CHARACTERIZING PEANUT RUST RESISTANCE: DETERMINING ITS MECHANISMS, AND THE GENETICS OF THE PEANUT HOST AND *PUCCINIA ARACHIDIS*

by

IMANA LEGISAH POWER

(Under the Direction of Albert K. Culbreath)

ABSTRACT

Peanut rust, caused by *Puccinia arachidis* Speg., is an important foliar disease of peanut (*Arachis hypogaea* L.) in tropical countries. Host resistance is the best option for disease management in these countries. Field, green house and growth chamber experiments were conducted to evaluate the response of peanut breeding lines with Bolivian genetic background, parents of mapping populations and peanut cultivars used in Georgia, U.S. to peanut rust. In field studies conducted over 2010-2013, several breeding lines developed in the UF150 project of the Peanut Collaborative Research and Support Program (Peanut CRSP) as part of the United States Agency for International Development (USAID) demonstrated varying levels of rust resistance, and a select few were resistant to late leaf spot, caused by *Cercosporidium personatum*, as well. The greenhouse and growth chamber assays revealed that infection frequency and percent diseased area can be used as indicators for field resistance, as genotypes with longer latent periods typically had low infection frequency at 7 days after inoculation and smaller percent diseased areas.

Newly developed CRSP breeding lines, plant introductions and commonly grown cultivars, were molecularly characterized using polymorphic SSR markers. These markers used detected

polymorphisms but were not able to distinguish resistant from susceptible peanut genotypes. None of the 22 private bands generated for the resistant population were absolute and no marker alleles could be exclusively linked to all resistant or all susceptible genotypes. This could be because the resistance observed in the genotypes may be explained by other partial resistance genes than previously identified. Highly resistant and highly susceptible genotypes did cluster; which may indicate that some of the resistant genotypes evaluated in this study may be identified with existing markers on molecular level.

Three loci of *P. arachidis* isolates collected from different regions in the U.S. and countries in Asia, South and Central America were sequenced to determine the genetic variation of *P. arachidis.* The loci 5.8S-ITS2-28S region, translongation elongation factor 1α, and *cytochrome b*, do not indicate high genetic variability among the populations: there was no clustering of isolates according to location or time collected.

INDEX WORDS: *Puccinia arachidis*, peanut rust, *Arachis hypogaea*, host resistance, field resistance, partial resistance, components of resistance, pathogen genetic variation, and durable resistance

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DEDICATION

To my family, my rock of love: Siegmien, Lesley, Moernisa, Milcar and Crystal.

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TABLE OF CONTENTS

Page

LIST OF TABLES

PAGE

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Peanut (*Arachis hypogaea* L.) is an important crop for much of the world and is used for human food, livestock feed and oil. Most peanut production is in tropical and subtropical regions of the world. Peanut production methods vary from modern large-scale mechanized commercial production to subsistence farms where there is little or no use of modern machinery, commercial fertilizers or pesticides. In the United States, Georgia, Florida, Alabama, North Carolina, Virginia, Oklahoma and Texas are the main peanut producers and account for 99% of the peanuts produced. Of these states, Georgia has the major proportion of 41%. In 2012, peanut was harvested from more than 292,000 ha in Georgia and yields averaged 5130 kg/ha with a total production of 1.5 billion kg with crop value that exceeded $586 million (49).

Across the range of production systems, plant diseases represent major constraints to production, through direct losses and/or cost of management inputs. Numerous pathogens reduce the quality and quantity of pods and seeds directly. Some examples are Aspergillus crown rot on seedlings caused by *Aspergillus niger* Tiegh., limb rot caused by *Rhizoctonia solani* (Kuhn), and stem rot caused by *Sclerotium rolfii* Sacc. (2, 9).

In addition to diseases that affect peanut stems, pods and seeds, there are major foliar diseases. *Tomato spotted wilt virus*, a tospovirus that causes tomato spotted wilt, *Cercospora arachidicola* S. Hori, a fungus that causes early leaf spot and *Cercosporidium personatum* (Berk & M. A. Curtis), a fungus that causes late leaf spot (2, 9) can cause considerable yield losses and consequently substantial economic losses. For example, in 2011 the total cost for damage and

control by the two leaf spots and tomato spotted wilt in Georgia were $29.3 and $3.1 million, respectively (52).

Yet another important foliar disease in peanut cultivation that is common in countries with warmer, tropical climates but typically does not cause extensive losses in the southeastern United States is peanut rust, caused by the fungus *Puccinia arachidis* Speg. (4, 46). The characteristic symptoms and signs of peanut rust are chlorotic to yellowish flecks on the upper side of the leaf and slightly raised orange pustules (the uredinia) on the underside of the peanut leaflets which, when ruptured, release reddish brown urediniospores (33, 42). Pustules can also form on petioles, stems and pegs, and in later stages of the disease, pustules may form on the upper sides of the leaflets. Depending on the susceptibility of the cultivar, the original pustules on the underside of the leaflets may be surrounded by colonies of secondary pustules. Heavily infected plants often appear pale green prior to death of the affected leaves (4, 42, 46). Although the infected leaves become necrotic and black, they remain attached to the plant (33, 42, 46).

Under normal cultivation conditions, yield losses due to peanut rust can be considerable (44, 45). Subrahmanyam et al. (42, 44) reported yield losses as high as 50% in India. Although peanut rust is primarily a disease of the tropics and subtropics and has been sporadic in occurrence in the southeastern United States, global climate change may result in greater problems with this disease in the United States either through greater frequency of tropical storms that move inoculum from sources in the Caribbean to peanut production areas in the U.S. or extending the range over which the pathogen can overwinter. Increased potential for rust epidemics should be addressed proactively, because most peanut cultivars in the United States currently grown have

little or no resistance to rust (4, 14, 44, 46, 47) or the level of resistance to the rust pathogen is not known.

In areas where rust causes frequent problems, management methods include cultural practices to reduce the inoculum source, such as eradicating volunteer plants and allowing fallow periods of at least one month between crops, and multiple fungicide applications throughout the season (4, 42, 46). However, chemical applications increase the production costs and moreover, the fungus may develop resistance with frequent fungicide applications (41). In addition, rust is problematic in numerous production areas where fungicide control is not an option due to cost or availability of fungicides. The use of resistant peanut cultivars is a promising alternative management approach, and can be beneficial to growers across a range of production levels. Many germplasm accessions have been screened, and several peanut genotypes with resistance to peanut rust have been identified, with sources for resistance mainly originating from Peru, Bolivia and India (4, 42, 53). However, very little new information on rust resistance from North or South America has become available in the last two decades.

Leaf spots are common in areas where peanuts are cultivated and occurrence of both the leaf spots and peanut rust in the same season will lead to economic losses (42). It is important that the peanut cultivars that are resistant to peanut rust have multiple disease resistance, but resistance to peanut leaf spots is a minimum.

Literature review

Peanut rust. Peanut rust, caused by the fungus *Puccinia arachidis* Speg., is one of the foliar diseases of peanut responsible for considerable damage in the peanut production (4, 46). Peanut

rust was first reported by Berkeley and Curtis in 1853 from a collection made in Surinam by Weigelt in the late 1820s (21). If the disease occurs early enough in the season, it may lead to two- to three-week earlier maturing pods, smaller seeds, increased pod detachment during digging, and decreased oil content of the kernels (33). This can lead to considerable yield losses, sometimes as high as 50% (42, 44). Peanut rust occurrences in the U.S. are less common and losses are typically localized, so little information on yield losses in the U.S. is available; Subrahmanyam (42) reported losses due to peanut rust in Texas of 50 and 70%.

Puccinia arachidis Speg. is a member of the Urediniomycetes where the Uredinales, the rusts, is a single order, containing approximately 5000 host-specific, obligate parasites (1, 25). They are among the most damaging of the world's plant diseases (25), with host specific races that only infect certain varieties within a species (1). Different races of *P. arachidis* have not been demonstrated (4). The characteristic symptoms and signs of peanut rust are chlorotic or yellowish flecks on the underside of the peanut leaflets in which slightly raised orange pustules (uredinia) appear as small spots. As the pustules mature, they rupture and release reddish-brown urediniospores. Most pustules are surrounded by a narrow chlorotic zone. Aside from leaflets, pustules can also form on petioles, stems and pegs, and in later stages of the disease, on the upper sides of the leaflets. Colonies of secondary pustules may surround the original pustules on the underside of the leaflets in highly susceptible cultivars (42). Pustules vary in size from 0.5 and 1.4 mm in diameter depending on the position (larger pustules on the underside) and the degree of crowding (smaller when more are present) (4, 42).

The general disease cycle of rusts includes different spore stages (1, 25), and rusts can be microcyclic or macrocyclic. The teliospores, which are the overseasoning spores for many rusts,

germinate and form basidiospores. The basidiospores can initiate infection when they land on a susceptible host which results in the production of pycniospores in pycnia. The pycniospores, functioning as spermatia now, fertilize female hyphae to form the aeciospores in the aecia, followed by the formation of urediniospores in uredinia. After that, teliospores are formed in the telia. Microcyclic or short-cycled rusts, and demicyclic rusts do not produce all spore stages, whereas macrocyclic or long-cycled rusts produce spermatia, aeciospores and urediniospores in addition to the teliospores and basidiospores. In some macrocyclic rusts the spermatia or urediniospores or both may be absent (1, 25). *Puccinia arachidis* is an example of a macrocyclic rust with at least absent spermatia. It is not known whether alternate hosts of the peanut rust pathogen exist and if the fungus produces pycnydia and aecia. Furthermore, telia and teliospores have been reported in South America, but have not been reported in the United States (4, 33, 42).

For peanut rust the infection process of a urediniospore from germination to invasion can take 16 to 20 h (33), under optimal temperatures of 22 to 25°C (5), low light and continued leaf wetness (33). The urediniospores germinate within 6 h by forming a germtube of variable length. Upon contact with a stoma, the germtube forms an appressorium, usually within 12 h after inoculation, from which a narrow infection peg grows through the stomatal aperture. The infection peg then swells and forms a vesicle, the substomatal vesicle, from which several infection hyphae form (7). Knoblike haustoria develop from these hyphae in mesophyl cells of the plant, which can germinate and continue as described. The germination and infection processes are similar for both the under and upper side of the peanut leaflets, as well as for young and old leaflets, but far less for the underside and old leaflets (7). Depending on host susceptibility, the incubation period varies from 7 to 20 days (42), and pustules usually appear three days after

symptom development (33). The peanut rust pathogen is very host specific; there are no reports of hosts outside of the *Arachis* genus (42). In the peanut producing regions with warm climates the pathogen survives on volunteer peanuts, and can survive up to one month on debris (25). The urediniospores cannot survive the winter temperatures in Georgia, but can be dispersed by rain (local) and wind (long distance) (33).

Peanut rust management: Cultural practices. Several methods are used to manage peanut rust. Reducing the inoculum source and preventing the spread of the disease to areas where it is not present are the main cultural practices to achieve this. In peanut producing areas where rust is common and a constant threat, crop rotations are effective (33, 42). In the United States, where the obligate pathogen has to be introduced every year, crop rotations are less effective, and eradicating volunteer plants and one-month fallow periods between crops are more effective in reducing the fungal inoculum. Hygienic measures are simple yet important measures to keep the disease out of the field or, once present, to prevent the spread of the disease to areas where it is not present (4, 42, 44, 46).

Peanut rust management: Chemical control. Chemical control of peanut rust (i.e., multiple fungicide applications throughout the season) has been successful in managing the disease, and several fungicides have been identified that provide control. In the past the Bordeaux mixture and several dust formulations of copper, mainly aimed at controlling the leaf spots, were relatively effective against rust (4, 42) and were regularly used in the United States until the 1960s (42). Regular chlorothalonil applications, combinations of mancozeb and zinc and several sterol-inhibiting fungicides and strobilurin fungicides have proven to be effective against peanut rust as well (42).

There are fungicides that are effective only against the leaf spots but exhibit no control activity against rust. Examples are benomyl and carbendazim and thiophanate methyl. Similarly, there are fungicides that are effective on peanut rust but ineffective against the leaf spots. One example is tridemorph (42). Finally, there are reports on fungicides that are effective on both rust and the leaf spots. Two examples are chlorothalonil and tebuconazole. This is important since the leaf spots are common in areas where peanuts are produced. In areas where both leaf spot and rust are present, a fungicide needs to be able to control both (42).

Peanut rust management: Host resistance. Since there is a substantial cost associated with fungicide applications and a risk of pathogen populations becoming resistant to the fungicides, host plant resistance is a desirable alternative for rust management in the U.S. (41). In many areas, fungicides are not available or are cost-prohibitive, so resistant cultivars are even more important for rust management. The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India screened thousands of germplasm accessions during the period from 1977 to 1992 that resulted in the identification of more than 120 germplasm lines with resistance to peanut rust. Most of these lines are either primitive land races with undesirable seed characters or wild *Arachis* spp. with high rust resistance but commercially unacceptable yields (4, 44, 48, 53).

In the mid-to-late 1970s, several peanut germplasm lines were developed and registered by the United States Department of Agriculture – Agricultural Research Service (USDA-ARS), in cooperation with the Coastal Plain Station of the University of Georgia, and ICRISAT. The lines, named Tifrust-1 through Tifrust-14, were evaluated for rust resistance in experiments that included standard cultivars from the United States, and the resistance to rust was greater than the standard cultivars available at that time. The lines contain common features such as growth and branching

habit, inflorescence and pod setting, but vary in plant size, vigor, maturity and the color of foliage, petals and pods (15-18). More recently, several breeding lines were developed with a Bolivian landrace as parent, in the UF150 project of the Peanut Collaborative Research and Support Program (Peanut CRSP) as part of the United States Agency for International Development (USAID).

Rust resistance in the peanut cultivars currently in production is low and involves polygenic minor genes that provide varying levels of partial resistance (42, 53). These cultivars are described as slow-rusting types, involving mechanisms such as an increased incubation period, decreased infection frequency, and reduced pustule size, spore production, and spore viability of the fungus (8, 42, 44, 53)). Furthermore, Cook (8) and Subrahmanyam et al. (44) related the age of the peanut leaflets and plants to peanut rust resistance, with less retention of the urediniospores on the leaf surface leading to a decrease in infection. According to Subrahmanyam et al. (42), rust resistance is environmentally stable in most genotypes.

Breeding for disease resistance in peanut. Cultivated peanut is an allotetraploid (2n = 4x = 40 chromosomes). It was formed through natural hybridization of two diploid Arachis species *A. duranensis* and *A. ipaensis*, followed by spontaneous chromosome doubling. This resulted in low genetic variation in cultivated peanut (13, 26, 51). Although the genetic variation is reported to be low, there are still many phenotypic features in peanut such as differences in seed size, seed coat color, root system, and response to disease, therefore the reported low genetic variation may be due to the availability of methods and techniques to detect polymorphism on molecular level. This low detectable genetic variation complicates crop improvement in general and thus breeding for peanut rust resistance in particular, especially when evaluation of breeding lines is based solely

on phenotypic traits. The low genetic polymorphism in peanut furthermore limits the development of genetic markers (12, 13, 23), however, continued research efforts have led to the development of different molecular markers that enable the identification of polymorphisms in peanut germplasm. In the last few years, hundreds of SSR markers have been developed by research groups including the University of Georgia and The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (10, 19, 20, 26, 36, 37, 39, 40, 50, 51).

The use of molecular markers in the breeding process (marker assisted selection or MAS) is beneficial, as it can increase efficiency of breeding efforts. For example, it can be used to identify variation that cannot be observed phenotypically. Evaluation of breeding lines using MAS speeds up the development of cultivars with traits of interest considerably. Chu et al. (6) were able to develop the high oleic Tifguard cultivar, by pyramiding the nematode resistance and the high oleic trait in less than three years by using molecular markers associated with these traits. Khedikar et al. (22), Mace et al. (26), Mondal et al. (35), and Varshney et al. (51) identified simple sequence repeat (SSR) markers that were able to detect high levels of polymorphism in peanut recombinant inbred lines (RILs) and peanut genotypes from different geographical regions, of which several were able to distinguish rust resistant from susceptible genotypes. Khedikar et al. (22) furthermore identified 12 quantitative trait loci (QTLs) for peanut rust resistance, of which one major QTL explained up to 55% of the phenotypic variation. Information on whether the newly developed polymorphic SSR markers are associated with peanut rust in the CRSP breeding lines, or whether these QTLs can also be detected in the CRSP breeding lines would be beneficial.

Population genetics. Knowledge of the population structure of plant pathogens is important for disease management strategies; for example the likelihood that a particular pathogen

will develop resistance to fungicides, or overcome host resistance (27-32, 34). Pathogens that reproduce sexually typically have a higher genetic variation than those that reproduce clonally, due to recombination during meiosis, and are therefore more likely to overcome disease management strategies, especially if resistance to a fungicide or virulence on a plant cultivar is governed by one or a few genes. These strategies furthermore impose high selection pressure on the pathogen population (27-32, 34).

There are numerous studies on genetic variation of economically important rust pathogens such as wheat stem rust pathogen *Puccinia graminis* (3) and wheat stripe rust pathogen *Puccinia triticina* (11, 24, 38), and the development of new pathogenic races. Breeding for durable resistance against these two pathogens is difficult, because of the high level of genetic variation, and the threat of the pathogen overcoming host resistance. For example, according to Kolmer et al. (24), up to 60 different virulence genotypes of *Puccinia triticina* can be found in North America each year. In the case of wheat stem rust, the in 1999 discovered pathogenic race of *P. graminis*, Ug99, is highly virulent on most wheat cultivars grown in the African continent and can lead to 100% yield loss (3).

Little is known about the diversity of *P. arachidis*, and little research is being conducted on this subject at the moment. One study (43) reported the possibility of pathogen adaptation to local environments, as higher infection frequencies of peanut rust in resistant genotype Tarapoto were observed in greenhouse studies compared to previous reports. Information on the molecular variability of *P. arachidis* will help reveal the population structure and evolution of the pathogen, and should help expedite breeding for resistance to peanut rust. That information should also

provide an indication of the likelihood of rust populations becoming resistant to new resistant cultivars.

Justification and goals

The goal of this research is to improve management of peanut rust by screening and characterizing available peanut breeding lines and cultivars for resistance to *Puccinia arachidis*. Areas of emphasis include identifying existing cultivars or genotypes with resistance to *P. arachidis* with potential for immediate use in low input peanut producing countries or that may be used as parents in breeding programs where developing cultivars with resistance to multiple foliar pathogens, including *P. arachidis*, is a primary objective. Information on components of rust resistance in peanut genotypes and cultivars should be useful in both scenarios. Knowledge of the molecular variability of *P. arachidis* will lay the groundwork for characterization of the population structure, and provide evidence of the evolutionary history of the pathogen. Information on the genetic variation of *P. arachidis* populations and the genetics of resistance to peanut rust should moreover enable effective breeding for resistance, and thus more effectively manage the disease.

The specific objectives are 1) to evaluate rust resistance in *Arachis hypogaea*; 2) to identify rust resistance genes in peanut genotypes using genetic markers; and 3) to assess the genetic variation among *P. arachidis* populations.

References

1. Agrios, G. N. 2005. Diseases caused by Basidiomycetes - The rusts. Pages 562-582 in: Plant Pathol. Academic Press, San Diego, CA.

2. Bellgard, S., and Ham, K. C. 2004. Common diseases of peanuts in the top end of the NT. Agnote Agdex No: 141/633.

3. Berlin, A., Djurle, A., Samils, B., and Yuen, J. 2012. Genetic Variation in *Puccinia graminis* Collected from Oats, Rye, and Barberry. Phytopathology 102:1006-1012.

4. Bromfield, K. R. 1971. Peanut rust: a review of literature. Journal of American Peanut Research and Education Association 3:111-121.

5. Butler, D. R., and Jadhav, D. R. 1991. Requirements of leaf wetness and temperature for infection of groundnut by rust. Plant Pathol. 40:395-400.

6. Chu, Y., Wu, C. L., Holbrook, C. C., Tillman, B. L., Person, G., and Ozias-Akins, P. 2011. Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. Plant Genome 4:110-117.

7. Cook, M. 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.

8. Cook, M. 1980. Peanut leaf wettability and susceptibility to infection by *Puccinia arachidis*. Phytopathology 70:826-830.

9. Damicone, J., and Melouk, H. A. Foliar diseases of peanut. Oklahoma State University, Division of Agricultural Sciencesand Natural Resources, Oklahoma Cooperative Extension Service EPP-7655.

10. Gautami, B., Pandey, M. K., Vadez, V., Nigam, S. N., Ratnakumar, P., Krishnamurthy, L., Radhakrishnan, T., Gowda, M. V. C., Narasu, M. L., Hoisington, D. A., Knapp, S. J., and Varshney, R. K. 2012. Quantitative trait locus analysis and construction of consensus

genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol. Breed. 30:757-772.

11. German, S. E., and Kolmer, J. A. 1994. Virulence phenotypes of *Puccinia recondita* f. sp. tritici in Uruguay. Plant Dis. 78:1139-1141.

12. Halward, T., Stalker, T., Larue, E., and Kochert, G. 1992. Use of single primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol.Biol. 18:315-325.

13. Halward, T. M., Stalker, H. T., Larue, E. A., and Kochert, G. 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. Genome 34:1013-1020.

14. Hammons, R. O. 1977. Groundnut rust in the United States and the Caribbean. PANS: pest articles and news summaries:300-304.

15. Hammons, R. O., Branch, W. D., Bromfield, K. R., Subrahmanyam, P., Rao, V. R., Nigam, S. N., and Gibbons, R. W. 1982. Registration of Tifrust-14 peanut germplasm. Crop Sci. 22:697.

16. Hammons, R. O., Branch, W. D., Bromfield, K. R., Subrahmanyam, P., Rao, V. R., Nigam, S. N., Gibbons, R. W., and Goldin, E. 1982. Registration of Tifrust-13 peanut germplasm. Crop Sci. 22:697.

17. Hammons, R. O., Nigam, S. N., Gibbons, R. W., Subrahmanyam, P., and Rao, V. R. 1982. Registration of eight peanut germplasm lines resistant to rust. Crop Sci. 22:452-453.

18. Hammons, R. O., Subrahmanyam, P., Rao, V. R., Nigam, S. N., and Gibbons, R. W. 1982. Registration of peanut germplasms Tifrust-1 to Tifrust-4. Crop Sci. 22:452.

19. He, G. H., Meng, R. H., Gao, H., Guo, B. Z., Gao, G. Q., Newman, M., Pittman, R. N., and Prakash, C. S. 2005. Simple sequence repeat markers for botanical varieties of cultivated peanut (*Arachis hypogaea* L.). Euphytica 142:131-136.

20. He, G. H., and Prakash, C. S. 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97:143-149.

21. Hennen, J. F., Subrahmanyam, P., and Figueiredo, M. B. 1987. The taxonomy, life history, and evolution of *Puccinia arachidis* Speg. Pages 145-155 in: Groundnut rust disease: Proceedings of a discussion group meeting, B. A. McDonald, P. Subrahmanyam and J. A. Wightman, eds. International Crop Research Institute for the Semi-Arid Tropics, ICRISAT Center, Patancheru, Anhra Pradesh, 502 324, India.

22. Khedikar, Y. P., Gowda, M. V. C., Sarvamangala, C., Patgar, K. V., Upadhyaya, H. D., and Varshney, R. K. 2010. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 121:971-984.

23. Kochert, G., Halward, T., Branch, W. D., and Simpson, C. E. 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81:565-570.

24. Kolmer, J. A. 1999. Virulence dynamics, phenotypic diversity, and virulence complexity in two populations of *Puccinia triticina* in Canada from 1987 to 1997. Can. J. Bot.-Rev. Can. Bot. 77:333-338.

25. Littlefield, L. J. 1981. Life cycle. Pages 15-31 in: Biology of plant rusts. An introduction. Iowa State University Press, Ames, Iowa.

26. Mace, E. S., Phong, D. T., Upadhyaya, H. D., Chandra, S., and Crouch, J. H. 2006. SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. Euphytica 152:317-330.

27. McDonald, B. 2010. How can we achieve durable disease resistance in agricultural ecosystems? New Phytol. 185.

28. McDonald, B. 2009. Which way will the agricultural research pendulum swing? Agrarforschung 16:3-3.

29. McDonald, B. A. 1997. The population genetics of fungi: Tools and techniques. Phytopathology 87:448-453.

30. McDonald, B. A. 2004. Population genetics of plant pathogens. The Plant Health Instructor DOI:10.1094/PHI-A-2004-0524-01.

31. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

32. McDonald, B. A., and McDermott, J. M. 1993. The population genetics of plant pathogenic fungi. BioScience 43:311-319.

33. Melouk, H. A., and Shokes, F. M. 1995. Peanut rust. Page 72 in: Peanut Health Management. American Phytopathological Society Press, St. Paul, MN.

34. Milgroom, M. G., and Fry, W. E. 1997. Contributions of population genetics to plant disease epidemiology and management. Pages 1-30 in: Advances in Botanical Research Incorporating Advances in Plant Pathology, vol. 24.

35. Mondal, S., Badigannavar, A. M., and D'Souza, S. F. 2012. Molecular tagging of a rust resistance gene in cultivated groundnut (*Arachis hypogaea* L.) introgressed from Arachis cardenasii. Mol. Breed. 29:467-476.

36. Mondal, S., Sutar, S. R., and Badigannavar, A. M. 2008. Comparison of RAPD and ISSR marker profiles of cultivated peanut genotypes susceptible or resistant to foliar diseases. J. Food Agric. Environ. 6:181-187.

37. Moretzsohn, M. C., Leoi, L., Proite, K., Guimaraes, P. M., Leal-Bertioli, S. C. M., Gimenes, M. A., Martins, W. S., Valls, J. F. M., Grattapaglia, D., and Bertioli, D. J. 2005. A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). Theor. Appl. Genet. 111:1060-1071.

38. Ordoñez, M. E., and Kolmer, J. A. 2009. Differentiation of molecular genotypes and virulence phenotypes of *Puccinia triticina* from common wheat in North America. Phytopathology 99:750-758.

39. Pandey, M. K., Gautami, B., Jayakumar, T., Sriswathi, M., Upadhyaya, H. D., Gowda, M. V. C., Radhakrishnan, T., Bertioli, D. J., Knapp, S. J., Cook, D. R., and Varshney, R. K. 2012. Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea*). Plant Breed. 131:139-147.

40. Ravi, K., Vadez, V., Isobe, S., Mir, R. R., Guo, Y., Nigam, S. N., Gowda, M. V. C., Radhakrishnan, T., Bertioli, D. J., Knapp, S. J., and Varshney, R. K. 2011. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 122:1119-1132.

41. Smith, N. B., and Littrell, R. H. 1980. Management of peanut foliar diseases with fungicides. Plant Dis. 64:356-360.

42. Subrahmanyam, P. 1997. Rust. Pages 31-33 in: Compendium of Peanut Diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith and P. Subrahmanyam, eds. American Phytopathological Society, St. Paul, MN.

43. Subrahmanyam, P., Hammons, R. O., Nigam, S. N., McDonald, D., Gibbons, R. W., Fan, M. Y., and Yeh, W. L. 1983. International cooperative screening for resistance of peanut to rust and late leaf spot. Plant Dis. 67:1108-1111.

44. Subrahmanyam, P., and McDonald, D. 1983. Rust Disease of Groundnut. in: Information bulletin no. 13. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Patancheru, Anhra Pradesh, India.

45. Subrahmanyam, P., McDonald, D., Gibbons, R. W., and Rao, P. V. S. 1983. Components of resistance to *Puccinia arachidis.* . Phytopathology 73:253-256.

46. Subrahmanyam, P., McDonald, D., Gibbons, R. W., and Reddy, L. J. 1985. Peanut rust: A major threat to peanut production in the semiarid tropics. Plant Dis. 69:813-819.

47. Subrahmanyam, P., Moss, J. P., and Rao, V. R. 1983. Resistance to peanut rust in wild *Arachis* species. Plant Dis. 67:209-212.

48. Subrahmanyam, P., Rao, V. R., McDonald, D., Moss, J. P., and Gibbons, R. W. 1989. Origins of resistances to rust and late leaf spot in peanut (*Arachis hypogaea*, Fabaceae). Econ. Bot. 43:444-455.

49. USDA-National Agricultural Statistics Service. 2013. Crop Production 2012 Summary. Report No. 1057-7823. Pages

http://usda.mannlib.cornell.edu/usda/nass/CropProdSu//2010s/2013/CropProdSu-2001-2011-2013.pdf.

50. Varshney, R. K., Bertioli, D. J., Moretzsohn, M. C., Vadez, V., Krishnamurthy, L., Aruna, R., Nigam, S. N., Moss, B. J., Seetha, K., Ravi, K., He, G., Knapp, S. J., and Hoisington, D. A. 2009. The first SSR-based genetic linkage map for cultivated groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 118:729-739.

51. Varshney, R. K., Mahendar, T., Aruna, R., Nigam, S. N., Neelima, K., Vadez, V., and Hoisington, D. A. 2009. High level of natural variation in a groundnut (*Arachis hypogaea* L.) germplasm collection assayed by selected informative SSR markers. Plant Breed. 128:486-494.

52. Williams-Woodward, J. 2011. Georgia Plant Disease Loss Estimates 2011. The University of Georgia Cooperative Extension Service, Athens, GA.

53. Wynne, J. C., Beute, M. K., and Nigam, S. N. 1991. Breeding for disease resistance in peanut (*Arachis hypogaea* L). Annu. Rev. Phytopathol. 29:279-303.

CHAPTER 2

FIELD RESISTANCE AND COMPONENTS OF PEANUT RUST RESISTANCE OF NEWLY DEVELOPED BREEDING LINES [1]

[1] Power, I. L., Tillman, B. L., and Culbreath, A. K. 2014. To be submitted to *Plant Disease*.

Abstract

Field, greenhouse and growth chamber experiments were conducted to determine the level of resistance to *Puccinia arachidis*, the cause of peanut rust, in newly developed breeding lines of peanut (*Arachis hypogaea* L.) developed in the UF150 project of the Peanut Collaborative Research and Support Program (Peanut CRSP) as part of the United States Agency for International Development (USAID). Field experiments were carried out in Citra, FL and Tifton, GA from 2010 to 2013. Genotypes Tifrust-10 and Tifrust-13, and CRSP breeding lines PTBOL3-3, 97x36-HO2-1-B2G-3-1-2-2 and BOL3-7 had the lowest stAUDPC and final disease severity score for rust. The CRSP breeding lines 97x36-HO2-1-B2G-3-1-2-2 and BOL3-7 also appeared to be highly resistant to late leaf spot, caused by *Cercosporidium personatum*. In growth chamber studies, genotypes with longer latent periods generally had lower infection frequencies at 7, 11 and 16 DAI, as well as smaller percent diseased areas. Latent period and percent diseased area were significantly correlated with stAUDPC. CRSP breeding lines 97x36-HO2-1-B2G-3-1-2-2 and BOL3-7, together with the previously reported resistant plant introductions PI562530, PI 568164 and PI298115 were among the genotypes with the lower scores for these components. These studies resulted in the identification of several genotypes with multiple disease resistance in different environments and under high disease pressure. Furthermore, these results indicate sources of rust resistance in the CRSP breeding lines, including several genotypes that could be used as parents in peanut germplasm enhancement programs. The studies moreover indicate that latent period, percent diseased area and lesion diameter may be used as indicators for rust resistance in growth chamber studies.

Introduction

Peanut (*Arachis hypogaea* L.) is an important crop for the United States and is especially important in Georgia. In 2011, peanut was harvested from more than 192,000 ha and yields averaged 4,170 kg/ ha with a total production value that exceeded $586 million (25). Peanut production can be threatened by a variety of diseases that can affect all parts of the peanut plant and reduce the quality and quantity of pods and seeds. Foliar diseases such as tomato spotted wilt, caused by the *Tomato spotted wilt tospovirus*, early and late leaf spot, caused by *Cercospora arachidicola* S. Hori and *Cercosporidium personatum* (Berk & M. A. Curtis), respectively (1, 6), can cause considerable yield losses and consequently substantial economic losses. Yield losses due to damage and increased management costs of these foliar diseases in 2011 in Georgia was estimated at $32.2 million (25).

Peanut rust caused by the fungus *Puccinia arachidis* Speg. is another damaging foliar disease in peanut cultivation that is common in countries with warm, tropical climates, but is relatively rare in the United States (3, 19). Peanut rust was first reported in Georgia in 1953, but it was indicated in that report that rust was not expected to become a serious problem in Georgia (12); the pathogen cannot survive the cold winter temperatures. Peanut rust became an important problem in southern Texas in 1965 (24). Under normal cultivation conditions, yield losses to peanut rust can be considerable. In India, yield losses as high as 50% have been reported (18, 20). Currently in the U.S., losses caused by peanut rust are typically localized, so little information on yield losses in the U.S. is available.

Management methods for peanut rust in regions where this disease is prevalent (developing countries) include cultural practices such as eradicating volunteer plants to reduce the inoculum

source, and allowing fallow periods of at least one month between crops (10, 15). Multiple fungicide applications throughout the season (3, 15, 19) with chlorothalonil, strobilurins and triazoles are effective; however, chemical control is often not an option in the developing countries due to increased production costs or lack of availability of fungicides. The pathogen may develop resistance with frequent fungicide applications (14) although the risk is not the same for the different chemical groups. The use of resistant peanut cultivars is thus a very desirable management approach. Many germplasm accessions were screened for resistance during the late 1970s through the early 1990s, resulting in the identification of several peanut accessions with resistance to peanut rust (3, 15, 22, 26). Sources of resistance mainly originated from Peru, Bolivia, and India (3, 10, 15, 22). However, very little new information on rust resistance has become available in the last two decades. Recently, several breeding lines have been developed in the UF150 project of the Peanut Collaborative Research and Support Program (Peanut CRSP) as part of the United States Agency for International Development (USAID). These breeding lines were developed from crosses with parents including a Bolivian landrace cultivar, BayoGrande, U.S. cultivars Hull and Florida MDR98, PI 656458, and the breeding lines VA98R and F79x4. These breeding lines are currently being screened for multiple disease resistance in the United States, and multiple peanut producing countries in the western hemisphere.

Resistance to *P. arachidis* typically is quantitative, where multiple components of resistance provide varying levels of partial resistance, leading to a reduced rate of the rust epidemic development. Components of peanut rust resistance that have been described include increased incubation period and latent period, and reduced infection frequency, pustule size, percent diseased area, spore production and spore germination (3, 5, 18). These components were

characterized in the 1980s and early 1990s (5, 23), but little work has been reported on the more recently developed breeding lines.

The objectives of this study were to determine the level of field resistance to peanut rust and components of resistance of 1) newly developed peanut CRSP breeding lines with Bolivian background, 2) commonly grown peanut cultivars in Georgia, and 3) parents of existing breeding populations. Part of this research has been reported previously (11).

Materials and methods

Field studies were conducted at the University of Florida, Plant Science and Education Unit, Citra, FL in 2010, 2011 and 2012, and at the University of Georgia, Coastal Plain Experiment Station, Tifton, GA in 2011, 2012 and 2013, to evaluate the field resistance of the breeding lines. A randomized complete block design with three replications was used for the experiments from 2010 through 2012, and four replications in 2013. Three and four replications were used because of the seed availability of the breeding lines that were still in the seed increasing phase of the breeding process. In Citra, 25 genotypes (Table 2.1) were planted on 25 May 2010, 1 June 2011 and 31 May in 2012. In Tifton, 19 genotypes (Table 2.1) were planted on 27 June 2011, and 20 genotypes (Table 2.1) were planted on 22 June 2012 and 11 June 2013.

Peanut seeds were planted at 20 seed per meter seeding rate in two-row plots bordered by cultivars Florida-07, Tufrunner-727 or Georgia-09B. The plots were 6 m long and 1.8 m wide with 0.91 m between rows. In both locations, leaf spot epidemics were suppressed by alternating sprays with flusilazole (Punch, DuPont de Nemours, Wilmington, DE) and thiophanate methyl (Topsin 4.5FL, United Phosphous, Inc., King of Prussia, PA). Applications were on a 14-day schedule

starting approximately 40 days after planting (DAP), for a total of 6 to 7 sprays per season. These fungicides have no activity against *P. arachidis* (personal communication, A. K. Culbreath). Peanuts in all experimental plots in both locations and in all three years were inoculated by brushing plants in the experimental plots with heavily rust-infected peanut plants collected from a nearby earlier planted field. In Citra plants were inoculated on 25 August 2010, 20 September 2011 and 29 August 2012, and in Tifton on 22, 23 September and 18 October 2011, 6 and 20 September 2012, and on 23 October 2013. Plants in all plots were also inoculated at night time in Tifton, when the leaves were closed, with a urediniospore suspension of less than 10.000 spores/ml on 25 August 2012 and 7 September 2013 and in Citra on 12 September 2012 during the day with a urediniospore suspension of about 1.000 spores/ml. The suspension used for the inoculation was prepared by adding vacuumed urediniospores from locally collected, heavily rust-infected peanut plants, to 0.005% Tween 20 solution. The suspension was sprayed using a pump-up air hand sprayer.

Rust severity was determined using a modified nine-point ICRISAT scale based on lesion density and leaf necrosis (16): 1 = no disease (0% severity); 2 = sparsely distributed lesions, primarily on lower leaves (1-5% severity); 3 = many lesions on the lower leaves with evident necrosis and very few lesions on middle and upper leaves (6-10% severity); 4 = numerous lesions on lower and middle and severe necrosis on lower leaves (11-20% severity); 5 = severe necrosis of middle and lower leaves and less severe lesions on top leaves (21-30% severity); 6 = extensive damage to lower leaves, lesions densely present on middle leaves with necrosis and lesions may be on top leaves as well (31-40% severity); 7 = severe damage to lower and middle leaves and lesions are densely distributed on top leaves (41-60% severity); 8 = 100% damage to lower and middle

leaves and lesions on top leaves with severe necrosis (61-80% severity) and 9 = almost all leaves are withering and bare stems are present (81-100% severity). In Citra, peanut rust severity was evaluated 108, 122, 136 DAP in 2010; 112, 118, 126 DAP in 2011; and 96, 110, 117, 124, 131, 138, DAP, in 2012. In Tifton, disease severity was assessed123, 130, 137 DAP in 2011; 101, 122, 129, 136, 140 DAP in 2012; and 134, 147, 154 DAP in 2013. Despite the fungicide applications for leaf spot suppression, late leaf spot pressure was high in Citra, so leaf spot severity was assessed on 136 DAP in 2010, 126 DAP in 2011 and 124, 131 and 138 DAP in 2012, using the 1-10 ICRISAT scale (2010) and the 1-10 Florida scale (2011 and 2012) (4).

Rust severity data were used to calculate area under the disease progress curve (AUDPC) for each plot (13). The number of days between disease assessments differed per year and per location, so AUDPC values were standardized (stAUDPC) by dividing the AUDPC by the number of days between the first and last assessment date. The effects of genotype on stAUDPC and final disease severity were analyzed using the Proc MIXED with ddfm = satterth option on the model statement (SAS v 9.3, SAS Institute Inc., Cary, NC). Fisher's protected LSD ($P \leq 0.05$) was used to determine significant differences in stAUDPC and final disease severity among genotypes.

Components of resistance. To assess the components of resistance, a detached leaf experiment was carried out as described by Cook (5). A single-pustule isolate was developed by harvesting urediniospores with a vacuum pump from peanut leaves collected from fields in Georgia, and inoculating healthy leaves with urediniospores from a single pustule. This cycle was repeated several times to ensure purified single-pustule isolates. Urediniospores were maintained on leaves of the susceptible cultivar Altika that were placed on 15-cm petri dishes with 10% water agar, and incubated at 25°C. Freshly produced urediniospores were collected from these leaves to

prepare a spore suspension of 40,000 spores/ml of 0.005% Tween 20, which was quantified using a hemacytometer. Percentage germination of the urediniospores was assessed 1 day before the start of the experiments, and on the day of the experiments. A spore suspension was sprayed on 10% water agar plates, and incubated at 25°C in darkness overnight. The next day, the number of germinated urediniospores out of 50 randomly chosen urediniospores was determined using a compound microscope at 100x magnification. Urediniospores were considered germinated if the germination tube was longer than the greatest diameter of the urediniospore.

The peanut genotypes used in this study included currently grown cultivars, CRSP breeding lines, ICRISAT plant introductions, and parents of existing recombinant inbred line (RIL) populations that are currently being screened for multiple disease resistance (Table 2.1). Peanut plants were grown from seed in the greenhouse at 25°C, in 15 cm pots filled with commercial potting soil (Sunshine Professional Growing Mix, Sun Gro Horticulture Distribution Inc, Bellevue, WA), and were watered as needed. Four or 5 seed, treated with Vitavex PC (a.i. captan, pentachloronitrobenzene and carboxin, Bayer CropScience LP, Research Triangle Park, NC). The youngest fully expanded leaves of 5- to 6-week-old plants were collected, the leaflets detached, and placed on sterile moistened filter paper in a petri dish (9 cm diameter) with the abaxial side up. The leaflets were inoculated by spraying them for 1 second using a compressed air sprayer containing the uredinial spore suspension. The experimental design was a randomized complete block with three replicates per genotype, and for each genotype a control was included by spraying leaves with sterile 0.005% Tween 20 solution. The petri dishes containing inoculated leaflets were incubated in darkness for 16 h at 25°C. After the 16-h dark period, the closed petri dishes were incubated at 25°C, with a 12h photoperiod for 16 days. The filter paper was kept

moist with sterile distilled water. The leaflets were examined on 7, 11 and 16 days after inoculation (DAI) for the development of pustules.

The components of resistance measured included: latent period, infection frequency, lesion diameter, and percent diseased area. The latent period was determined by counting the number of days between DAI and spore production of at least one pustule. The infection frequency was determined as the number of pustules per square centimeter of leaf area, and lesion diameter was determined by measuring the lesion area of 10 arbitrarily selected lesions, and calculating the mean lesion diameter from the formula area = $\pi d^2/4$. Leaf area, lesion area, and percent diseased area were measured from digital images of leaves, taken 16 DAI, using the ASSESS 2.0 Image Analysis Software for plant disease quantification (APS Press, St. Paul, MN). For ASSESS analyses, the leaflets were glued to blue paper background, covered with a sheer plastic sheet, scanned at 300 dpi, and stored as .TIFF files. The detached leaf experiment was done three times.

The effects of genotype on the components of resistance were analyzed using Proc MIXED with ddfm = satterth option on the model statement (SAS v 9.3, SAS Institute Inc., Cary, NC). Fisher's protected LSD ($P \leq 0.05$) was used to determine significant differences in the components of resistance among genotypes. The correlation of these components from the growth chamber assays with stAUDPC and the final disease severity from the field experiments was determined by calculating the correlation coefficient at ($P \leq 0.05$) with the Proc CORR procedure.

Greenhouse evaluations. The same genotypes used in the components of resistance study were used for this study. A randomized complete block design was used with four replicates per genotype. The experiment was repeated twice. Peanut was planted as described previously and 3-

to 4-weeks after planting individual plants were replanted to planting cones. The plants were inoculated 5- to 6-weeks after planting by spraying leaves (with above described inoculum) until covered completely with the spore suspension. Effort was made to direct sprays primarily on undersides of the leaves. The inoculated plants were placed in a moist chamber constructed of PVC pipe and covered with black plastic, and incubated in darkness for 16 h at 25°C. Humidifiers were used to keep the leaves wet and the humidity high (> 90%). The youngest fully developed leaf was labeled and at 21 DAI, the labeled leaves were processed for ASSESS 2.0 analysis as described above. The components of resistance measured for the labeled leaves were infection frequency, lesion diameter and percent diseased area, and they were determined as described above.

The effects of genotype on the components of resistance were analyzed using Proc MIXED with ddfm = satterth option on the model statement (SAS v 9.3, SAS Institute Inc., Cary, NC). Fisher's protected LSD ($P \leq 0.05$) was used to determine significant differences in the components of resistance among genotypes.

Results

Field evaluations of rust resistance. There were significant year × genotype interactions for rust severity and stAUDPC among years, and the experiments in the different locations were not identical with respect to genotypes included, so each experiment was analyzed separately. Rust was present in both locations and in each experimental year, but severity of the epidemics varied among years and between locations. In Citra, rust was first observed approximately 90-95 DAP in 2010 through 2012, while in Tifton rust epidemics started 60 to 70 DAP in 2011 through 2013.

There were significant differences ($P \leq 0.05$) in stAUDPC and final rust severity among the genotypes (Table 2.2, Table 2.3). In Citra, the disease pressure was high in 2010 and 2012 due to favorable weather conditions. In 2011, disease pressure was low in Citra and Tifton, due to very dry and hot weather conditions. This resulted in few differences among genotypes for either final rust severity or stAUDPC. Moreover, the epidemic started too late in 2011 in Tifton so the final disease severity was too low to distinguish among genotypes. Due to wet and cold weather in Tifton in 2013, final disease severity was too low to distinguish resistant from susceptible genotypes. No immunity to rust was noted, as all genotypes were infected in all experimental years and in both locations.

In Citra, genotypes Tifrust-10, Tifrust-13 and PTBOL3-3 were among the genotypes with the lowest stAUDPC and final disease severity in all 3 years; however, they had high late leaf spot severity ratings (*Cercosporidium personatum*) (Table 2.2). CRSP breeding lines 97x36-HO2-1-B2G-3-1-2-2 and BOL3-7 appeared to be among the most resistant to both rust and late leaf spot, as these breeding lines had low stAUDPC and low final disease severity values for both diseases in all 3 years. Cultivars Altika and BayoGrande, and CRSP breeding line BOL19-b5 had the highest stAUDPC for rust in 2010 and 2012. These genotypes had high final leaf spot severity as well. In Tifton in 2012, Tifrust-10, Tifrust-13, PTBOL3-3, 97x36-HO2-1-B2G-3-1-2-2 and BOL3-7 had the lowest stAUDPC for rust (Table 2.3).

Components of rust resistance. Germination of the urediniospores was higher than 90% for all three trials. Pustules developed on all inoculated leaves, and no pustules were present on the control leaves in any of the repeated studies. There were significant trial × genotype interactions for infection frequency at 7 and 16 DAI, percent diseased area, lesion diameter and

latent period, so each experiment was analyzed separately. There were significant differences ($P \leq 0.05$) among the genotypes for all of the components measured in trial 1 (Table 2.4) and trial 2 (Table 2.5). In Differences among the genotypes were significant ($P \leq 0.05$) for infection frequency at 7, 11 and 16 DAI, percent diseased area, and latent period in the third trial, but not for lesion diameter ($P = 0.25$) (Table 2.6). In general, genotypes that had high infection frequencies at 7 DAI also had high infection frequencies at the end of the experiment, higher percent diseased area and shorter latent periods (Table 2.10). On the other hand, genotypes with longer latent periods generally had lower infection frequencies at 7, 11 and 16 DAI and smaller percent diseased areas. Genotypes Georgia-09B, NC3033, BayoGrande and C99R were among the genotypes with higher infection frequencies, larger percent diseased areas and shorter latent periods in most of the experimental trials (Table 2.4). In trials 2 and 3 (Table 2.5, Table 2.6), SPT-06-06, and Florida-07 also were among genotypes with higher infection frequencies, larger percent diseased areas and shorter latent periods. Tifrust-13, PI 568164, PI562530, Tarapoto, Georgia-03L, and GT-C20, typically were among those with lower infection frequencies, smaller percent diseased areas and longer latent periods.

Greenhouse evaluations of rust resistance. There were significant trial × genotype interactions for the components between repeated trials, so each experiment was analyzed separately. In all three trials, pustules developed on all inoculated leaves, except for Tarapoto in the third trial. There were no significant differences among genotypes for infection frequency ($P = 0.21$), lesion diameter ($P = 0.06$) and percent diseased area ($P = 0.08$) in the first trial. Differences among genotypes were significant for infection frequency ($P = 0.05$), but not for lesion diameter ($P = 0.31$) and percent diseased area ($P = 0.34$) in the second trail. In the third trial differences

among genotypes were significant for lesion diameter (P = 0.005) and percent diseased area (P = 0.009), but not for infection frequency (P = 0.34). Although not all genotype differences were significant, the trend was similar to that observed in the detached leaf studies, in which genotypes NC3033, Georgia-09B, and C99R were among those with the highest infection frequency 21 DAI in all three trials and percent diseased area in the first and third trial. Furthermore, Tarapoto, PI562530, PI568164, 99x33-1-B2G-2-2-2 were among genotypes with the lowest infection frequency and percent diseased area 21 DAI. Resistance ratings were inconsistent for field-resistant CRSP breeding lines BOL3-7, 97x36-HO2-1-B2G3-1-2-2 and 99x33-1-B2G-13-1-1, and rust-resistant standards Tifrust-13 and PI298115; infection frequencies and percent diseased areas were highly variable for these genotypes in all the experiment trials.

Correlations between field resistance and components of resistance. Infection frequencies at 7, 11 and 16 DAI were highly positively correlated ($P \leq 0.05$) with each other ($r > 0.8$) and infection frequencies at 7 and 16 DAI were also positively correlated with lesion diameter and percent diseased area ($P \leq 0.05$), but negatively correlated with latent period ($P \leq 0.05$). The general trend observed was that genotypes with short latent periods had high infection frequencies and large percent diseased area, whereas genotypes with long latent periods had low infection frequencies and small percent diseased areas. However, infection frequencies were not correlated ($P > 0.05$) with field observation. Standardized AUDPC was correlated with lesion diameter (P = 0.04) and percent diseased area (P = 0.02), however the correlation coefficient was low ($r \leq 0.21$). There were no correlations between final disease severity and any of the components ($P > 0.05$). Correlations between stAUDPC and final severity were high ($r > 0.8$, $P \leq 0.05$).

Discussion

In this study newly developed CRSP breeding lines with Bolivian background, commonly grown peanut cultivars in Georgia, and parents of existing breeding populations were compared for their response to peanut rust by determining the level of field resistance and components of resistance in growth chamber and greenhouse experiments. Peanut rust severity in the field varied each year. Development of epidemics depends on the introduction of inoculum, usually by tropical storms, and subsequent environmental conditions that are conducive for disease development, such as warm temperatures, rainfall and high humidity. This was the case in 2010 and 2012, but not in 2011 and 2013, in both Citra and Tifton.

In three experiments where the rust epidemics were sufficiently severe to distinguish resistance from susceptibility, breeding lines 97x36-HO2-1-B2G3-1-2-2, PT910-2-8-11, 99x33-1-B2G-13-1-1, BOL3-7, 99x8-1-B2G-3-1-1, PTBOL3-3 and 98x116-5-1-1-1-2-1 were among the genotypes with the lowest rust severity ratings. Of these, several were also among the genotypes with the lowest disease severity ratings for late leaf spot (*Cercosporidium personatum*), for example genotypes 97x36-HO2-1-B2G3-1-2-2, 99x33-1-B2G-13-1-1, BOL3-7 and 98x116-5-1-1-1-2-1. These genotypes show potential to be developed into cultivars, since they appear to have multiple disease resistance. There were a few genotypes that had low rust severity ratings, but high leaf spot severity ratings, with more than 90% defoliation at harvest. Although these show little promise for use for peanut production, they may of use in breeding for rust resistance. Tifrust-10 and Tifrust-13, two rust-resistant standards (7, 8), and PTBOL3-3, a CRSP breeding line, are examples of lines with resistance to rust but susceptibility to leaf spot. Genotypes Altika and BOL11-b7 had high severity ratings for both rust and leaf spot.

The environmental conditions (frequent rains and hot summer temperatures) led to very conducive conditions for peanut rust development in both Citra and Tifton in 2012, resulting in relatively high disease pressure by the end of the season. The resistance in some of the breeding lines was not as effective under this high disease pressure; however, breeding lines 97x36-HO2-1-B2G3-1-2-2, BOL3-7 and 98x116-5-1-1-1-2-1 maintained resistance in both Citra and Tifton in 2012. Breeding line PTBOL3-3 had a low final rust severity rate in Citra, but had one of the higher scores in Tifton in that same year.

Cultivars Southern Runner (2) and York (personal communications B. L. Tillman) are partially resistant to rust, and Tifguard is moderately resistant as well (personal communications A. K. Culbreath). Georgia-03L, a cultivar observed to have some resistance to rust in Nicaragua and Haiti (field experiments in 2010, personal communications T. B. Brenneman), appeared to be very susceptible in Tifton in 2012. Georgia-07W, a cultivar currently grown in Georgia with resistance to stem rot (*Sclerotium rolfsii*), did not appear to be resistant to rust, as it had high stAUDPC and final disease severity scores. Although there were correlations between field resistance and the components of resistance, the correlation coefficients were low. There are several factors that may explain the low or lack of correlation. One explanation may be that there are environmental factors in the field situations related to the growth habit of a genotype that may affect susceptibility of the genotypes to rust, and enhance or reduce disease development. For example, Tifrust-13 has a vine-like growth habit, which may enable it to escape spore deposition, and/or reduce the humidity necessary for successful peanut rust inoculations.

High values were obtained for infection frequency and percent disease area very early on in the growth chamber experiments; infection frequency in the majority of the genotypes reached

about 50% by 7 DAI, even though these pustules were very young at that time point. In contrast to these findings, Mehan et al. (9) and Subramanyam et al. (23) reported incubation periods (time from inoculation to 50% of the pustules developed) ranging from 8.5 or 9 days for the highly susceptible genotypes, to 18 or 19 days for the resistant ones. The differences between these results and theirs may be due to inconsistencies in the Tween concentration used to prepare the inoculum. Mehan et al. (9) used a 0.1% Tween solution and Subramanyam et al. (23) added "a few drops of Tween 80" to sterile water. In earlier experiments in this study (11) a 0.1% Tween 20 solution was used for the inoculum suspension and few lesions developed in the growth chamber studies at 20 and 31 DAI. These studies corroborate previous reports of rust resistance in genotypes such as Tarapoto, PI298115, PI568164, PI562530, Tifrust-10 and Tifrust-13 (7, 8, 17, 21). These genotypes were among those with the lower infection frequencies and percent disease areas, and smaller lesion diameters.

Several peanut mapping populations are being evaluated for multiple traits, including disease resistance (personal communications A. K. Culbreath). Information on the response of the parents to peanut rust would be beneficial, because these populations may then be screened for peanut rust resistance as well to enable the identification of QTLs for rust resistance. Together with the availability of microsatellite markers, this could enable marker-assisted breeding for peanut rust resistance. Eight parental lines were evaluated, but they were all among the genotypes with high infection frequencies at 16 DAI in the growth chamber studies.

In conclusion, these studies resulted in the identification of several genotypes with multiple disease resistance in different environments and under high disease pressure. Furthermore, these results indicate sources of rust resistance in the CRSP breeding lines, including several genotypes

that could be used as parents in peanut germplasm enhancement programs. These studies moreover indicate that latent period, percent diseased area and lesion diameter may be used as indicators for rust resistance in growth chamber studies.

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References

1. Bellgard, S., and Ham, K. C. 2004. Common diseases of peanuts in the top end of the NT. Agnote Agdex No: 141/633.
2. Black, M. C., and Smith, D. H. 1987. Spotted wilt and rust reactions in south Texas among selected peanut genotypes. Proc. Am. Peanut Res. Ed. Soc. 19:31 (Abstr.).
3. Bromfield, K. R. 1971. Peanut rust: a review of literature. Journal of American Peanut Research and Education Association 3:111-121.

4. Chiteka, Z. A., Kucharek, T. A., Knauft, D. A., Gorbet, D. W., and Shokes, F. M. 1988. Components of resistance to late leafspot in peanut. I. Levels and variability--implications for selection. Peanut Science 15:25-30.

5. Cook, M. 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.

6. Damicone, J., and Melouk, H. A. Foliar diseases of peanut. Oklahoma State University, Division of Agricultural Sciencesand Natural Resources, Oklahoma Cooperative Extension Service EPP-7655.

7. Hammons, R. O., Branch, W. D., Bromfield, K. R., Subrahmanyam, P., Rao, V. R., Nigam, S. N., Gibbons, R. W., and Goldin, E. 1982. Registration of Tifrust-13 peanut germplasm. Crop Sci. 22:697.

8. Hammons, R. O., Nigam, S. N., Gibbons, R. W., Subrahmanyam, P., and Rao, V. R. 1982. Registration of eight peanut germplasm lines resistant to rust. Crop Sci. 22:452-453.

9. Mehan, V. K., Reddy, P. M., Rao, K. V., and McDonald, D. 1994. Components of rust resistance in peanut genotypes. Phytopathology 84:1421-1426.

10. Melouk, H. A., and Shokes, F. M. 1995. Peanut rust. Page 72 in: Peanut Health Management. American Phytopathological Society Press, St. Paul, MN.

11. Power, I. L., Culbreath, A. K., and Tillman, B. L. 2013. Characterization of resistance of peanut to *Puccinia arachidis*. Plant Health Progress. doi:10.1094/PHP-2013-1125-02-RS.

12. Rankin, H. W. 1953. Peanut rust in Georgia. Plant Disease Reporter 37:528.

13. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

14. Smith, N. B., and Littrell, R. H. 1980. Management of peanut foliar diseases with fungicides. Plant Dis. 64:356-360.

15. Subrahmanyam, P. 1997. Rust. Pages 31-33 in: Compendium of Peanut Diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith and P. Subrahmanyam, eds. American Phytopathological Society, St. Paul, MN.

16. Subrahmanyam, P. 1995. Screening methods and sources of resistance to rust and late leaf spot of groundnut. Report No. 9290663197. Pages 1-14. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India.

17. Subrahmanyam, P., Hammons, R. O., Nigam, S. N., McDonald, D., Gibbons, R. W., Fan, M. Y., and Yeh, W. L. 1983. International cooperative screening for resistance of peanut to rust and late leaf spot. Plant Dis. 67:1108-1111.

18. Subrahmanyam, P., McDonald, D., Gibbons, R. W., and Rao, P. V. S. 1983. Components of resistance to *Puccinia arachidis.* . Phytopathology 73:253-256.

19. Subrahmanyam, P., McDonald, D., Gibbons, R. W., and Reddy, L. J. 1985. Peanut rust: A major threat to peanut production in the semiarid tropics. Plant Dis. 69:813-819.

20. Subrahmanyam, P., McDonald, D., and Rao, P. V. S. 1983. Influence of host genotype on uredospore production and germinability in *Puccinia arachidis*. Phytopathology:726-729.

21. Subrahmanyam, P., McDonald, D., Waliyar, F., Reddy, L. J., Nigam, S. N., Gibbons, R. W., Ramanatha Rao, V., Singh, A. K., Pande, S., Reddy, P. M., and Subba Rao, P. V. 1995. Screening methods and sources of resistance to rust and late leaf spot of groundnut. Pages 1-14 in: Information bulletin no. 47 International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India.

22. Subrahmanyam, P., Rao, V. R., McDonald, D., Moss, J. P., and Gibbons, R. W. 1989. Origins of resistances to rust and late leaf spot in peanut (*Arachis hypogaea*, Fabaceae). Econ. Bot. 43:444-455.

23. Subrahmanyam, P., Subba Rao, P. V., Gibbons, R. W., and McDonald, D. 1983. Components of resistance to *Puccinia arachidis* in peanuts Phytopathology 73:253-256.

24. Van Arsdel, E. P., and Harrison, A. L. 1972. Possible origin of peanut rust epidemics in Texas. Phytopathology 62:794.

25. Williams-Woodward, J. 2011. Georgia Plant Disease Loss Estimates 2011. The University of Georgia Cooperative Extension Service, Athens, GA.

26. Wynne, J. C., Beute, M. K., and Nigam, S. N. 1991. Breeding for disease resistance in peanut (*Arachis hypogaea* L). Annu. Rev. Phytopathol. 29:279-303.

Table 2.1. Characteristics of the peanut genotypes studied in field and growth chamber experiments

Genotype	Origin/background	Status/generation	Experiment[a]	
96x72-HO1-9-1-1-1-1-2-1	((89xOL2-)x(84x28-))	CRSP UF150	F (Citra, Tifton)	
97x34-HO3-1-B2G-3-1-1-1	[(89xOL28-)x(87x8-)]	CRSP UF150	F (Citra, Tifton)	
97x36-HO2-1-B2G-3-1-2-2	((89xOL28-)xBayoGrande)	CRSP UF150	F (Citra, Tifton)	C
98x116-5-1-1-1-2-1	((DP-1)x(89xOL28-))	CRSP UF150	F (Citra, Tifton)	
99x33-1-B2G-12-2-1	BayoGrandexHull	CRSP UF150	F (Citra, Tifton)	C
99x33-1-B2G-13-1-1	BayoGrandexHull	CRSP UF150	F (Citra, Tifton)	C
99x33-1-B2G-2-2-2	BayoGrandexHull	CRSP UF150	F (Citra, Tifton)	C
99x8-1-B2G-3-1-1	BayoGrandexHull	CRSP UF150	F (Citra, Tifton)	
Altika	F393-7-1xGeorgia 119-20	Cultivar	F (Citra)	C
Bailey High O/L	USA	Released cultivar/RIL parent		C
BayoGrande	Bolivia	Landrace	F (Citra, Tifton)	C
BOL11-b7	Overo Chiquitano/(F84x23)	CRSP UF150	F (Citra)	
BOL19-b5	FLMDR98/BG	CRSP UF150	F (Citra)	
BOL20-b5	FLMDR98/BG	CRSP UF150	F (Citra)	
BOL21-b5	FLMDR98/BG	CRSP UF150	F (Citra)	
BOL22-b5	FLMDR98/BG	CRSP UF150	F (Citra)	
BOL3-7	(F79x4)/PI656458	CRSP UF150	F (Citra, Tifton)	C
C-99R	USA	Released cultivar		C
DP-1	(80202x81206]	CRSP UF150	F (Citra)	
Florida-07	USA	Released cultivar/RIL parent	F (Tifton)	C
Georgia-03L	USA	Released cultivar	F (Tifton)	C
Georgia06G	USA	Cultivar		C
Georgia-07W	USA	Released cultivar	F (Tifton)	C

Table 2.1. continued

Genotype	Origin/background	Status/generation	Experiment	
Georgia-09B	USA	Cultivar		C
GT-C20	China	Breeding line		C
Guyana Jumbo	Guyana	Cultivar		C
NC3033	USA	Released germplasm/RIL parent		C
NC94022	USA	Breeding line/RIL parent		C
PI298115	Israel	Plant introduction		C
PI562530	India	Plant introduction		C
PI568164	India	Plant introduction		C
PT910-2-8-11	VA98R/BayoGrande	CRSP UF150	F (Citra)	
PTBOL3-3	(F79x4)/PI656458	CRSP UF150	F (Citra, Tifton)	
PTBOL3-4	(F79x4)/PI656458	CRSP UF150	F (Citra, Tifton)	
RP-97F2-B-9-2-2-1-b3-B	[MDR98xBGrande]	CRSP UF150	F (Citra)	
Southern Runner	USA	Released cultivar	F (Citra, Tifton)	C
SPT-06-06	USA	RIL parent		C
SunOleic 97R	USA	Released cultivar/RIL parent		C
Tarapoto	Peru	Plant introduction		C
Tifguard	USA	Released cultivar	F (Tifton)	C
Tifrunner	USA	Released cultivar/RIL parent		C
Tifrust-10		Tifton ARS	F (Citra, Tifton)	
Tifrust-13		Tifton ARS	F (Citra, Tifton)	C
York	USA	Released cultivar	F (Citra, Tifton)	C

[a] F = field experiments and C = components of resistance study.

Table 2.2. Disease response of peanut genotypes to rust and leaf spot in Citra, Florida (FL) in 2010, 2011 and 2012

Genotype	stAUDPC [a]			Final severity [b]			Leaf spot severity [c]		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
96x72-HO1-9-1-1-1-1-2-1	29.35 e-i	11.82 c-f	52.12 f-l	3.08 d-g	2.00 d-h	3.25 c-f	3.50 f-i	3.92 f-i	4.58 g-l
97x34-HO3-1-B2G-3-1-1-1	23.25 g-i	13.29 b-f	55.12 d-k	2.50 e-i	2.42 b-e	3.42 b-f	2.92 g-i	4.00 e-i	4.25 i-m
97x36-HO2-1-B2G-3-1-2-2	15.83 i	8.79 e-f	46.49 j-l	1.58 g-i	1.42 f-h	2.25 g-i	2.33 i	3.00 i	3.25 m-n
98x116-5-1-1-1-2-1	30.98 e-i	15.50 a-d	54.08 e-k	2.75 e-i	2.83 a-d	2.75 e-g	2.42 h-i	3.67 h-i	3.00 n
99x33-1-B2G-2-2-2	30.40 e-i	14.43 b-e	53.29 e-k	2.75 e-i	2.83 a-d	3.25 c-f	2.42 h-i	3.83 g-i	4.00 j-n
99x33-1-B2G-12-2-1	36.38 d-g	14.33 b-e	66.59 b-f	2.92 d-h	2.33 c-f	3.25 c-f	2.75 g-i	4.08 e-i	3.83 k-n
99x33-1-B2G-13-1-1	26.71 f-i	11.62 c-f	63.90 b-g	2.25 f-i	2.08 c-h	3.33 c-f	2.33 i	3.58 h-i	3.83 k-n
99x8-1-B2G-3-1-1	32.00 e-h	16.35 a-c	63.65 b-h	2.25 f-i	3.00 a-c	3.75 a-d	2.33 i	3.67 h-i	4.25 i-m
Altika	43.79 b-e	9.91 d-f	112.89 a	3.58 c-f	1.67 f-h	4.17 a-c	6.17 c-d	6.50 a	7.50 a
BayoGrande	50.92 a-d	21.27 a	67.90 b-e	3.92 a-e	3.67 a	3.58 a-e	3.08 g-i	3.58 h-i	4.42h-l
BOL11-b7	58.52 ab	11.73 c-f	75.76 b-c	5.42 a	1.75 e-h	3.92 a-c	5.67 c-e	6.08 a-b	6.58 a-c
BOL19-b5	61.42 a	13.31 b-f	77.62 b	5.42 a	2.42 b-e	4.42 a	4.75 e-f	4.67 d-h	5.50 d-g
BOL20-b5	58.44 ab	10.27 d-f	74.88 b-c	5.17 a-c	2.00 d-h	4.08 a-c	5.75 c-e	4.08 e-i	5.00 f-j
BOL2b5	49.35 a-d	13.42 b-f	56.74 d-j	4.42 a-d	2.08 c-h	3.58 a-e	5.33 d-e	4.67 d-h	5.17 f-i
BOL22b5	52.54 a-c	12.19 b-f	69.53 b-d	5.25 a-b	2.25 c-g	4.33 a-b	5.42 d-e	4.08 e-i	5.33 e-h
BOL3-7	17.83 h-i	8.79 e-f	48.78 h-l	2.25 f-i	1.42 f-h	2.50 f-h	2.92 g-i	4.92 c-g	4.25 i-m
DP1	38.83 c-f	18.01 a-b	47.72 i-l	3.00 d-g	3.33 a-b	2.92 d-g	4.00 f-g	4.17 e-h	4.33 h-l
PT910-2-8-11	40.44 c-f	12.85 b-f	48.44 i-l	2.67 e-i	1.83 e-h	2.25 g-i	6.08 c-d	5.08 b-e	5.83 c-f
PTBOL3-3	25.35 f-i	7.69 f	49.19 g-l	1.67 g-i	1.33 g-h	1.50 i-j	6.92 b-c	5.67 a-d	6.42 b-d
PTBOL3-4	33.00 e-g	12.35 b-f	50.21 g-l	3.42 d-f	1.92 d-h	3.67 a-e	3.83 f-g	4.92 c-g	5.17 f-i
RP-97F2-B-9-2-2-1-b3-B	37.63 c-g	11.82 c-f	61.75 c-i	2.92 d-h	1.92 d-h	3.83 a-d	3.75 f-g	5.08 b-e	3.67 l-n
Southern Runner	32.25 e-h	13.96 b-e	53.70 e-k	2.67 e-i	2.08 c-h	2.58 f-h	5.92 c-e	5.00 b-f	6.33 b-e

Table 2.2. continued

Genotype	stAUDPC [a,d]			Final severity [b,d]			Leaf spot severity [c,d]		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
Tifrust-10	23.50 g-i	12.87 b-f	41.72 k-l	1.33 g-i	2.00 d-h	1.67 h-j	8.42 a	5.83 a-c	7.25 a-b
Tifrust-13	16.38 i	7.67 f	37.88 l	1.25 i	1.17 h	1.00 j	8.00 a-b	6.67 a	7.25 a-b
York	36.02 d-g	10.64 c-f	56.45 d-k	3.67 b-f	2.25 c-g	3.58 a-e	3.67 f-h	4.17 e-h	4.83 f-k
LSD ($P \leq 0.05$)	15.15	6.02	14.94	1.63	0.94	0.99	1.30	1.11	1.07

[a] Means of standardized area under the disease progress curve (stAUDPC) of 3 replications.
[b] Means of final rust severity of 3 replications, assessed using the 1-9 ICRISAT scale.
[c] Means of final leaf spot severity of 3 replications, assessed using the 1-10 ICRISAT (2010) or 1-10 Florida scale (2011 and 2012).
[d] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.3. Disease response of peanut genotypes to rust in Tifton, Georgia (GA) in 2011, 2012 and 2013

Genotype	stAUDPC [a, c]			Final Severity [b, c]		
	2011	2012	2013	2011	2012	2013
96x72-HO1-9-1-1-1-1-2-1	12.67 b-d	68.90 b-e	18.05 a-d	1.92 a-f	3.92 d-g	1.81 a-d
97x34-HO3-1-B2G-3-1-1-1	15.75 a-b	82.99 a-d	19.76 a	2.25 a-d	4.83 b-e	2.19 a
97x36-HO2-1-B2G-3-1-2-2	12.27 c-d	50.68 e	16.30 c-f	1.67 c-f	2.42 g	1.44 c-f
98x116-5-1-1-1-2-1	16.13 a	84.75 a-c	20.53 a	2.50 a	4.83 b-e	1.94 a-b
99x33-1-B2G-2-2-2	11.10 c-e	97.60 a	19.29 a-c	1.92 a-f	5.00 b-d	1.81 a-d
99x33-1-B2G-12-2-1	12.15 c-d	84.15 a-c	19.27 a-c	2.42 a-b	4.83 b-e	1.88 a-c
99x33-1-B2G-13-1-1	11.60 c-e	70.72 b-e	16.37 c-f	1.50 e-f	4.50 b-f	1.69 b-e
99x8-1-B2G-3-1-1	16.06 a	86.51 a-c	18.03 a-e	2.33 a-c	5.00 b-d	1.69 b-e
BayoGrande[d]	.	83.25 a-d	20.53 a	.	4.25 c-g	1.94 a-b
BOL3-7	9.79 d-e	50.97 e	16.31 c-f	1.67 c-f	2.42 g	1.44 c-f
PTBOL3-3	11.92 c-d	86.24 a-c	15.89 d-f	1.58 e-f	5.00 b-d	1.31 e-f
PTBOL3-4	10.10 d-e	55.12 e	15.89 d-f	1.75 b-f	3.25 d-g	1.31 e-f
Southern Runner	13.60 a-c	61.54 c-e	17.57 a-e	2.17 a-e	2.92 f-g	1.56 b-f
Tifrust-10	10.10 d-e	57.74 d-e	18.01 a-e	1.67 c-f	3.08 e-g	1.63 b-e
Tifrust-13	8.77 e	49.28 e	15.03 e-f	1.25 f	3.17 d-g	1.13 f
York	10.69 c-e	72.43 a-e	14.67 e	1.58 e-f	3.92 d-g	1.38 d-f
Florida-07	12.00 c-d	89.85 a-b	16.72 c-f	1.75 b-f	6.33 a-b	1.44 c-f
Georgia-03L	11.35 c-e	90.29 a-b	16.73 b-f	1.83 a-f	7.58 a	1.56 b-f
Georgia 07W	11.06 c-e	83.66 a-d	19.21 a-c	1.83 a-f	5.92 a-c	1.56 b-f
Tifguard	12.02 c-d	61.75 c-e	19.71 a-b	1.92 a-f	3.58 d-g	2.00 a-b
LSD ($P \leq 0.05$)	3.12	26.09	3.01	0.70	1.88	0.49

[a] Means of standardized area under the disease progress curve (stAUDPC) of 3 replications.
[b] Means of final rust severity of 3 replications in 2011 and 2012, and 4 replications in 2013, assessed using the 1-9 ICRISAT scale.
[c] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.
[d] Genotype BayoGrande was not included in the experiments in 2011.

Table 2.4. Components of peanut rust resistance from growth chamber detached leaf assays, trial 1

Genotype	Infection frequency 7 DAI [a, e]	Infection frequency 16 DAI [b, e]	Lesion diameter 16 DAI (mm) [c, e]	Percent diseased area 16 DAI (%) [d, e]
Altika	6.87 a-c	9.92 a	0.70 g	9.05 a-f
Bailey high O/L	3.57e-f	6.67 c-g	0.78 c-g	6.41 c-g
BayoGrande	6.51 a-d	7.97 a-e	0.79 c-g	7.36 b-g
BOL3-7	7.42 a-b	9.18 a-c	0.78 d-g	9.13 a-f
C99R	7.95 a	6.33 c-g	0.88 b-e	11.56 a-b
97x36-HO2-1-B2G-3-1-2-2	3.45 e-f	5.70 d-g	0.78 d-g	4.71 f-g
99x33-1-B2G-12-2-1	5.83 a-e	6.82 b-g	0.85 b-g	9.63 a-e
99x33-1-B2G-13-1-1	3.69 e-f	5.38 e-g	0.86 b-f	6.87 c-g
99x33-1-B2G-2-2-2	5.25 a-e	6.30 d-g	0.75 e-g	5.57 e-g
Florida-07	4.43 c-e	5.73 d-g	0.84 b-g	7.60 b-g
Georgia 03L	4.62 b-e	5.74 d-g	0.85 b-g	8.57 a-g
Georgia 06G	1.62 f	2.37 h	1.14 a	4.68 f-g
Georgia 07W	5.19 a-e	6.27 d-g	0.92 b-d	9.62 a-e
Georgia 09B	7.43 a	8.08 a-e	0.94 b-c	12.34 a
GT-C20	5.96 a-e	8.49 a-d	0.82 b-g	7.22 b-g
NC3033	7.63 a	8.27 a-d	0.85 b-g	9.49 a-e
NC94022	6.72 a-c	7.89 a-e	0.81 b-g	8.30 a-g
PI298115	4.62 b-e	6.82 b-g	0.85 b-g	5.82 d-g
PI562530	1.49 f	4.69 g-h	0.71 f-g	4.20 g
PI568164	3.75 d-f	5.03 f-h	0.96 b	10.08 a-d
Southern Runner	5.90 a-e	7.66 a-f	0.83 b-g	10.86 a-c
SPT-06-06	6.14 a-e	8.10 a-e	0.83 b-g	9.18 a-f
SunOleic 97R	5.74 a-e	7.70 a-f	0.73 e-g	6.89 c-g
Tarapoto	3.53 e-f	6.13 d-g	0.80 c-g	5.76 d-g
Tifguard	4.50 c-e	6.91 b-g	0.78 c-g	7.75 b-g
Tifrust-13	4.62 b-e	7.35 a-g	0.78 d-g	8.56 a-g
Tifrunner	7.58 a	9.59 a-b	0.80 c-g	9.06 a-f
LSD	2.81	2.85	0.15	4.50

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[c] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[d] Means of percent diseased area (%) of 3 replications, 16 DAI.
[e] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.5. Components of peanut rust resistance from growth chamber detached leaf assays, trial 2

Genotype	Infection frequency 7 DAI [a, g]	Infection frequency 11 DAI [b, g]	Infection frequency 16 DAI [c, g]	Lesion diameter 16 DAI (mm) [d, g]	Percent diseased area 16 DAI (%) [e, g]	Latent period (days) [f, g]
Altika	6.32 a-e	7.80 a-d	8.5 a-d	0.87 c-i	7.03 d-f	9.00 e
Bailey high O/L	5.15 a-h	6.90 a-e	7.94 a-e	0.97 a-f	11.18 a-c	9.00 e
BayoGrande	5.05 a-h	6.83 a-f	7.47 b-f	0.99 a-f	7.26 c-f	9.00 e
BOL3-7	3.18 h-j	4.35 f-g	5.22 f-g	1.01 a-e	5.64 e-h	9.33 d-e
C99R	5.91 a-g	8.2 a-b	7.92 a-e	0.97 a-g	6.52 d-g	9.00 e
97x36-HO2-1-B2G-3-1-2-2	4.58 b-h	5.67 c-g	6.1 d-g	0.93 a-h	5.98 d-h	9.00 e
99x33-1-B2G-12-2-1	6.24 a-e	7.26 a-d	8.13 a-e	0.77 g-i	7.54 c-f	9.00 e
99x33-1-B2G-13-1-1	3.95 d-i	5.43 d-g	6.36 c-g	1.07 a-c	8.27 b-f	9.33 d-e
99x33-1-B2G-2-2-2	5.83 a-g	6.95 a-e	7.5 b-ef	1.05 a-c	9.4 b-e	9.00 e
Florida-07	6.80 a-b	7.79 a-d	8.49 a-d	0.84 d-i	9.2 b-e	9.00 e
Georgia-03L	3.70 e-j	5.42 d-g	6.37 c-g	1.11 a	6.23 d-g	9.33 d-e
Georgia-06G	7.64 a	9.04 a	9.96 a	1.02 a-d	11.94 a-b	9.00 e
Georgia-07W	6.46 a-d	7.25 a-d	7.78 a-e	0.90 b-h	7.77 c-f	9.00 e
Georgia-09B	5.42 a-h	6.98 a-e	7.5 b-f	1.08 a-b	13.41 a	9.00 e
Guyana Jumbo	7.36 a	6.34 b-f	8.04 a-e	0.95 a-h	7.41 c-f	9.00 e
GT-C20	3.34 g-j	5.46 d-g	7.02 b-f	0.93 a-h	8.35 b-f	10.00 b-c
NC3033	5.35 a-h	5.68 b-g	6.00 e-g	0.88 b-i	7.49 c-f	9.00 e
NC94022	6.64 a-c	8.17 a-c	7.90 a-e	1.03 a-d	9.71 a-d	9.33 d-e
PI298115	3.55 f-j	4.68 e-g	6.77 b-f	0.92 a-h	4.55 f-h	9.67 c-d
PI562530	1.09 j	1.77 h	4.00 g	0.83 d-i	3.01 g-h	10.67 a
PI568164	4.04 c-i	6.06 b-g	7.02 b-f	0.91 a-h	7.51 c-f	10.00 b-c
Southern Runner	6.37 a-e	7.62 a-d	8.80 ab	0.93 a-h	8.75 b-e	9.00 e
SPT-06-06	7.16 a-b	8.94 a	9.99 a	0.81 e-i	12.1 a-b	9.00 e
SunOleic 97R	5.60 a-h	6.32 b-f	7.15 b-f	0.82 e-i	6.59 d-g	9.00 e
Tarapoto	5.32 a-h	6.81 a-f	7.89 a-e	1.00 a-e	7.94 c-f	9.67 c-d
Tifguard	6.08 a-f	6.93 a-e	7.58 a-f	0.96 a-h	8.99 b-e	9.00 e
Tifrust-13	1.77 i-j	3.56 g-h	4.17 g	0.70 i	2.16 h	10.33 a-b
Tifrunner	5.77 a-h	6.56 a-f	7.85 a-e	0.76 h-i	7.21 d-f	9.00 e
York	5.23 a-h	7.72 a-d	8.53 a-c	0.79 f-i	7.25 d-f	9.00 e
LSD	2.65	2.52	2.42	0.20	3.93	0.66

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%)of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.6. Components of peanut rust resistance from growth chamber detached leaf assays, trial 3

Genotype	Infection frequency 7 DAI [a, g]	Infection frequency 11 DAI [b, g]	Infection frequency 16 DAI [c, g]	Lesion diameter 16 DAI (mm) [d, g]	Percent diseased area 16 DAI (%) [e, g]	Latent period (days) [f, g]
Altika	4.56 b-f	5.58 a-d	6.52 a-d	0.90 b-d	13.19 a-c	8.33 f-g
Bailey high O/L	3.48 c-h	4.28 b-h	5.66 b-g	0.96 b-d	8.85 b-gh	10.00 c-e
Bayo Grande	3.33 d-h	5.07 a-e	6.1 a-e	1.01 b-d	9.08 b-g	9.00 d-g
BOL3-7	2.85 f-j	3.96 c-i	5.33 b-g	0.92 b-d	7.57 d-i	8.33 f-g
C99R	4.48 b-f	6.24 a-b	6.97 a-c	0.97 b-d	12.53 a-d	9.67 d-f
97x36-HO2-1-B2G-3-1-2-2	4.59 b-f	5.07 a-e	5.83 b-f	1.12 a-b	11.72 a-d	9.00 d-g
99x33-1-B2G-12-2-1	4.10 b-g	5.39 a-e	6.25 a-d	1.06 a-d	11.55 a-d	8.00 g
99x33-1-B2G-13-1-1	3.84 b-h	4.57 a-g	5.26 b-g	0.87 c-d	9.63 b-f	8.00 g
99x33-1-B2G-2-2-2	3.68 c-h	5.31 a-e	6.38 a-d	1.12 a-b	12.2 a-d	8.00 g
Florida-07	5.13a-d	5.98 a-c	7.13 a-b	0.92 b-d	12.75 a-d	8.00 g
Georgia-03L	2.89 e-j	3.55 d-i	3.85 e-h	1.00 b-d	5.26 e-i	9.33 d-g
Georgia-06G	5.6 a-b	6.49 a	7.18 a-b	1.10 a-c	11.13 a-d	8.00 g
Georgia-07W	3.88 b-h	5.83 a-c	6.42 a-d	1.03 a-d	11 b-d	8.00 g
Georgia-09B	6.94 a	6.29 a-b	8.39 a	0.90 b-d	16.31 a	8.00 g
GT-C20	3.96 b-g	4.9 a-f	6.08 b-e	1.00 b-d	10.51 b-e	8.67 e-g
NC3033	4.70 b-e	5.82 a-c	6.72 a-c	1.10 a-c	13.84 ab	8.00 g
NC94022	2.29 g-j	3.38 e-i	4.41 d-h	0.91 b-d	5.51 e-i	11.33 a-c
PI298115	2.43 g-j	4.03 c-i	4.93 b-h	0.83 d	4 g-i	11.67 a-b
PI562530	1.46 i-j	2.81 f-i	3.38 g-h	0.96 b-d	3.77 h-i	12.00 a
PI568164	1.12 j	2.21 h-i	2.72 h	1.08 a-c	3.23 i	12.00 a
Southern Runner	4.26 b-f	5.80 a-c	6.56 a-d	1.03 a-d	12.51 a-d	8.00 g
SPT-06-06	5.19 a-c	5.90 a-c	6.70 a-d	0.90 b-d	9.48 b-f	8.00 g
SunOleic 97R	3.50 c-h	4.81 a-g	6.23 a-d	1.03 a-d	14.16 a-b	8.33 f-g
Tarapoto	2.08 h-j	2.73 g-i	3.59 f-h	0.97 b-d	4.60 f-i	10.33 b-d
Tifguard	3.20 e-i	4.20 b-h	5.29 b-g	1 bcd	10.11 b-e	10.33 b-d
Tifrust-13	1.19 j	2.03 i	3.53 g-h	0.84 d	10.33 b-e	12.00 a
Tifrunner	4.46 b-f	5.7 a-d	6.65 a-d	0.98 b-d	12.79 a-d	9.00 d-g
York	3.79 b-h	4.45 a-g	4.68 c-h	1.25 a	8.29 c-i	8.00 g
LSD	1.83	2.16	2.30	0.25	5.31	1.37

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%)of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.7. Components of peanut rust resistance from greenhouse studies, trial 1

Genotype	Infection frequency 21 DAI [a, d]	Percent diseased area 21 DAI (%) [b, d]	Lesion diameter 21 DAI (mm) [c, d]
Altika	4.45 a-c	4.76 a-d	0.92 a-b
Bailey high O/L	3.01 b-c	4.08 b-d	0.97 a
BayoGrande	6.14 a-b	4.47 a-d	0.93 a-b
BOL3-7	6.41 a-b	5.25 a-d	0.80 a-c
C99R	5.58 a-c	6.46 a-c	0.93 a-b
97x36-HO2-1-B2G-3-1-2-2	4.72 a-c	2.98 c-d	0.79 a-c
99x33-1-B2G-13-1-1	4.70 a-c	4.51 a-d	0.93 a-b
99x33-1-B2G-2-2-2	1.82 b-c	2.08 c-d	0.67 a-d
Florida-07	3.00 b-c	3.82 b-d	0.91 a-b
Georgia-03L	3.56 b-c	3.12 c-d	1.00 a
Georgia-07W	2.71 b-c	2.02 c-d	0.91 a-b
Georgia-09B	6.23 a-b	9.68 a	1.02 a
GT-C20	5.20 a-c	4.60 a-d	0.81 a-c
NC3033	9.24 a	8.75 a-b	0.88 a-b
NC94022	3.77 b-c	3.94 b-d	0.75 a-d
PI298115	4.18 b-c	2.87 c-d	0.71 a-d
PI562530	2.58 b-c	1.34 c-d	0.44 c-d
PI568164	1.75 b-c	0.85 d	0.38 d
Southern Runner	2.35 b-c	2.17 c-d	1.01 a
SPT-06-06	3.66 b-c	4.03 b-d	0.67 a-d
SunOleic 97R	6.07 a-b	4.71 a-d	0.88 a-b
Tarapoto	0.70 c	1.32 c-d	0.58 b-d
Tifguard	4.18 b-c	3.38 c-d	0.83 ab
Tifrust-13	5.37 a-c	4.10 b-d	0.84 a-b
Tifrunner	2.85 b-c	2.72 c-d	0.96 a
LSD	5.01	5.30	0.38

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 21 days after inoculation (DAI).
[b] Means of percent diseased area (%) of 3 replications, 21 DAI.
[c] Means of lesion diameter (mm) of 3 replications, 21 DAI.
[d] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.8. Components of peanut rust resistance from greenhouse studies, trial 2

Genotype	Infection frequency 21 DAI [a, d]	Percent diseased area 21 DAI (%) [b, d]	Lesion diameter 21 DAI (mm) [c, d]
Altika	2.36 d-g	0.90 c-e	0.60 a-e
Bailey high O/L	0.96 g	0.44 d-e	0.32 e
Bayo Grande	5.59 a-e	3.12 a-e	0.70 a-d
BOL3-7	1.46 f-g	0.61 c-e	0.61 a-e
C99R	3.68 c-g	4.26 a-e	0.51 c-e
97x36-HO2-1-B2G-3-1-2-2	4.02 b-g	3.8 a-e	0.73 a-d
99x33-1-B2G-12-2-1	4.34 b-g	1.89 b-e	0.69 a-d
99x33-1-B2G-13-1-1	8.93 a	4.83 a-d	0.67 a-d
99x33-1-B2G-2-2-2	1.77 e-g	0.96 c-e	0.70 a-d
Florida-07	2.67 c-g	1.74 b-e	0.51 c-e
Georgia-03L	3.34 c-g	5.46 a-b	0.82 a-b
Georgia-06G	6.52 a-c	4.77 a-d	0.83 a-b
Georgia-07W	2.36 d-g	1.23 b-e	0.70 a-d
Georgia-09B	2.44 c-g	1.32 b-e	0.49 d-e
GT-C20	2.75 c-g	2.53 a-e	0.78 a-d
NC3033	3.50 c-g	1.75 b-e	0.71 a-d
NC94022	2.68 c-g	1.09 b-e	0.70 a-d
PI298115	6.44 a-d	4.98 a-c	0.73 a-d
PI562530	3.66 c-g	2.42 a-e	0.66 a-d
PI568164	3.37 c-g	2.18 b-e	0.80 a-c
Southern Runner	5.29 a-f	2.93 a-e	0.62 a-e
SPT-06-06	6.42 a-d	4.24 a-e	0.73 a-d
SunOleic 97R	7.91 a-b	6.88 a	0.87 a
Tarapoto	0.50 g	0.09 e	0.54 b-e
Tifguard	4.54 b-g	2.71 a-e	0.78 a-d
Tifrust-13	2.92 c-g	2.02 b-e	0.66 a-d
Tifrunner	2.88 c-g	1.95 b-e	0.77 a-d
York	4.32 b-g	3.82 a-e	0.72 a-d
LSD	4.09	4.49	0.31

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 21 days after inoculation (DAI).
[b] Means of percent diseased area (%) of 3 replications, 21 DAI.
[c] Means of lesion diameter (mm) of 3 replications, 21 DAI.
[d] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.9. Components of peanut rust resistance from greenhouse studies, trial 3

Genotype	Infection frequency 21 DAI [a, d]	Percent diseased area 21 DAI (%) [b, d]	Lesion diameter 21 DAI (mm) [c, d]
Altika	1.96 a-e	1.42 b-e	0.89 a-c
Bailey high O/L	1.38 b-f	0.88 d-e	0.81 a-c
Bayo Grande	1.23 d-f	0.64 e	0.76 a-c
BOL3-7	1.26 d-f	0.66 e	0.72 b-c
C99R	3.20 a-c	3.46 a-c	0.88 a-c
97x36-HO2-1-B2G-3-1-2-2	1.96 a-e	1.28 b-e	0.75 a-c
99x33-1-B2G-12-2-1	2.08 a-e	1.43 b-e	0.74 a-c
99x33-1-B2G-13-1-1	1.94 a-e	0.92 d-e	0.76 a-c
99x33-1-B2G-2-2-2	2.58 a-e	0.86 d-e	0.69 b-d
Florida-07	1.37 c-f	0.91 d-e	0.79 a-c
Georgia-03L	1.78 a-f	2.24 b-e	0.93 a-b
Georgia-06G	1.43 a-f	1.08 d-e	0.6 c-d
Georgia-07W	2.84 a-d	1.97 b-e	0.73 b-c
Georgia-09B	3.32 a	3.53 a-b	0.89 a-c
GT-C20	1.62 a-f	0.94 d-e	0.73 b-c
NC3033	3.29 a-b	5.18 a	1.04 a
NC94022	1.33 c-f	1.42 b-e	0.68 b-d
PI298115	1.85 a-f	3.10 a-d	0.59 c-e
PI562530	0.83 e-f	0.30 e	0.3 e-f
PI568164	1.50 a-f	1.6 b-e	0.89 a-c
Southern Runner	2.03 a-e	1.41 b-e	0.74 a-c
SPT-06-06	1.78 a-f	1.05 d-e	0.79 a-c
SunOleic 97R	1.55 a-f	1.44 b-e	0.4 d-e
Tarapoto	0 f	0 e	0 f
Tifguard	1.63 a-f	1.18 c-e	0.88 a-c
Tifrust-13	0.71 e-f	0.55 e	0.65 b-d
Tifrunner	1.54 a-f	1.16 c-e	0.81 a-c
York	2.22 a-e	1.36 b-e	0.85 a-c
LSD	1.91	2.35	0.30

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 21 days after inoculation (DAI).
[b] Means of percent diseased area (%) of 3 replications, 21 DAI.
[c] Means of lesion diameter (mm) of 3 replications, 21 DAI.
[d] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2.10. Pearsons correlation coefficients between stAUDPC values, final peanut rust severity ratings and peanut rust resistant components of 29 peanut genotypes

	stAUDPC [a]	Final rust severity [a]	Infection frequency 7 DAI [b]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [b]	Lesion diameter 16 DAI (mm) [b]	Percent diseased area 16 DAI (%) [b]	Latent period 16 DAI (days) [b]
stAUDPC [c]								
Final rust severity [d]	0.82332							
	<.0001							
Infection frequency 7 DAI [e]	0.01115	0.16315						
	0.9026	0.0714						
Infection frequency 11 DAI [f]	-0.02881	0.17309	0.88408					
	0.7947	0.1154	<.0001					
Infection frequency 16 DAI [g]	-0.02233	0.11393	0.81339	0.95774				
	0.8064	0.2096	<.0001	<.0001				
Lesion diameter 16 DAI (mm) [h]	0.18408	0.14636	-0.20307	-0.1406	-0.23475			
	0.0424	0.1077	0.0249	0.2049	0.0093			
Percent diseased area 16 DAI (%) [i]	0.20585	-0.00174	0.24858	0.25668	0.3881	0.20915		
	0.0229	0.9848	0.0058	0.0192	<.0001	0.0208		
Latent period (days) [j]	-0.20342	-0.15673	-0.30198	-0.31482	-0.28392	-0.31262	-0.34714	
	0.0635	0.1545	0.0052	0.0035	0.0089	0.004	0.0013	

[a] Observations from field experiments.
[b] Observations from components of resistance study.
[c] Standardized area under disease progress curve (stAUDPC).
[d] Final peanut rust severity rating using the I-9 ICRISAT scale.
[e] Number of lesions per leaf area (cm^2), 7 days after inoculation (DAI).
[f] Number of lesions per leaf area (cm^2), 11 DAI.
[g] Number of lesions per leaf area (cm^2), 16 DAI.

[h] Lesion diameter (mm), 16 DAI.
[i] Percent diseased area (%), 16 DAI.
[j] Number of DAI until at least one lesion produced spores.
[k] The top number is the Pearson's correlation coefficient (*r* value) and the bottom number is the level of significance (*P* value).

CHAPTER 3

THE INFLUENCE OF HOST DEVELOPMENTAL STAGE ON PEANUT RUST RESISTANCE [1]

[1] Power, I. L., Tillman, B. L., and Culbreath, A. K. 2014. To be submitted to *Phytopathology*.

Abstract

The response of peanut genotypes at different developmental stages on infection by the rust pathogen (*Puccinia arachidis*) was evaluated in this study. The results indicate that peanut rust resistance is related to the developmental stage of the peanut plant. The general trends were that the youngest fully expanded leaves of 12-week-old plants were more resistant than the youngest fully expanded leaves of 3- or 6-week-old peanut. Although these trends were similar for resistant and susceptible peanut genotypes, the susceptible cultivar Altika had the highest infection frequencies and larger percent diseased area than the resistant standard Tifrust-13. Since all the leaves used in this study were of the same age, but collected from plants at different ages, the observed plant age-related resistance does not appear to be due to tissue maturation. These results would support the recommendation of maintaining a peanut-free period prior to planting peanut in areas where *P. arachidis* can overwinter on volunteer or multiple cropping, since the presence of inoculum at the time peanut plants are young, would allow for more time for the epidemic to develop, but would also be present when the plant is more susceptible to infection.

Introduction

Knowledge of when peanut (*Arachis hypogaea* L.) plants or individual leaves are most vulnerable to infection by *Puccinia arachidis* Speg., the causal agent of peanut rust, could aid in control of this disease. Information on when plants are more or less susceptible to infection could aid in timing fungicidal applications. For characterizing host resistance, information on vulnerable host tissues or stages would be useful for developing resistant cultivars. Identifying the right tissues or developmental stages to use for resistance screening of breeding lines could help ensure accurate

assessment of relative levels of resistance in lines being evaluated. Such knowledge might also be useful in discerning mechanisms of resistance to the pathogen.

The effect of host development on susceptibility for disease has been studied for several host pathogen systems (1, 4, 7-11, 17). Developmental resistance is any change in susceptibility to a pathogen in relation to host development. The most common developmental resistance is adult plant resistance, also referred to as ontogenic or age related resistance, and is resistance expressed when the host is resistant in the adult phase but not in the juvenile stage. Resistance can also be related to transition from one developmental stage to another, for example transitions from juvenile to adult stage, from vegetative to generative stage, or the fruit ripening or flowering stages (1, 5, 10, 11, 18).

When peanut is most vulnerable to infection may be important for evaluating breeding lines for peanut rust resistance. Cook (4), Gremillion (9), and Wang et al. (17) determined that leaf age influences susceptibility to infection by *P. arachidis*. Cook (4) evaluated the wettability of different peanut leaf ages and found that leaves became more resistant to peanut rust infection as the leaves aged. She related the susceptibility of leaves to leaf wettability, i.e., younger leaves were more susceptible due to the higher wettability of the young leaves, whereas older leaves were more resistant due to the low wettability of these leaves. Wang and Lin (17) modeled the relationship between leaf position, a measure for leaf age, and the numbers of pustules produced per leaflet in peanut. They reported a decline in pustules per leaflet as the leaflets aged. Gremillion et al. (9) compared rust incidence of greenhouse-grown peanut plants at two different plant developmental stages, after exposure to *P. arachidis* inoculum in a peanut field with severe rust epidemics. Although results were not consistent, they observed a trend of lower peanut rust incidence for

older plants. In that study, inoculum was not quantified or standardized, so variability in inoculum exposure may have accounted for some of the inconsistency.

The objective of this study was to use detached leaf assays to determine whether plant age affects susceptibility of peanut leaves to infection by *P. arachidis.* Of particular interest was whether any observed effects of plant age were consistent across genotypes with a range of partial resistance to *P. arachidis.*

Materials and methods

Four developmental stages were selected to determine if response of peanut to infection by the rust pathogen differed as peanut plants developed. Seed of all genotypes were planted at staggered intervals so that 3- (S1), 6- (S2), 9- (S3), and 12-week-old (S4) plants were inoculated with *P. arachidis* at the same time. These developmental stages corresponded with the "leaf development" stage (3-week-old plants), "side shoot formation" stage (6-week-old plants), "flowering" stage (9-week-old plants) and the "pod development" stage (12-week-old plants) of the BBCH scale (13). These stages also corresponded to the vegetative and reproductive stages described by Boote (2); with the vegetative stages being S1 and S2 (V-1 – V-n), and the reproductive stages corresponding with the R1 and R3 stages, or beginning bloom and beginning pod, respectively. Five genotypes differing in resistance to peanut rust were used in this study: Altika and Tifrust-13 as the susceptible and resistant standards, two resistant CRSP breeding lines (97x36-HO2-1-B2G3-1-2-2 and BOL3-7) and the moderately susceptible cultivar and parental breeding line Florida-07. Peanut plants were grown from seed in the greenhouse with the temperature set at 25°C, in 15 cm pots filled with commercial potting soil (Sunshine Professional

Growing Mix, Sun Gro Horticulture Distribution Inc, Bellevue, WA), and were watered as needed. Four or 5 seed, treated with Vitavex PC (a.i. captan, pentachloronitrobenzene and carboxin, Bayer CropScience LP, Research Triangle Park, NC).

Inoculum was obtained from a single-pustule isolate collected from a field in Georgia. Urediniospores were collected from one pustule using a dissecting needle, healthy leaves were re-inoculated with urediniospores from a single pustule. This cycle was repeated several times to ensure purified single-pustule isolates. Urediniospores were maintained on leaves of susceptible cultivar Altika that were placed in 15-cm petri dishes with 10% water agar and incubated at 25°C until needed. Freshly produced urediniospores were collected from these leaves, using a spore collector connected to a vacuum pump, to prepare a spore suspension of 40,000 spores/ml of 0.005% Tween 20, which was quantified using a hemacytometer.

Germination percentage of the urediniospores was assessed 1 day before the start of the experiments and on the day of the inoculations. A spore suspension was sprayed on 10% water agar plates, and incubated at 25°C in darkness overnight. The next day, the number of germinated urediniospores out of 50 randomly chosen urediniospores was determined using a compound microscope at 100x magnification. Urediniospores were considered germinated if the germination tube was longer than the greatest diameter of the urediniospore.

The effect of host age on peanut rust resistance was determined by assessing the components of resistance using the detached leaf method described by Cook (3). The youngest fully expanded leaves of plants at the selected developmental stages were collected, in order to examine resistance of different plant ages but the same leaf ages. The leaflets were detached and placed on sterile moist filter paper in a petri dish (9 cm diameter) with the abaxial side up. The

leaflets were then inoculated by spraying them for 1 sec using an aerosol sprayer containing a single-pustule spore suspension of 40,000 spores/ml of 0.005% Tween 20. The petri dishes containing inoculated leaflets were incubated in darkness for 16 h at 25°C. After the 16-h dark period, the closed petri dishes were incubated at 25°C, with a 12-h photoperiod for 16 days. The filter paper was kept moist with sterile distilled water. Five genotypes and four plant ages were arranged factorially in a randomized complete block design. There were three replications per genotype and for each genotype a control was included by spraying leaves with 0.005% Tween 20 solution. The leaflets were examined on 7, 11 and 16 days after inoculation (DAI) for the development of pustules.

The components of resistance measured included: latent period, infection frequency, lesion diameter, and percent diseased area. The latent period was determined by counting the number of days between DAI and spore production of at least 1 pustule. The infection frequency was determined as the number of pustules per cm^2 of leaf area, and lesion diameter was determined by measuring the area of 10 arbitrarily selected lesions, and calculating the mean lesion diameter from the formula area = $\pi d^2/4$. Leaf area, lesion area, and percent diseased area were measured from digital images of leaves, taken 16 DAI, using the ASSESS 2.0 Image Analysis Software for plant disease quantification (APS Press, St. Paul, MN). For ASSESS analyses, the leaflets were glued to blue paper background, covered with a sheer plastic sheet, scanned at 300 dpi, and stored as .TIFF files. The detached leaf experiment was done three times.

The effects of genotype and plant age on the components of resistance were analyzed using Proc MIXED with ddfm = satterth option on the model statement (SAS v 9.3, SAS Institute Inc., Cary, NC). Trial, replication, and interactions of trial with genotype and plant age were

considered random effects. Genotype and plant age were considered fixed effects. Regression analysis was used to determine response of the variables to plant age. Fisher's protected LSD was used to determine significant differences in the components of resistance among genotypes and plant age.

Results

Germination of the urediniospores was higher than 90% for all three trials, and pustules developed on all and only on inoculated leaves in the repeated studies. There were significant trial × genotype × plant age interactions for infection frequency at seven days after inoculation. Therefore each trial was analyzed independently. In trial 1 and trial 2, there was a significant genotype × plant age effect in addition to significant main effects for genotype and plant age. In those trials infection frequency decreased linearly or according to quadratic functions with increasing plant age for all genotypes (Fig. 3.1). In trial 1 there were significant differences among genotypes for plant ages 3 weeks ($P = 0.04$) and 6 weeks ($P < 0.01$), but not for 9 weeks ($P = 0.49$) or 12 weeks ($P = 0.38$). Among plants that were 3 weeks old infection frequency was lower in CRSP breeding line 97x36-HO2-1-B2G3-1-2-2 than in any genotype except Altika (Fig. 3.1). Among plants that were 6 weeks old, infection frequency was higher in Altika than in Tifrust-13 or 97x36-HO2-1-B2G3-1-2-2, with Florida-07 and BOL3-7 intermediate (Fig. 3.1). Infection frequencies were low for all genotypes in plants that were 9 or 12 weeks old, and there were no differences among genotypes (Fig. 3.1). There was a linear reduction in infection frequency with increasing plant age for all genotypes except Altika (Table 3.1).

In Trial 2, there were no significant genotype effects ($P > 0.29$) within any age group Fig. 3.1. In each genotype, infection frequency declined with increasing age as described by linear or quadratic functions (Table 3.1). Infection frequency was not determined at 11 DAI in Trial 1, but was measured in Trials 2 and 3. There were no interactions of trial ($P > 0.34$) with genotype, plant age, or genotype × plant age for infection frequency at 11 DAI. Therefore analysis was done across trials. Across trials, the interaction of genotype × plant age was not significant ($P > 0.15$), so analysis was done for each main effect factor across levels of the other. Main effects of genotype ($P < 0.01$) and plant age ($P < 0.01$) were significant. Across plant ages, infection frequency was lower for Tifrust-13 than for any other genotype, and infection frequencies were similar for the other genotypes. Across plant ages, infection frequency was 5.3, 4.9, 5.3, 5.0, and 3.0 for Altika, BOL3-7, 97x36-HO2-1-B2G3-1-2-2, Florida-07 and Tifrust-13, respectively (LSD = 0.8, $P = 0.05$). Across genotypes, infection frequency decreased with plant age according to a quadratic function (Fig. 3.2).

For infection frequency at 16 DAI, there were no interactions of trial ($P > 0.21$) with genotype, plant age, or genotype × plant age; therefore analysis was done across trials. Across trials, genotype ($P = 0.06$) and plant age ($P < 0.01$) were significant, but genotype × plant age was not ($P > 0.21$). Across plant ages, infection frequency was lower for Tifrust-13 than for any other genotype, and infection frequencies were similar for the other genotypes. Across plant ages, infection frequency was 5.9, 5.3, 5.4, 5.6, and 4.2 for Altika, BOL3-7, 97x36-HO2-1-B2G3-1-2-2, and Tifrust-13, respectively (LSD = 1.0, $P = 0.05$). Across genotypes, infection frequency 16 DAI decreased with plant age according to a quadratic function (Fig. 3.2)

Interactions of trial with genotype, plant age, or genotype × plant age were not significant ($P > 0.16$) for lesion diameter at 16 DAI. Therefore analysis was conducted across trials. There were no significant genotype ($P > 0.19$) or genotype × plant age interaction ($P > 0.73$) effects, but plant age effects were significant, ($P = 0.02$). Across plant ages, average lesion diameter ranged from 0.86 mm in Tifrust-13 to 0.97 mm for Florida-07. However, there was no main significant genotype effect ($P > 0.19$). Across genotypes, there was a significant reduction in lesion diameter with increasing plant age, as described by a quadratic function (Fig.), but the model accounted for only a small amount of the variability in lesion diameter (Fig 3.3).

There were no significant interactions for trial with genotype, plant age or genotype × plant age ($P > 0.10$). Therefore, data presented represent results from the pooled analysis. Genotype ($P = 0.08$) and plant age ($P < 0.01$) main effects on diseased area were significant, but genotype × plant age effects were not ($P > 0.34$). Across plant ages, percent diseased area was 8.7, 8.1, 7.6, 8.1 and 4.9% for Altika, BOL3-7, 97x36-HO2-1-B2G3-1-2-2, Florida-07 and Tifrust-13, respectively (LSD = 2.2, $P = 0.05$). Across genotypes, percent diseased area decreased with increasing plant age according to the quadratic function shown in Fig. 3.3.

There were no significant ($P > 0.14$) interactions of genotype, plant age or genotype × plant age for latent period. Main effects of genotype ($P > 0.10$) and plant age ($P > 0.54$) were not significant, but genotype × plant age effects were significant ($P < 0.01$). Within plants 3 weeks old, latent period in Tifrust-13 was longer than for Altika or BOL3-7 (Fig. 3.4). Within plants 6 weeks old, latent period in Tifrust-13 was longer than for Altika or BOL3-7 (Fig. 3.4). In both those cases, latent periods of the other genotypes were intermediate. There were no differences among genotypes for latent period for plants that were 9 or 12 weeks old (Fig. 3.4). There was significant

latent response to plant age only for Altika and BOL3-7. In both those genotypes, there was no response between 3 and 6 weeks of age, but latent period increased linearly with plant age after plants were six weeks old (Fig. 3.4).

Discussion

In general, adult plant resistance may be attributed to maturation of host tissue, mostly anatomical features. Examples are the thickening of cell walls, formation of pectic structures (5, 18). Findings by Cook (4), that older peanut leaves are more resistant to infection by *P. arachidis* than younger leaves, may be explained by differences is wax content, as the older more resistant leaves appeared to have reduced leaf wettability.

Adult plant resistance may also be explained by the production of antifungal compounds, phytoalexins, toxins, or hormones by mature tissues. There are several studies on the production of phytoalexins related to infection of peanut leaves by *P. arachidis* (6, 14-16). For example, Sankara et al. (15) reported an increased production of isoflavanones in a resistant peanut genotype within 2 days after infection by P. arachidis, compared to a susceptible rust-infected peanut genotype.

These data indicate that peanut rust resistance is related to the developmental stage of the peanut plant. General trends were observed that the youngest fully expanded leaves of 12-week-old plants were more resistant than the youngest fully expanded leaves of 3- or 6-week-old peanut. These results corroborate findings by Gremillion et al. (9). The plant age-related resistance observed in the current study would not be due to tissue maturation since leaves of the same age

were used, collected from plants at different ages. All leaves used in these experiments were the youngest fully unfolded leaves.

Regardless of the mechanism involved, these results indicate that once the developmental resistance occurs, the host plant did not revert back to susceptible during the age range evaluated in this study. This is promising for the indeterminate growing peanut plant. Although newly developed leaves may be more susceptible to infection by *P. arachidis* (4), once the peanut plant has passed the flowering stage, and especially in the pod development stage, even new young leaves will be more resistant to infection. These results would support the recommendation of maintaining a peanut-free period prior to planting peanut in areas where *P. arachidis* can overwinter on volunteer or multiple crops. Based on these results, not only would the presence of inoculum at the time peanut plants are young allow for more time for the epidemic to develop, but also would be present when the plant is more susceptible to infection. In Georgia, circumstantial evidence indicates that urediniospores typically must be introduced each growing season. If those introductions do not occur early in the season, peanut plants may be in a more resistant stage. Miller et al. (12) reported that removal of flower buds resulted in less injury by late leaf spot (*Cercosporidium personatum*). It has also been hypothesized that peanut plants become more prone to damage by both early and late leaf spot pathogens during the final weeks of the growing season when plant physiology is concentrated on filling and maturing the kernels (A. K. Culbreath personal communication). The relationship between plant age beyond 12 weeks and rust susceptibility of peanut has not been characterized and additional changes in susceptibility to *P. arachidis* with later growth stages, particularly as a high percentage of pods and kernels near maturity should be considered.

The results from this study underscore the complexity of factors involved in quantitative resistance and that a combination of several physical and physiological factors may be involved in peanut rust resistance.

Acknowledgments

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References

1. Ando, K., Hammar, S., and Grumet, R. 2009. Age-related resistance of diverse cucurbit fruit to infection by *Phytophthora capsici*. J. Am. Soc. Hortic. Sci. 134:176-182.
2. Boote, K. J. 1982. Growth stages of peanut (*Arachis hypogaea* L.). Peanut Science 9:35-39.
3. Cook, M. 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.
4. Cook, M. 1980. Peanut leaf wettability and susceptibility to infection by *Puccinia arachidis*. Phytopathology 70:826-830.
5. Develey-Rivière, M.-P., and Galiana, E. 2007. Resistance to pathogens and host developmental stage: A multifaceted relationship within the plant kingdom. New Phytol. 175:405-416.

6. Edwards, C., Strange, R. N., and Cole, D. L. 1995. Accumulation of isoflavonoid phytoalxins in leaves of *Arachis hypogaea* differing in reaction to rust (*Puccinia arachidis*) and early leaf spot (*Cercospora arichidicola*). Plant Pathol. 44:573-579.

7. Ficke, A., Gadoury, D. M., and Seem, R. C. 2002. Ontogenic resistance and plant disease management: a case study of grape powdery mildew. Phytopathology 92:671-675.

8. Gadoury, D. M., Seem, R. C., Ficke, A., and Wilcox, W. F. 2001. The epidemiology of powdery mildew on Concord grapes. Phytopathology 91:948-955.

9. Gremillion, S. K. 2007. Contributions to management of diseases of peanut (*Arachis hypogaea*) through Bolivian-derived host resistance, integrated disease management and knowledge of pathogen variability. PhD Dissertation University of Georgia, Athens, GA.

10. Kus, J. V., Zaton, K., Sarkar, R., and Cameron, R. K. 2002. Age-related resistance in Arabidopsis is a developmentally regulated defense response to *Pseudomonas syringae*. The Plant Cell 14:479-490.

11. Mellano, H. M., Munnecke, D. E., and Endo, R. M. 1970. Relationship of seedling age to development of *Pythium ultimum* on roots of *Antirrhinum majus*. Phytopathology 60:935-942.

12. Miller, I. L., Norden, A. J., Knauft, D. A., and Gorbet, D. W. 1990. Influence of maturity and fruit yield on susceptibility of peanut to *Cercosporidium personatum* (Late leaf spot pathogen). Peanut Science 17:52-58.

13. Munger, P., Bleiholder, H., Hack, H., Hess, M., Stauss, R., van den Boom, T., and Weber, E. 1998. Phenological growth stages of the peanut plant (*Arachis hypogaea* L.): Codification and description according to the BBCH scale. J. Agron. Crop Sci.-Z. Acker Pflanzenbau 180:101-107.

14. Rao, P. V. S., Einhorn, J., Geiger, J. P., Malosse, C., Rio, B., and Ravise, A. 1991. Alkyl bis phenyl ethers, New phytoalexins produced by *Arachis hypogaea* L. infected with *Puccinia arachidis* Speg. Oleagineux 46:501-507.

15. Sankara, P., Rao, P. V. S., and Strange, R. N. 1996. Differential accumulation of phytoalexins in leaves of susceptible and resistant genotypes of groundnut (*Arachis hypogaea* L.) inoculated with *Puccinia arachidis* Speg. J. Phytopathol.-Phytopathol. Z. 144:527-532.

16. Strange, R. N., Edwards, C., and Richards, S. 1988. Phytoalexins of groundnuts and their role in resistance to fungal parasites. Phytoparasitica 16:202-203.

17. Wang, Z. Z., and Lin, K. H. 2000. Modeling leaf age-related susceptibility and rust eruption dynamics in peanut. Peanut Science 27:7-10.

18. Whalen, M. C. 2005. Host defence in a developmental context. Mol. Plant Pathol. 6:347-360.

Table 3.1. Regression analysis of the effect of plant age in weeks on infection frequency of peanut rust in 5 peanut genotypes

Genotype	Regression equation	R^2	*P* value
Trial 1			
Altika	NS	-	0.06
BOL3-7	4.6 – 0.35 ×age[a]	0.63	0.002
97x36-HO2-1-B2G-3-1-2-2	6.7 – 0.22 × age	0.48	0.01
Florida-07	5.1 – 0.39 × age	0.59	0.003
Tifrust-13	5.02 – 0.44 × age	0.62	0.002
Trial 2			
Altika	6.4 – 0.04 × age^2	0.59	0.003
BOL3-7	6.1 – 0.03 × age^2	0.51	0.008
97x36-HO2-1-B2G-3-1-2-2	6.0 – 0.03 × age^2	0.50	0.009
Florida-07	7.0 – 0.38 × age	0.34	0.05
Tifrust-13	5.08 – 0.32 × age	0.44	0.02

[a] Plant age at which the plants were inoculated: Stage 1= 3 weeks after planting (WAP); Stage 2= 6 WAP; Stage 3= 9 WAP; Stage 4= 12 WAP

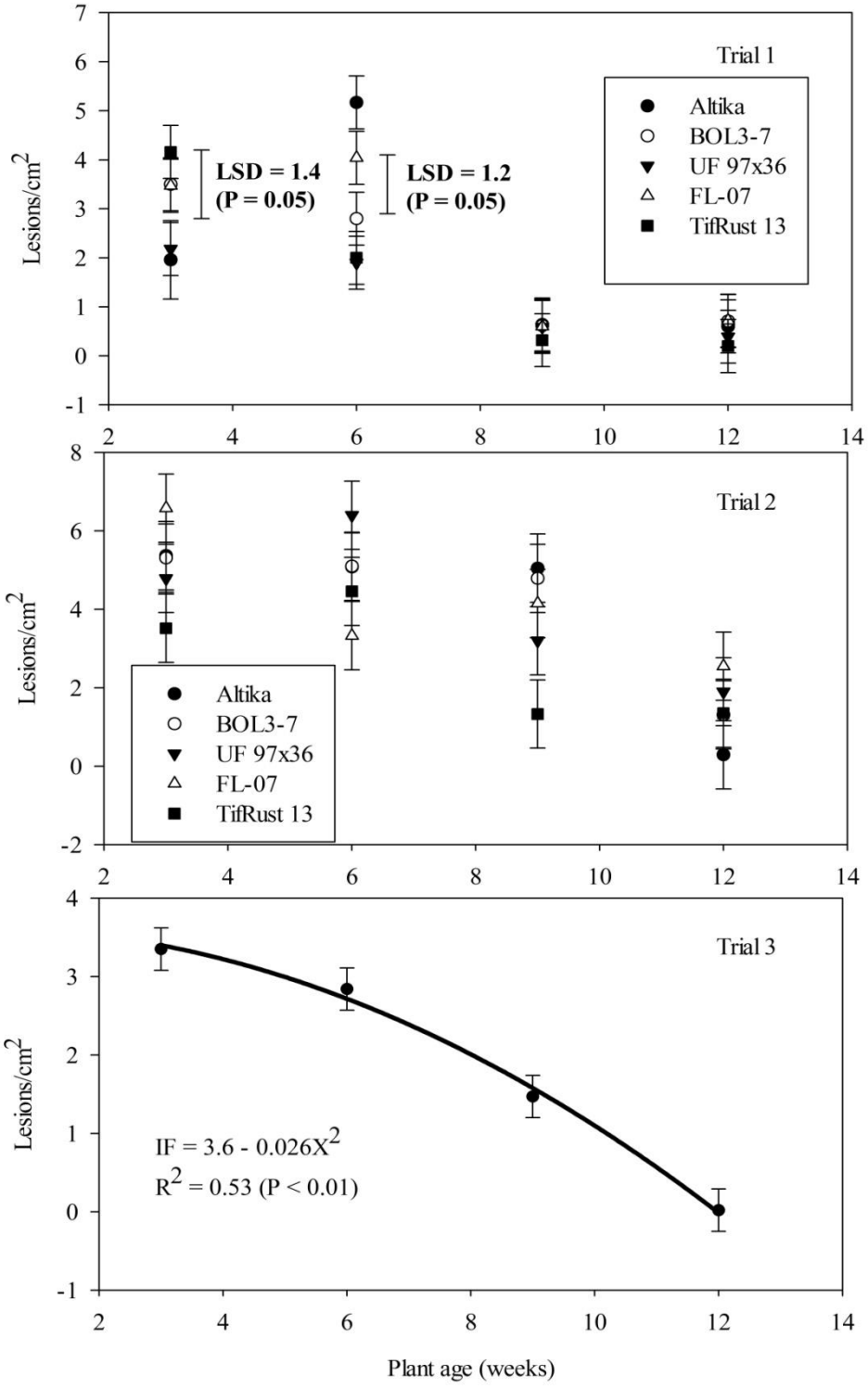

Fig. 3.1. Effect of peanut genotype and plant age on infection frequency of peanut rust, 7 days after inoculation. CRSP breeding line 97x36-HO2-1-B2G-3-1-2-2 = UF 97x36, Florida-07= FL-07, and Tifrust-13 = TifRust 13. Data points represent the means of 3 replications. Error bars represent the standard errors of the mean.

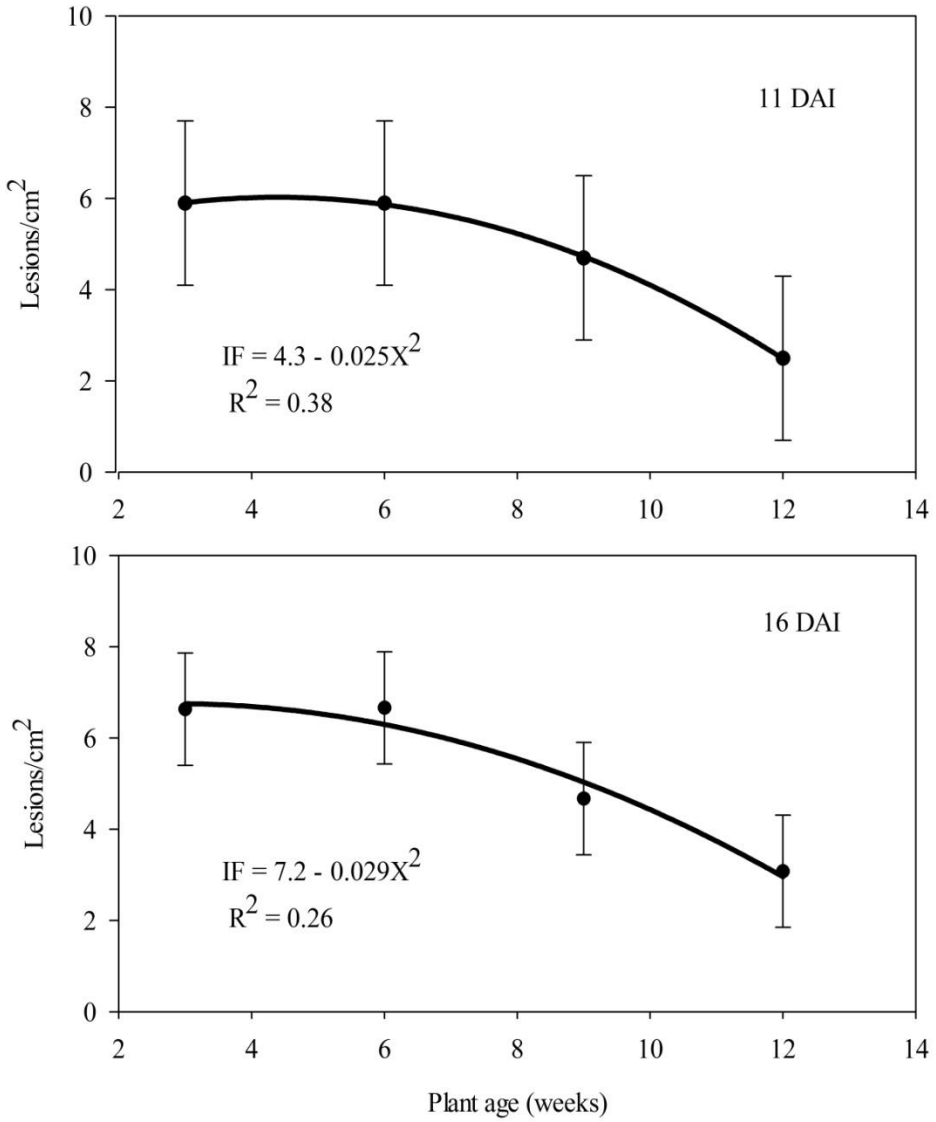

Fig. 3.2. Effect of peanut genotype and plant age on infection frequency of peanut rust 11 and 16 days after inoculation. Data points represent the means of 3 replications of 5 genotypes. Error bars represent the standard errors of the mean.

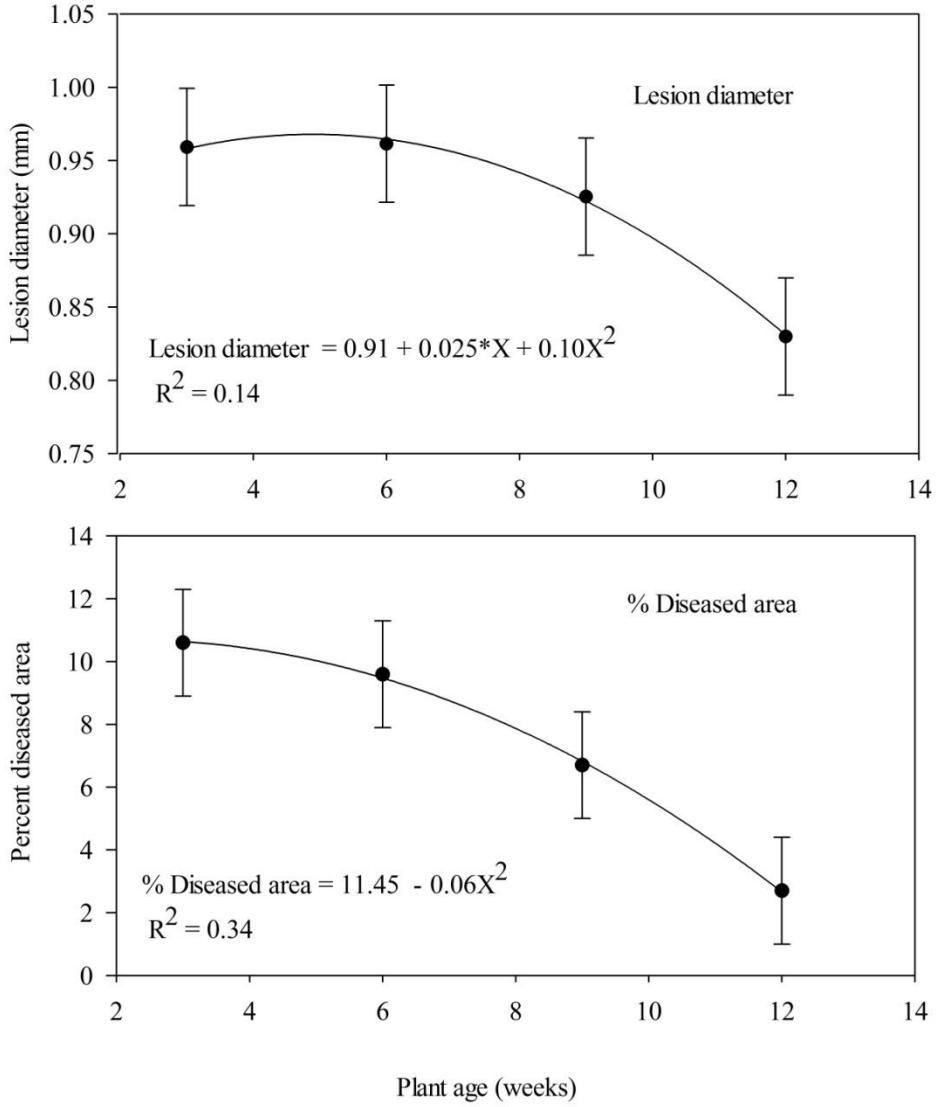

Fig. 3.3. Effect of peanut genotype and plant age on lesion diameter and percent diseased area of peanut rust 16 days after inoculation. Data points represent the means of 3 replications of 5 genotypes. Error bars represent the standard errors of the mean.

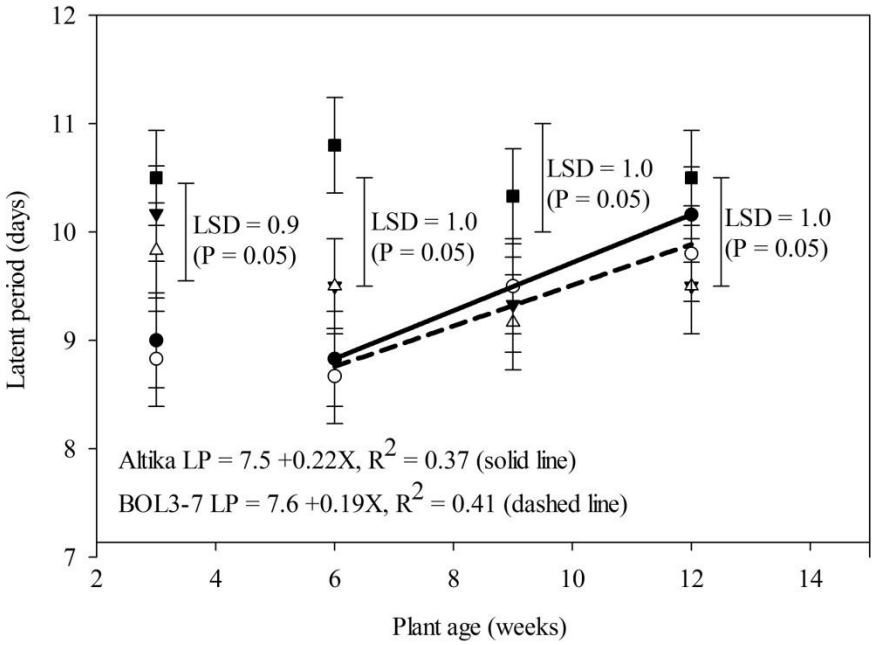

Fig. 3.4. Effect of peanut genotype and plant age on latent period (LP) (days until sporulation) of peanut rust. (Altika: closed circles, BOL3-7: open circles, 97x36-HO2-1-B2G-3-1-2-2 (UF 94 x 36): open triangles, Florida-07: closed triangles, and Tifrust-13: closed squares). Data points represent the means of 3 replications. Error bars represent the standard errors of the mean.

CHAPTER 4

DISTINGUISHING RUST-RESISTANT AND -SUSCEPTIBLE PEANUT GENOTYPES, USING PREVIOUSLY DEVELOPED POLYMORPHIC MICROSATELLITE MARKERS [1]

[1] Power, I. L., Tillman, B. L., and Culbreath, A. K. 2014. To be submitted to *Peanut Science*.

Abstract

The ability of previously identified polymorphic microsatellite markers to distinguish rust resistant genotypes from susceptible genotypes was studied. Newly developed CRSP breeding lines, plant introductions and commonly grown cultivars, were molecularly characterized using these polymorphic SSR markers. The markers used detected polymorphisms but were not able to distinguish resistant from susceptible peanut genotypes. None of the 22 private bands generated for the resistant population were absolute and no marker alleles could be exclusively linked to all resistant or all susceptible genotypes. This could be because the resistance observed in the genotypes may be explained by other partial resistance genes than previously identified. Highly resistant and highly susceptible genotypes did cluster. This may indicate that some of the resistant genotypes evaluated in this study may be identified with existing markers, on molecular level.

Introduction

Peanut rust caused by *Puccinia arachidis* is an important foliar disease of peanut (*Arachis hypogaea* L.), primarily in countries with warm, tropical climates. This pathogen infects leaves primarily, but can infect stems, petioles and other above-ground parts as well. If left unmanaged, yield loss can be devastating; estimates of up to 50% have been reported (30, 32). Applications of fungicides such as chlorothalonil, triazoles and strobilurins are effective management methods; however, access to fungicides is often limited in the developing world, where this disease is more prevalent. In these countries, host resistance is the best option for management of peanut rust.

Peanut rust resistance typically is polygenic: several minor genes provide varying levels of partial resistance (30, 35) evidenced in one or more components of peanut rust resistance. These

include an increased incubation and latent period, decreased infection frequency, and reduced pustule size, spore production and spore viability (3, 30, 32, 35). Thousands of germplasm accessions have been screened for rust resistance by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and many rust resistant lines have been identified. However, most of these lines are either primitive land races with undesirable seed characters or wild *Arachis* spp. with high rust resistance but commercially unacceptable yields (1, 30, 35). Rust resistance in the peanut cultivars currently in production is low to moderate, and involves polygenic minor genes that provide varying levels of partial resistance. These cultivars are described as slow-rusting types, due to one or more of the components of resistance described earlier (30, 35).

Breeding for improvement of peanut, for example increased rust resistance, is hampered by the relatively low genetic variability in cultivated peanut. This is believed to be the result of the recent hybridization (8, 9) of two diploid *Arachis* species *A. duranensis* and *A. ipaensis*, followed by spontaneous chromosome doubling (9, 15, 18, 20, 34). The use of molecular markers for marker assisted selection (MAS) can increase efficiency of efforts and resources in the breeding process. For example, MAS may allow identification of genes responsible for traits that cannot be observed phenotypically, or reduce the time needed for germplasm evaluation. For example, Chu et al. (2) developed the high oleic Tifguard cultivar by pyramiding the nematode resistance and the high oleic trait in less than three years by using molecular markers associated with these traits. The development of genetic markers for MAS in peanut breeding is also limited by the low genetic variation in peanut (8, 9, 16). However, in the last few years hundreds of SSR markers have been developed by research groups including the University of Georgia and The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (5, 6, 12, 13, 18, 19, 21, 28, 34).

Khedikar et al. (15), Mace et al. (18), Mondal et al. (20), and Varshney et al. (34) furthermore identified simple sequence repeat (SSR) markers with which they were able to detect high levels of polymorphism in peanut recombinant inbred lines (RILs) and peanut genotypes from different geographical regions, of which several were used successfully to distinguish rust resistant from susceptible genotypes. Khedikar et al. (15) also identified twelve quantitative trait loci (QTLs) for peanut rust resistance, of which one major QTL explained up to 55% phenotypic variation. However, these markers have not been evaluated for their utility for differentiating susceptibility or resistance to *P. arachidis* in breeding lines and cultivars from the southeastern U.S.

The objective of this study was to evaluate whether the previously identified polymorphic SSR markers could be used to distinguish rust resistant genotypes from susceptible genotypes in rust resistance characterization programs in the southeastern U.S., and thus if these markers could potentially be used in MAS in breeding for peanut rust resistance.

Methods

Plant material. For this study, 32 SSR markers (Table 4.1) were used to screen 43 genotypes (Table 4.2) of cultivated peanut with varying levels of peanut rust resistance. The selected genotypes included currently grown cultivars, CRSP breeding lines, ICRISAT plant introductions and parents of existing recombinant inbred line (RIL) populations that are currently being screened for multiple disease resistance. These were obtained from USAID Peanut CRSP, USDA-ARS, The Plant Genetic Resources Conservation Unit (Griffin), The University of Georgia Coastal Plain Experiment Station, in Tifton, GA, and the University of Florida.

DNA isolations. Total genomic DNA of the genotypes was extracted from fresh unfolded leaves of 8-week-old greenhouse-grown plants, following the CTAB protocol described by Murray and Thompson (22). DNA quality and quantity were determined with nanodrop and the DNA concentration was adjusted to 5 ng/µl for amplification.

PCR reactions. PCR amplifications were performed in 10-µl total volumes containing 0.5 µl DNA template, 0.5 µl of each forward and reverse primer, 1 µl of 10x Taq buffer, 0.8 µl of 2.5 mM dNTPs, 0.6 µl 25mM $MgCl_2$, 1 µl 10% PVP, 0.1 µl of 10 µg/µl BSA, 0.1 µl Jumpstart Taq polymerase and 4.9 µl water. The Touchdown PCR amplification was as follows: an initial denaturing step at 94°C for 1 min followed by 6 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 30 s, by which the annealing temperature decreased 1°C every cycle. The program then continued with 30 additional cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 7 min. The PCR products were checked on a 1% agarose gel to confirm amplification, diluted 40-fold, and sent to the University of Georgia Genomics Facility (Athens, GA) for analysis.

Data analyses. Peak analysis was conducted using GeneMapper software v4.0 (Applied Biosystems, Foster City, CA). The alleles of each SSR locus from the 43 peanut genotypes were scored as binary data: presence (1) or absence (0) of the allele. Analysis of molecular variance (AMOVA), estimates of genetic distances between the genotypes, and principal component analysis were calculated with GenAlEx6.4 (27) to analyze differences between the resistant and susceptible peanut genotypes. Estimates of genetic distances and principal component analysis were also calculated for highly resistant and highly susceptible genotypes.

Results and discussion

A total of 142 polymorphic alleles were generated for the 32 SSR markers across the 43 peanut genotypes, with an average of 4.4 alleles per locus. All of the markers used detected polymorphisms, however, none was able to distinguish resistant from susceptible peanut genotypes among the lines evaluated in this experiment. In the principal component analysis (Fig. 4.1), where the first and second principal coordinates accounted for 22.22% and 19.95% of the total genetic variation, respectively, a similar result was observed: there are no specific clusters for rust resistant or susceptible genotypes. The analysis of molecular variation (AMOVA) of the 43 peanut genotypes (Table 4.3) showed a low percentage of the genetic variation associated with disease resistance or susceptibility, as only 2% of the observed variation is accounted for by among resistant and susceptible populations, whereas 98% is accounted for within resistant and susceptible populations. The observed low polymorphism is consistent with previous reports (15, 18, 20, 34).

One possible explanation for failure of the markers to distinguish rust-resistant from -susceptible genotypes may be that the resistance observed in the genotypes is explained by other partial resistance genes than previously identified (15, 18, 20, 34). The resistance observed in the mapping population used by Khedikar et al. (15), who identified 12 QTLs for peanut rust resistance, including one major QTL, is derived from peanut germplasm from India. The parents of those mapping populations are high yielding and leading peanut varieties in India. In this study, a collection of mostly unrelated genotypes was used to evaluate the microsatellites. The CRSP breeding lines have the Bolivian landrace BayoGrande as a common parent. According to studies by Khedikar et al. (15), the SSR markers GM005 and GM624 are associated with rust

resistance. In their study, marker TC1B02 (GM005) was associated with QTLrust04, which was mapped to linkage group 3, and marker GM624 was associated with QTLrust09 which was mapped to linkage group 9. In this study, none of the alleles of these markers could be exclusively linked to a resistant genotype.

Although the markers were not able to separate rust resistant from susceptible genotypes, there were 22 private bands present for the resistant population. However, these loci were not absolute as they were not present in all, or the majority of the resistant genotypes. For example the allele at 194 bp of marker GM011, one of the 22 private bands, was only present in moderately resistant cultivar Georgia-03L, and the allele at 288 bp of marker GM421 was only present in resistant genotypes PI544351 and PI556992. Furthermore, there was one private band for the susceptible population however it was not absolute either: allele 167 of GM19 was only present in susceptible cultivar NC3033. In this study, no marker alleles could be exclusively linked to all resistant or all susceptible genotypes.

When considering only the highly resistant and highly susceptible genotypes, these two populations were grouped into three separate clusters in the principal component analysis (Fig. 4.2.). Here the first principal coordinate accounted for 29.47% and the second for 19.33% of the total genetic variation. The highly resistant genotypes grouped together in one separate group, while the highly susceptible separated into two groups. This is encouraging as it seems promising that some of the resistant genotypes evaluated in this study may be identified with existing markers. In addition, the genotypes used in this study that proved to be resistant were from different origins. If that resistance is not associated with alleles detected by markers used, the resistance may be imparted by genes other than those for which markers were available. If

genotypes with alleles previously reported to provide resistance to rust are present, variability in the pathogen population would be one factor that should be considered.

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References

1. Bromfield, K. R. 1971. Peanut rust: a review of literature. Journal of American Peanut Research and Education Association 3:111-121.
2. Chu, Y., Wu, C. L., Holbrook, C. C., Tillman, B. L., Person, G., and Ozias-Akins, P. 2011. Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. Plant Genome 4:110-117.
3. Cook, M. 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.
4. Ferguson, M. E., Burow, M. D., Schulze, S. R., Bramel, P. J., Paterson, A. H., Kresovich, S., and Mitchell, S. 2004. Microsatellite identification and characterization in peanut (A-hypogaea L.). Theor. Appl. Genet. 108:1064-1070.
5. Gautami, B., Fonceka, D., Pandey, M. K., Moretzsohn, M. C., Sujay, V., Qin, H. D., Hong, Y. B., Faye, I., Chen, X. P., BhanuPrakash, A., Shah, T. M., Gowda, M. V. C., Nigam, S. N., Liang, X. Q., Hoisington, D. A., Guo, B. Z., Bertioli, D. J., Rami, J. F., and Varshney,

R. K. 2012. An International Reference Consensus Genetic Map with 897 Marker Loci Based on 11 Mapping Populations for Tetraploid Groundnut (Arachis hypogaea L.). PLoS One 7:7 doi:10.1371/journal.pone.0041213.

6. Gautami, B., Pandey, M. K., Vadez, V., Nigam, S. N., Ratnakumar, P., Krishnamurthy, L., Radhakrishnan, T., Gowda, M. V. C., Narasu, M. L., Hoisington, D. A., Knapp, S. J., and Varshney, R. K. 2012. Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol. Breed. 30:757-772.

7. Guohao, H., Meng, R., Newman, M., Guoqing, G., Pittman, R. N., and Prakash, C. S. 2003. Microsatellites as DNA markers in cultivated peanut (Arachis hypogaea L.). BMC Plant Biol. 3:1-6.

8. Halward, T., Stalker, T., Larue, E., and Kochert, G. 1992. Use of single primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol.Biol. 18:315-325.

9. Halward, T. M., Stalker, H. T., Larue, E. A., and Kochert, G. 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. Genome 34:1013-1020.

10. Hammons, R. O., Branch, W. D., Bromfield, K. R., Subrahmanyam, P., Rao, V. R., Nigam, S. N., Gibbons, R. W., and Goldin, E. 1982. Registration of Tifrust-13 peanut germplasm. Crop Sci. 22:697.

11. Hammons, R. O., Nigam, S. N., Gibbons, R. W., Subrahmanyam, P., and Rao, V. R. 1982. Registration of eight peanut germplasm lines resistant to rust. Crop Sci. 22:452-453.

12. He, G. H., and Prakash, C. 2001. Evaluation of genetic relationships among botanical varieties of cultivated peanut (*Arachis hypogaea* L.) using AFLP markers. Genet. Resour. Crop Evol. 48:347-352.

13. He, G. H., and Prakash, C. S. 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97:143-149.

14. Hopkins, M. S., Casa, A. M., Wang, T., Mitchell, S. E., Dean, R. E., Kochert, G. D., and Kresovich, S. 1999. Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci. 39:1243-1247.

15. Khedikar, Y. P., Gowda, M. V. C., Sarvamangala, C., Patgar, K. V., Upadhyaya, H. D., and Varshney, R. K. 2010. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 121:971-984.

16. Kochert, G., Halward, T., Branch, W. D., and Simpson, C. E. 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81:565-570.

17. Krishna, G. K., Zhang, J. F., Burow, M., Pittman, R. N., Delikostadinov, S. G., Lu, Y. Z., and Puppala, N. 2004. Genetic diversity analysis in Valencia peanut (*Arachis hypogaea* L.) using microsatellite markers. Cell. Mol. Biol. Lett. 9:685-697.

18. Mace, E. S., Phong, D. T., Upadhyaya, H. D., Chandra, S., and Crouch, J. H. 2006. SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. Euphytica 152:317-330.

19. Mace, E. S., Varshney, R. K., Mahalakshmi, V., Seetha, K., Gafoor, A., Leeladevi, Y., and Crouch, J. H. 2008. In silico development of simple sequence repeat markers within the

aeschynomenoid/dalbergoid and genistoid clades of the Leguminosae family and their transferability to *Arachis hypogaea*, groundnut. Plant Sci. 174:51-60.

20. Mondal, S., Badigannavar, A. M., and D'Souza, S. F. 2012. Molecular tagging of a rust resistance gene in cultivated groundnut (*Arachis hypogaea* L.) introgressed from Arachis cardenasii. Mol. Breed. 29:467-476.

21. Moretzsohn, M. C., Leoi, L., Proite, K., Guimaraes, P. M., Leal-Bertioli, S. C. M., Gimenes, M. A., Martins, W. S., Valls, J. F. M., Grattapaglia, D., and Bertioli, D. J. 2005. A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). Theor. Appl. Genet. 111:1060-1071.

22. Murray, M. G., and Thompson, W. F. 1980. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res. 8:4321-4325.

23. Nagy, E. D., Chu, Y., Guo, Y. F., Khanal, S., Tang, S. X., Li, Y., Dong, W. B. B., Timper, P., Taylor, C., Ozias-Akins, P., Holbrook, C. C., Beilinson, V., Nielsen, N. C., Stalker, H. T., and Knapp, S. J. 2010. Recombination is suppressed in an alien introgression in peanut harboring Rma, a dominant root-knot nematode resistance gene. Mol. Breed. 26:357-370.

24. Nagy, E. D., Guo, Y. F., Tang, S. X., Bowers, J. E., Okashah, R. A., Taylor, C. A., Zhang, D., Khanal, S., Heesacker, A. F., Khalilian, N., Farmer, A. D., Carrasquilla-Garcia, N., Penmetsa, R. V., Cook, D., Stalker, H. T., Nielsen, N., Ozias-Akins, P., and Knapp, S. J. 2012. A high-density genetic map of *Arachis duranensis*, a diploid ancestor of cultivated peanut. BMC Genomics 13:469.

25. Nigam, S. N., Dwivedi, S. L., Nambiar, P. T. C., and Gibbons, R. W. 1991. Registration of Five Nonnodulating Peanut Germplasm Lines. Crop Sci. 31:855-856.

26. Nigam, S. N., Reddy, L. J., Subrahmanyam, P., Reddy, A. G. S., McDonald, D., and Gibbons, R. W. 1992. Registration of ICGV 87157, an elite peanut germplasm with multiple resistance to diseases. Crop Sci. 32:837-837.

27. Peakall, R., and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

28. Ravi, K., Vadez, V., Isobe, S., Mir, R. R., Guo, Y., Nigam, S. N., Gowda, M. V. C., Radhakrishnan, T., Bertioli, D. J., Knapp, S. J., and Varshney, R. K. 2011. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 122:1119-1132.

29. Reddy, L. J., Nigam, S. N., Rao, R. C. N., and Reddy, N. S. 2001. Registration of ICGV 87354 peanut germplasm with drought tolerance and rust resistance. Crop Sci. 41:274-275.

30. Subrahmanyam, P. 1997. Rust. Pages 31-33 in: Compendium of Peanut Diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith and P. Subrahmanyam, eds. American Phytopathological Society, St. Paul, MN.

31. Subrahmanyam, P., Gibbons, R. W., Nigam, S. N., and Rao, V. R. 1980. Screening methods and further sources of resistance to peanut rust. Peanut Science 7:10-12.

32. Subrahmanyam, P., and McDonald, D. 1983. Rust Disease of Groundnut. in: Information bulletin no. 13. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Patancheru, Anhra Pradesh, India.

33. Subrahmanyam, P., Rao, V. R., McDonald, D., Moss, J. P., and Gibbons, R. W. 1989. Origins of resistances to rust and late leaf spot in peanut (*Arachis hypogaea*, Fabaceae). Econ. Bot. 43:444-455.

34. Varshney, R. K., Mahendar, T., Aruna, R., Nigam, S. N., Neelima, K., Vadez, V., and Hoisington, D. A. 2009. High level of natural variation in a groundnut (*Arachis hypogaea* L.) germplasm collection assayed by selected informative SSR markers. Plant Breed. 128:486-494.

35. Wynne, J. C., Beute, M. K., and Nigam, S. N. 1991. Breeding for disease resistance in peanut (*Arachis hypogaea* L). Annu. Rev. Phytopathol. 29:279-303.

Table 4.1. Simple Sequence Repeat (SSR) markers used in the present study to characterize genotypes varying in resistance to peanut rust

SSR Marker	Product Length (bp)	Reference
GM005	239-301	(21)
GM007	220-295	(21)
GM011	122-152	(21)
GM019	135-245	(21)
GM028	162-201	(21)
GM036	124-187	(21)
GM038	240-425	(21)
GM039	226-255	(21)
GM048	147-162	(21)
GM056	121-178	(21)
GM059	188-310	(21)
GM071	247-302	(21)
GM076	102-139	(21)
GM081	88-173	(21)
GM087	103-142	(21)
GM089	181-269	(21)
GM218	200-210	(21)
GM237	105-115	(21)
GM245	242-366	(21)
GM339	79-130	(14)
GM346	93-161	(17)
GM386	212-245	(7)
GM393	141-145	(7)
GM402	95-101	(7)
GM403	113-156	(7)
GM408	173-214	(7)
GM421	206-294	(4)
GM525	215-294	(4)
GM624	185-238	(23)
GM629	320-352	(23)
GM1954	115	(24)
GM2009	107	NCBI reference number GO339476.1

Table 4.2. Peanut genotypes used in the present study and their reaction to rust, caused by *Puccinia arachidis*

Genotype	Response to peanut rust
96x72-HO1-9-1-1-1-1-2-1	Resistant (chapter 2)
97x34-HO3-1-B2G-3-1-1-1	Resistant (chapter 2)
97x36-HO2-1-B2G-3-1-2-2	Resistant (chapter 2)
98x116-5-1-1-1-2-1	Resistant (chapter 2)
99x33-1-B2G-12-2-1	Resistant (chapter 2)
99x33-1-B2G-13-1-1	Resistant (chapter 2)
99x33-1-B2G-2-2-2	Resistant (chapter 2)
99x8-1-B2G-3-1-1	Resistant (chapter 2)
Altika	Susceptible (chapter 2)
Bailey High O/L	Unknown
BayoGrande	Susceptible (chapter 2)
BOL19-b5	Susceptible (chapter 2)
BOL3-7	Resistant (chapter 2)
C-99R	Susceptible (Field observations)
Florida-07	Unknown/ moderately susceptible
Georgia Green	Unknown
Georgia-03L	Moderately Resistant (T. B. Brenneman Personal communication)
Georgia-07W	Unknown
GT-C20	Resistant (Personal communication)
Guyana Jumbo	Susceptible (Field observations)
NC3033	Susceptible (Greenhouse observations)
NC94022	Unknown
PI478856	Resistant (31)
PI298115	Resistant (33)
PI314817	Resistant (33)
PI478856	Resistant (31)
PI562530	Resistant (31)
PI568164	Resistant (31)
PTBOL3-3	Resistant (chapter 2)
PTBOL3-4	Resistant (chapter 2)
Southern Runner	Resistant (chapter 2)
SPT-06-06	Unknown
SunOleic97R	Unknown
Tarapoto	Resistant (29, 33)
Tifguard	Unknown
Tifrunner	Unknown
Tifrust-10	Resistant (11)
Tifrust-13	Resistant (10)

Table 4.2. continued

Genotype	Response to peanut rust
TMV2	Susceptible (18)
York	Resistant (chapter 2)
PI544349	Resistant (25)
PI544351	Resistant (25)
PI556992	Resistant (26)

Table 4.3. Results from the analysis of molecular variance (AMOVA) for rust resistance in 43 peanut genotypes based on genetic differences in resistance to peanut rust, calculated from 32 SSR markers

Source of variation	df [a]	Sums of squares [b]	Variance components [c]	Percentage of variation accounted for
Among resistant and susceptible populations	2	83.808	0.700 [d]	2%
Within resistant and susceptible populations	40	1353.820	33.846 [e]	98%

[a] Degrees of freedom.
[b] Sum of squared deviations.
[c] Significance of the variance components.
[d] Significance among groups.
[e] Significance within groups.

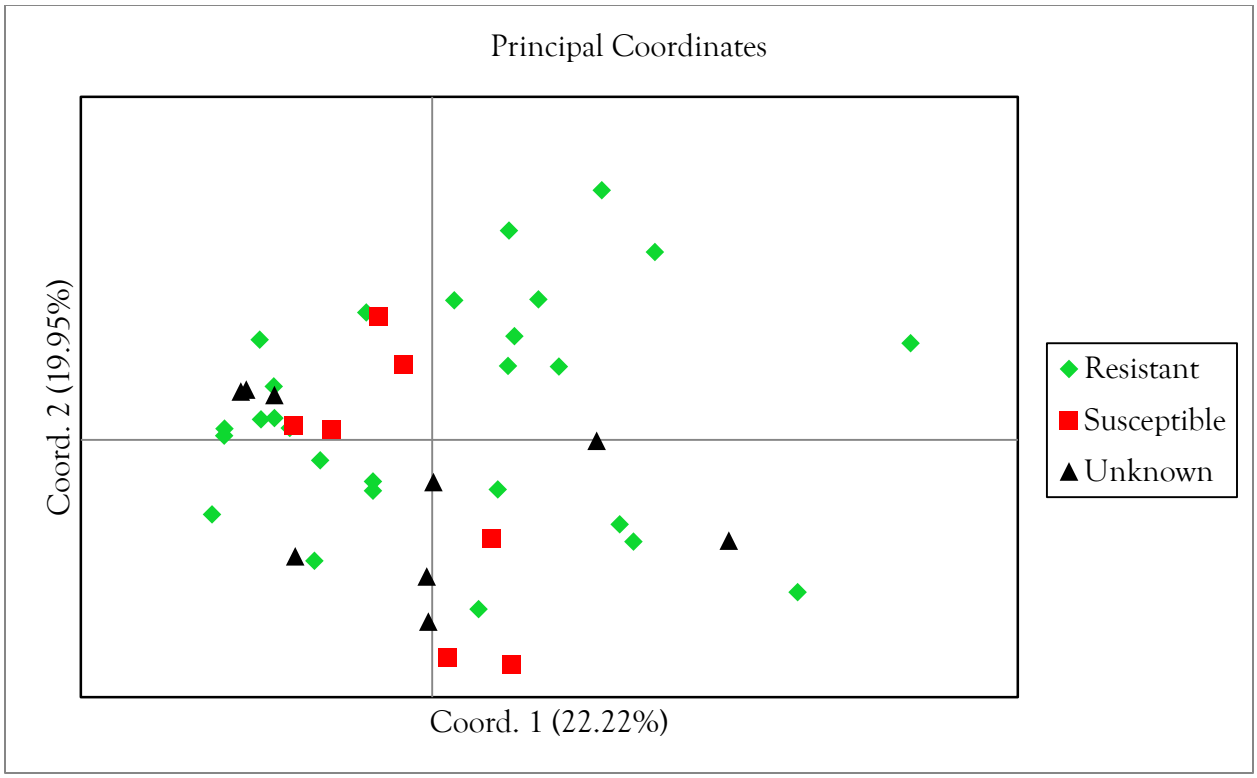

Fig. 4.1. Principal Component Analysis plot of 43 cultivated peanut genotypes based on genetic differences in resistance to peanut rust, calculated from 32 SSR markers.

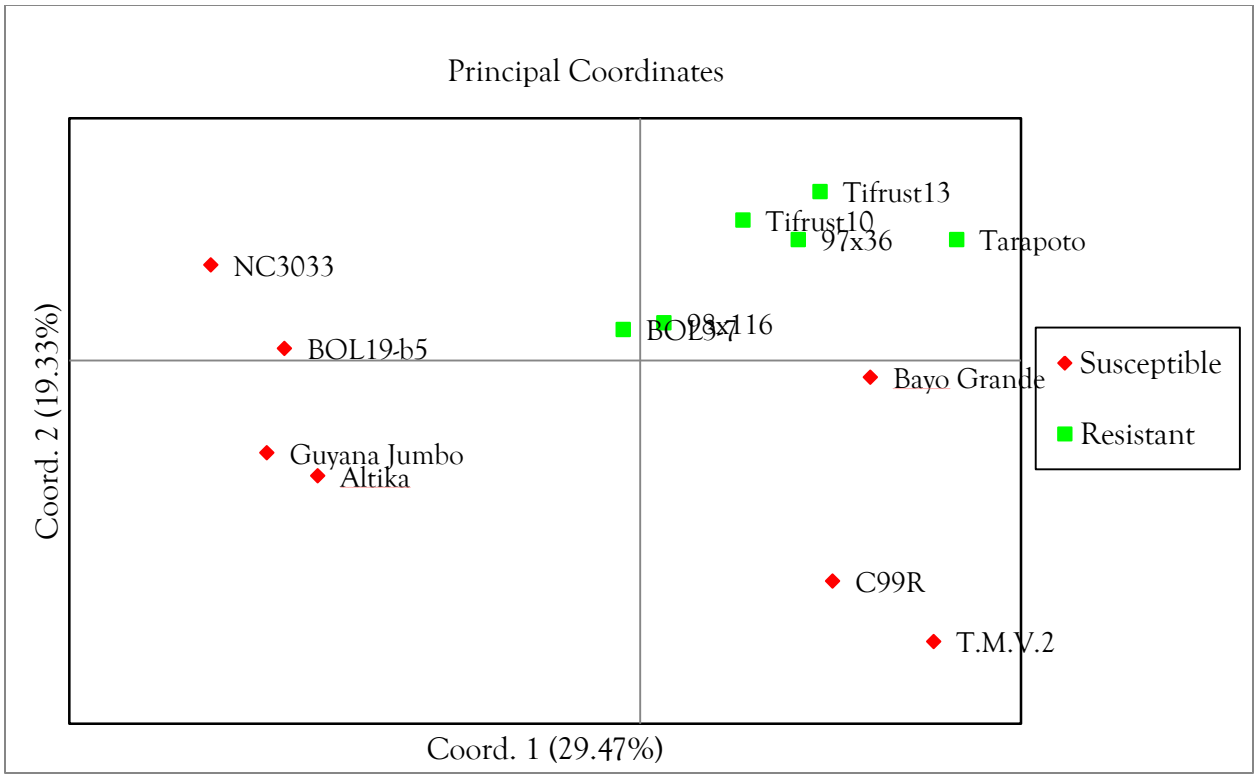

Fig 4.2. Principal Component Analysis plot of 6 highly rust resistant and 7 highly susceptible cultivated peanut genotypes. 97x36 = CRSP breeding line 97x36-HO2-1-B2G-3-1-2-2, and 98x118 = 98x116-5-1-1-1-2-1.

CHAPTER 5

MULTILOCUS PHYLOGENY OF GEOGRAPHICALLY DIVERSE *P. ARACHIDIS* ISOLATES[1]

[1] Power, I. L., Arias., R., and Culbreath, A. K. 2014. To be submitted to *Phytopathology*.

Abstract

Puccinia arachidis, is the causal agent of the economically important peanut rust disease. Yield losses due to peanut rust can be substantial, due to the ability of the fungus to complete multiple disease cycles throughout a crop season. Yield losses of 50% have been reported, however, despite the ability of *P. arachidis* to cause huge yield losses, little is known about the genetic variability of this pathogen, and little sequence information is available. In this first study on population genetics of *P. arachidis*, the 5.8S-ITS2-28S region, translongation elongation factor 1α, and cytochrome b of *P. arachidis* isolates collected from different regions in the U.S. and countries in Asia, South and Central America were sequenced to determine the genetic variation of the pathogen. These loci do not indicate high genetic variability among the populations: there was no clustering of isolates according to location or time collected.

Introduction

Puccinia arachidis Speg. is the causal agent of the economically important peanut rust disease. Yield losses due to peanut rust can be substantial due to the ability of the fungus to complete multiple disease cycles throughout a crop season; Subramanyam et al. (33) reported yield losses of 50%. Management of this disease usually requires multiple fungicide applications throughout the season however this will lead to increased production costs. Moreover, the pathogen may develop resistance with frequent fungicide applications (30). Host resistance is thus considered the most cost effective management method for in the developing countries where this disease is prevalent.

Puccinia arachidis reproduces asexually, through continued multiplication of the urediniospores. There are no reports of the existence of basidiospores, pycniospores or aeciospores, and teliospores have been rarely observed; no sexual stage of this fungus has been observed (7, 31, 32). The genetic variation in clonally reproducing organisms is typically lower than those that reproduce sexually, due to the lack of recombination during meiosis (20, 22-25). The likelihood for clonally reproducing plant pathogens to overcome disease management strategies, such as overcoming host resistance is therefore lower (20-26). However, it has been reported that asexually reproducing plant pathogens can overcome disease management strategies because of selection pressure. There are several reports (6, 15, 27) that the asexually reproducing *Puccinia triticina* develops different virulence genotypes in North America each year because of a combination of the different cultivars used as well as the inoculum that is blown into the area from different locations. Selection pressure due to fungicide application is another example of mutations in asexually reproducing due to disease management strategies. Schmitz et al. (29) reported the reduced sensitivity of *Phakopsora pachyrhizi* isolates to demethylation inhibiting (DMI) fungicides in Brazil, as a result of several amino acid substitutions in the *cyp51* gene.

Despite the huge yield losses that *P. arachidis* can cause, little is known about the genetic variability of this pathogen, and little DNA sequence information is available and little research is being conducted on this subject at the moment. Information on genetic diversity of pathogenic fungi can provide important information on origin, spread and evolution of the pathogen and is therefore important in developing effective and durable disease management strategies. Furthermore, knowledge of population genetics can provide information about whether or not the pathogen reproduces sexually or asexually. Greater knowledge of the genetic variability of the *P.*

arachidis populations and the genetics of resistance to peanut rust will therefore enable us to effectively breed for resistance and thus effectively manage the peanut rust disease on the long run.

In this study, one mitochondrial and two nuclear loci were analyzed to determine the genetic variation among isolates of *P. arachidis*, collected in 6 countries, between 2010 and 2013. This is the first study on population genetics of *P. arachidis*. Part of this research has been reported previously (28).

Materials and methods

Collection of *P. arachidis* isolates. *Puccinia arachidis* isolates were collected in North America, South America, Central America and Asia (Table 5.1), in different years and over different growing seasons. Isolates collected outside of Tifton, GA were collected using FTA cards (Whatman, Thermo Fisher Scientific. Inc., Wilmington, DE), and isolates collected from fields in Tifton, GA were collected using a vacuum pump.

DNA isolation. Genomic DNA of urediniospores was extracted from 10 to 25 mg of urediniospores per isolate, by grinding the spores with glass beads in a bead beater for 5 min, followed by the Omniprep for fungi extraction kit (G-Biosciences, St. Louis, MO) according to the instructions. DNA of isolates collected using FTA cards was extracted using the Qiagen REPLI-g Ultrafast mini kit (Qiagen, Valencia, CA), as described by Wang et al. (35) with minor revisions: Several spores per isolate were added to a 2.5-µl mixture that contained 1 µl phosphate buffered saline (PBS) and 1.5 µl denaturing buffer (D2). After a 10-min ice-incubation, 1.5 µl of stop solution was added. A 16-µl mixture containing 15 µl REPLI-g UltraFast reaction buffer and 1 µl REPLI-g UltraFast DNA polymerase was then added to the denatured DNA and incubated at

30°C for 16 h. The DNA polymerase was inactivated by heating the sample at 65°C for 3 min. The buffers, stop solution and polymerase were included in the kit. After each addition of buffer, stop solution and polymerase, the samples were vortex-mixed and centrifuged briefly. DNA was cleaned by precipitating with ethanol and NaAc. DNA quality was examined on a 1% agarose gel, and the quantity was determined with nanodrop. The DNA concentration was adjusted to 10 ng/ µl for amplification.

DNA amplification. Two nuclear loci, ITS and translongation elongation factor 1α (TEF), and one mitochondrial gene, *cytochrome b* (Cytb), were amplified and sequenced. Rust2inv (1) and LR6 (34) were used to amplify the complete ribosomal 5.8S subunit, the internal transcribed spacer region 2 (ITS2) and the 28S subunit, yielding a 1400-bp product. For TEF, a 600-bp portion was amplified using primers PaEF1F (AAAGTTCGAGAAGGAAGCTGC) and PaEF1R (AGGGACAAAGGGAATGCTTT). The Cytb sequence was obtained by amplifying a 200-bp product using primer pairs PaCytb1F (CCTCTAGGGGTGACTGGGAA) and PaCytb1R (GGTACGATCGAAGCTGGAGT). Primers PaCytb1F and PaCytb1R; and PaEF1F and PaEF1R were designed using the primer design tool from National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR amplifications for all primer combinations were performed in 25-µl total volumes containing 10 µl DNA template, 1.25 µl of each forward and reverse primer (10 µM), and 25 µl of PCR Master Mix (Promega Corp., Madison, WI). PCR conditions for ITS2 included an initial denaturing step at 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 57°C for 1 min and 72°C for 1.5 min, and a final extension at 72°C for 7 min. For TEF and Cytb the amplification conditions were an initial denaturing step

at 94°C for 2 min followed by 40 cycles of 94°C for 30s, 57°C for 1 min and 72°C for 1.5 min, and a final extension at 72°C for 7 min.

Sequencing. The PCR products were separated on a 1% agarose and visualized by staining with ethidium bromide. Single band PCR products were purified with the QIAquick PCR Purification kit (Qiagen, Valencia, CA). If more than one band was produced during amplification, the correct band was excised from the gel, and purified with the QIAquick Gel Extraction kit (Qiagen, Valencia, CA). Cleaned PCR products were sequenced with the same primers used for PCR amplification.

Phylogenetic analysis. To confirm rust specificity, all DNA sequences were subjected to BLAST search. Sequences were edited and assembled manually, aligned using Geneious alignment (93% similarity) in the Geneious R7 software program (Biomatters Ltd., www.geneious.com), using the genetic distance model Tamura-Nei. The Neighbor Joining tree building method was used to construct the phylogenetic trees.

Results and discussion

Based on sequences of genomic DNA, PCR-amplified and sequenced ribosomal 5.8S-ITS2-28S region (ITS2), the translongation elongation factor 1-α (TEF) and the cytochrome b (Cytb) regions there was no indication of variability among the isolates that was attributable to geographic location or collection date. None of the sequenced loci showed distinct, well-supported grouping (Fig. 5.1- Fig. 5.3). This high degree of genetic similarity is expected from for a pathogen that reproduces clonally.

Plant pathogens often are capable of adapting to their environment for survival. There are multiple reports each year of plant pathogens that have overcome host resistance (9-14, 16-19) or that have developed resistance to specific fungicides (2, 5, 29). This is more often the case in sexually reproducing pathogens that recombine during meiosis, leading to the development of new races, or when new more virulent pathogenic isolates are introduced in a different region.

Genetic variation is also known to occur in asexually reproducing organisms. Crop management activities can lead to selection of less fit individuals in a population. One example is directional selection due to the continued use of fungicides. Schmitz et al. (29) reported pathogenic isolates of the soybean rust causing fungus *Phakopsora phachyrhizi*, collected from fields in Brazil, that are less sensitive to DMI fungicides. The reduced sensitivity was due to multiple amino acid substitutions in the *cyp51* gene. In developing countries where peanut rust is a significant problem, management of the disease with fungicides is usually restricted due to the limited access to these chemistries. New isolates that might arise in the U.S. due to selection by fungicides used in the peanut production would likely not survive the cold winter temperatures.

Another crop management activity that can lead to the development of different races of plant pathogens would be selection due to cultivar use. Kolmer et al. (9-14, 16-19) reported the identification of several genetically different races of the wheat leaf rust fungus *P. striiformis*, every year from fields in North America. They suggested this could be due to the combination of cultivars grown in the different regions, and to inoculum that is blown into the area from different locations. The peanut cultivars usually grown in tropical countries are typically landraces consisting of different genetic background with less selection pressure for individuals in a population.

Yet another way asexually reproducing fungi can differ genetically is due to regional isolation. If this would be the case with *P. arachidis*, isolates collected from fields in Asia would likely be genetically different from isolates collected in North America. However, there was no distinction between Asian isolates and those from the Americas in any of the three loci. Peanut was introduced into Asia only after exploration of South America, and introduced even later into North America via Africa (4). Presumably, *P. arachidis* would not have existed in those areas before peanut was grown, and likely was introduced considerably later. Therefore, there has been a relatively short time for regional isolation compared to a pathogen like *Puccinia graminis*.

The genes, ITS, Cytb and TEF, examined in this study are essential for basic cellular function and fungal survival, and these "housekeeping genes" may be too conserved to provide an estimate of genetic variability. Mutations in conserved regions of these loci are often lethal. ITS regions are therefore often used in systematics studies to determine relationships at the genus level, since these are highly conserved regions. However, although highly conserved, these regions evolve faster than other conserved regions such as the mitochondrial cytochrome b gene or the nuclear translongation elongation factors. For this reason, the ITS regions have been useful for identifying molecular variability within populations of the same species (8). There are furthermore several multilocus studies using Cytb, TEF and beta-tubulin (BTUB) gene regions, where genetic variation was detected in different pathogenic isolates from different parts of the world. For example, Brewer et al. (3) sequenced ITS BTUB and TEF of *Erysiphe necator* isolates, the causal agent of grape powdery mildew, and found different numbers of haplotypes in all three loci. Furthermore, they were able to distinguish introduced populations from native U.S. isolates.

Although housekeeping genes have been successfully used to identify molecular variation in organisms, the use of microsatellite markers would be more useful for detecting variation if present. A SSR library for *P. arachidis* has been developed *de novo* and selected a subset of markers to be used on the collected isolates. The data from the microsatellite study may provide more information about the population structure of *P. arachidis*. Little information on the population structure of *P. arachidis* based on sequence characterization has been reported.

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References

1. Aime, M. C. 2006. Toward resolving family-level relationships in rust fungi (Uredinales). Mycoscience (Springer Science & Business Media B.V.) 47:112-122.
2. Boukef, S., McDonald, B. A., Yahyaoui, A., Rezgui, S., and Brunner, P. C. 2012. Frequency of mutations associated with fungicide resistance and population structure of *Mycosphaerella graminicola* in Tunisia. Eur. J. Plant Pathol. 132:111-122.

3. Brewer, M. T., and Milgroom, M. G. 2010. Phylogeography and population structure of the grape powdery mildew fungus, *Erysiphe necator*, from diverse *Vitis* species. BMC Evol. Biol. 10:268-281.

4. Coffelt, T. A., and Simpsom, C. E. 1997. Origin of the peanut. Page 2 in: Compendium of Peanut Diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith and P. Subrahmanyam, eds. American Phytopathological Society, St. Paul, Minnestoa.

5. Drabesova, J., Rysanek, P., Brunner, P., McDonald, B. A., and Croll, D. 2013. Population genetic structure of *Mycosphaerella graminicola* and Quinone Outside Inhibitor (QoI) resistance in the Czech Republic. Eur. J. Plant Pathol. 135:211-224.

6. German, S. E., and Kolmer, J. A. 1994. Virulence phenotypes of *Puccinia recondita* f. sp. tritici in Uruguay. Plant Dis. 78:1139-1141.

7. Hennen, J. F., Subrahmanyam, P., and Figueiredo, M. B. 1987. The taxonomy, life history, and evolution of *Puccinia arachidis* Speg. Pages 145-155 in: Groundnut rust disease: Proceedings of a discussion group meeting, B. A. McDonald, P. Subrahmanyam and J. A. Wightman, eds. International Crop Research Institute for the Semi-Arid Tropics, ICRISAT Center, Patancheru, Anhra Pradesh, 502 324, India.

8. Iwen, P. C., Hinrichs, S. H., and Rupp, M. E. 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. Med. Mycol. 40:87-109.

9. Kolmer, J. A. 1996. Physiologic specialization of *Puccinia recondita* f sp *tritici* in Canada in 1994. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 18:300-302.

10. Kolmer, J. A. 1988. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1987. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 10:354-358.

11. Kolmer, J. A. 1994. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1992. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 16:61-63.

12. Kolmer, J. A. 1998. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1996. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 20:176-181.

13. Kolmer, J. A. 2001. Physiologic specialization of *Puccinia triticina* in Canada in 1998. Plant Dis. 85:155-158.

14. Kolmer, J. A. 1999. Physiologic specialization of *Puccinia triticina* in Canada in 1997. Plant Dis. 83:194-197.

15. Kolmer, J. A. 1999. Virulence dynamics, phenotypic diversity, and virulence complexity in two populations of *Puccinia triticina* in Canada from 1987 to 1997. Can. J. Bot.-Rev. Can. Bot. 77:333-338.

16. Kolmer, J. A., and Chong, J. 1993. Distribution of virulence in 2 populations of *Puccinia coronata* f. sp. *avenae* in Canada. Can. J. Bot.-Rev. Can. Bot. 71:946-950.

17. Kolmer, J. A., and Hughes, M. E. 2013. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2011. Plant Dis. 97:1103-1108.

18. Kolmer, J. A., Long, D. L., and Hughes, M. E. 2012. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2010. Plant Dis. 96:1216-1221.

19. Kolmer, J. A., Long, D. L., Kosman, E., and Hughes, M. E. 2003. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2001. Plant Dis. 87:859-866.

20. McDonald, B. 2010. How can we achieve durable disease resistance in agricultural ecosystems? New Phytol. 185.

21. McDonald, B. 2009. Which way will the agricultural research pendulum swing? Agrarforschung 16:3-3.

22. McDonald, B. A. 1997. The population genetics of fungi: Tools and techniques. Phytopathology 87:448-453.

23. McDonald, B. A. 2004. Population genetics of plant pathogens. The Plant Health Instructor DOI:10.1094/PHI-A-2004-0524-01.

24. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

25. McDonald, B. A., and McDermott, J. M. 1993. The population genetics of plant pathogenic fungi. BioScience 43:311-319.

26. Milgroom, M. G., and Fry, W. E. 1997. Contributions of population genetics to plant disease epidemiology and management. Pages 1-30 in: Advances in Botanical Research Incorporating Advances in Plant Pathology, vol. 24.

27. Ordoñez, M. E., and Kolmer, J. A. 2009. Differentiation of molecular genotypes and virulence phenotypes of *Puccinia triticina* from common wheat in North America. Phytopathology 99:750-758.

28. Power, I. L., Culbreath, A. K., and Tillman, B. L. 2013. Characterization of resistance of peanut to *Puccinia arachidis*. Plant Health Progress. doi:10.1094/PHP-2013-1125-02-RS.

29. Schmitz, H. K., Medeiros, C. A., Craig, I. R., and Stammler, G. 2014. Sensitivity of *Phakopsora pachyrhizi* towards quinone-outside-inhibitors and demethylation-inhibitors, and corresponding resistance mechanisms. Pest Manag. Sci. 70:378-388.

30. Smith, N. B., and Littrell, R. H. 1980. Management of peanut foliar diseases with fungicides. Plant Dis. 64:356-360.

31. Subrahmanyam, P. 1997. Rust. Pages 31-33 in: Compendium of Peanut Diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith and P. Subrahmanyam, eds. American Phytopathological Society, St. Paul, MN.

32. Subrahmanyam, P., and McDonald, D. 1983. Rust Disease of Groundnut. in: Information bulletin no. 13. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Patancheru, Anhra Pradesh, India.

33. Subrahmanyam, P., Subba Rao, P. V., Gibbons, R. W., and McDonald, D. 1983. Components of resistance to *Puccinia arachidis* in peanuts Phytopathology 73:253-256.

34. Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172:4238-4246.

35. Wang, Y. C., Zhu, M. Q., Zhang, R., Yang, H. L., Wang, Y., Sun, G. Y., Jin, S. L., and Hsiang, T. 2009. Whole genome amplification of the rust *Puccinia striiformis* f. sp *tritici* from single spores. J. Microbiol. Methods 77:229-234.

Table 5.1. Characteristics of *Puccinia arachidis* isolates studied

Isolate nr	Country	State/ Place	Location	Collection date
1	Bolivia	Quirusillas	Research field	Mar-11
2	Bolivia	Saveedro	Research field	Mar-11
3	Bolivia	Puerto Fernandez	ANAPO Research field	Mar-11
4	Bolivia	Santa Cruz	ANAPO Research station sp2	Dec-11
5	Bolivia	Santa Cruz	ANAPO Research station	Apr-12
6	Bolivia	Puerto Fernandez	ANAPO Research field	Apr-12
7	Bolivia	26 de Augusto	ANAPO Research field	Apr-12
8	Bolivia	Santa Cruz	ANAPO Research station wild arachis	Apr-12
9	Bolivia	Santa Cruz	ANAPO Research station Greenhouse	Apr-12
10	Bolivia	Santa Cruz	ANAPO Research station sp1	Dec-11
11	Guyana	Annai	CRSP demoplot	Sep-10
12	Guyana	Annai	Farmer's field, Mr. Hamilton	Sep-10
13	Guyana	Annai	Farmer's field, Mr. Carlyle	May-11
14	Guyana	Annai	Farmer's field, Mr. Carlyle	Aug-11
15	Guyana	Annai	Farmer's field, Mr. Hamilton	Sep-11
16	Guyana	Annai	CRSP demoplot	Sep-11
17	Guyana	Annai	CRSP demoplot	Sep-12
18	Guyana	Annai	CRSP demoplot Gregory	Sep-11
19	Guyana	Annai	CRSP demoplot BOL3-7	Sep-11
20	Guyana	Annai	CRSP demoplot	Sep-11
21	Haiti	Tovar	Farmer's field	Sep-11
22	Haiti	Limbe	Ag School	Sep-11
23	Haiti	Bas Limbe	Farmer's field	May-12
24	Haiti	Isle Adam	Ag School	Sep-11
25	Haiti	Terrier Rouge	Ag School	Sep-11
26	Nicaragua	La Libertad	Farmer's field	Sep-11
27	Nicaragua	Malpaislo	Farmer's field	Sep-11

Table 5.1. continued

Isolate nr	Country	State/ Place	Location	Collection date
28	Nicaragua	San Antonio,	Farmer's field	Sep-11
29	Nicaragua	San Jose	Farmer's field	Sep-11
30	Nicaragua	El Ojachal	Farmer's field	Sep-11
31	Nicaragua	Ceiba Mocha	Farmer's field	Sep-11
32	Nicaragua	Lourdes	Farmer's field	May-12
33	Nicaragua	Bella Vista	Farmer's field	May-12
34	The Philippines	Bukidnon	Farmer's field	May-12
35	The Philippines	Bukidnon2	Farmer's field	May-12
36	The Philippines	Bukidnon Manok Porti	Farmer's field	May-12
37	USA	Gainesville, FL	CITRA research station	Aug-10
38	USA	Gainesville, FL	CITRA research station	Aug-11
39	USA	Attapulgus, GA	UGA Tifton research station	Sep-11
40	USA	Attapulgus, GA	UGA Tifton research station	Sep-12
41	USA	Tifton, GA	UGA Tifton research station, Gibbs farm	Sep-10
42	USA	Tifton, GA	UGA Tifton research station, Lang farm	Sep-12
43	USA	Tifton, GA	UGA Tifton research station, RDC farm	Sep-12
44	USA	Tifton, GA	UGA Tifton research station, Gibbs farm #919	Sep-12
45	USA	Tifton, GA	UGA Tifton research station, Gibbs farm #969	Sep-12
46	USA	Tifton, GA	UGA Tifton research station, Gibbs farm #1059	Sep-12
47	USA	Tifton, GA	UGA Tifton research station, Gibbs farm	Sep-12
48	USA	Tifton, GA	UGA Tifton research station, Ponder farm	Sep-12
49	USA	Plains, GA	UGA Tifton research station	Sep-12
50	USA	Tifton, GA	UGA Tifton research station, Lang farm, single-pustule isolate	Sep-12

Table 5.1. continued

Isolate nr	Country	State/ Place	Location	Collection date
51	USA	Attapulgus, GA	UGA Tifton research station urediniospore isolate	Sep-11
52	USA	Attapulgus, GA	UGA Tifton research station Greenhouse urediniospore isolate	Sep-11
53	USA	Tifton, GA	UGA Tifton research station, Lang farm urediniospore isolate	Sep-12
54	USA	Tifton, GA	UGA Tifton research station, Gibbs farm urediniospore isolate	Sep-10
55	USA	Tifton, GA	UGA Tifton research station, Lang farm urediniospore isolate	Sep-13
56	USA	Gainesville, FL	CITRA research station	Aug-12
57	USA	Tifton, GA	UGA Tifton research station, Lang farm	Sep-10
58	USA	Tifton, GA	UGA Tifton research station, Gibbs farm #916	Sep-12

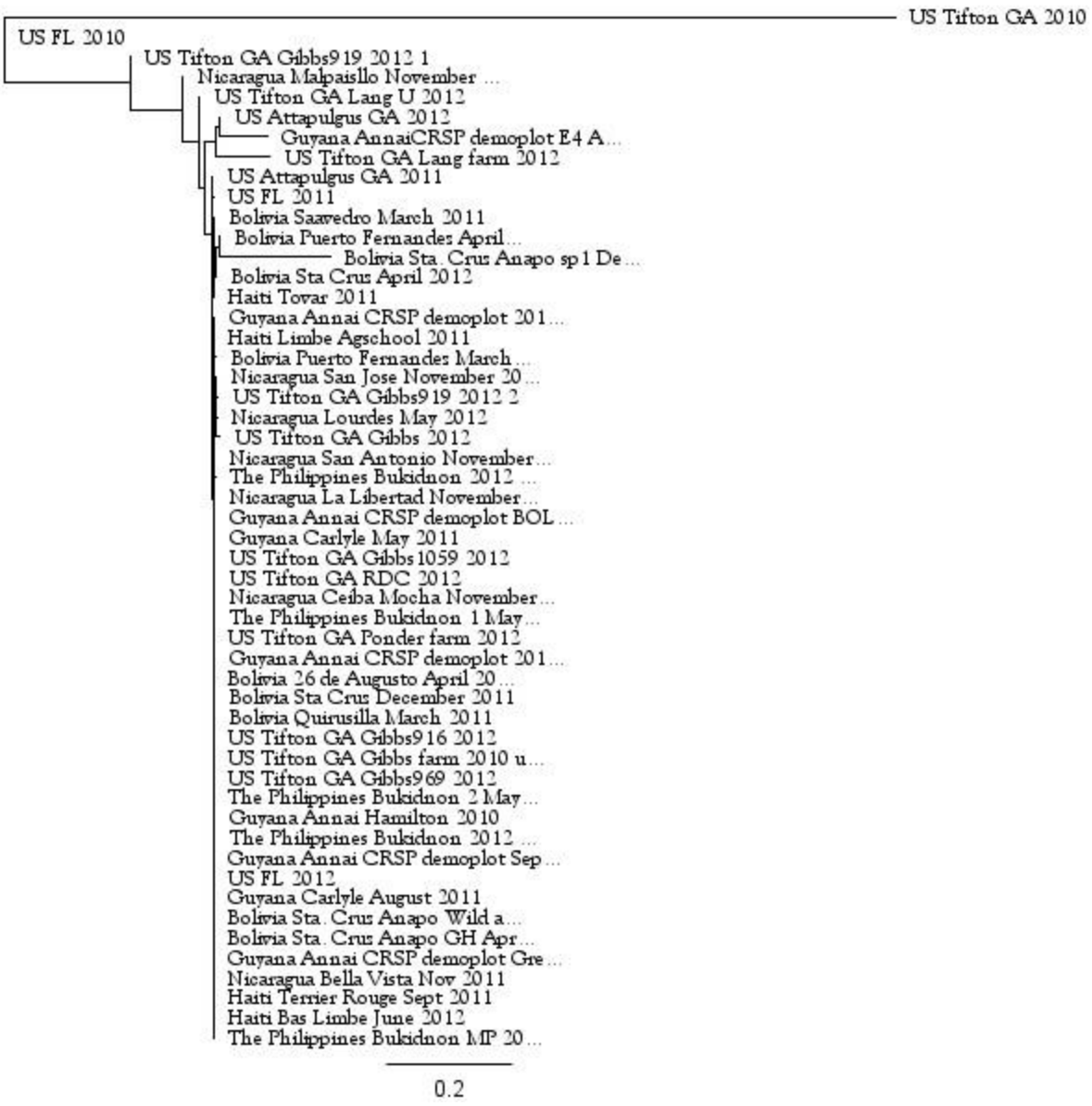

Fig. 5.1. Phylogenetic relation of the 5.8S-ITS2-28S region of 52 *Puccinia arachidis* isolates collected from cultivated and wild peanut, in the U.S., Bolivia, Guyana, Haiti, Nicaragua and the Philippines, as derived from neighbor joining analysis of the ribosomal 5.8S-ITS2-28S region, after multiple alignment with Geneious. The confidence level of the nodes was tested by bootstrapping 1000 replications. Scale bar indicates a distance of 0.2 (2 base pair changes per 10 nucleotide positions).

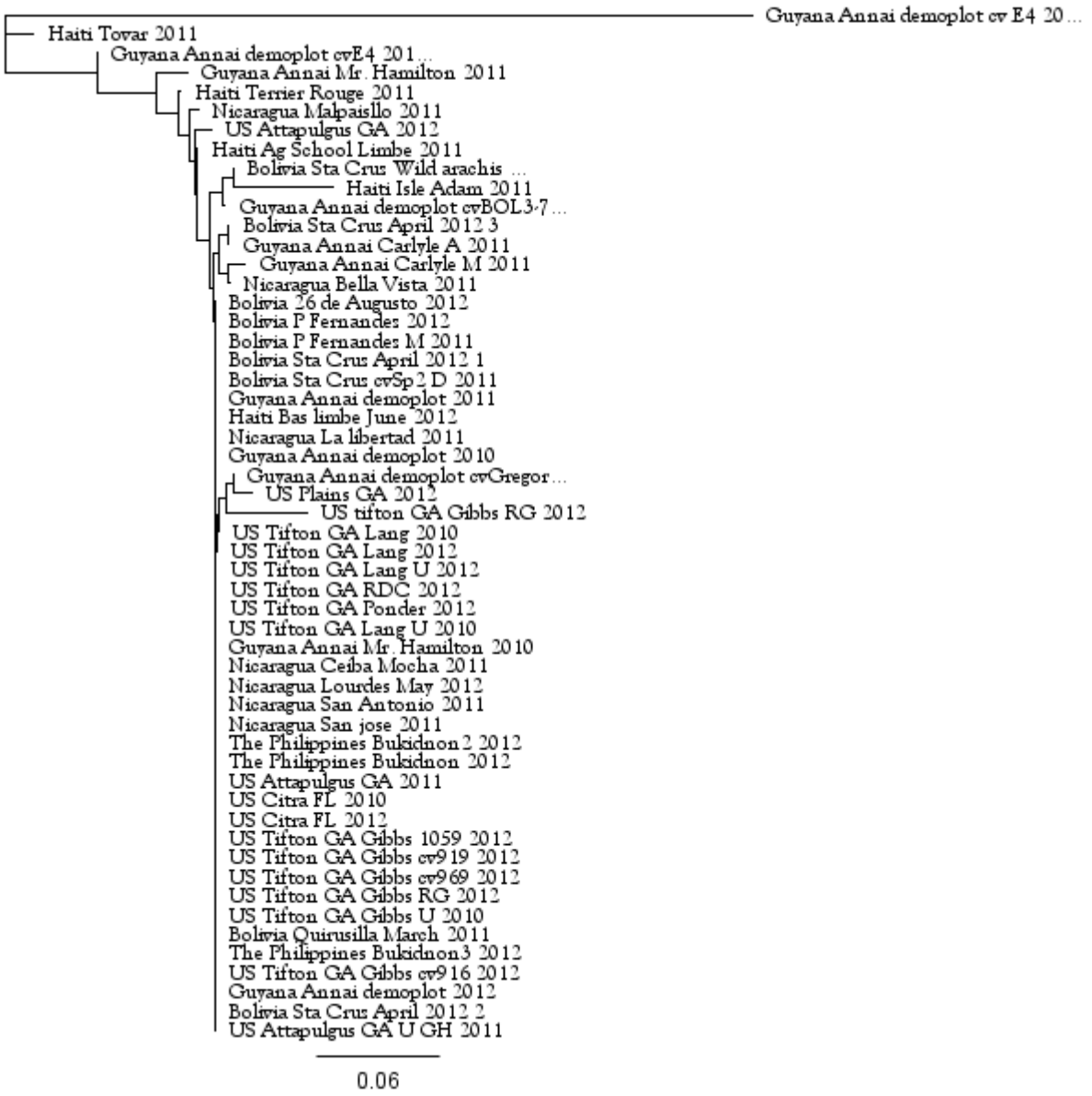

Fig. 5.2. Phylogenetic relation of Cytochrome b (Cytb) of 53 *Puccinia arachidis* isolates collected from cultivated and wild peanut, in the U.S., Bolivia, Guyana, Haiti, Nicaragua and the Philippines, as derived from neighbor joining analysis of Cytb, after multiple alignment with Geneious. The confidence level of the nodes was tested by bootstrapping 1000 replications. Scale bar indicates a distance of 0.06 (6 base pair changes per 100 nucleotide positions).

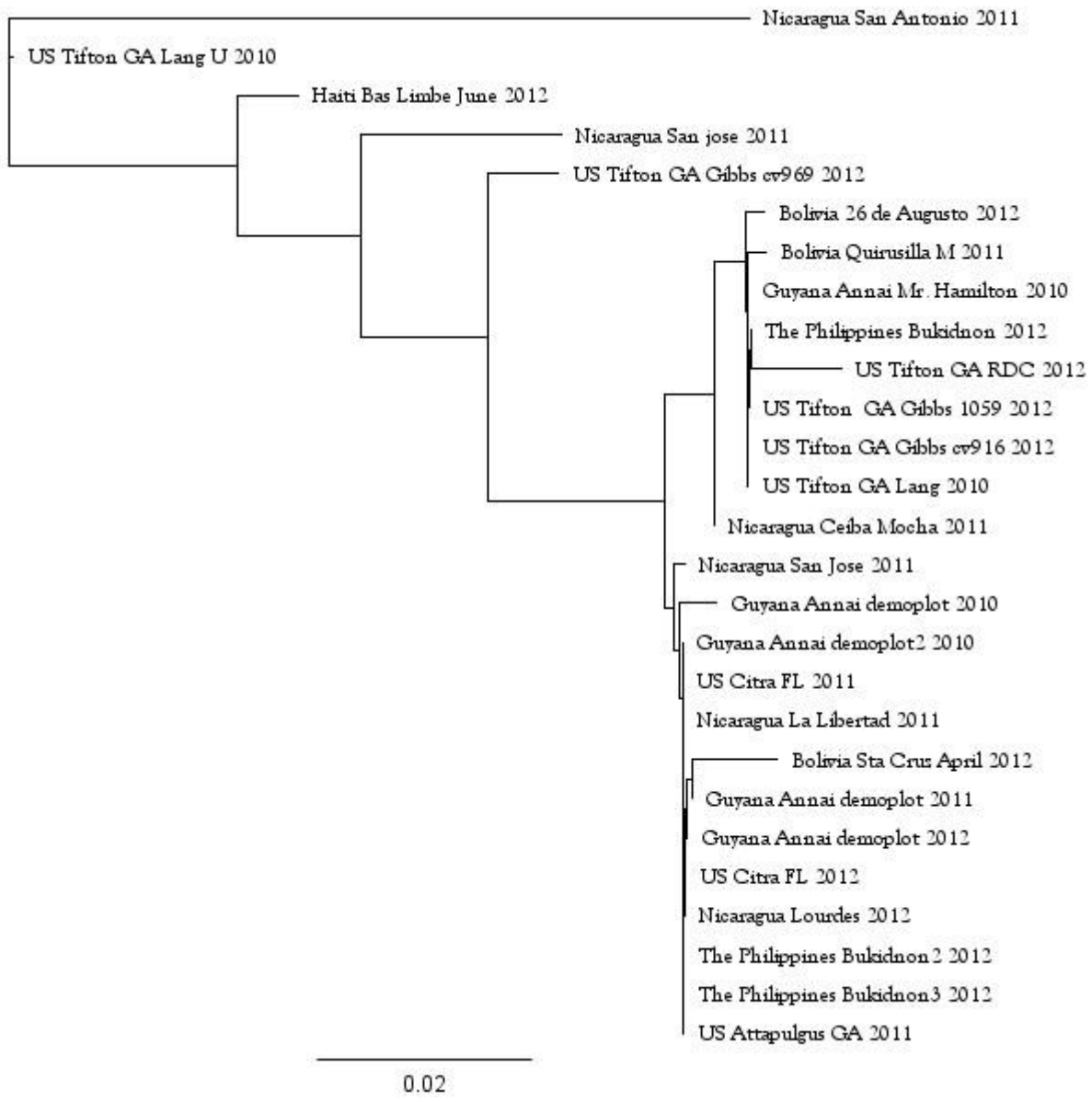

Fig. 5.3. Phylogenetic relation of Translongation elongation factor 1-α (TEF) of 53 *Puccinia arachidis* isolates collected from cultivated and wild peanut, in the U.S., Bolivia, Guyana, Haiti, Nicaragua and the Philippines, as derived from neighbor joining analysis of TEF, after multiple alignment with Geneious. The confidence level of the nodes was tested by bootstrapping 1000 replications. Scale bar indicates a distance of 0.02 (2 base pair changes per 100 nucleotide positions).

CHAPTER 6

Conclusions

Peanut rust, caused by *Puccinia arachidis* Speg, is an important foliar disease of peanut (*Arachis hypogaea* L.) in tropical countries. Host resistance is the best option for disease management in these countries. The goal of this research was to improve management of peanut rust by screening and characterizing available peanut breeding lines and cultivars for resistance to *Puccinia arachidis*. The focus was on identifying existing cultivars or genotypes with resistance to *P. arachidis* with potential for immediate use in low input peanut producing countries or that may be used as parents in breeding programs where developing cultivars with resistance to multiple foliar pathogens, including *P. arachidis*, is a primary objective. Information on components of rust resistance in peanut genotypes and cultivars should be useful in both scenarios.

Field, green house and growth chamber experiments were conducted to evaluate the response of peanut breeding lines with Bolivian genetic background, parents of mapping populations and peanut cultivars used in Georgia, U.S. to peanut rust. In field studies conducted over 2010-2013, several CRSP breeding lines demonstrated varying levels of rust resistance, and a select few were resistant to late leaf spot, caused by *Cercosporidium arichidicola*, as well. The greenhouse and growth chamber assays revealed that infection frequency and percent diseased area can be used as indicators for field resistance, as genotypes with low infection frequency at 7 days after inoculation, typically had smaller percent diseased areas and longer latent periods.

The use of molecular markers can be beneficial in the breeding process, as it can increase efficiency of breeding efforts, however, the genetic variation in cultivated peanut low. This low genetic variation complicates crop improvement as it limits the development of genetic markers. Still, continued research efforts have led to the development of different molecular markers, able to identify polymorphisms in peanut germplasm, and hundreds of SSR markers have been developed by research groups including the University of Georgia and The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) that were able to detect high levels of polymorphism in peanut genotypes. Information on whether the newly developed polymorphic SSR markers are associated with peanut rust in the CRSP breeding lines, or whether these QTLs can also be detected in the CRSP breeding lines would be beneficial. Newly developed CRSP breeding lines, plant introductions and commonly grown cultivars, were molecularly characterized using polymorphic SSR markers. These markers used detected polymorphisms but were not able to distinguish resistant from susceptible peanut genotypes. None of the 22 private bands generated for the resistant population were absolute and no marker alleles could be exclusively linked to all resistant or all susceptible genotypes. This could be because the resistance observed in the genotypes may be explained by other partial resistance genes than previously identified. Highly resistant and highly susceptible genotypes did cluster; which may indicate that some of the resistant genotypes evaluated in this study may be identified with existing markers.

Knowledge on the population structure of plant pathogens is important for disease management strategies; for example the likelihood that a particular pathogen will develop resistance to fungicides, or the likelihood that the pathogen will overcome host resistance Pathogens that reproduce sexually typically have a higher genetic variation than those that

reproduce clonally, due to recombination during meiosis, and are therefore more likely to overcome disease management strategies, especially if resistance to a fungicide or virulence on a plant cultivar is governed by one or a few genes. These strategies furthermore impose high selection pressure on the pathogen population

Information on the molecular variability of *P. arachidis* will lay the groundwork in the population structure and evolution of the pathogen, and should help to effectively breed for resistance and thus effectively manage the peanut rust disease. That information should also provide an indication of the likelihood of rust populations becoming resistant to new resistant cultivars. Three loci of *P. arachidis* isolates collected from different regions in the U.S. and countries in Asia, South and Central America were sequenced to determine the genetic variation of *P. arachidis.* The loci 5.8S-ITS2-28S region, translongation elongation factor 1α, and *cytochrome b*, do not indicate high genetic variability among the populations: there was no clustering of isolates according to location or time collected.

The findings of this study are significant as they indicate that once a peanut genotype with resistance to rust is identified there is a high likelihood for durable resistance, because several minor genes of resistance are involved in slowing down the epidemic rate of the pathogen. The resistance will furthermore be durable due to the low genetic variation of the pathogen.

Emphasis of future activities may be on evaluating the CRSP breeding lines of interest in multiple locations to test whether the lines would be able to uphold the resistance in different environments. Since infection frequency, latent period and percent diseased area are correlated to field resistance, they could be used to phenotype the existing peanut mapping populations. This

could enable the development of more linkage maps with the possibility to identify additional QTLs for rust resistance.

Regarding the population structure of the peanut rust pathogen, work is being undertaken to screen the collected isolates with a number of prescreened SSR markers from a SSR library of *P. arachidis* that has been developed *de novo*. Efforts are furthermore undertaken to collect more isolates, including from new locations.

ADDENDUM

Comparing detached leaf assays for rust resistance

Identifying peanut germplasm for rust resistance typically involves field screening, however, there is one peanut season per year which means that field screenings can be carried out only once per year. Furthermore, the peanut rust pathogen needs to be reintroduced every year, which, together with weather conditions, may lead to disease pressure that may not be adequate to distinguish resistant from susceptible genotypes. It is therefore important to have a reliable and rapid greenhouse or growth chamber technique to screen peanut breeding lines for resistance to rust that is correlated with field resistance. The objective of this study is to compare different growth chamber methods for their ability to accurately and efficiently distinguish rust resistance peanut genotypes. Three growth chamber experiments were carried out to test the efficiency for screening for peanut rust resistance

Detached leaf method comparison. The methods that were compared were the beaker method, the tube method, agar plate method, filter plate method and living plants. A single-pustule isolate was developed by harvesting urediniospores from an isolate collected from fields in Georgia with a vacuum pomp, and re-inoculating healthy leaves with urediniospores from a single pustule. This cycle was repeated several times to ensure a purified single-pustule isolate. The urediniospores from the single-spore isolate were collected from the re-inoculated leaves to prepare a spore suspension of 40,000 spores/ml of 0.005% Tween 20, which was quantified using a hemacytometer. The peanut genotypes used in this study were Tifrust-13 and Altika. Peanut

plants were grown from seed in the greenhouse at 25°C, in 15 cm pots filled with commercial potting soil (Sunshine Professional Growing Mix, Sun Gro Horticulture Distribution Inc, Bellevue, WA), and were watered as needed. Four or 5 seed, treated with Vitavex PC (a.i. captan, pentachloronitrobenzene and carboxin, Bayer CropScience LP, Research Triangle Park, NC). For the detached leaf methods (beaker, tube, agar- and filter-plate), the youngest fully expanded leaves of 5- to 6-week-old plants were collected. For the beaker and tube methods, the ends of the petioles were dipped in rooting powder, and placed in beakers containing sterile damp sand (beaker method) or in test tubes containing sterile distilled water (tube method). For the plate methods, leaflets were detached, and placed in a petri dish (9 cm diameter) on sterile damp filters (filter-plate method) or water agar (agar-plate method) with the abaxial side up. For the living plants method, individual plants were replanted in cones containing potting soil 3- to 4-weeks after planting. The youngest fully expanded leaves were marked for inoculation, on the day of inoculation, when the plants were 5- to 6-week-old.

Leaf side comparison. Inoculation of peanut leaves on the abaxial (under side) or adaxial (upper side) side were compared. The youngest fully expanded leaves of 5- to 6-week-old plants were collected, the leaflets detached, and detached leaflets of two leaves were placed in 15-cm petri dishes containing sterile damp filters, one leaf with the adaxial up and the other leaf with the abaxial side up.

Surfactants. In this experiment several adjuvants were compared for their ability to enable rust urediniospores to infect peanut leaves more efficiently. The adjuvants that were compared were Tween 20, Vegetoil, Active-it, Induce and Silicon. Detached leaflets were placed on sterile moistened filter paper in 9-cm petri dishes with the abaxial side up, and inoculated with spore

suspensions of 40,000 spores/ml of 0.005% adjuvant. The spore suspensions were prepared as described above.

Inoculation and incubation. The leaves and leaflets were inoculated by spraying them for one second using a compressed air sprayer containing the uredinial spore suspension. There were three replicates per genotype and for each genotype a control was included by spraying leaves with 0.005% of the appropriate adjuvant/ solution. The petri dishes of the leaf side and surfactant comparison experiments, and the beakers, tubes, petri dishes and living plants of the methods comparison experiments, were arranged in a randomized complete block, and incubated in darkness for 16 h at 25°C, followed by incubation at 25°C, 12h photoperiod for 16 days in the incubator. In the methods comparison experiments, humidifiers were used to keep the leaves wet and the humidity high (> 90%), in the incubator. Incubation of the third trial of the methods comparison experiment was done in the greenhouse, to increase infection: The beakers, tubes, petri dishes containing the inoculated leaves and the living plants were placed in a moist chamber constructed of PVC pipe and covered with black plastic, and incubated in darkness for 16 h at 25°C. Humidifiers were used to keep the leaves wet and the humidity high (> 90%). From 7 days after inoculation (DAI) on, the leaflets were examined daily for the development of symptoms and signs. These experiments were done 3 times.

Data analysis. The components of resistance were measured as described in chapter 2. The effects of genotype on the components of resistance were analyzed using the Proc GLM procedure (SAS v 9.2, SAS Institute Inc., Cary, NC). Fisher's LSD ($P \leq 0.05$) was used to determine significant differences among genotypes and detached leaf assays for the components of resistance.

Results detached leaf method comparison. There were significant differences ($P < 0.05$) among the different methods for several of the components measured in trial 1 and trial 2. In both trials, the plate methods had the highest infection frequencies, lesion diameter and percent diseased area for both Altika and Tifrust-13. Infection frequency at 7 DAI was not significant for Altika ($P = 0.058$) in trial 1. The main

challenge for the beaker, tube and living plant was to maintain continuous leaf wetness during the first 16-h darkness incubation, and high relative humidity throughout the experiment, necessary for successful inoculation and development of signs. Therefore all plates, beakers, tubes and living plants were incubated in the greenhouse in a moist chamber constructed of PVC pipe and covered with black plastic. This resulted in fewer differences among methods: if leaf wetness and relative humidity can be maintained in the 16-h darkness incubation period, the beaker method, tube method and both plate methods have similar result. Choice of method for resistance screening would then depend on ease of data scoring.

Results leaf side comparison. There were few significant differences ($P < 0.05$) between inoculating the upper and underside of leaves for the components measured in all 3 trials. Differences were significant for infection frequency at 7 DAI and percent diseased area in trial 1, infection frequency at 7, 11, and 16 DAI and percent diseased area in trial 2 and for latent period in trial 3 for Altika. For Tifrust-13, differences were significant for infection frequency at 7, 11, and 16 DAI and percent diseased area in trial 1, and for percent diseased area and latent period in trial 3. The general trend was that inoculation on the underside of the leaves resulted in more disease development.

Results surfactants. There were significant differences ($P < 0.05$) among the surfactants for the components measured in all 3 trials: infection frequency at 7 DAI and lesion diameter were not significant for Altika ($P = 0.06$) in trial 3 and infection frequency at 16 DAI ($P = 0.15$) and percent diseased area ($P = 0.11$) were not significant for Tifrust-13 in trial 3. In all trials, Tween 20 and Silicon had the highest infection frequencies, lesion diameter and percent diseased area for both Altika and Tifrust-13. Surfactants Active it, Induce, and Vegetoil may have an inhibitory effect on spore germination; there was variable low (Active it trial 3, Induce trials 2 and 3, Vegetoil trials 1,2 and 3), or no (Active it trial 2, Induce trial 1) infection in the different trials.

Table A.1. Detached leaf method comparison, trial 1

Genotype	Method	Infection frequency 7 DAI [a]	Infection frequency 16 DAI [b]	Lesion diameter 16 DAI (mm) [c]	Percent diseased area 16 DAI (%) [d]
Altika	Agar-plate	2.48 ab [e]	5.72 a	0.96 a	8.20 a
	Beakers	0.33 b	1.00 b	0.73 a	1.26 b
	Filter-plate	3.19 a	5.58 a	0.90 a	7.27 a
	Living plants	0.10 b	0.10 b	0.24 b	1.16 b
	Tubes	0.07 b	0.12 b	0.87 a	0.12 b
LSD		2.56	2.20	0.49	5.07
Tifrust-13	Agar-plate	2.55 a	5.62 a	0.87 a	12.69 a
	Beakers	0.20 b	0.65 b	0.81 a	1.83 b
	Filter-plate	2.88 a	5.21 a	0.87 a	8.58 a
	Living plants	0.09 b	0.00 b	0.00 b	0.00 b
	Tubes	0.13 b	0.00 b	0.00 b	0.00 b
LSD		1.45	2.42	0.08	6.16

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[c] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[d] Means of percent diseased area (%) of 3 replications, 16 DAI.
[e] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.2. Detached leaf method comparison, trial 2

Genotype	Method	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]
Altika	Agar-plate	2.88 a [f]	6.00 a	6.73 a	0.99 a	11.27 a
	Beakers	0.00 b	0.03 b	0.03 b	0.16 b	0.02 b
	Filter-plate	2.83 a	6.07 a	6.57 a	1.08 a	11.95 a
	Living plants	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
	Tubes	0.00 b	0.01 b	0.01 b	0.05 b	0.00 b
LSD		1.05	1.31	1.24	0.21	3.61
Tifrust-13	Agar-plate	2.03 a	4.92 a	6.27 a	0.89 a	5.30 b
	Beakers	0.00 b	0.01 b	0.01 b	0.02 b	0.01 c
	Filter-plate	3.17 a	6.12 a	7.72 a	0.97 a	11.2 a
	Living plants	0.00 b	0.00 b	0.00 b	0.00 b	0.00 c
	Tubes	0.00 b	0.01 b	0.01 b	0.03 b	0.00 c
LSD		1.47	2.69	2.02	0.09	5.08

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.3. Detached leaf method comparison, trial 3

Genotype	Method	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]	Latent period (days) [f]
Altika	Agar-plate	1.38 a-b [g]	4.77 a	5.99 a	1.15 a	13.17 a	9.67 a
	Beakers	2.65 a	7.02 a	7.56 a	1.08 a	11.39 a	9.33 a
	Filter-plate	1.45 a-b	3.81 a-b	5.08 a	1.14 a	8.92 a	9.33 a
	Living plants	0.03 b	0.70 b	1.16 b	0.86 a	0.89 b	10.33 a
	Tubes	2.73 a	4.95 a	5.81 a	1.02 a	9.49 a	9.00 a
LSD		2.61	3.27	2.79	0.36	5.75	1.35
Tifrust-13	Agar-plate	1.37 a	6.04 a	2.35 a-b	0.84 a	2.26 b-c	10.67 a
	Beakers	0.46 a-b	1.68 b	2.68 a-b	0.85 a	6.02 a	10.67 a
	Filter-plate	1.15 a	2.23 b	4.73 a	0.88 a	5.57 ab	10.33 a
	Living plants	0.02 b	0.39 b	0.80 b	0.75 a	0.39 c	10.67 a
	Tubes	0.57 a-b	0.77 b	2.64 a-b	0.78 a	2.43 b-c	10.67 a
LSD		0.95	2.32	2.86	0.22	3.32	1.14

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%)of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.4. Leaf side comparison, trial 1

Genotype	Leaf side	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]
Altika	Under	0.59 a [f]	3.62 a	4.67 a	0.90 a	6.29 a
	Upper	0.00 b	1.08 a	1.54 a	0.96 a	2.68 b
LSD		0.49	5.44	5.12	0.37	1.85
Tifrust-13	Under	0.95 a	4.24 a	5.32 a	0.75 a	5.41 a
	Upper	0.00 b	0.32 b	1.42 b	0.70 a	1.27 b
LSD		0.55	0.69	2.74	0.1	3.68

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%)of 3 replications, 16 DAI.
[f] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.5. Leaf side comparison, trial 2

Genotype	Leaf side	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]
Altika	Under	3.64 a [f]	6.37 a	5.73 a	1.09 a	12.58 a
	Upper	0.00 b	1.58 b	2.36 b	1.17a	3.80 b
LSD		1.40	3.53	1.35	0.47	3.40
Tifrust-13	Under	1.21 a	2.48 a	3.50 a	0.86 a	2.89 a
	Upper	0.00 b	0.13 a	0.56 a	0.72 a	0.76 a
LSD		1.51	4.2	3.55	0.35	4.27

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.6. Leaf side comparison, trial 3

Genotype	Leaf side	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]	Latent period (days) [f]
Altika	Under	2.43 a [g]	3.42 a	4.27 a	1.00 a	7.93 a	8.33 b
	Upper	0.52 a	1.30 a	2.17 a	0.88 a	2.20 a	10.33 a
LSD		2.29	3.38	2.78	0.18	6.77	0.00
Tifrust-13	Under	0.23 a	0.34 a	0.80 a	0.73b	0.51 a	10.00 b
	Upper	0.00 a	0.10 a	0.38 a	0.92 a	0.14 b	11.00 b
LSD		0.59	0.31	0.84	0.17	0.11	0.00

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.7. Surfactants, trial 1

Genotype	Surfactant	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]
Altika	Active it *	.	.	.	.	.
	Induce	0.00 b [f]	0.00 c	0.00 c	0.00 b	0.00 c
	Silicon	2.60 a	6.33 a-b	6.06 a	0.75 a	12.74 a
	Tween20	2.81 a	6.84 a	6.82 a	0.87 a	14.14 a
	Vegetoil	0.47 b	2.15 b-c	2.98 b	0.75 a	5.31 b
LSD		1.74	4.43	2.72	0.24	5.30
Tifrust-13	Active it	.	.	.	.	
	Induce	0.00 c	0.00 c	0.00 d	0.00 b	0.00 b
	Silicon	2.94 a	6.11 a	6.66 a	0.86 a	10.18 a
	Tween20	1.81 b	4.38 ab	4.64 b	0.74 a	5.38 a-b
	Vegetoil	0.10 c	1.99 b-c	2.61 c	0.78 a	2.04 b
LSD		1.07	2.42	2.01	0.18	5.88

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.
* Surfactant active it was not included in trial 1.

Table A.8. Surfactants, trial 2

Genotype	Surfactant	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]	Latent period (days) [f]
Altika	Active it	0.00 b [g]	0.00 b	0.00 b	0.00 c	0.00 b	0.00 b
	Induce	0.00 b	0.15 b	0.24 b	0.84 b	0.25 b	9.33 a
	Silicon	1.65 a	2.73 a	3.30 a	1.05 a-b	6.09 a	9.33 a
	Tween20	1.17 a	2.27 a	3.06 a	1.13 a	4.74 a	9.50 a
	Vegetoil	0.00 b	0.46 b	0.65 b	1.04 a-b	1.06 b	10.00 a
LSD		1.11	1.16	1.16	0.22	2.32	0.75
Tifrust-13	Active it	0.00 b	0.00 b	0.00 b	0.00 d	0.00 b	0.00 b
	Induce	0.03 b	0.10 b	0.19 b	0.70 b-c	0.573 b	9.67 a
	Silicon	1.26 a	1.75 a	2.50 a	0.93 a	3.13 ab	9.33 a
	Tween20	1.77 a	2.63 a	3.87 a	0.91 a-b	4.72 a	9.67 a
	Vegetoil	0.00 b	0.07 b	0.15 b	0.48 c	0.16 b	10.00 a
LSD		1.02	1.09	1.52	0.22	3.55	0.91

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table A.9. Surfactants, trial 3

Genotype	Surfactant	Infection frequency 7 DAI [a]	Infection frequency 11 DAI [b]	Infection frequency 16 DAI [c]	Lesion diameter 16 DAI (mm) [d]	Percent diseased area 16 DAI (%) [e]	Latent period (days) [f]
Altika	Active it	0.20 c [g]	0.46 b	0.92 b	1.01 a	1.18 b	9.00 a-b
	Induce	0.07 c	0.15 b	0.24 b	0.90 a	1.16 b	10.00 a
	Silicon	2.50 a	3.96 a	4.34 a	1.00 a	14.09 a	8.00 b
	Tween20	2.29 a-b	3.71 a	3.54 a	1.13 a	9.56 a	8.00 b
	Vegetoil	0.39 b-c	0.67 b	1.03 b	1.14 a	1.60 b	9.00 ab
LSD		2.08	2.61	1.80	0.43	7.51	1.19
Tifrust-13	Active it	0.01 b-c	0.02 b	0.17 a-b	0.39 b	0.39 b	11.00 a
	Induce	0.00 c	0.00 b	0.05 b	0.17 b	0.41 b	11.00 a
	Silicon	0.42 a-b	0.84 a-b	1.77 a	0.85 a	1.44 a-b	10.00 b
	Tween20	0.56 a	1.25 a	1.14 a-b	0.85 a	2.31 a	10.00 b
	Vegetoil	0.05 b-c	0.07 b	0.15 a-b	0.40 b	0.24 b	11.00 a
LSD		0.41	0.85	1.64	0.35	1.79	0.84

[a] Means of number of lesions per leaf area (cm^2) of 3 replications, 7 days after inoculation (DAI).
[b] Means of number of lesions per leaf area (cm^2) of 3 replications, 11 DAI.
[c] Means of number of lesions per leaf area (cm^2) of 3 replications, 16 DAI.
[d] Means of lesion diameter (mm) of 3 replications, 16 DAI.
[e] Means of percent diseased area (%) of 3 replications, 16 DAI.
[f] Means of 3 replications of the number of DAI until at least one lesion produced spores.
[g] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.