low resistance to leaf spot. Florida MDR-98 and C-99R were later released with better resistance to early leaf spot (7, 32, 33) and late leaf spot (7, 22, 23), and similar or better resistance to TSW (13, 16, 18, 19, 21, 45) compared to GG.

To utilize the leaf spot and TSW suppressive characteristics of strip tillage and host resistance, an integrated disease management system (IDM) system including these components has been evaluated. In two studies, the IDM system allowed an increase in days between fungicide applications, resulting in fewer overall fungicide sprays required to control *C. arachidicola* (7, 32, 33). Although strip tillage was found to have a greater impact on leaf spot than genotype (7), the potential for improved disease management with the addition of cultivars with greater leaf spot resistance exists (7, 32, 33). As U.S. breeding programs produce new cultivars with better leaf spot and TSW resistance, the strength of these IDM systems should increase and contribute to reducing fungicide inputs to greater extents that previously observed.

Recent screening of several landrace cultivars from Bolivia, South America, where the peanut is believed to have originated (30), is noted by Holbrook and Stalker (2003) as a center for both early and late leaf spot resistance in cultivated peanut (25). The Bolivian land-race cultivar "Bayo Grande" (BG) was tested in the U.S. and showed moderate resistance to the leaf spot pathogens (J.W. Todd, *unpublished data*). Subsequently, BG was used as a parent in crosses with MDR-98, and the resulting progeny, the "CRSP" breeding lines, have shown improved leaf spot resistance compared to GG (Chapter 3 of this dissertation). The resistance of the CRSP lines and parent, BG, has potential to contribute to IDM systems that include reduced fungicide regimes and conservation tillage. The objective of this study was to determine the response of BG and the CRSP breeding lines to reduced fungicide applications alone and in combination with strip tillage as part of a leaf spot and TSW IDM system aimed at disease control and reductions in fungicide applications.

Materials and Methods

Experimental design. Two sets of field experiments were conducted to assess the disease resistance and yield response of BG and CRSP breeding line progeny under two IDM programs. In the first set of experiments, known as the "fungicide response" experiments, BG and the CRSP lines were tested under varying number of applications of tebuconazole. The experiment was conducted at the University of Georgia (UGA) Coastal Plain Experiment Station, Lang Farm in 2002-03. The soil type was characterized a Tifton, loamy sand. Field sites followed a rotation of cotton (*Gossypium hirsutum*) the previous year and peanut two years prior. A split-plot design with three replications was used. Fungicide regimes represented the whole plot treatments and included: i) non-treated control (0 sprays); ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at 0.138 kg a.i./ha (TEB) with initial applications at the first observation of leaf spot symptoms followed by a second spray 14 days later (2 sprays); iii) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and iv) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date). The fungicide rate was selected to mimic practices of Bolivian farmers who typically spray at reduced rates to lower production costs.

Sub-plot treatments consisted of the late maturing genotypes including BG, MDR-98 and seven late maturing progeny lines including CRSP-01, CRSP-08, CRSP-14, CRSP-15, CRSP-19, CRSP-20 and CRSP-22. The seven CRSP lines, developed from crosses of Bayo Grande and Florida MDR-98, were in the F5 generation in 2002 and F6 in 2003. With susceptibility to leaf spot and moderate field resistance to TSWV, GG, the predominant medium-maturing cultivar grown in the southeastern U.S., was included to serve as a susceptible control for disease comparisons. Planting dates were 21 May 2002 and 10 June

2003. Seeding rate was 16.4 seed/m of row. Plots were 1.8 m × 6.1 m and consisted of two rows, 0.9 m apart. Once seedlings emerged, plots were reseeded manually as necessary to ensure an adequate stand. Calcium sulfate (gypsum) was applied broadcast as a calcium source for the pods (112 kg/ha in 2002 and 560 kg/ha in 2003), and plots were irrigated as needed. Once medium maturing genotypes were harvested, no additional fungicide applications were applied on the remaining genotypes.

In the second set of experiments, known as the "tillage and fungicide response" experiments, BG and the CRSP lines were evaluated under the combinations of two tillage treatments and a varying number of fungicide applications. The experiment was conducted at the UGA Coastal Plain Experiment Station Rigdon Farm in 2002-3. Soil type at this location was characterized as Tifton, loamy sand. Field sites followed a rotation of cotton. A split-split plot experimental design with three replications was used. Two tillage treatments represented the whole plots and included conventional and strip tillage. A cover crop of winter wheat (*Triticum aestivum*) was planted on the entire experimental site in the fall before each planting season. The cover crop was killed by an application of glyphosate (Roundup 4 EC, Monsanto, Kansas City, MO) 4.8 kg a.i./L. To establish strip tillage plots, a subsoil shank attached to a strip-till implement (Kelley Manufacturing Company, Tifton, GA) was used to loosen the plow pan approximately 33 cm beneath each row. The implement tilled strips 20 to 25 cm wide, and the remaining soil and crop residue of the strip-till plots was left undisturbed. Peanut seed were then planted in the tilled strips among the existing wheat cover crop. Soil was prepared by conventional tillage methods that included turning soil 20-25 cm deep with a moldboard plow, thereby burying debris from the winter cover crop. Soil was subsequently shaped with a disk bedder into beds 0.9 m wide for planting.

Fungicide regimes represented the sub-plot treatments and were similar to those previously described with i) non-treated control (0 sprays); ii) TEB at the first observation of

leaf spot (1 spray); iii) TEB at the first observation of leaf spot followed by a second spray 14 days later (2 sprays); iv) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and v) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date). The fungicide rate was selected to mimic practices of Bolivian farmers who typically spray at reduced rates to lower production costs.

Genotypes made up the sub-sub plot treatments and included the cultivars GG, BG and MDR-98, and the breeding lines CRSP-01, CRSP-08, CRSP-14, and CRSP-20. The U.S. cultivar, C-99R, was also included to represent a moderate level of resistance to *C. arachidicola*, *C. personatum* and TSWV (7, 10, 11, 19, 22, 23, 32, 45). Fields were planted on May 20, 2002 and May 21, 2003. Seeding rate and plot size, and general plot maintenance were as previously described.

Disease and yield assessments. Leaf spot intensity was assessed using the Florida 1-10 scale where 1=no disease (0% defoliation); 2=very few lesions, more on upper canopy (0% defoliation); 3=few lesions, very few on upper canopy (0% defoliation); 4=some lesions with more on upper canopy, noticeable defoliation (5% defoliation); 5=lesions noticeable even on upper canopy, noticeable defoliation (20% defoliation); 6=lesions numerous and very evident on upper canopy, significant defoliation (50% defoliation); 7=lesion numerous on upper canopy with much defoliation (75% defoliation); 8=upper canopy covered with lesions with high defoliation (90% defoliation); 9=very few leaves remain and those covered with lesions, some plants completely defoliated (98% defoliation); and 10=dead plants (100% defoliation), completely defoliated and killed by leaf spot (11). Area under the disease progress curve (AUDPC) based on % defoliation was calculated for each replication of tillage, fungicide and genotype treatment (40) and standardized by dividing AUDPC by the number

of days between the first and final LS rating (4). In the fungicide response experiment, leaf spot intensity was assessed at 53, 66, 80, 89, 100, 107 and 120 DAP in 2002 and 79, 86, 93, 100, 106, 114, 120, 128 and 143 DAP in 2003. In the tillage and fungicide response experiments, leaf spot intensity was assessed at 86, 108, 116, 121 and 140 DAP in 2002 and 58, 63, 70, 77, 84, 91, 98, 105, 112, 119, 126 and 145 DAP in 2003.

Tomato spotted wilt incidence was assessed calculated as the percentage of the total row length of plants with severe TSW symptoms (%TSW) (16). In the fungicide response experiments, TSW evaluations were assessed at 119 DAP for all genotypes in 2002 and at 90 DAP for GG and 97 DAP for other genotypes in 2003. In the tillage and fungicide response experiments, TSW incidence was assessed at 124 DAP for all genotypes in 2002 and at 118 DAP in 2003. Intensity of southern stem rot (SSR), caused by *Sclerotium rolfsii*, was recorded at digging as the percentage of 31-cm sections of row with sign or symptom of infection.

Yields of in-shell peanuts were recorded at harvest as kilograms per hectare (kg/ha). In the fungicide response experiments, GG was dug at 140 DAP and the other genotypes at 153 DAP in 2002. In 2003, GG was dug at 134 DAP and the other genotypes 143 DAP. In the tillage and fungicide response experiments, GG was dug at 126 DAP and the other genotypes 140 DAP in 2002. In 2003, GG was dug 133 DAP and the other genotypes at 145 DAP. Also, total sound mature kernals (TSMK) were recorded as percentage of total sound mature kernals in a 500 g yield sample.

Statistical analyses. The effects of tillage, fungicide and genotype on AUDPC, %TSW, %SSR and yield were analyzed using SAS Proc MIXED (SAS v 9.1, SAS Institute, Inc. Cary, NC). The "Satterth" option was used for determining degrees of freedom. Significant interactions were included in the model random effects statement. Non-significant interactions ($P \leq 0.05$) were removed from the model. If non-significant interactions

contributed substantially to variation (F-value > 1.00), they were included as random effects. The "pdiff" option was included in each main effect and significant interaction LSMEAN statement, and Proc MIXED was executed again to obtain new standard errors (SE) and degrees of freedom (df). Significant differences in AUDPC, %TSW and yield among treatments were determined based on Fisher's LSD ($P \leq 0.05$). Significance levels reported in the text are $P \leq 0.05$ unless otherwise indicated.

Results

Leaf spot resistance. In the fungicide response experiments, early and late leaf spot were present in both years with early leaf spot as the more prevalent disease. Defoliation began earlier in 2002 than in 2003 (Fig. 4.1). Epidemics were more severe in 2003 (final defoliation mean across all genotypes = 88.9%) than in 2002 (final defoliation mean across all genotypes = 78.9%). In the tillage and fungicide response experiments, early and late leaf spot occurred in similar proportions in 2002, whereas early leaf spot was more common in 2003. Defoliation began earlier in 2003 (Fig. 4.2) and was more severe by the end of the season (2002 final defoliation mean across tillage and genotypes = 30.8%, 2003 final defoliation mean across tillage and genotypes = 53.6%) compared to epidemics in 2002.

In the fungicide response experiment, genotype significantly affected AUDPC in 2002 (Table 4.1). Bayo Grande and the CRSP lines had lower AUDPC levels compared to GG and MDR-98. Under the two- and three-spray fungicide regimes, leaf spot levels were statistically similar to that of the full season, six-spray regime. In 2003, the fungicide by genotype interaction was significant for AUDPC (Table 4.2). Georgia Green and MDR-98 had lower AUDPC values under the two and three-spray regimes compared to the non-sprayed control, but were significantly higher under the six-spray, full season regime. In most cases, BG and CRSP lines had AUDPC values under the two or three fungicide applications that were comparable to the same genotypes under the full season regime. Leaf spot levels in

CRSP-08 and CRSP-14 were not affected by fungicide application; AUDPC values under no fungicide applications were not significantly different from those under the full season regime, saving six sprays.

In the tillage and fungicide response experiment in 2002, late onset of leaf spot resulted in a short epidemic duration that only allowed time for the completion of two sprays for the planned three-spray fungicide regime. Data for this abbreviated regime was averaged with data from the two-spray regime; therefore, no results will be shown for the three-spray regime for that year. In 2003, the two and three spray regimes received an extra spray each, making the totals, 3 and 4 sprays, respectively.

A tillage by fungicide by genotype interaction was significant for AUDPC in both years. In 2002, under conventional tillage, only BG treated with one or two fungicide applications showed a decrease in leaf spot intensity comparable to the full season regime (Table 4.3). No other genotype under reduced fungicide regimes had AUDPC values comparable to the full season regime. In the strip till plots, leaf spot intensity in all genotypes, except C-99R, was comparable between the non-sprayed control and the full season region. In 2003, in conventionally tilled plots, BG and all CRSP lines treated with three and four reduced fungicide applications resulted in leaf spot AUDPC comparable to the same genotypes under the full season regime (Table 4.4). With the addition of strip tillage, the response of BG and the other CRSP lines was similar, and reductions in leaf spot levels occurred in MDR-98 and C-99R. Georgia Green under the three and four fungicide spray regimes resulted in decreased leaf spot levels compared to the non-sprayed control, but was not comparable to the full season regime.

TSW resistance. Tomato spotted wilt pressure was low across tests and years with a mean of 7.4% 2002 and 1.3% in 2003 in fungicide response experiments, and 17.1% in 2002 and 3.4% in 2003 in the tillage and fungicide response experiments. In the fungicide response

experiment in 2002, BG and the CRSP lines had lower TSW incidence than GG and incidence equivalent to MDR-98, except for BG and CRSP-14 which had TSW incidence levels lower than MDR-98 (Table 4.1). There was no significant difference in TSW incidence among genotypes in 2003, likely due to negligible disease pressure.

In the tillage and fungicide response experiment, the tillage by genotype interaction was significant for TSW incidence in both years. In 2002, TSW incidence was significantly lower in the strip tilled plots than in the conventionally tilled plots planted with MDR-98 (Fig. 4.3). The other genotypes followed this trend numerically, except for CRSP-01, but the differences were not statistically significant. In 2003, results were similar for MDR-98 but TSW incidence for CRSP-20 under conventional tillage was significantly lower than under strip tillage (Fig. 4.4). This trend was also observed, numerically, within CRSP-01, but the differences were not statistically significant. Means of genotypes across tillage and fungicide revealed numerically lower TSW levels in BG and the CRSP lines (15.4-18.2% in 2002, 1.4-3.8% in 2003) compared to GG (21.3% in 2002, 4.0% in 2003) and MDR-98 (19.6% in 2002 and 6.80% in 2003), but not better than C-99R (11.3% in 2002, 2.3% in 2003).

Yield. Yield of in-shell peanuts varied across locations and years with the mean yield in the fungicide response experiments 2508 kg/ha in 2002 and 3116kg/ha in 2003, and 3062 kg/ha in 2002 and 4509 kg/ha in 2003 in the tillage and fungicide response experiments. In the fungicide response experiment, BG and all CRSP lines produced higher yields than GG and MDR-98 in both years, except for CRSP-01 which yielded similarly to MDR-98 in 2003 (Table 4.1). Fungicide did not significantly improve yields in 2002, but in 2003 yields were higher with two and three fungicide applications compared to the control. However, yields under the reduced regimes were significantly less than the yields of the full season, six-spray regime.

In the tillage and fungicide response experiments, the tillage by genotype interaction was significant in 2002. Across fungicide and tillage treatments, BG and the CRSP lines (ranging from 2969-3389 kg/ha) had higher yields than GG (2596 kg/ha) and MDR-98 (2871 kg/ha), and only CRSP-14 (3389 kg/ha) out-produced C-99R (3354 kg/ha). Yields in strip tillage plots were significantly lower than those of conventional plots for the genotypes BG, CRSP-01 and CRSP-08 (Fig. 4.5). This trend was also observed in the remaining genotypes, although differences were not significant. In 2003, genotype, fungicide and tillage all significantly affected yield (Table 4.5). Yields of the breeding lines CRSP-08 and CRSP-14 and the cultivar C-99R were the highest. Yields of BG, GG and the remaining CRSP lines were intermediate and MDR-98 had the lowest yield. Yields for a single fungicide application were similar to the non-sprayed control. An increase in yield was observed as the number of applications increased from one to three and again from three to four. All reduced fungicide regimes had significantly lower yields than the full season, eight-spray regime. Yields were lower in strip tilled plots compared to conventionally tilled plots.

Discussion

Results from this study indicate that BG and the CRSP breeding lines have potential as components of leaf spot and TSW IDM systems composed of reduced fungicide regimes or strip tillage coupled with reduced fungicide regimes. The leaf spot resistance observed in these genotypes allowed for reductions of either three or four sprays per season. Furthermore, in 2003, CRSP-08 and CRSP-14 did not require any fungicides to suppress leaf spot compared to the full season regime. In general, leaf spot epidemics were suppressed by strip tillage compared to conventional tillage. This result is consistent with previous reports (5, 7, 32, 33, 38).

In two recent studies, resistance coupled with strip tillage allowed for a longer fungicide interval between applications, resulting in fewer applications over a growing season (7, 32,

33). In the present study, the response of BG and the CRSP lines to strip tillage resulted in lower leaf spot intensity but, in some cases, the response was not as great as that of host resistance. In 2002, all CRSP lines responded to strip tillage with equal leaf spot suppression in non-sprayed control plots compared to full season fungicide regime plots, a reaction not observed under conventional tillage. The combination of BG and one fungicide application produced leaf spot levels similar to the full spray regime under conventional tillage, while the addition of strip tillage eliminated the need for the one spray. In 2003 under conventional tillage, leaf spot levels in BG and the CRSP lines under three or four fungicide applications were comparable to full season regimes, and the addition of strip tillage did not result in additional leaf spot suppression in those genotypes. This lack of tillage effect during 2003 may indicate the relative importance of resistance when leaf spot epidemics were more severe than in 2002, and the response of tillage was expected to be greater. These results suggest that improved host resistance may be more influential in leaf spot suppression than tillage when disease pressure is high.

In the fungicide response tests, TSW field resistance was found to be better in BG and the CRSP lines compared to the moderately resistant genotype GG and equal to or better in MDR-98. Trends in 2003 were numerically similar, except for BG which had the most %TSW, but differences were not statistically significant. In addition to host resistance, previous work has indicated that TSW is reduced under strip tillage (2, 5, 7, 26, 32). The mechanism of this suppression is not known, but it has been suggested that thrips are unable to detect peanuts in field with the addition of cover crop residue (14). In the both years of the current study, the interactive effects of tillage and genotype on TSW did not corroborate results of previous studies; in both years %TSW was suppressed under strip tillage for MDR-98, but in the second year, CRSP-20 resulted in higher %TSW in strip tiled plots and similar, numerical trends were observed with CRSP-01 in both years, although differences were not

significant. Reasons for this outcome are unknown. Personal observations of BG and many of the CRSP lines in the field indicated increased crop recovery, or formation of new leaves in place of those lost to defoliation, in response to defoliation by leaf spot (Gremillion, unpublished data). It is possible that CRSP-01 and CRSP-20, in response to more severe leaf spot in conventional plots, produced a greater amount of new, healthy foliage, than the other genotypes tested, thus masking the overall appearance of TSW, making disease levels appear to be less in conventional tillage than in strip tillage. The cultivar, Southern runner, is known to recover from defoliation caused by late leaf spot by refoliating, but at the cost of lower assimilates in pod development (36, 37). Even if BG and the CRSP lines are refoliating and reducing their assimilates, their overall yields remain equal to or higher than that of GG. More data is needed to test for crop recovery in these new lines, especially under severe TSW conditions when treatment effects may be more distinct.

Yields of BG and most of the CRSP lines were typically higher than that of MDR-98. This result was observed with GG, except in 2003 in the tillage and fungicide experiment in which CRSP-08 and CRSP-14 were the only genotypes to produce higher yields than GG. At this location in this year, yields were high for all genotypes tested, likely due to ideal growing conditions and low TSW pressure. Cantonwine et al. (2006) found no significant differences among yields under four- or five-spray programs compared to a full season, seven-spray program across tillage and genotype treatments (7). The reduced fungicide applications of the current study were not statistically similar to the higher yields of the full season regime. Yields were lower under strip tillage compared to conventional tillage for the genotypes BG, CRSP-01 and CRSP-08 in 2002 and generally lower across all genotypes in 2003 compared to yields under conventional tillage. Yield inconsistencies under strip tillage are well-documented (7, 24, 32, 33, 38). However, it has been suggested that peanuts planted to fields with no previous history of conservation tillage, such as in this current study, may not show

equivalent yields under strip tillage compared to conventional tillage until three years of peanut-cotton rotations have been completed (7).

While this study supports an IDM system composed of BG or the CRSP breeding lines coupled with strip tillage and/or half-rate, reduced fungicide applications will help growers in the southeastern U.S. control *C. arachidicola* and *C. personatum*, the effect of this management strategy does not address the potential impact of soilborne pathogens, if present. Southern stem rot (SSR) and Rhizoctonia limb rot, cause by *Rhizoctonia solani*, are two important soilborne fungal pathogens of peanut in GA (27, 28). Rhizoctonia limb rot was not observed in any field in either years, but epidemics of white mold did occur. Incidence of SSR was significantly lower in the lines CRSP-08, CRSP-15 and CRSP-22 than in Florida MDR-98 (data not shown), a cultivar with some resistance to *S. rolfsii* (15, 19). While strip tillage has not been shown to affect SSR (26, 32) , a significant tillage by genotype interaction showed that SSR incidence was significantly higher in BG under strip tillage compared to conventional tillage (data not shown). Further evaluation of resistance of BG and the CRSP lines to *S. rolfsii* and *R. solani* under both conventional and strip tillage is needed before recommendations of this IDM system can be made to growers with regards to both foliar and soilborne disease control.

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Figure 4.1. Peanut leaf spot disease progress curves of genotypes in the fungicide response experiment in (a) 2002; (b) 2003. Leaf spot is caused by *Cercospora arachidicola*, causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot. Genotypes included Bolivian Bayo Grande (BG) and MDR-98 and the breeding lines CRSP-01, CRSP-08, CRSP-14, CRSP-15, CRSP-19, CRSP-20, and CRSP-22. Georgia Green (GG) served as the control due to its high susceptibility to *C. arachidicola* and *C. personatum*. Plots of GG, a medium maturing cultivar, were harvested 7-10 days before the remaining genotypes. Percent (%) defoliation was determined based on corresponding ratings of the Florida 1-10 Intensity scale (Chiteka et al. 1988a). Disease progress curves are represented across all fungicide regimes.

Figure 4.1.

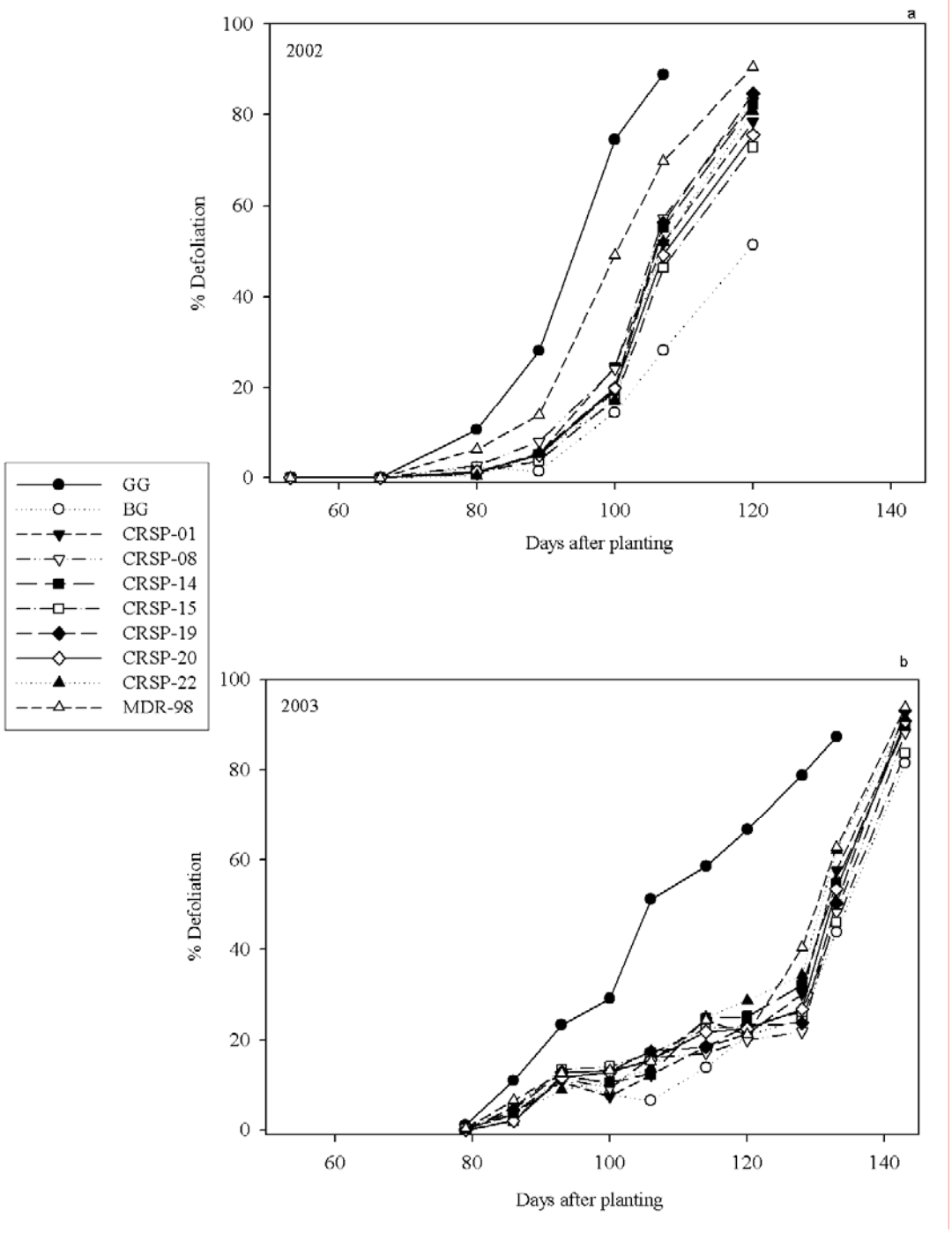
a
2002
% Defoliation
Days after planting
b
2003
% Defoliation
Days after planting
GG
BG
CRSP-01
CRSP-08
CRSP-14
CRSP-15
CRSP-19
CRSP-20
CRSP-22
MDR-98

Figure 4.2. Peanut leaf spot disease progress curves of the tillage and fungicide response experiment in (a) conventional tillage in 2002, (b) strip tillage in 2002, (c) conventional tillage in 2003, and (d) strip tillage in 2003. Leaf spot is caused by *Cercospora arachidicola,* causal agent of early leaf spot, and *Cercosporidium personatum*, causal agent of late leaf spot. Genotypes include Georgia Green (GG), Bayo Grande (BG), CRSP-01, CRSP-08, CRSP-14, CRSP-20, C-99R and MDR-98. Georgia Green (GG) served as the control due to its high susceptibility to *C. arachidicola* and *C. personatum*. Plots of GG, a medium maturing cultivar, were harvested 7-10 days before the remaining genotypes. Percent (%) defoliation was determined based on corresponding ratings of the Florida 1-10 Intensity scale (Chiteka et al. 1988a). Disease progress curves are represented across all fungicide regimes.

Figure 4.2.

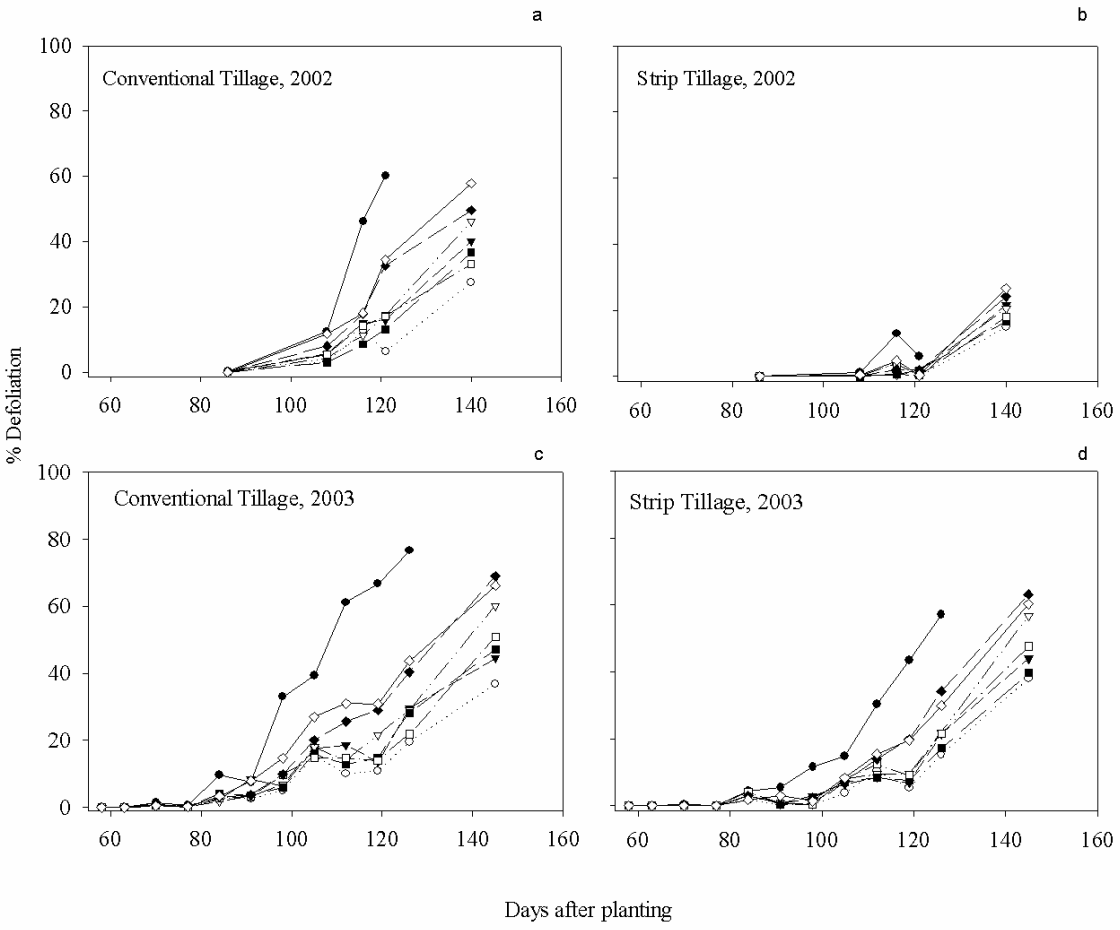
a
b
c
d
Conventional Tillage, 2002
Strip Tillage, 2002
Conventional Tillage, 2003
Strip Tillage, 2003
% Defoliation
Days after planting
0
20
40
60
80
100
60
80
100
120
140
160

Figure 4.3. A tillage by genotype interaction for Tomato spotted wilt (TSW) in the tillage and fungicide response experiment in 2002. Dark bars represent conventional tillage and grey bars represent strip tillage. Genotypes include Georgia Green (GG), Bayo Grande (BG), CRSP-01, CRSP-08, CRSP-14, CRSP-20, C-99R and MDR-98. Georgia Green (GG) has moderate TSW resistance and served as a control. Percent TSW was calculated as the percentage of the total row length of plants with severe TSW symptoms (Culbreath et al. 1997a). Asterick (*) indicates a significant difference among tillage treatments within a genotype. LSD=least significant difference.

Figure 4.3.

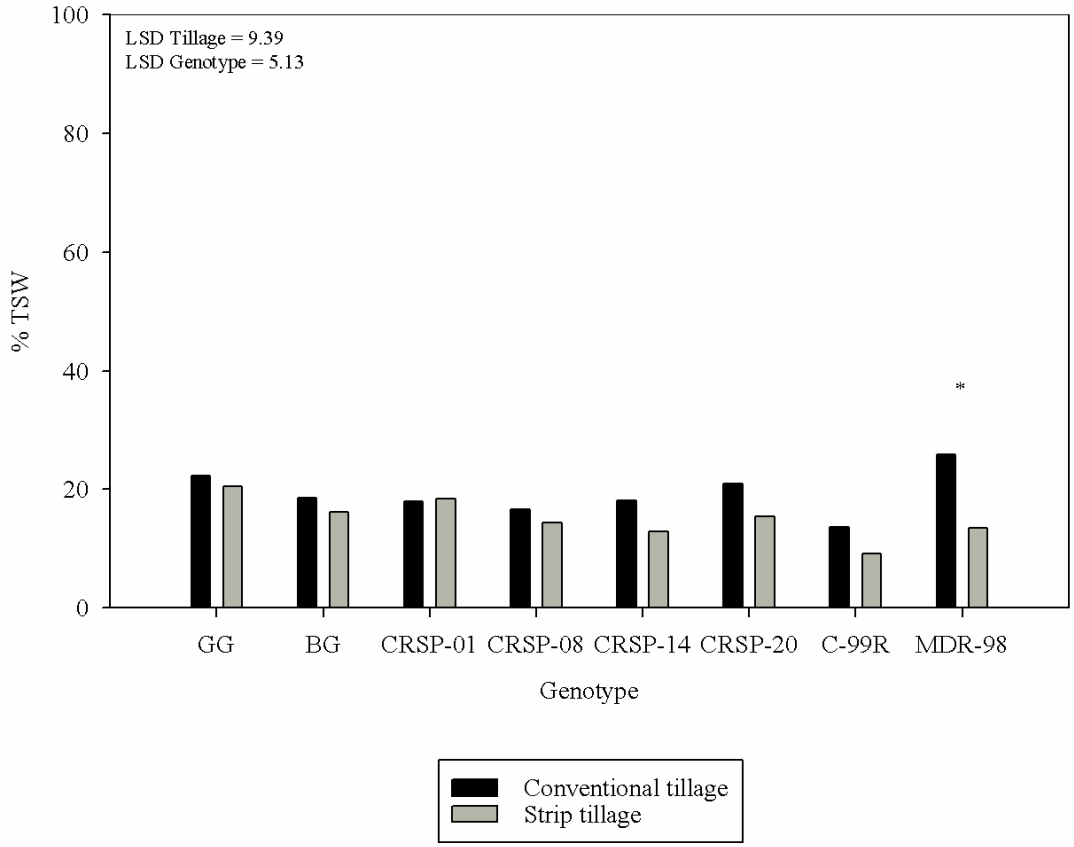
LSD Tillage = 9.39
LSD Genotype = 5.13
% TSW
100
80
60
40
20
0
*
GG
BG
CRSP-01
CRSP-08
CRSP-14
CRSP-20
C-99R
MDR-98
Genotype
Conventional tillage
Strip tillage

Figure 4.4. A tillage by genotype interaction for Tomato spotted wilt (TSW) in the tillage and fungicide response experiment in 2003. Dark bars represent conventional tillage and grey bars represent strip tillage. Genotypes include Georgia Green (GG), Bayo Grande (BG), CRSP-01, CRSP-08, CRSP-14, CRSP-20, C-99R and MDR-98. Georgia Green (GG) has moderate TSW resistance and served as a control. Percent TSW was calculated as the percentage of the total row length of plants with severe TSW symptoms (Culbreath et al. 1997a). Asterick (*) indicates a significant difference among tillage treatments within a genotype. LSD=least significant difference.

Figure 4.4.

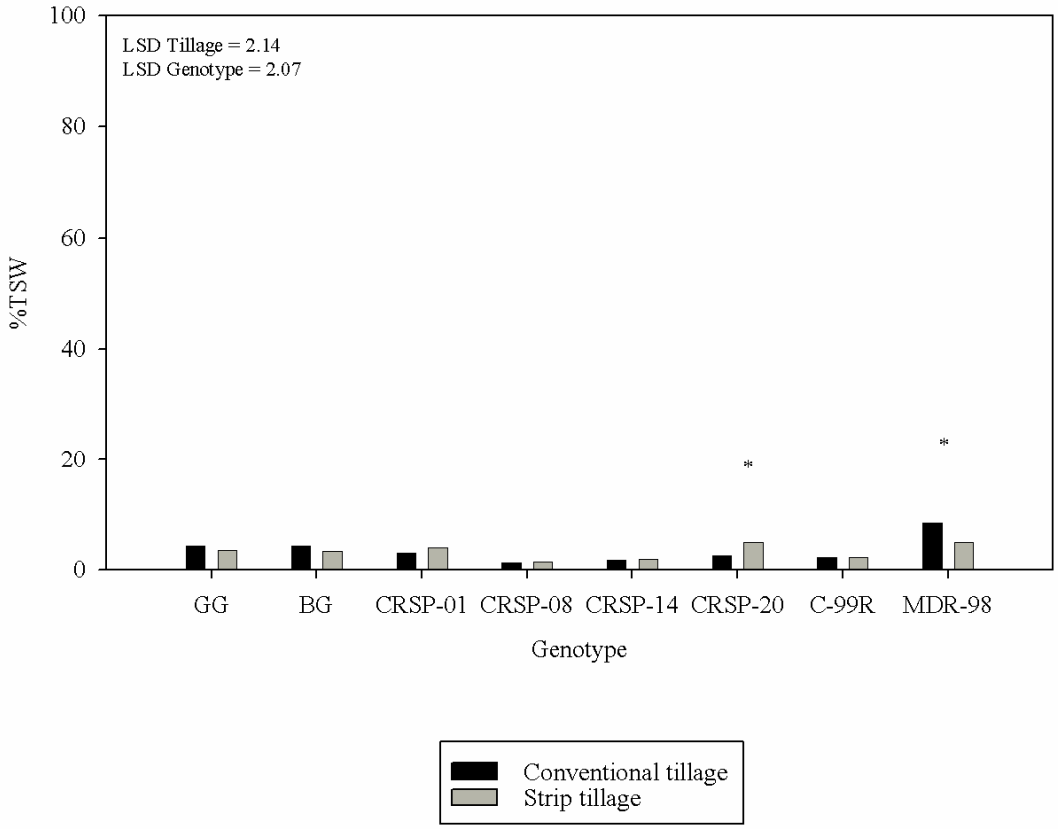
LSD Tillage = 2.14
LSD Genotype = 2.07
%TSW
100
80
60
40
20
0
*
*
GG
BG
CRSP-01
CRSP-08
CRSP-14
CRSP-20
C-99R
MDR-98
Genotype
Conventional tillage
Strip tillage

Figure 4.5. A tillage by genotype interaction for yield (kg/ha) in the tillage and fungicide response experiment in 2002. Dark bars represent conventional tillage and grey bars represent strip tillage. Genotypes include Georgia Green (GG), Bayo Grande (BG), CRSP-01, CRSP-08, CRSP-14, CRSP-20, C-99R and MDR-98. Georgia Green (GG) is the standard cultivar grown in the southeastern U.S. and served as a control. Asterick (*) indicates a significant difference among tillage treatments within a genotype. LSD=least significant difference.

Figure 4.5.

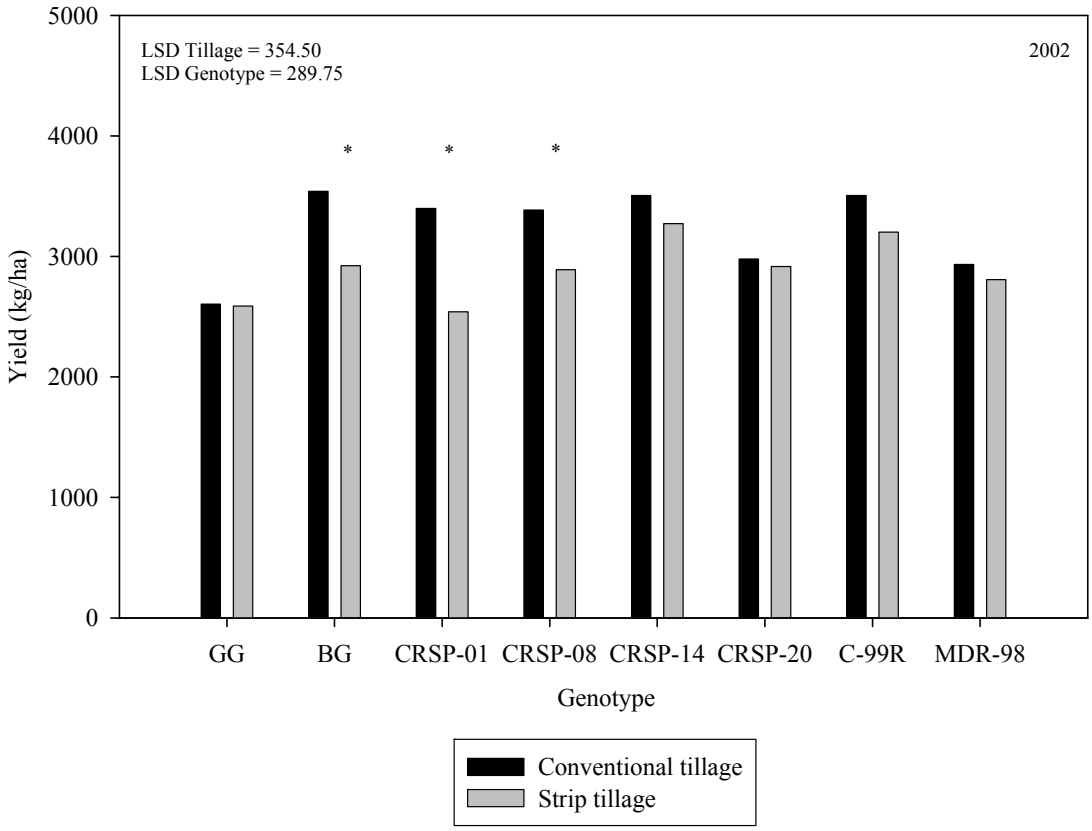
LSD Tillage = 354.50
LSD Genotype = 289.75
2002
5000
4000
3000
2000
1000
0
Yield (kg/ha)
*
*
*
GG
BG
CRSP-01
CRSP-08
CRSP-14
CRSP-20
C-99R
MDR-98
Genotype
Conventional tillage
Strip tillage

Table 4.1. The effect of genotype and number of fungicide sprays on AUDPC of leaf spot, %TSW and yield in the fungicide response experiment in 2002-03.
[a]Least square means from Proc MIXED of area under the progress curve (AUDPC) based on % defoliation due to leaf spot.
[b]Least square means from Proc MIXED of %TSW ((# of 0.3 m sections of row with plants severely affected by TSW / total length of row per plot)*100))
[c]Least square means from Proc MIXED of yield of in-shell peanut (kg/ha).
[d]No AUDPC is shown for 2003 due to a fungicide by genotype interaction, data of which is shown in another table.
Different letters denote a significant difference between treatments (P≤0.05).NS= not significant

	2002			2003[d]	
Treatment	AUDPC [a]	TSW (%) [b]	Yield (kg/ha) [c]	TSW (%) [b]	Yield (kg/ha) [c]
Genotype					
Georgia Green	25.66 b	11.96 a	1810.81 d	1.55	1990.53 d
Bayo Grande	11.73 f	4.75 d	3302.86 a	1.88	3421.54 a
CRSP-01	19.61 cde	8.29 bc	2543.27 c	1.33	3007.84 bc
CRSP-08	21.49 c	7.13 bcd	2394.06 c	1.13	3438.50 a
CRSP-14	19.75 cde	4.92 d	2875.59 b	1.08	3397.81 a
CRSP-15	17.10 e	6.71 bcd	2441.54 c	0.58	3197.74 ab
CRSP-19	20.32 cd	8.96 b	2407.63 c	1.33	3248.60 ab
CRSP-20	18.32 de	6.88 bcd	2685.69 bc	1.21	3479.19 a
CRSP-22	18.77 cde	6.50 cd	2916.28 b	0.96	3282.51 ab
MDR-98	28.93 a	8.58 bc	1702.29 d	1.63	2692.47 c
LSD, df	*3.17 99*	*2.44 27*	*329.04 99*	*NS*	*342.54 27*
Fungicide applications					
0	23.40 a	7.37	2371.00	1.25	2689.76 c
2	20.51 ab	7.83	2471.38	1.02	3192.99 b
3	20.38 ab	6.87	2688.40	1.45	2993.60 b
6	16.41 b	7.80	2501.22	1.35	3586.35 a
LSD, df	*4.24 6*	*NS*	*NS*	*NS*	*303.95 6.04*

Table 4.2. The effect of a fungicide by genotype interaction on AUDPC in the fungicide response experiment in 2003 [a]

[a]Least square means from Proc MIXED of area under the progress curve (AUDPC) based on % defoliation due to leaf spot.

Different upper case letters indicate a significant difference among fungicide treatment within the same genotype, LSD=6.79, df =55.8 ($P \leq 0.05$).

Different lower case letters indicate a significant difference among genotypes within the same fungicide treatment, LSD=6.20, df=72.0 ($P \leq 0.05$).

GG=Georgia Green.

BG=Bayo Grande.

	Fungicide Applications			
Genotype	**0**	**2**	**3**	**6**
GG	62.57 Aa	44.85 Ba	45.88 Ba	24.52 Ca
BG	28.90 Acde	20.32 Bd	20.25 Bd	14.60 Bc
CRSP-01	32.24 Abcde	24.30 Bcd	23.87 Bbcd	19.09 Babc
CRSP-08	26.17 Ae	24.21 Acd	22.73 Acd	19.83 Aabc
CRSP-14	34.39 Abc	24.38 Bcd	25.70 Bbcd	20.22 Babc
CRSP-15	32.98 Abcd	25.87 Bbcd	22.82 BCcd	18.09 Cbc
CRSP-19	27.68 Ade	27.96 Abc	27.27 Abc	18.34 Babc
CRSP-20	26.91 ABde	28.95 Abc	25.25 ABbcd	20.77 Babc
CRSP-22	31.36 Abcde	30.81 Ab	26.12 ABbcd	22.63 Bab
MDR-98	37.12 Ab	29.62 Bbc	29.62 Bb	19.33 Cabc

Table 4.3. The effect of tillage by fungicide by genotype interaction on AUDPC in the tillage and fungicide response experiment in 2002 [ab]

[a]Least square means from Proc MIXED of area under the progress curve (AUDPC) based on % defoliation due to leaf spot

[b]Fungicide programs includes i) non-treated control (0 sprays); ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at 0.138 kg a.i./ha (TEB) at the first observation of leaf spot (1 spray); iii) TEB at the first observation of leaf spot followed by a second spray 14 days later (2 sprays); iv) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and v) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date).

Asterick (*) indicates a significant different between tillage treatments within the same fungicide regime and genotype, LSD = 10.82, df =6.33 ($P \leq 0.05$).

Different upper case letters denote a significant different between fungicide regimes within the same genotype and tillage treatment, LSD=6.42, df=43.7 ($P \leq 0.05$).

Different lower case letters denote a significant different between genotypes within the same tillage and fungicide treatment, LSD=4.79, df=112 ($P \leq 0.05$).

Conv=conventional tillage. Strip=strip tillage.

GG=Georgia Green, BG=Bayo Grande.

	Fungicide Application											
	0				**1**				**2**			
	Conv		**Strip**		**Conv**		**Strip**		**Conv**		**Strip**	
Genotype												
GG	27.89	Aa*	5.50	Abc	18.95	Bc*	3.71	Aa	24.30	ABb*	4.25	Aa
BG	15.71	Ab*	3.98	Ac	8.84	Be	4.65	Aa	7.36	Bf	3.62	Aa
CRSP-01	19.07	Ab*	5.12	Abc	17.67	Acd*	2.74	Aa	15.39	Ade	5.48	Aa
CRSP-08	18.17	Ab*	6.65	Abc	18.05	Acd*	3.08	Aa	18.26	Acd*	3.36	Aa
CRSP-14	15.19	Ab	5.20	Abc	13.61	Ade	2.93	Aa	13.38	Ae	3.08	Aa
CRSP-20	19.84	Ab*	5.02	Abc	13.90	Ad	4.32	Aa	15.10	Ade*	3.18	Aa
C-99R	31.09	Aa	10.97	Aa	25.62	Ab*	4.06	Ba	21.17	Abc*	3.18	Ba
MDR-98	28.43	Aa*	9.28	Aab	31.07	Aa*	6.37	ABa	29.46	Aa*	2.74	Ba

Table 4.4. The effect of tillage by fungicide by genotype interaction on AUDPC in the tillage and fungicide response experiment in 2003 [a]

[a] Least square means from Proc MIXED of area under the progress curve (AUDPC) based on % defoliation due to leaf spot

[b]Fungicide programs includes i) non-treated control (0 sprays); ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at 0.138 kg a.i./ha (TEB) at the first observation of leaf spot (1 spray); iii) TEB at the first observation of leaf spot followed by a second spray 14 days later (2 sprays); iv) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and v) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date).

Asterick (*) indicates a significant different between tillage treatments within the same fungicide regime and genotype, LSD = 5.97, df =123 ($P \leq 0.05$).

Different upper case letters denote a significant different between fungicide regimes within the same genotype and tillage treatment, LSD = 5.97, df = 123 ($P \leq 0.05$).

Different lower case letters denote a significant different between genotypes within the same tillage and fungicide treatment, LSD = 5.40, df = 140 ($P \leq 0.05$).

Conv=conventional tillage.

Strip=strip tillage.

GG=Georgia Green.

BG=Bayo Grande

	Fungicide Applications											
	0				**1**				**3**			
	Conv		**Strip**		**Conv**		**Strip**		**Conv**		**Strip**	
Genotype												
GG	37.37	Aa*	23.51	Aab	37.32	Aa*	22.50	Abc	28.02	Ba*	14.20	Babc
BG	18.67	Ad	12.15	ABd	15.23	Ad	15.46	Ad	8.49	Bd	8.91	BCcd
CRSP-01	26.58	Ab*	14.29	ABd	16.20	Bd	17.62	Acd	12.63	BCcd	10.74	BCbcd
CRSP-08	24.77	Abc*	16.72	Acd	22.19	ABc	17.63	Acd	16.70	BCc	13.36	ABa-d
CRSP-14	20.43	Acd*	14.43	Ad	18.63	Acd	14.59	Ad	15.25	ABc*	8.28	Bd
CRSP-20	21.21	Abcd	19.95	Abc	20.61	Acd	17.42	Acd	12.06	Bcd	10.19	Bcd
C-99R	33.99	Aa*	26.86	Aa	31.05	Ab	25.19	Aab	22.01	Bb	16.24	Ba
MDR-98	34.17	Aa*	24.58	Aab	38.74	Aa*	28.63	Aa	16.85	Bc	16.09	Bab

Table 4.4 (continued).
[a] Least square means from Proc MIXED of area under the progress curve (AUDPC) based on % defoliation due to leaf spot
[b]Fungicide programs includes i) non-treated control (0 sprays); ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at 0.138 kg a.i./ha (TEB) at the first observation of leaf spot (1 spray); iii) TEB at the first observation of leaf spot followed by a second spray 14 days later (2 sprays); iv) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and v) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date).
Asterick (*) indicates a significant different between tillage treatments within the same fungicide regime and genotype, LSD = 5.97, df =123 ($P \leq 0.05$).
Different upper case letters denote a significant different between fungicide regimes within the same genotype and tillage treatment, LSD = 5.97, df = 123 ($P \leq 0.05$).
Different lower case letters denote a significant different between genotypes within the same tillage and fungicide treatment, LSD = 5.40, df = 140 ($P \leq 0.05$).
Conv=conventional tillage.
Strip=strip tillage.
GG=Georgia Green.
BG=Bayo Grande

	Fungicide Applications			
	4		**8**	
	Conv	**Strip**	**Conv**	**Strip**
Genotype				
GG	25.35 Ba*	8.92 Bab	4.59 Ca	2.53 Ca
BG	7.56 Bc	3.93 CDbc	5.38 Ba	2.14 Da
CRSP-01	12.72 BCbc*	6.75 CDabc	7.49 Ca	4.04 Da
CRSP-08	11.95 CDbc	8.27 BCabc	8.09 Da	6.16 Ca
CRSP-14	11.55 BCbc	6.35 Babc	6.27 Ca	3.80 Ba
CRSP-20	9.82 Bbc	5.85 Babc	6.57 Ba	4.81 Ba
C-99R	13.73 Cb	9.41 Ca	7.35 Da	4.17 Ca
MDR-98	20.92 Ba*	3.30 Cc	7.62 Ca	5.29 Ca

Table 4.5. The effect of genotype, number of fungicide applications and tillage on yield (kg/ha) in the tillage and fungicide response experiment in 2003[ab]
[a]Least square means from Proc MIXED of yield of in-shell peanuts (kg/ha).
[b]Fungicide programs includes i) non-treated control (0 sprays); ii) tebuconazole (Folicur 3.6F, Bayer CropScience, Research Triangle Park, NC) applied at 0.138 kg a.i./ha (TEB) at the first observation of leaf spot (1 spray); iii) TEB at the first observation of leaf spot followed by a second spray 14 days later (2 sprays); iv) TEB at the first observation of leaf spot symptoms followed by second spray 14 days and a third spray 28 days later (3 sprays); and v) TEB applied at approximately 30 days after planting (DAP) and subsequent applications made at 14-day intervals until harvest of GG (5 to 8 total sprays depending on digging date).
Different letters indicate a significant difference between genotype treatments ($P \leq 0.05$).

Treatment	**Yield (kg/ha)**	
Genotype		
Georgia Green	4290.86	b
Bayo Grande	4516.85	b
CRSP-01	4399.51	b
CRSP-08	5036.35	a
CRSP-14	5029.97	a
CRSP-20	4337.36	b
C-99R	5157.07	a
MDR-98	3305.57	c
LSD, df	*332.4*	*203*
Fungicide applications		
0	3912.74	d
1	3827.77	d
3	4438.85	c
4	4962.77	b
8	5403.85	a
LSD, df	*332.36*	*20*
Tillage		
Conventional tillage	4867.37	a
Strip tillage	4151.02	b
LSD, df	*489.51*	*2*

APPENDIX OF CHAPTER 4

Index Table 4A. The effect of genotype and number of fungicide applications on SSR intensity and TSMK in the fungicide response experiment in 2002-2003[a]

[a]No %TSMK was recorded for 2002.

[b] Least square means from Proc MIXED of Southern stem rot (SSR) intensity (percentage of 31-cm sections of row with sign or symptom of infection) caused by *Sclerotium rolfsii*.

[c]Least square means from Proc MIXED of %TSMK (percentage of total sound mature kernals in a 500 g sample).

Different letters denote a significant difference between treatments ($P \leq 0.05$).

NS=not significant.

	2002[a]		**2003**			
	SSR (%)[b]		**TSMK (%)[c]**		**SSR (%)[b]**	
Genotype						
Georgia Green	11.67	a	79.27	a	2.04	a
Bayo Grande	2.29	c	77.25	de	0.83	bc
CRSP-01	6.04	b	77.88	bcd	1.04	bc
CRSP-08	3.33	c	78.18	b	0.54	c
CRSP-14	2.92	c	78.38	b	0.75	bc
CRSP-15	2.92	c	77.43	cd	0.67	c
CRSP-19	5.63	b	78.10	bc	1.00	bc
CRSP-20	5.63	b	77.81	bcd	0.67	c
CRSP-22	2.92	c	76.72	e	0.92	bc
MDR-98	6.25	b	78.45	b	1.32	b
LSD, df	*2.36*	*99*	*0.68*	*105*	*0.58*	*97.4*
Fungicide applications						
0	4.75		77.64	b	1.14	
2	5.42		77.89	b	0.83	
3	3.75		77.87	b	1.10	
6	5.92		78.39	a	0.83	
LSD, df	*NS*		*0.43*	*105*	*NS*	

Index Table 4B. The effect of tillage by fungicide by genotype interaction on TSMK in the tillage and fungicide response experiment in 2002 [a]

[a]Least square means from Proc MIXED of total sound mature kernals (TSMK) measured as the percentage of total sound mature kernals per 500 g sample.

Dash (--) indicates that no TSMK data was collected for Georgia Green (GG) for the tillage and fungicide response experiment in 2002.

Asterick (*) indicates a significant different between tillage treatments within the same fungicide regime and genotype, LSD= 3.12, df =92.6 ($P \leq 0.05$).

Different upper case letters denote a significant different between fungicide regimes within the same genotype and tillage treatment, LSD=3.02, df =96.2 ($P \leq 0.05$).

Different lower case letters denote a significant different between genotypes within the same tillage and fungicide treatment, LSD=2.90, df =92.8 ($P \leq 0.05$).

Conv=conventional tillage.

Strip=strip tillage.

	Fungicide Application							
	0				**1**			
	Conv		**Strip**		**Conv**		**Strip**	
Genotype								
Georgia Green	--		--		--		--	
Bayo Grande	73.75	Acd*	67.52	Bc	72.12	ABc	70.47	ABbc
CRSP-01	76.71	Aab*	72.53	Aab	75.70	Aab*	67.90	Bc
CRSP-08	77.83	Aa*	73.96	Aa	76.25	Aab	74.02	Aa
CRSP-14	75.31	Aabc*	70.95	Bbc	73.96	Abc	72.19	ABab
CRSP-20	72.31	ABd	74.12	Aa	74.72	Aabc	72.10	Aab
C-99R	74.77	Abcd	74.35	Aa	76.91	Aa*	72.03	Aab
MDR-98	73.98	Abcd	72.32	Aab	75.44	Aab*	72.30	Aab

Index Table 4B (continued)
[a]Least square means from Proc MIXED of total sound mature kernals (TSMK) measured as the percentage of total sound mature kernals per 500 g sample.
Dash (--) indicates that no TSMK data was collected for Georgia Green (GG) for the tillage and fungicide response experiment in 2002.
Asterick (*) indicates a significant different between tillage treatments within the same fungicide regime and genotype, LSD= 3.12, df =92.6 ($P \leq 0.05$).
Different upper case letters denote a significant different between fungicide regimes within the same genotype and tillage treatment, LSD=3.02, df =96.2 ($P \leq 0.05$).
Different lower case letters denote a significant different between genotypes within the same tillage and fungicide treatment, LSD=2.90, df =92.8 ($P \leq 0.05$).
Conv=conventional tillage.
Strip=strip tillage.

	Fungicide Application							
	2				**7**			
	Conv		**Strip**		**Conv**		**Strip**	
Genotype								
Georgia Green	--		--		--		--	
Bayo Grande	70.30	Bd	70.00	ABb	73.03	ABabc	71.35	Ac
CRSP-01	72.30	Bcd	72.60	Aab	74.21	ABabc	71.59	Ac
CRSP-08	77.80	Aa	75.30	Aa	75.51	Aa	74.53	Aab
CRSP-14	74.20	Abc	73.10	ABa	72.83	Abcd	75.10	Aa
CRSP-20	74.40	ABbc	72.80	Aab	71.64	Bc	72.69	Aabc
C-99R	75.70	Aab	73.60	Aa	74.56	Aab	72.25	Aabc
MDR-98	74.80	Abc	73.70	Aa	74.34	Aabc	72.16	Abc

Index Table 4C. The effect of a tillage by genotype interaction on SSR in the tillage and fungicide response experiment in 2002[ab]

[a]Least square means from Proc MIXED of Southern stem rot (SSR) intensity (percentage of 31-cm sections of row with sign or symptom of infection) caused by *Sclerotium rolfsii*.

[b]No SSR data was collected for Georgia Green for the tillage and fungicide response experiment in 2002.

Asterick (*) indicates a significant different between tillage within the same genotype treatment, LSD=1.18, df =23.6 ($P \leq 0.05$).

Different lower case letters denote a significant different between genotypes within the same tillage treatment, LSD=1.02, df =53.1 ($P \leq 0.05$) .

	2002			
	Conventional tillage		**Strip tillage**	
Genotype				
Georgia Green	--		--	
Bayo Grande	4.04	ab*	6.33	a
CRSP-01	3.04	ab	4.79	b
CRSP-08	3.33	b	3.62	c
CRSP-14	4.29	ab	4.12	bc
CRSP-20	4.00	a	4.87	b
C-99R	4.08	ab	5.00	b
MDR-98	3.45	a	4.83	b

Index Table 4D. The effect of tillage by genotype interaction on %TSMK in the tillage and fungicide response experiment in 2003[a]

[a]Least square means from Proc MIXED of percentage of total sound mature kernals (%TSMK) measured as the percentage of total sound mature kernals per 500 g sample.
Asterick (*) indicates a significant different between tillage within the same genotype treatment, LSD=1.10, df =28.5 ($P \leq 0.05$).
Different letters indicate a significant different between genotypes within the same tillage treatment, LSD=1.03, df =61.2 ($P \leq 0.05$).

	2003			
	Conventional tillage		**Strip tillage**	
Genotype				
Georgia Green	75.90	cd*	73.78	e
Bayo Grande	76.04	bcd	76.04	bcd
CRSP-01	76.48	bcd	75.50	cd
CRSP-08	78.42	a	77.43	a
CRSP-14	76.03	bcd	76.39	c
CRSP-20	76.81	b	75.89	cd
C-99R	76.97	b	77.01	ab
MDR-98	75.57	d	75.15	d

CHAPTER 5

GENETIC VARIABILITY OF *CERCOSPORA ARACHIDICOLA* POPULATIONS IN NORTH AND SOUTH AMERICA[1]

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Early and late leaf spot, caused by *Cercospora arachidicola* S. Hori (*Mycosphaerella arachidis* Deighton) and *Cercosporidium personatum* (Berk. & M.A. Curtis) (*Mycosphaerella berkeleyii* Jenk.), respectively, are reoccurring diseases of peanut (*Arachis hypogaea*) around the world. Selection forced by management practices such as fungicides and resistant host cultivars can lead to an increase in frequency of resistant pathogen isolates, which, over time can render control strategies ineffective. To predict the potential for resistance in populations of *C. arachidicola*, the predominant pathogen in Georgia peanut fields in recent years, genetic diversity was measured in populations of *C. arachidicola* from the U.S. and Bolivia. Genetic structure was described by DNA sequence of partial regions of the β-tubulin and calmodulin genes. Sequences of both loci were highly homogeneous and therefore uninformative. No morphological differences were seen among individual isolates or among populations of the U.S. and Bolivia. Genetic and phenotypic results indicate that populations of *C. arachidicola* are not highly diverse.

Keywords: *Passalora*, early leaf spot, *Arachis hypogaea*, β-tubulin, calmodulin

Introduction

Early and late leaf spot, caused by *Cercospora arachidicola* S. Hori (*Mycosphaerella arachidis* Deighton) and *Cercosporidium personatum* (Berk. & M.A. Curtis) (*Mycosphaerella berkeleyii* Jenk.), respectively, are reoccurring diseases of peanut (*Arachis hypogaea*) around the world. Fields infected with one or both pathogens can experience yield losses up to 70% if disease is not managed (34). In GA, these pathogens are easily controlled with multi-season fungicide sprays (17), moderately resistant host cultivars (5, 11, 12, 25, 26), and cultural practices like conservation tillage which has been shown to suppress early leaf spot (3, 5, 25, 26, 32), likely due to delaying epidemics (4).

Despite current leaf spot management, the potential for breakdown of host resistance in both leaf spot pathogens is a looming threat to peanut production. Populations of pathogens are not static groups in terms of genetics. Changes in the genetic structure of populations occur through the natural processes of mutations, gene flow, genetic drift and mating, and, in agricultural settings, selection by human-implemented disease control methods. In *C. arachidicola* and *C. personatum*, selection by management practices, especially fungicides and host cultivars, may leave behind resistant individuals, which, over time can increase in number and render control strategies ineffective. It has been suggested that predicting the potential for resistance in a pathogen population is possible and has been described as a function of genetic variation (21, 24), with populations of high genetic diversity having a greater evolutionary potential to overcome resistance (21).

Many plant pathology-related population genetic studies have been conducted on fungi (20, 22, 23), and only within the last decade, have the number of such studies expanded in regards to the genus *Cercospora* and its teleomorph, *Mycosphaerella* (7, 16, 27, 30, 31). Using neutral molecular markers like DNA fingerprinting and DNA sequences, genetic diversity within populations of *C. beticola* was found to be high within and between four

locations in Greece, indicating substantial movement of the pathogen throughout the sugarbeet region (27). A study of ten isolates within a single symptomatic lesion revealed high genetic diversity and varying reactions to fungicides, offering proof that *C. beticola*, a pathogen believed to predominately reproduce asexually, may be undergoing sexual reproduction (28).

Mycosphaerella fijiensis populations on banana from the Australasian-Pacific region had moderate genetic diversity within populations in most locations, and moderate to high diversity as populations became for geographically separated (15). All populations were characterized as being in gametic equilibrium, indicating that recombination, or sexual reproduction was occurring and likely contributing to diversity. The region of Papua New Guinea had the most diverse population structure and was suggested to be the center of origin of *Mycosphaerella fijiensis*.

A global study of 14 populations of *M. graminicola* from four continents correlated high genetic diversity within populations with increased age, according to the spread of wheat with developing civilizations; the most diversity was seen in populations from the Middle East, the oldest known area of civilization and wheat cultivation. Moderate diversity was noted in Europe, and the lowest diversity in the North and South America and Australia, areas considered younger or "New World" civilizations.

In *C. zeae-maydis*, a study of U.S. populations described the presence of two, genetically distinct sibling species within the country, group I and II (39). Later studies revealed that the genetic structure of group II populations were similar to African populations, suggesting that an African isolate migrated to the U.S. and established the group II (7, 30). The appearance of two sibling species suggests that speciation is occurring within global populations of *C. zeae-maydis*, a process that will affect future breeding and disease management.

From South American and Asian collections of *C. kikuchii*, Imazaki et al. (2006), found three genetically distinct population lineages from Brazil and Argentina and two unique to Japan (16). An additional two additional lineages were shared among both locations, suggesting that the pathogen may have been introduced to South America from Japan where soybean originated. Fungicide sensitive isolates were also documented within lineages to identify populations in which fungicide selection will have the most impact.

The presence and distribution of the mating types of various *Cercospora* species from around the world have been studied by amplifying the loci, *MAT1-1* and *MAT1-2* in individual isolates using PCR (13). Populations of *C. apii, C. apiicola*, *C. beticola*, *C. zeae-maydis*, and *C. zeina* were found to have both mating types, indicating the potential for sexual reproduction, a process that can lead to increased genetic diversity. Knowledge of mating types within a population may also indicate differences in pathogenicity, as seen among global isolates *Mycosphaerella graminicola* (40).

Despite the breadth of population genetic work with the aforementioned *Cercospora* and *Mycosphaerella* species, no work has explored population structures of *C. arachidicola* or *C. personatum*. In recent years, *C. arachidicola* has been the prominent pathogen in fields in GA. Knowledge of the genetic variability and frequency of mating types of this pathogen may give insight into the origin and spread of *C. arachidicola* as well as reveal evolutionary forces acting upon these populations. In addition to genetic evaluation, assessments of fungal morphology such as spore size, may provide evidence of genetic changes in populations of *C. arachidicola*. Both genotypic and phenotypic information will lead to the support of more informed selections of control strategies and overall stronger durability of management programs (2, 8, 14, 24).

The objective of this study was to describe the genetic structure and spore phenotype of *Cercospora arachidicola* populations in Bolivia and the United States. Our specific

objectives were i) to determine if partial DNA sequence data from β-tubulin, calmodulin, cytochrome B oxidase (Cytb), cytochrome C oxidase 1 (COX1), elongation 1-α and histone 3 genes are appropriate for measuring genetic diversity at the population level, ii) to document the distribution of mating types of *C. arachidicola* populations from North and South America and iii) to determine if fungal spore length corresponds to genetic diversity.

Materials and Methods

Fungal isolations. All *C. arachidicola* isolates used in this study are shown in Table 1. Peanut leaves showing early leaf spot symptoms were collected in the United States and Bolivia during 2004-2006. Leaves were air-dried for 48 hrs and stored in dry, plastic containers. Fungal isolations were conducted using a modified method by Cantonwine et al. (4). Leaves were sealed in a ziplock with a moist paper towel and placed under a light source with a 12 hr photoperiod at room temperature (20 to 24 C). After 24 hrs, one visibly sporulating lesion per leaf was excised, and the adaxial surface was spread across a plate of water agar (40g Bacto brand agar/L water). Plates were then placed under a continuous light source at room temperature. After 24 hrs, plates were viewed at 100X magnification, and single *C. arachidicola* spores with one or more germ tubes were removed with a sterilized needle and placed on potato dextrose agar (PDA) (Difco brand, 28g/L water) or V8 (180 g V8 juice, 2g $CaC0_3$, 20 g agar). One spore per lesion was isolated, and double parafilm was used to secure plates and prevent moisture evaporation. Isolate colonies were maintained for months under these conditions.

DNA isolation. DNA from each isolate culture was extracted by grinding frozen fungal tissue in liquid nitrogen in a chilled mortar and pestal, and then isolated with a DNeasy Plant Mini kit (Qiagen, Valencia, CA) using manufacturer's protocol.

Genetic variation and mating type. To measure genetic variation among isolates, nucleotide sequence data were compared. Genomic regions of both mitochondrial DNA

(mtDNA) and nuclear DNA (nDNA) were targeted. Degenerate primers for the mtDNA genes cytochrome B oxidase (Cytb) and the cytochrome C oxidase 1 (COX1) were created based on highly conserved regions of Cytb sequences [*F. oxysporum* (AY945289) and *A. japonicus* (AB020009)] found in GENBANK and COX1 sequences *Aspergillus japonicus* (AF123600)] and [*Fusarium oxysporum* (AY945289). Primers were designed using Primer3 (33). A total of fifteen forward and reverse primer pairs for COX1 and Cytb were constructed. Nuclear DNA genes evaluated included β-tubulin using the primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (10), Histone 3 using the primers H31a (5'-ACTAAGCAGACCGCCCGCAGG-3') and H31b (5'-GCGGGCGAGCTGGATGTC CTT-3') (10), elongation 1-α using the primers EF1-728F (5'-CATCGAGAAGTTCGAG AAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3') (6), and calmodulin using the primers CAL-228F (5'-GAGTTCAAGGAGGCCTTCTCCC-3') and CAL-737R (5'-CATCTTTCTGGCCATCATGG-3') (6).

Mating type primers included the MAT1-1 primers CercosporaMat1f (5'-CTTGC AGTGAGGACATGG-3') and CercosporaMat1r (5'-GAGGCCATGGTGAGTGAG-3') and the MAT1-2 primers CercosporaMat2f (5'-GATNTACCNTCTCGACCTC-3') and CercosporaMat2r (5'-CTGTGGAGCAGTGGTCTC-3') (13). The MAT1-1 primers MAT1-1_P1F (5'-CTTCACCACACCCAAAC-3') and MAT1-1_P4R (5'-TGTTCGGTG TCGTGATG-3'), and the MAT1-2 primers MAT1-2_P1F (5'-CTGCCAGTTCTGCTTT G-3') and MAT1-2_P4R (5'-TCCACGTCGAAGTAGAG-3') were also used (36).

Genomic and mating type regions were amplified with PCR using PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences) with a reaction mixture of 2.0 µl of DNA, 0.5 µl of forward and reverse primers (25 pmol/µl) and 22 µl of dH_2O for a total reaction volume of 25 µl. Amplification using β-tubulin, COX1, Ctyb, histone 3, elongation 1-α and

calmodulin primers was tested using the following PCR profile with a range of annealing temperatures depending on the primer set: initial preheating at 95°C for 3 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at 50-60°C for 1 min and elongation at 72°C for 1.5 min with a final elongation step at 72°C for 10 min. Amplification using CercosporaMat1f/CercosporaMat1r and CercosporaMat2f/ CercosporaMat2r was tested using the following PCR profile with a range of annealing temperatures: initial preheating at 94°C for 5 min, then 40 cycles of denaturation at 94°C for 20 sec, annealing at 58-62°C for 30 sec and elongation at 72°C for 50 sec with a final elongation step at 72°C for 5 min. For the mating type primers MAT1-1_P1F/MAT1-1_P4R and MAT1-2_P1F /MAT1-2_P4R the following PCR profile with a range of annealing temperatures: initial preheating at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 sec, annealing at 50-55°C for 90 sec and elongation at 68°C for 30 sec with a final elongation step at 68°C for 7 min.

The amplicons of specific gene regions were viewed by electrophoresis of 8.0 μl of sample on a 0.5% agarose gel stained with ethidium bromide for 30 min at 90 V. Gels were viewed under UV light to ensure results of single bands. The QIAquick PCR Purification Kit (Qiagen, Valencia, CA) was used to purify PCR samples following manufacturer's directions. Samples were then sequenced by Davis Sequencing (Davis, California) using Applied Biosystems 3730 DNA analyzer. DNA of the isolates USVA05-8 and BLV06-16 were extracted and sequenced twice for β-tubulin to ensure repeatability (data not shown). DNA of the isolates BLV06-SC19 and USOK06-4 were extracted and sequenced twice for calmodulin to ensure repeatability (data not shown).

Nucleotide sequences were aligned in groups of similar geographical origin (e.g. U.S. and Bolivia) using CLUSTALX (38). An outgroup species for each genetic region was selected by a BLAST search in GENBANK using a randomly selected *C. arachidicola* isolate sequence. Alignments were then combined and realigned by sight in MEGA3 (19). Neighbor-

joining trees were created using Jukes-Cantor with complete deletion selected for gaps/missing data and rooted by the outgroup. Statistical analyses included bootstrap values calculated using 1000 replications.

Morphological characterization. The isolates BLV06-SC2, BLV06-SC10, BLV06-SC12, USTX05-SV6, USTX06-Y2 and USVA05-12 were selected for spore dimension assessments. To induce sporulation, three samples of approximately 0.5 cm^3 of tissue per isolate were ground in 1.0 ml of dH_2O for 10 sec using a homogenizer (TissueMiser: Fisher Scientific, Pittsburgh, PA). Each homogenate was spread across separate plates of V8 media. Plates were allowed to dry under sterile conditions and were parafilmed and placed in a light box at room temperature under a 12 hr photoperiod. After seven days, spores were collected by 10.0 ml washes with 0.005% Tween 20 solution using a flame-sterilized glass hockey stick rod to agitate spores into the wash. A 1.0 ml sample from the wash was collected, and 50 spores per sample were selected for measuring. Measurements were taken with a Nikon DS Camera Control Unit DS-L1, DS Camera Head DS-5M, and DS Cooled Camera Head DS-5Mc. Spore length and width (μm) was recorded for one spore per replicated sample. The effect of origin on spore length and width was determined separately for each variable using SAS Proc MIXED SAS Proc MIXED (SAS v 9.1, SAS Institute, Inc. Cary, NC). The "Satterth" option was used for determining degrees of freedom. Significance among origins was defined by Fisher's LSD ($P \leq 0.05$). Significance levels reported in the text are $P \leq 0.05$ unless otherwise indicated.

Results

Measuring genetic variation. At the PCR annealing temperature range tested, the COX1, Cytb, histone 3 and elongation 1-α primers resulted in the appearance of multiple bands or an absence of bands (data not shown); therefore, no data resulted from their use. Both β-tubulin and calmodulin primers amplified a single band. All isolates included in this

study were selected for DNA sequence comparisons (Fig, 5.1). Outgroup sequences were retrieved from GENBANK and included the closely-related fungi, *Cercospora beticola* (AY840422.1) and *C. piaropi* (AF146116.1) for calmodulin- and β-tubulin-phylogenetic trees, respectively. Both revealed very little diversity among isolates (Fig. 5.1 and Fig. 5.2, respectively), and; therefore, no distinct lineages were apparent and no information on genetic structure of *C. arachidicola* could be extrapolated.

Morphological characterization. Spore dimensions of four Bolivian and three U.S. isolates were evaluated (Table 5.2). All isolates produced spores within previously documented length for *C. arachidicola* of 61-153 μm (35). Statistical analyses revealed that spore length was not significant among individual isolates or isolates grouped according to country.

Discussion

The genetic structure of populations of *Cercospora arachidicola* was described by the β-tubulin and calmodulin loci in this study. Sequences from both genes revealed low genetic diversity. This result corresponds to minimal calmodulin sequence variation seen among isolates of *Sclerotinia sclerotorium* (6). However, high diversity has been reported in the β-tubulin locus in studies of *Fusarium graminearum* (29), *Aspergillus flavus* (9), *Mycosphaerella graminincola* (1) and *Cercospora kikuchii* (16). The popularity of the β-tubulin region in phylogenetic studies is suggested to be a result of high genetic variation within introns, or non-coding regions of DNA, and conserved exons where PCR primers can be developed (1). Results of the spore length study revealed that no difference in phenotype is evident within region or country populations of *C. arachidicola*.

Low genetic diversity may indicate young pathogen populations in this study (1, 37). However, because the cultivated peanut is believed to have originated in Bolivia, (18), it is likely that *C. arachidicola* evolved there with its host, and it is expected that populations of

that area would be more genetically diverse than those in secondary origins, like the U.S. Low genetic diversity may also indicate either a lack of sexual reproduction or high genotype flow. The asexual cycle is predominant and polycyclic during the disease cycle of *C. arachidicola* while sexual reproduction is only known to occur in overwintering crop debris at the beginning of peanut growing seasons, and it is not clear how often this actually takes place. Also, genotype flow among populations in the U.S. and Bolivia may occur through the long distance dispersal of conidia of *C. arachidicola* or movement of peanut materials among production regions. This flow may to low genetic diversity, although little data of this nature has been collected.

Further studies including more genetically variable genomic regions, an increased number and geographic range of isolates and inclusion of mating type loci will help support hypothesis brought up in this current study. Techniques of DNA fingerprints such as amplified fragment length polymorphisms (AFLPs) have been utilized to define populations of other fungal pathogens (16, 30, 31). While primers for COX1, Cytb, histone 3, and elongation 1-α did not produce good results in this study, developing effective primers for these regions as well as others may lead to multilocus support of genetic diversity. A survey of isolates from other peanut producing regions would help clarify the story of *C. arachidicola* and contribute to a better overall picture of global genetic diversity.

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Figure 5.1. Neighbor-joining tree derived from partial nucleotide sequences of the β-tubulin gene of 50 *C. arachidicola* isolates. Distances were determined by the Juke-Cantor parameter. Scale bar indicates a distance of 0.02 (2 base pair changes per 100 nucleotide positions). Values on tree branches represent the percent appearance of a given branch in 1,000 bootstrap replications. The outgroup 'Cercospora piaropi' β-tubulin sequence retrieved from GENBANK (AF146116.1).

Figure 5.1

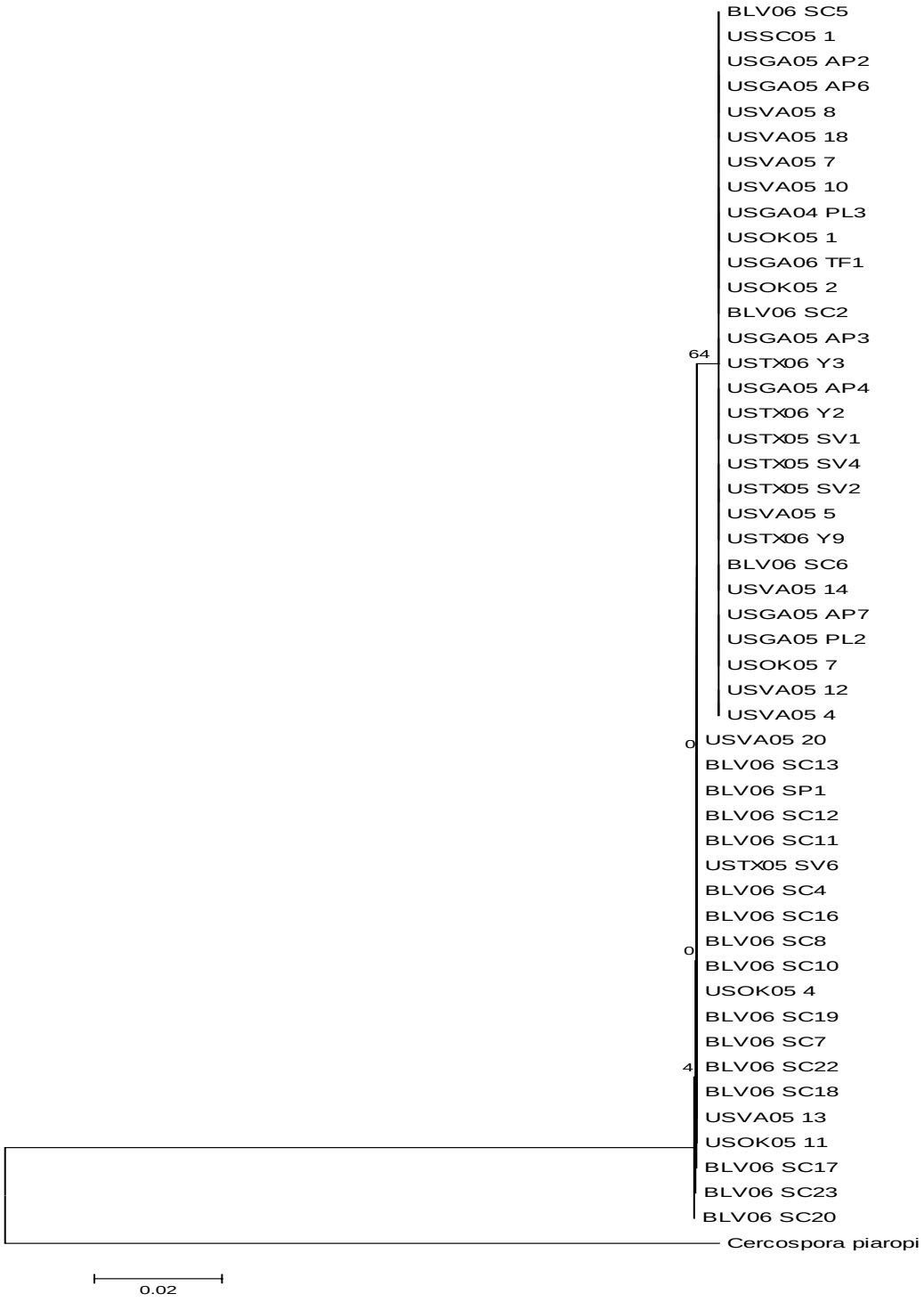
BLV06 SC5
USSC05 1
USGA05 AP2
USGA05 AP6
USVA05 8
USVA05 18
USVA05 7
USVA05 10
USGA04 PL3
USOK05 1
USGA06 TF1
USOK05 2
BLV06 SC2
USGA05 AP3
64
USTX06 Y3
USGA05 AP4
USTX06 Y2
USTX05 SV1
USTX05 SV4
USTX05 SV2
USVA05 5
USTX06 Y9
BLV06 SC6
USVA05 14
USGA05 AP7
USGA05 PL2
USOK05 7
USVA05 12
USVA05 4
0
USVA05 20
BLV06 SC13
BLV06 SP1
BLV06 SC12
BLV06 SC11
USTX05 SV6
BLV06 SC4
BLV06 SC16
BLV06 SC8
0
BLV06 SC10
USOK05 4
BLV06 SC19
BLV06 SC7
4
BLV06 SC22
BLV06 SC18
USVA05 13
USOK05 11
BLV06 SC17
BLV06 SC23
BLV06 SC20
Cercospora piaropi
0.02

Figure 5.2. Neighbor-joining tree derived from partial nucleotide sequences of the calmodulin gene of 50 *C. arachidicola* isolates. Distances were determined by the Juke-Cantor parameter. Scale bar indicates a distance of 0.02 (2 base pair changes per 100 nucleotide positions). Values on tree branches represent the percent appearance of a given branch in 1,000 bootstrap replications. The outgroup 'Cercospora beticola' sequence retrieved from GENBANK (AY840422.1).

Figure 5.2

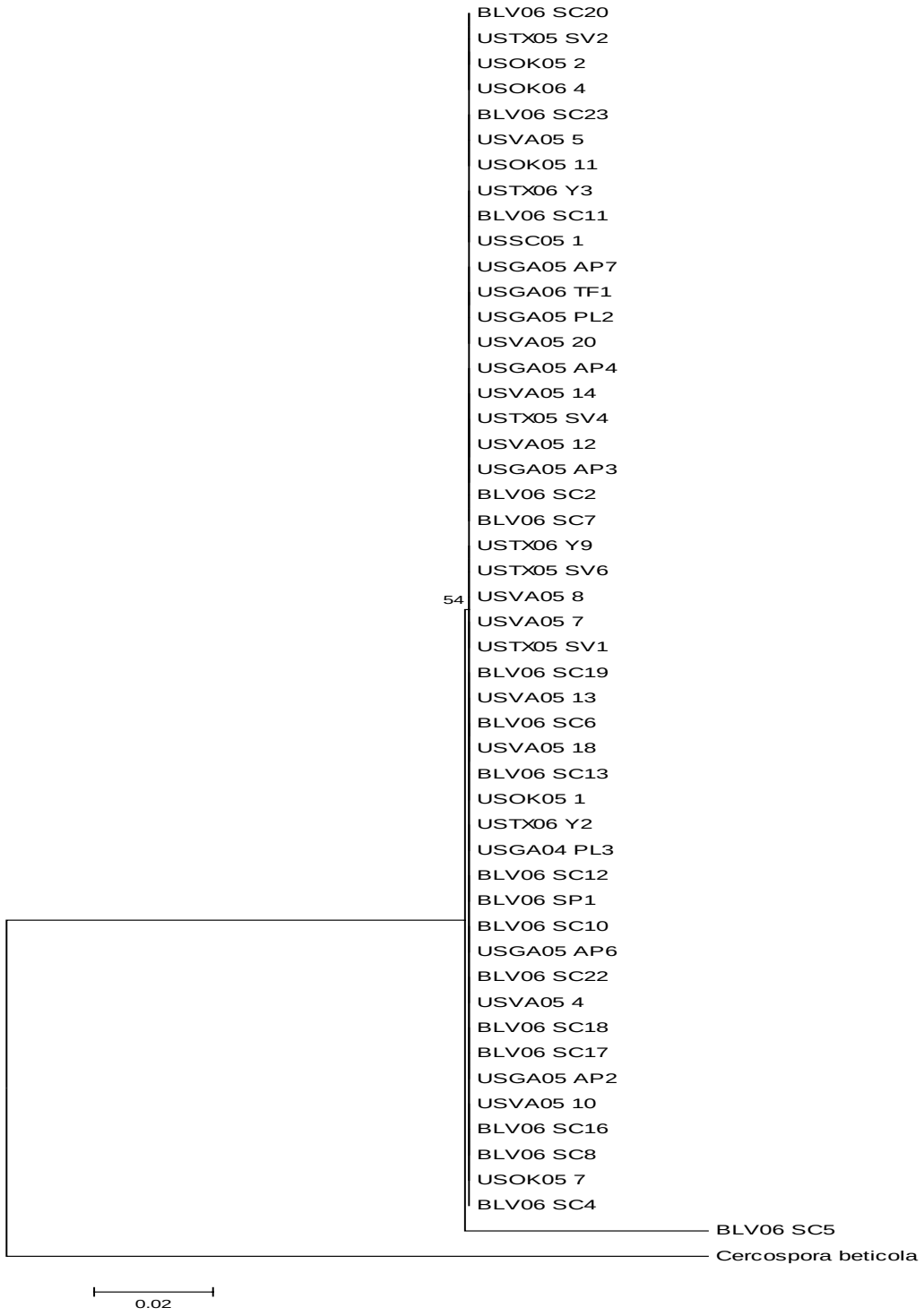
BLV06 SC20
USTX05 SV2
USOK05 2
USOK06 4
BLV06 SC23
USVA05 5
USOK05 11
USTX06 Y3
BLV06 SC11
USSC05 1
USGA05 AP7
USGA06 TF1
USGA05 PL2
USVA05 20
USGA05 AP4
USVA05 14
USTX05 SV4
USVA05 12
USGA05 AP3
BLV06 SC2
BLV06 SC7
USTX06 Y9
USTX05 SV6
54
USVA05 8
USVA05 7
USTX05 SV1
BLV06 SC19
USVA05 13
BLV06 SC6
USVA05 18
BLV06 SC13
USOK05 1
USTX06 Y2
USGA04 PL3
BLV06 SC12
BLV06 SP1
BLV06 SC10
USGA05 AP6
BLV06 SC22
USVA05 4
BLV06 SC18
BLV06 SC17
USGA05 AP2
USVA05 10
BLV06 SC16
BLV06 SC8
USOK05 7
BLV06 SC4
BLV06 SC5
Cercospora beticola
0.02

Table 5.1. Geographical origin of fifty *Cercospora arachidicola* isolates used in this study

Fungal Isolate Name	Geographical Origin	Year Isolated
BLV06-SC2	Santa Cruz, Bolivia	2006
BLV06-SC4	Santa Cruz, Bolivia	2006
BLV06-SC5	Santa Cruz, Bolivia	2006
BLV06-SC6	Santa Cruz, Bolivia	2006
BLV06-SC7	Santa Cruz, Bolivia	2006
BLV06-SC8	Santa Cruz, Bolivia	2006
BLV06-SC10	Santa Cruz, Bolivia	2006
BLV06-SC11	Santa Cruz, Bolivia	2006
BLV06-SC12	Santa Cruz, Bolivia	2006
BLV06-SC13	Santa Cruz, Bolivia	2006
BLV06-SC16	Santa Cruz, Bolivia	2006
BLV06-SC17	Santa Cruz, Bolivia	2006
BLV06-SC18	Santa Cruz, Bolivia	2006
BLV06-SC19	Santa Cruz, Bolivia	2006
BLV06-SC20	Santa Cruz, Bolivia	2006
BLV06-SC22	Santa Cruz, Bolivia	2006
BLV06-SC23	Santa Cruz, Bolivia	2006
BLV06-SP1	San Pedro, Bolivia	2006
USGA04-PL3	Plains, Georgia, United States	2004
USGA05-AP2	Attapulgus, Georgia, United States	2005
USGA05-AP3	Attapulgus, Georgia, United States	2005
USGA05-AP4	Attapulgus, Georgia, United States	2005
USGA05-AP6	Attapulgus, Georgia, United States	2005
USGA05-AP7	Attapulgus, Georgia, United States	2005
USGA05-PL2	Plains, Georgia, United States	2005
USGA06-TF1	Tifton, Georgia, United States	2006
USOK05-1	Perkins, Oklahoma, United States	2005
USOK05-2	Perkins, Oklahoma, United States	2005
USOK06-4	Perkins, Oklahoma, United States	2006
USOK06-7	Perkins, Oklahoma, United States	2006
USOK06-11	Perkins, Oklahoma, United States	2006
USSC05-1	Barnwell County, South Carolina, United States	2005
USTX05-SV1	Stephenville, Texas, United States	2005
USTX05-SV2	Stephenville, Texas, United States	2005
USTX05-SV3	Stephenville, Texas, United States	2005
USTX05-SV4	Stephenville, Texas, United States	2005
USTX05-SV6	Stephenville, Texas, United States	2005
USTX06-Y2	Yoakum, Texas, United States	2006
USTX06-Y3	Yoakum, Texas, United States	2006
USTX06-Y9	Yoakum, Texas, United States	2006
USVA05-4	Suffolk, Virginia, United States	2005

Table 5.1 (continued)

Fungal Isolate Name	Geographical Origin	Year Isolated
USVA05-5	Suffolk, Virginia, United States	2005
USVA05-7	Suffolk, Virginia, United States	2005
USVA05-8	Suffolk, Virginia, United States	2005
USVA05-10	Suffolk, Virginia, United States	2005
USVA05-12	Suffolk, Virginia, United States	2005
USVA05-13	Suffolk, Virginia, United States	2005
USVA05-14	Suffolk, Virginia, United States	2005
USVA05-18	Suffolk, Virginia, United States	2005
USVA05-20	Suffolk, Virginia, United States	2005

Table 5.2 Spore length of six *C. arachidicola* isolates from Bolivia and the U.S.
[a]Averaged over three replicated measurements of 50 spores each.

Fungal Isolate Name	**Spore length[a]** (μm)
BLV06-SC2	103.82
BLV06-SC10	116.43
BLV06-SC12	104.44
USTX05-SV6	104.96
USTX06-Y2	109.98
USVA05-12	108.60
Standard Error	0.36
LSD, df	*NS*
Bolivia	107.41
U.S.	107.85
Standard Error	0.11
LSD, df	*NS*

CHAPTER 6

CONCLUSIONS

The goal of this dissertation thesis was to contribute to management of peanut diseases through newly-incorporated Bolivian host resistance, integrated disease management and knowledge of pathogen variability. The CRSP breeding lines along with the parent cultivar, BG, showed improved leaf spot resistance as measured by percent defoliation and rates of epidemic progression in field trials in the U.S, but not in Bolivia. The components of resistance specific to *C. arachidicola* that were most associated with U.S. field resistance of BG and the CRSP lines are suggested to be lower maximum percent sporulating lesions and lesion size. A lack of consistency in results indicates a need for more work in this area to confirm the mechanisms of genetic resistance to this pathogen. Rust evaluations in the fields of Bolivia and in greenhouses in the U.S. revealed no improved rust resistance among any genotypes tested, but ontogenic resistance was observed across all genotypes as plants matured from the flowering stage to the beginnings of maturity. Yield in the U.S. indicated that BG and the breeding lines were capable similar are better production than other genotypes tested. In Bolivia, BG and the CRSP lines had equal or better yields than other genotypes when leaf spot pressure was high.

As part of an integrated disease management system (IDM) of conventional and strip tillage and/or full and various half-rate, reduced fungicide regimes, BG and the CRSP lines had better leaf spot and tomato spotted wilt (TSW) resistance than the standard southeastern U.S. cultivar, Georgia Green (GG). This improvement in resistance in these lines allowed for a reduction in the rate and number of fungicide sprays required to suppress leaf spot. A savings of

three or four applications, and in some cases, applications numbers equivalent with the full season spray regimes. The use of strip tillage to reduce leaf spot negated the need for fungicides in most genotypes in one year, but was not influencing on suppressing leaf spot in the following year. The effect of strip tillage on %TSW was conflicting across years, lacking in correlation with previous studies. Bayo Grande and the CRSP lines increased yields compared to GG in three of the four locations while reduced fungicides did not supported yields comparable to production under the full season fungicide regime. Strip tillage reduced yields in one year and in specific cultivars in the next. Overall, the use of Bolivian resistance in a reduced fungicide and/or strip tillage IDM system will reduce fungicide use compared to standard production practices but may result in less yields.

The directional selection placed upon *C. arachidicola* populations by management practices such as fungicides and host cultivars increase the number of resistant isolates and the possibility for future populations unaffected by control strategies. To predict the potential for resistance development the genetic diversity was measured in populations from the U.S. and Bolivia using DNA sequence of partial regions of β-tubulin and calmodulin genes. Little genetic diversity was observed at both loci. Spore length measurements also indicated that there is low diversity among the two populations of North and South America. Processes such as a lack of sexual recombination, high genotype flow and young age of population establishment may be the cause for the low diversity observed in U.S. populations.