GENETICS OF TOMATO SPOTTED WILT VIRUS RESISTANCE IN PEANUT (*Arachis hypogaea L.*)

By

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To Monica and Victoria.

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS 4

LIST OF TABLES 7

LIST OF FIGURES 9

ABSTRACT 11

CHAPTER

1 EFFECT OF PLANTING DATE ON SPOTTED WILT EXPRESSION IN DIFFERENTIALLY SUSCEPTIBLE PEANUT (ARACHIS HYPOGAEA L.) CULTIVARS 13

Introduction 13
Material and Methods 15
Field Trials 15
Trait Measurements 17
Statistical Analysis 17
Variables and factors 18
Anaylses performed 18
Linear mixed analyses 19
Calculation of Genetic Correlations 26
Calculation of Repeatability 28
Results 28
Location Effect 29
Planting Date Effect 30
Location by Planting date (cell) effect 31
Phenotypic and Genetic Correlations 31
Repeatability 32
Discussion 33
Conclusions 36

2 HERITABILITY AND BREEDING VALUES OF TSWV RESISTANCE IN POPULATIONS FROM PEANUT (ARACHIS HYPOGAEA L.) CROSSES 46

Introduction 46
Material and Methods 49
Results 55
Observed Variance Components 57
Heritability 58
Phenotypic and Genetic Correlations 58
Breeding Values (BLUPs) 59
Generation BLUPs 59

Individual BLUPs....60
Family BLUPs....61
Discussion....61
Conclusion....67

3 ARTIFICIAL INOCULATION STUDIES IN THE TSWV-PEANUT PATHOSYSTEM....89

Introduction....89
Materials and Methods....91
Plant Culture....91
Inoculum Preparation....92
Sap Inoculation....92
Description of Tests....92
Test 1: Effect of elapsed time from preparation to inoculation on infection frequency....92
Test 2: Determining the importance of amount of rubbing on infection rate....93
Test 3: Evaluating the influence of inoculum concentration on infection rate....93
Imposing Treatments....93
Evaluation of Inoculated Plants and Analysis of Data....94
Results....96
Test 1....96
Test 2....98
Test 3....99
Discussion....100
Test 1....100
Test 2....101
Test 3....102
Conclusions....103

LIST OF REFERENCES....106

BIOGRAPHICAL SKETCH....112

LIST OF TABLES

Table		page
1-1	Layout of data collection for planting date studies of peanut in Marianna and Citra, Florida in 2005.	38
1-2	Means and standard errors for tomato spotted wilt disease intensity ratings at different planting dates at Citra and Marianna, Florida in 2005.	38
1-3	Location effect and variance ratios for bivariate analysis performed on transformed spotted wilt disease intensity ratings at three planting dates assessed in different times at Citra and Marianna, Florida in 2005.	39
1-4	Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for planting dates assessed at different times at Citra and Marianna, Florida in 2005.	39
1-5	Planting date effect and variance ratios for univariate analysis performed on transformed spotted wilt disease intensity ratings at four planting dates assessed in different times at Citra and Marianna, Florida in 2005.	40
1-6	Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for at four planting dates assessed in different times at Citra and Marianna, Florida in 2005.	40
1-7	Location by planting date combination (cell) effect and variance ratios for multivariate analysis performed on transformed spotted wilt disease intensity ratings at different planting dates assessed at different times at Citra and Marianna, Florida in 2005.	40
1-8	Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for location by planting date combinations (cells) assessed at different times in Citra and Marianna, Florida in 2005. Each assessment date was analyzed separately.	41
1-9	Phenotypic and genetic correlations among transformed spotted wilt disease intensity ratings at four planting dates assessed in different times at Citra and Marianna, Florida in 2005. Values in brackets are standard errors.	41
1-10	Type B genetic correlations and [standard errors] for transformed spotted wilt disease intensity ratings at four planting dates assessed in different times in Citra and Marianna, Florida in 2005. [a]	42
1-11	Entry-mean repeatability estimates and their [standard errors] for transformed spotted wilt intensity ratings at three planting dates assessed three times in Citra and Marianna, Florida in 2005. Values for each location are separated by a slash, Citra being on the left and Marianna on the right.	42

2-1 Sowing date, replication number and design of tests assessing performance against spotted wilt in five peanut crosses in Florida.....69

2-2 Mean (S.D.) score for each spotted wilt symptom at 30, 60 and 120 days after planting, at each of five field tests in which five peanut populations were evaluated in Florida.....69

2-3 REML variance estimates for stunting and foliar symptoms caused by TSWV in populations derived from five peanut crosses tested at Citra, Florida in 2005 and 2006, Marianna, Florida in 2006 and 2007 and Quincy, Florida in 2007.....70

2-4 Heritability (S.E.) estimates for stunting and foliar symptoms caused by TSWV on peanut populations from five crosses at different assessment dates in five tests in Floirida. Estimates were calculated using univariate Animal Models.....72

2-5 Phenotypic and genetic correlation (S.E.) estimates between stunting and foliar symptoms caused by TSWV on peanut populations from five crosses at different assessment dates in five tests in Florida. Estimates were calculated using univariate Animal Models.....72

3-1 Effect of elapsed time after preparation of Tomato spotted wilt virus inoculum on the number of peanut plants declared infected by visual examination or serological means. 105

3-2 Maximum Likelihood Estimates for time and inoculum batch effects on artificial inoculation of Georgia Green peanut.....105

3-3 Effect of inoculum dilution on the number of peanut plants declared infected by ELISA. 105

LIST OF FIGURES

Figure		page
1-1	Change of spotted wilt disease intensity ratings over time at Citra and Marianna, Florida in 2005 (averaged over all planting dates and genotypes)	43
1-2	Spotted wilt disease intensity ratings at 112 days after planting for ten genotypes planted at different dates at Marianna, Florida in 2005.	44
1-3	Spotted wilt disease intensity ratings at 112 days after planting for ten genotypes planted at different dates at Citra, Florida in 2005.	45
2-1	Incidence of spotted wilt symptoms at 120 days after planting in populations from five peanut crosses tested in five Florida environments.	73
2-2	Percentage of plants displaying spotted wilt symptoms in five peanut crosses in five field tests assessed at three dates in five Florida environments.	74
2-4	Spotted wilt incidence in four peanut genotypes at three assessment dates at Marianna, Florida in 2006	76
2-5	Spotted wilt incidence in four peanut genotypes at three assessment dates at Marianna, Florida in 2007	77
2-6	Observed stunting severity among different populations from five peanut crosses, at three different dates at Marianna, Florida in 2007	78
2-7	Frequency distributions for TSWV-induced stunting scores among parents of five peanut crosses field tested at Marianna, Florida in 2007.	79
2-8	Frequency distributions for TSWV-induced stunting scores among F2 populations from four peanut crosses field tested at Marianna, Florida in 2007.	80
2-9	Observed vs. estimated overall severity in stunting and foliar symptoms caused by TSWV on five peanut crosses in five field tests in Florida.	81
2-10	Best linear unbiased predictors (relative to test average) for TSWV-induced stunting in parents of five peanut crosses assessed in five field tests in Florida.	82
2-11	Breeding values' reliability for TSWV-induced stunting among individuals in segregating populations derived from five peanut crosses evaluated in five field tests.	83
2-12	Generation-mean best linear unbiased predictors for TSWV-induced stunting in populations from five peanut crosses tested at Quincy, Florida in 2007 (all reliabilities were above 0.9).	84

2-13 Generation-mean best linear unbiased predictors for TSWV-induced stunting in populations from five peanut crosses tested at Marianna, Florida in 2007 (all reliabilities were above 0.9). 85

2-15 Variability of best linear unbiased predictors for TSWV-induced stunting in the F_2 and F_3 generations of five peanut crosses tested at Quincy, Florida in 2007. 87

2-16 Percentage of individuals and *(number of F_3 families)* displaying BLUPs for TSWV-induced stunting above their best parent, in each of five peanut crosses tested at Marianna and Quincy, Florida in 2007. 88

Abstract of Dissertation Presented to the Graduate School
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(Arachis hypogaea L.)

By

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Major: Agronomy

Tomato spotted wilt virus (TSWV, *Bunyaviridae:Tospovirus*) is a major peanut pathogen in the USA. Its management involves, among other factors, the use of resistant cultivars and recommended planting dates.

Ten genotypes with varied degrees of resistance were field tested in two locations and four planting dates with the following objectives: 1) to ascertain the importance of planting dates and location as determining factors of spotted wilt epidemic intensity, 2) t evaluate the consistency in the performance of an array of genotypes with contrasting spotted wilt resistance assessed at different times, and 3) to provide an estimation of how much genotypic consistency can be ascribed to genetic causes. Results indicated that location was a significant factor in determining the spotted wilt damage, while planting date was significant only under a light epidemic or late in the season under a heavy epidemic. The high correlation between assessment dates implied that genotypic performance was perceived early and differences persisted until harvest. High Type B genetic correlation and repeatability suggested a strong genetic determination of resistance.

Heritability is a genetic parameter of paramount importance for efficient plant breeding but no estimates have been published for resistance to TSWV in peanuts. To provide such estimates and assess resistance sources, five populations from three resistant and a susceptible parent were field tested in five environments in Florida, USA. Approximately 36,300 total plants were individually assessed three times for five spotted wilt symptoms using a six level scale. Each environment was individually analyzed using an Animal Model containing block, plot, additive and non-additive terms. High phenotypic (0.80-0.93) and genetic (0.88-0.99) correlation estimates between stunting and spots/mosaic were obtained. Individual-basis heritability estimates showed a wide range (0.01-0.71) although values most frequently were in the low-medium range. This suggests individual selection for resistance to spotted wilt should not be applied in early generations within the tested populations. The resistant parents produced populations with similar breeding values when crossed to the susceptible parent, while the population from a cross between resistant parents exhibited the best breeding values for resistance to spotted wilt.

A published inoculation method was used to study if inoculum age, viral concentration, and extent of rubbing during inoculation affected the frequency of infection. Results showed that neither number of rubbings nor inoculum concentration were important factors. Inoculum showed better infectivity 10 minutes after preparation than at zero or twenty minutes after preparation. Inoculum batch was an important factor; highlighting the fact that viral titer is highly variable even when collected from similar plant tissues. The overall low infection rates suggest that additional work is necessary for mechanical inoculation to be a reliable research tool.

CHAPTER 1
EFFECT OF PLANTING DATE ON SPOTTED WILT EXPRESSION IN DIFFERENTIALLY SUSCEPTIBLE PEANUT (ARACHIS HYPOGAEA L.) CULTIVARS

Introduction

Peanut spotted wilt caused by Tomato spotted wilt virus (TSWV, *Bunyaviridae:Tospovirus*) can cause significant losses in the Southeastern USA. Epidemics of TSWV are highly variable among locations and even from year to year at a single location (Culbreath et al., 2003).

Resistant cultivars are the single most important factor in the management of this disease (Brown et al., 2007). Although lack of genotype by environment (GxE) interaction under a wide range of conditions has been reported in the literature (McKeown et al., 2001), this is not the norm. Tillman et al. (2007) and Murakami et al. (2006) reported significant genotype x year and genotype x planting date interactions for final spotted wilt ratings. Culbreath et al. (1997) also found genotype by year interaction for some locations but not others for final spotted wilt intensity ratings. Similarly, in a two year study, Culbreath et al. (2005) found that genotypes interacted with locations, although similar trends were observed across locations and years. As more factors enter the equation, multiple interactions can sometimes occur (Hurt et al., 2005).

GxE interaction can be caused by genotype cross-over, a change in the relative ranking across environments, due to the interaction among pathogenicity factors and resistance genes (Develey-Riviere & Galiana, 2007). However, no genotype by isolate interaction has been reported so far in the TSWV-peanut pathosystem (Mandal et al., 2006). GxE interaction can also have statistical causes. Heterocedasticity (unequal variances) is a violation of the ANOVA assumptions commonly found in biological experiments (Eisen and Saxton, 1983). This is caused by the relationship between variance and mean which is usually referred to as “scale effects” (Falconer and MacKay, 1996).

These scale effects cause the interaction term in the ANOVA to become significant (Eisen and Saxton, 1983). A way to cope with this biological fact is to move away from the usual approach of classical linear models towards a mixed linear models approach which provides the flexibility of modeling data means, variances and covariances by specifying the correct structure of variances and errors (Gilmour et al., 2006).

Genetic correlations among traits indicate the degree of change in one trait as a result of a change in another trait (Zobel and Talbert, 1984). Estimates of type B genetic correlations are also used as quantitative measures of genotype by environment interactions (Lu et al., 2001). Type B is the genetic correlation of the same trait measured on the same individual at different environments (Yamada, 1962).

Several methods can be used to estimate Type B genetic correlation. The simplest ones are called generically "univariate" because by using univariate linear models they calculate genetic correlations according different procedures. They are easy to calculate but can be biased if data are severely unbalanced or variances are very different (Lu et al., 2001).

With the increase in computational power of computers, statistical software using restricted maximum likelihood (REML) techniques has become widely available and provides a means to calculate Type B genetic correlations referred generically as "multivariate analysis". These methods can estimate genetic variances and covariances simultaneously using a REML approach (Holland, 2006). For these methods, the traits being correlated (the same trait in different environments) are handled with attention to their variance-covariance structure, thus solving the main limitation of the univariate methods. The REML approach is better for handling unbalanced data for the purpose of variance component estimation (Searle et al., 1992). Multivariate methods

can also apply constraints to estimates of genetic variances and covariances so that estimates of genetic correlation remains within the theoretical parameter space (Lu et al., 2001).

Type B genetic correlations range between 0 and 1. Its value indicates the correlation of genotypic performance across sites, thus assessing GxE interaction. High values indicate reduced GxE interaction and high genotypic determination for the trait studied (Lu et al., 2001, Lynch and Walsh, 1998).

An additional rough estimate regarding the importance of genotype in the observed performance across environments is the repeatability, which is the intraclass correlation among observations of the same trait at different moments. It provides the theoretical upper limit for the heritability (Falconer & Mackay, 1996).

For spotted wilt resistance a better distinction of the genotypic performance has usually been observed under strong epidemics (Culbreath et al., 2003). A better separation of a wide array of responses allows more accurate estimations of repeatability (Betrán et al., 2006).

In order to better understand the nature of the GxE interaction frequently reported in the TSWV-pathosystem, the present study pursued the following objectives: 1) To ascertain the importance of planting date and location as determining factors of spotted wilt epidemic intensity, 2) To evaluate the consistency in the performance of an array of genotypes with contrasting spotted wilt resistance assessed at different times, and 3) To provide an estimation of how much of the genotypic consistency can be ascribed to genetic causes.

Material and Methods

Field Trials

Two field tests were conducted at the University of Florida Plant Science Research and Education Unit in Citra, Florida on a Candler Sand (Hyperthermic, uncoated Typic Quartzipsamments) and at the North Florida Research and Education Center near Marianna,

Florida on a Chipola loamy sand (Loamy, kaolinitic, thermic Arenic Kanhapludults), during the summer of 2005.

Eight cultivars and two breeding lines with variable maturity, belonging to three market groups (spanish, runner, virginia) and having varied response to TSWV were tested. Based on previous research the genotypes were considered to have three different reactions to TSWV: susceptible, moderately resistant (intermediate) and resistant. The susceptible group included F-435HO (Gorbet, pers. comm.), NemaTAM (Simpson et al., 2003) and SunOleic 97R (Gorbet & Knauft, 2000). Georgia Green (Branch, 1996) and ANorden (Gorbet, 2007a) constituted the intermediate group while C-99R (Gorbet and Shokes 2002), NC94002 (Gorbet, pers. comm.), DP-1 (Gorbet, 2003), AP-3 (Gorbet, 2007b) and Georgia-02C (Branch 2003) formed the resistant group.

The planting window at both locations was divided so that a similar number of days elapsed between successive planting dates. At Citra the planting dates were 03/29/2005, 04/19/2005, 05/10/2005 and 06/2/2005 while at Marianna they were 4/19/05, 5/04/05, 5/19/05 and 6/06/05.

The experimental design was a 2 x 4 x 10 (location x planting date x cultivar) factorial which was planted in a randomized complete block, split-split-plot design, with location being the whole-plot, planting date the sub-plot and cultivar the sub-sub-plot. Plots consisted of two 4.5 m long rows spaced 0.91 m apart. The planting density was 18 seeds/m. The first planting date at Citra didn't include F435HO, NemaTAM or NC94002.

After sowing, plots were maintained according to commercial peanut production practices for the region with fertilizer, herbicide, fungicide, insecticide and irrigation applied as recommended by the University of Florida extension guidelines.

Trait Measurements

Natural TSWV-infected thrips populations were relied upon to cause spotted wilt epidemics. Although asymptomatic infections of TSWV in peanut have been reported (Culbreath et al., 1992) all discussion about spotted wilt incidence in the present study refers to symptomatic plants.

A disease intensity rating (DIR) representing a combination of incidence and severity was calculated by counting the number of foci of plants severely affected by spotted wilt for each plot divided by the number of potential foci (Culbreath et al., 1997). A focus represented 0.31 m or less of linear row with plants severely stunted, killed, or showing intense chlorosis due to TSWV. Strong reduction (<50% than healthy) in height or width of the peanut row was required for a row portion to be considered severely affected with regard to stunting and subsequently declared as a focus. If a portion showed less than severe reductions, it was counted as one half of a "focus".

The DIR were measured at the four assessment dates (ADs) observed in Table 1-1, expressed as days after planting (DAP), along with other details.

Samples were taken from symptomatic plants and tested by DAS-ELISA (SRA 30400/0096 kit, AGDIA, Elkhart, IN) to confirm TSWV infection.

Statistical Analysis

All the analyses carried out in this study employed linear mixed models which were performed using ASREML (Gilmour et al, 2006). In short, and following the notation presented by the authors, the software uses the following linear mixed model.

If y denotes an n x 1 vector of observations, the linear mixed model (lato sensu) can be expressed as

$$y = X\tau + Zu + e \qquad \text{(Eq. 1-1)}$$

where τ is the p x 1 vector of fixed effects, X is an n x p design matrix of full column rank which associates observations with the appropriate combination of fixed effects, u is the q x 1 vector of random effects, Z is the n x q design matrix which associates observations with the appropriate combination of random effects, and e is the n x 1 vector of residual errors.

The model (Eq. 1-1) is called a linear mixed model or linear mixed effects model. It is assumed:

$$\begin{bmatrix} u \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \theta\begin{bmatrix} G(\gamma) & 0 \\ 0 & R(\phi) \end{bmatrix}\right)$$

where the matrices G and R are functions of parameters γ and ϕ, respectively. The parameter θ is a variance parameter which is usually referred to as the scale parameter.

Both G and R matrices can assume different structures depending on the inherent structure of the data. It's necessary, in consequence, to find the right matrix structure so that a correct and meaningful analysis of the data can be performed.

Variables and factors

The variable under study in all the analyses was the DIR. The ratings were transformed to arcsine(square root(DIR)) to improve normality of the original variable.

The factors included in the analyses were Genotype, Location and Planting Date (PD). Both Location and PD were considered fixed effects while the Genotype was considered random (regarded as a sample of the population of genotypes that could be grown in Southeastern USA).

Anaylses performed

The fact that not all PD were evaluated on all the AD caused imbalance in the design (Table 1-1). Consequently, analyses involving the three factors at the same time (location, PD

and genotype) were only performed for two different ADs and not all the PDs. Simpler analyses were also performed according to data availability. In most of the analyses, the effects of fixed factors (location, PD or their interaction) were evaluated. When statistical significance was below 5%, predicted marginal means and overall standard errors were obtained as suggested by Gilmour et al. (2004). Additionally, different types of genetic correlation were obtained for DIR from different ADs in every analysis.

Linear mixed analyses

The data analysis began by building simple linear mixed models (including genotypes plus either Location or PD) to test for differences in levels of the fixed effects and then progressed towards more complex models containing location, planting date and genotype at the same time. The general progression in complexity followed this order:

i) Univariate analysis for location. It studied, for each PD and AD, the effect of Locations.

ii) Bivariate analysis through G structure specification. It applied a bivariate model containing location and genotype with specification of G structures by means of which the correlation between DIR at each location was estimated. This analysis was performed for each PD separately.

iii) Univariate analysis for PDs. It studied, for each location and AD, the effect of PDs.

iv) Multivariate analysis through G structure specification. This applied a multivariate approach. The DIR from each Loc*PD combination (cell) was regarded as a variable through the specification of the corresponding G structure. Consequently, a correlation among the DIR was obtained.

v) Bivariate analysis for different ADs. This was designed to address the correlation among DIR from different ADs for every available Location by PD combination (see Table 1-1). Additionally, the effect of PD on each DIR in the pair being analyzed, was determined.

A detailed explanation of each analysis follows:

i) Univariate analysis for location

This analysis was performed for each planting date separately to test the difference in the DIR between locations. For example, for PD 2, the only score available at both locations was the one obtained at 132 AD. Thus, this data subset was analyzed and from that the differences among Locations were tested.

The arcsine(square root(DIR) values observed in a PD at a determined AD were modeled as

$$y = \mu + X_l \tau_l + Z_v u_v + Z_{lv} u_{lv} + Z_{lb} u_{lb} + e \qquad \text{(Eq. 1-2)}$$

where y is a vector containing the arcsine(square root(DIR) values, τ_l is the p x 1 vector of a constant and fixed effect Location, Xl is an n x p design matrix of full column rank which associates observations with the appropriate combination of the fixed effect. The u vectors (u_v, u_{lv} and u_{lb}) are q x 1 vectors for the random terms variety, Location by variety and Location by block while Z matrices (Z_v, Z_{lv} and Z_{lb}) are n x q design matrices for those random effects mentioned above. The random effects and error are assumed to be independent Gaussian variables with zero means and variance structures $\text{var}(u_i) = \sigma^2_i I_{bi}$ (where b_i is the length of u_i; i = 1….3) and $\text{var}(e) = \sigma^2 I_n$.

The random terms were assumed to have a unique G structure while the error variance was described by specifying an Identity R structure for each Location.

This analysis also provided an estimate of Type B genetic correlation (Yamada II) among DIR at certain AD from different Locations.

ii) Bivariate analysis through G structure specification

This analysis tested the differences in DIR among locations at certain AD and PD. The data were modeled as

$$y = X_l \tau_l + Z_{lg} u_{lg} + Z_{lb} u_{lb} + e \qquad \text{(Eq. 1-3)}$$

where y is a vector containing the arcsine(square root(DIR) for the corresponding AD, τ_l is the p x 1 vector of a constant and fixed effect Location, Xl is an n x p design matrix of full column rank which associates observations with the appropriate combination of fixed effect. The u vectors (u_{lg} and u_{lb}) are q x 1 vectors for the random terms Location by variety and Location by block effects while Z matrices (Z_{lg} , and Z_{lb}) are n x q design matrices for those random effects mentioned above. The random effects and error are assumed to be independent Gaussian variables with zero means. The random terms were assumed to have different variance structures: diagonal G structure for blocks (no correlations among blocks from different locations)

$$\text{var}_{block} = \begin{bmatrix} \sigma^2_{b_1} & 0 & 0 \\ 0 & \sigma^2_{b_2} & 0 \\ 0 & 0 & \sigma^2_{b_3} \end{bmatrix}$$

and a correlation G structure for the genotypes (there is covariance among genotypes between locations):

$$\text{var}_{genotype} = \begin{bmatrix} \sigma_1^2 + \sigma_C^2 & \sigma_1^2 \\ \sigma_1^2 & \sigma_1^2 + \sigma_M^2 \end{bmatrix}$$

while the error variance was described by specifying an Identity R structure for each Location.

This analysis also provided an estimate of Type B genetic correlation (bivariate REML estimation) among DIR from different locations at each AD by modeling the G structure of the interaction between locations and genotypes. The correlation among genotypes was obtained at the genotypic rather than the phenotypic level because through the specification of a correlation model applied to the G structure for the Genotype factor it's possible to obtain a true estimation of the genotypic correlation (Gilmour et al., 2006).

Estimates of Repeatability were also obtained at each Location.

iii) Univariate analysis for planting dates

This analysis was performed for each location separately to test the difference in the DIR among PDs. For example, for Marianna, the DIRs obtained at AD 90 which were available only for PDs 2 & 3, (Table 1-1) were analyzed and the difference between those PDs was tested.

The arcsine(square root(DIR) observed in a Location at an AD were modeled as

$$y = \mu + X_p \tau_p + Z_v u_v + Z_{pv} u_{pv} + Z_{pb} u_{pb} + e \quad \text{(Eq. 1-4)}$$

where y is a vector containing the arcsine(square root(DIR), τ_p is the p x 1 vector of a constant and fixed effect PD, X is an n x p design matrix of full column rank which associates observations with the appropriate fixed effect level. The u vectors (u_v, u_{pv} and u_{pb}) are q x 1 vectors for the random terms variety, PD by variety and PD by block effects while Z matrices (Z_v, Z_{pv} and Z_{pb}) are n x q design matrices for those random effects mentioned above. The random

effects and error are assumed to be independent Gaussian variables with zero means and variance structures var(u_i) = $\sigma^2_i I_{bi}$ (where b_i is the length of u_i; i = 1….3) and var(e) = $\sigma^2 I_n$. The G structure was assumed unique and an Identity R structure was specified for each PD.

This analysis also allowed the estimation of Type B genetic correlation (Yamada II) among DIR from different PDs at each AD.

iv) Multivariate analysis through G structure specification

This analysis was performed analyzing both locations at the same time. The PDs analyzed were those in which both locations have DIRs for the same AD. Thus, for AD112, PDs 3 & 4 for both locations were analyzed while for AD132 PDs 2 & 3 were the ones tested.

For this analysis, the data structure was modified by creating a new factor (cell) which was the combination of the levels of the factors Location and PD. Consequently, when analyzing AD112 the four "cells" were Citra-PD3, Citra-PD4, Marianna-PD3 and Marianna-PD4 while in analyzing AD132 the "cells" were Citra-PD2, Citra-PD3, Marianna-PD2 and Marianna-PD3.

The arcsine(square root(DIR) observed in a Location at each of two planting dates at an AD were modeled as

$$y = X_c \tau_c + Z_{cg} u_{cg} + Z_{cb} u_{cb} + e \qquad \text{(Eq. 1-5)}$$

where y is a vector containing the arcsine(square root(DIR) for the corresponding AD, τ_c is the p x 1 vector of a constant and fixed effect Cell, X_c is an n x p design matrix of full column rank which associates observations with the appropriate combination of fixed effect. The u vectors (u_{cg} and u_{cb}) are q x 1 vectors for the random terms Cell by variety and Cell by block while Z matrices (Z_{cg} , and Z_{cb}) are n x q design matrices for those random effects mentioned above. The random effects and error are assumed to be independent Gaussian variables with zero

means. The random terms were assumed to have different variance structures: diagonal G structure for blocks (no correlations among blocks from different cells) and unstructured correlation G structure for the genotypes (there is covariance among genotypes between cells) while the error variance was described by specifying an Identity R structure for each Cell.

v) Bivariate analysis for different assessment dates

For multivariate linear mixed methods, measurements from different environments are treated as different variable with different variance and covariance structures which are simultaneously estimated using the REML approach (Schaeffer & Wilton, 1978). Consequently, the main weakness of univariate methods (i.e. heterogeneous variances) is properly addressed (Lu, 2001).

A bivariate analysis (a special case of multivariate) was used here to estimate the genetic correlation between DIR by jointly analyzing two AD's from the same location.

In Marianna, DIRs at each AD were taken in at least two different PDs (see Table 1-1). This also allowed testing the effects of PDs on both AD DIRs being analyzed bivariately. In the case of Citra, each combination of AD DIRs being analyzed was taken only in one PD so no testing for PD effect was possible.

The bivariate model for the Marianna data subset can be written as

$$y = (I_2 \otimes X_p)\,\tau_p + (I_2 \otimes Z_g)\,u_g + (I_2 \otimes Z_{pg})\,u_{pg} + (I_2 \otimes Z_{pb})\,u_{pb} + e \qquad \text{(Eq. 1-6)}$$

where $y = (y'_{ADa}, y'_{ADb})'$; $u = (u'_{g_{ADa}}, u'_{g_{ADb}})'$; $u = (u'_{pg_{ADa}}, u'_{pg_{ADb}})'$; $u = (u'_{pb_{ADa}}, u'_{pb_{ADb}})'$ and $e = (e'_{ADa}, e'_{ADb})'$. In turn y'_{ADa} = the vector containing the arcsine(square root(DIR) for AD "a"; while τ_p is the 1 x p vector of a constant and fixed effect PD, X_p is an *p x n* design matrix of full column rank which associates observations with the appropriate combination of the fixed effect, the *u* vectors ($u'_{g_{ADa}}, u'_{pg_{ADa}}$ and $u'_{pb_{ADa}}$) are 1 x q vectors for the random terms variety, PD by

variety and PD by block while Z matrices (Z_g, Z_{pg} and Z_{pb}) are n x q design matrices for those random terms mentioned above.

The random effects and error were assumed to be independent Gaussian variables with zero means and variance structures:

$$\text{var}(u_g) = \sigma^2_g I_{10},\ var(u_{pg}) = \sigma^2_{pg} I_{20},\ var(u_{pb}) = \sigma^2_{pbj} I_3 \text{ and } var(e_j) = \sigma^2 I_{60}.$$

Thus, the random terms were assumed to have a variance resulting from the direct product of three G correlation structures (one for each random term) while the error variance was described by specifying a unique unstructured correlation R structure.

In addition to the previous assumptions, the bivariate analysis also involves the following ones:

$$\text{cov}(u_{g_{ADa}})u'_{g_{ADb}} = \sigma_{g_{ADaADb}} I_{10},\ \text{cov}(u_{pg_{ADa}})u'_{pg_{ADb}} = \sigma_{pg_{ADaADb}} I_{20},\ \text{cov}(u_{pb_{ADa}})u'_{pb_{ADb}} = \sigma_{pb_{ADaADb}} I_3$$

and $\text{cov}(e_{ADa})e'_{ADb} = \sigma_{ADaADb} I_{60}$. Thus random effects and errors are correlated between variables (DIRs from different ADs).

The bivariate model for the Citra data subset can be written as

$$y = (I_2 \otimes X)\,\tau + (I_2 \otimes Z_g)\,u_g + (I_2 \otimes Z_b)\,u_b + e \qquad \text{(Eq. 1-7)}$$

where $y = (y'_{ADa}, y'_{ADb})'$; $u = (u'_{g_{ADa}}, u'_{g_{ADb}})'$; $u = (u'_{b_{ADa}}, u'_{b_{ADb}})'$ and $e = (e'_{ADa}, e'_{ADb})'$. In turn, y'_{ADa} = the vector containing the arcsine(square root(DIR) for AD "a"; while τ_p is the 1 x p vector of a constant, X_p is an p x n design matrix of full column rank which associates observations with the constant, the u vectors ($u'_{g_{ADa}}$ and $u'_{b_{ADa}}$) are 1 x q vectors for the random terms variety and block while Z matrices (Z_g and Z_b) are n x q design matrices for those random terms mentioned above.

The random effects and error were assumed to be independent Gaussian variables with zero means and variance structures:

$\text{var}(u_g) = \sigma^2_g I_{10}$, $var(u_b) = \sigma^2_b I_3$ *and* $var(e_j) = \sigma^2_j I_{60}$.

Thus, the random terms were assumed to have a variance resulting from the direct product of two G structures, an unstructured correlation for Genotypes and a general correlation for blocks while the error variance was described by specifying a unique unstructured correlation R structure.

In addition to the previous assumptions, the bivariate analysis also involves the following ones: $\text{cov}(u_{g_{ADa}})u'_{g_{ADb}} = \sigma_{g_{ADaADb}} I_{10}$, $\text{cov}(u_{b_{ADa}})u'_{b_{ADb}} = \sigma_{b_{ADaADb}} I_3$ and $\text{cov}(e_{ADa})e'_{ADb} = \sigma_{ADaADb} I_{60}$. Thus random effects and errors are correlated between variables (DIRs from different ADs).

The bivariate analysis also provided an estimate of "true" Type A genetic correlation (two traits measured on the same experimental unit) through bivariate REML estimation among DIR obtained at different AD (Gilmour et al., 2006).

Calculation of Genetic Correlations

Traditional (Type A) genetic correlation was calculated among DIRs obtained on the same plot at different ADs. Using the genotypic variance and covariance component estimates obtained from the corresponding linear mixed model described under section v) Bivariate analysis for different ADs. The genotypic correlation between DIRs from AD_j and AD_k was estimated as

$$r_g = \frac{\text{cov}_{g_{jk}}}{\sqrt{\sigma^2_{g_j} \sigma^2_{g_k}}} \qquad \text{(Eq. 1-8)}$$

where $\mathrm{cov}_{g_{jk}}$ is the estimated genotypic covariance between DIRs j and k and $\sigma^2_{g_j}$ is the estimated genotypic variance for the score obtained at AD_j.

For the same trait, for example, a score obtained at AD_j but assessed in different experimental units (i.e. different PD or Location), a Type B correlation, model II (Yamada, 1962) was calculated by using the corresponding ratio of variances, depending on the nature of the performed analysis (univariate or bivariate). If calculated from variance components obtained through the univariate analyses described under sections i) Univariate analysis for Location or iii) Univariate analysis for PDs, the Type B genetic correlation was estimated as

$$r_{B_{II}} = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_{gf}} \qquad \text{(Eq. 1-9)}$$

where σ^2_g is the estimated genotypic variance for the score obtained at that Location or PD and σ^2_{gf} is the corresponding interaction term between genotype and either Location or PD.

When calculated from bivariate or multivariate analyses, as shown under sections ii) Bivariate analysis through G structure specification and iv) Multivariate analysis through G structure specification, the Type B genetic correlation was estimated as

$$r_{B_{II}} = \frac{\mathrm{cov}_{g_{c1c2}}}{\sqrt{\sigma^2_{g_{c1}} + \sigma^2_{g_{c2}}}} \qquad \text{(Eq. 1-10)}$$

where $\mathrm{cov}_{g_{c1c2}}$ is the estimated genotypic covariance between DIRs from locations one and two and $\sigma^2_{g_{c1}}$ is the estimated genotypic variance for the score obtained at Location number 1.

Standard errors of the correlations were calculated using the Taylor Series Expansion method (Gilmour et al., 2006).

Calculation of Repeatability

An entry-mean repeatability of performance in each environment was calculated as an intra-class correlation using the corresponding variance components from the linear mixed models described under section ii) Bivariate analysis through G structure specification, according to the formula (Holland et al., 1998):

$$R = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}} \qquad \text{(Eq. 1-11)}$$

where, σ_g^2 , σ_e^2 and "r" are the genotypic variance, the error variance and the number of replications at that location, respectively.

Results

There was a clear difference in the intensity of the epidemic between locations. By 132 DAP the highest DIR in Citra was less than half the smallest DIR recorded at Marianna (Table 1-2).

The epidemic progression was slow but steady at Citra while at Marianna it was fast and abrupt, reaching "final" intensity as early as 90 DAP (Fig. 1-1). The slight reduction in intensity observed in the figure was caused by the harvest of susceptible genotypes prior to the assessment at 132 DAP, reducing the overall intensity DIRs for that AD. Among genotypes, a clearer separation of resistance groups was observed at Marianna, under a heavy epidemic compared to Citra (Figs. 1-2 & 1-3). The groups' conformation was as expected, the susceptible genotypes comprising F-435HOL, NemaTAM and SunOleic 97R, the resistant genotypes comprising

NC94002, AP-3, Georgia-02C, DP-1 and C-99R while two genotypes showed intermediate values (Georgia Green and ANorden).

Location Effect

The univariate analysis first tried (described under point "i' of Material and Methods), is similar to the usual ANOVA plus the specification of different error terms for each location. It provided variance components of the interaction Location by PD that were rather important most of the time (data not shown) and were due to heterogeneous variances among planting dates. Consequently the decision was made to model a variance for each combination of location and planting date as described under point "ii" of Material and Methods. With this model, the location effect was found significant in all the PD and AD (Table 1-3) with Marianna exhibiting higher predicted values (Table 1-4). In the most extreme case, PD3 at AD90, the Marianna predicted value for transformed DIR was five times larger.

The ratio of error variance for Marianna to the error variance for Citra was mostly above one (Table 1-3), suggesting higher data variability at Marianna. An extreme value of 4.5 for this ratio was observed at PD 3, AD112.

In Marianna, the ratio of the block variance component to the error variance ranged from 0 to 0.7. At Citra, the DIR were extremely close to zero (data not shown), denoting the rather minor importance of block as a variability source.

The ratio of genotypic variance to the biggest location error variance ranged from 2.5 to 20.2. In every case, the error variance for Marianna was used as the denominator in the ratio. It was clear that genotypic variance was the main source of data variability.

Planting Date Effect

i) Univariate analysis for planting dates

The importance of planting date as a factor determining the DIR was variable according to the location. In Citra it ranged from highly significant (p=0.006) to slightly non-significant (p=0.054) depending on the genotypes and planting dates included in each analysis (Table 1-5).

Earlier AD (70 & 90) showed greater significant differences than later ones (112 & 132), although different PDs were compared in each analysis. At 70AD and 132AD, the later PD exhibited higher predicted DIR (Table 1-6), while at 90AD the opposite was true.

In Marianna, planting dates were statistically different only at 132AD, with the PDs ranking 3>4>2 in DIR.

The ratios of planting date by genotype interaction variance to the biggest error variance were most of the time close to zero (Table 1-5). The only exception was Marianna at 132AD where the ratio was 0.8, yet small compared to the genotypic variance. In comparison, Genotypes seemed far more important as a variability source with the ratios of genotypic variance to the biggest error variance ranging from 0.4 to 1.7 at Citra and from 2.4 to 4.4 at Marianna. In order to compare ADs, when this ratio was calculated only from PD 2 and 3, its value went from 1.5 at AD90 to 3.3 at AD112 (data not shown), suggesting increasing variability over time.

In Citra, the variance component for the PD by block interaction varied from small, 1/10 of the smallest error variance, at AD112 to negligible at the other three ADs (data not shown). Meanwhile, in Marianna it ranged from rather small, 1/4 of the smallest error variance, to important (1.5 times the smallest error variance).

The ratio of error variances among PDs was quite similar among ADs at Citra, ranging from 1 to 1.4 while at Marianna they varied widely from 1.5 to 7.6.

Location by Planting date (cell) effect

v) Multivariate analysis through G structure specification

The analysis using common combinations of planting dates and assessment dates between both locations (referred herein as "cells") showed that the cell effect was highly significant at both ADs (Table 1-7), with Citra showing smaller predicted values than Marianna (Table 1-8).

Cells of different location were always significanlty different. At 112 DAP, within-location cells were only different for citra. At 132 DAP, within-location cells were only different for Marianna.

Cells showed different variability with Marianna's cells displaying the most variability (data not shown). The ratio of the greatest error variance to the smallest (Marianna at PD4 : Citra at PD 3) was 8.8 for 112AD while it was 1.2 (Marianna at PD 3 : Citra at PD 3) for 132AD (data not shown).

The block variance had a negligible value at AD112 for PD 3 and at AD132 for both PDs at Citra (data not shown). In Marianna the block variance had some importance, but not in Citra. The ratio of block variance for a cell to its error variance at 112AD was highest for PD 3 at Marianna, while for 132AD the highest ratio was observed for PD 2 in Marianna.

The ratio of genotypic variance within a location to the error variance for that location ranged from 0.7 to 20.2 with 5 out of 8 values above 2 (data not shown), showing that the genotypic variance was the largest variability source for spotted wilt intensity DIRs.

Phenotypic and Genetic Correlations

The DIRs a plot received at different assessment dates were strongly correlated. Phenotypic correlations were medium to high (Table 1-9). In Citra the values ranged wider and below (0.43-0.89) those in Marianna (0.85-1).

Genotypic correlations were high at both locations, ranging from 0.85 to 1 (Table 1-9) with rather small standard errors. Many coefficients were fixed at the maximum theoretical value of 1 by constraining the covariance matrix.

There was a moderate and significant correlation among the phenotypic and genotypic correlation coefficients (0.76, p=0.04) by Spearman's rank correlation but due to the reduced number of data pairs, this significance should be taken cautiously.

A high within location consistency of genotypic performance for DIR at different PDs and ADs was observed. Type B genetic correlation coefficients were high, ranging from 0.83 to 1 with modest standard errors, with Citra displaying larger errors (Table 1-10). There was also consistency of genotypic performance across locations at the same PD and AD. In three out of five cases, the correlation coefficient was 1 while in the remaining two cases it was above 0.7 (Table 1-10).

When calculated from the combination of locations x planting date (cell), the correlation coefficient between transformed DIR was very high, with all but one value above 0.9 (Table 1-10). All the coefficients for AD132 had a value of one, suggesting that the performance of the evaluated genotypes in a certain environment (combination of location x planting date) was predictable based upon its performance in another environment.

Irrespective of the type of genetic correlation calculated, the general results showed that the genotypic performances in the tested environments were highly correlated between locations and among ADs and PDs. Additionally, the modeling of variance structures allowed better estimations of these correlations through elimination of scale effects and error shrinkage.

Repeatability

Repeatability values for the transformed DIR at different PDs and AD were high (Table 1-11) and their precision good (small standard errors). All but one of the values was above 0.75

showing that the theoretical upper limit for the heritability of the intensity was quite high. In general, the repeatabilities at Citra were smaller than at Marianna.

Discussion

Some locations tend to have stronger spotted wilt epidemics than others (Culbreath et al., 2003). Marianna has usually displayed severe epidemics since the late '90s (Tillman et al., 2007; Culbreath et al., 2005) while spotted wilt is a lesser problem at Citra (Gorbet, pers. comm.). The epidemics observed in 2005 followed a similar trend, with spotted wilt being consistently more prevalent in every PD and AD in Marianna. This was reflected not only in the final DIR but also on the fact that they peaked earlier in the season in every planting date.

The epidemic seemed to almost reach its peak at Marianna at around 90 days after planting as there was little disease progression afterwards. This rapid establishment of near maximal DIR at such an early assessment date is uncommon in the literature. Even the worse epidemics reported so far have reached final intensities at later assessment dates (Murakami et al., 2006, Culbreath et al., 1997). This was especially important because early TSWV infection typically leads to more damage inflicted by the virus (Culbreath et al., 2003). In contrast to Marianna, the progression of the epidemic at Citra showed a typical steady increase similar to mild epidemics reported elsewhere (Murakami et al., 2006, Culbreath et al., 1997).

In the present study, the level of spotted wilt intensity recorded for well known genotypes resembled those of severe epidemics described by some authors (Culbreath et al., 2005, Tillman et al., 2007). These authors reported intensity ratings above 0.8 for susceptible genotypes such as SunOleic 97R and variable ratings for the moderately susceptible Georgia Green. These values are in agreement with ours, suggesting that final intensity ratings were in the expected range for a wide array of genotypes of varying resistance levels.

Coinciding with published research, resistance differences among genotypes were most noticeable under the severe epidemics in Marianna (Culbreath et al., 1997; Tillman et al., 2007). The observed resistance grouping was in good agreement with the information provided by the present version of the Peanut Disease Risk Index (Brown et al, 2007).

Murakami et al. (2006) reported that planting dates diverged in spotted wilt incidence as the season progressed under a mild epidemic but were similar under a severe one. However, in the present study rather the opposite was true.

Under a mild epidemic in Citra, differences in spotted wilt intensity between PDs were easily observed early in the season (PD2 vs. PD4 at AD70), but they tended to diminish as the season advanced. Meanwhile, in Marianna the effect of PD was not important until late in the season (132 AD) where all the PDs differed in the intensity DIRs. Curiously, the spotted wilt intensity spiked at the third PD which coincides with the extension recommendation for plantings with reduced spotted wilt risk (Brown et al., 2007). This further underscores the seasonal unpredictability of spotted wilt epidemics and the need to plant resistant varieties. For example, Tillman and coworkers (2007) reported that June plantings were less conducive to severe spotted wilt in most of the genotypes tested in the Florida Panhandle. In the present study, however, early May planting seemed best for reduction of spotted wilt damage.

Differing from results reported by Hurt et al. (2005), in the present study the interactions among genotypes, location and planting dates were not important. This was so even when all the three factors were jointly analyzed, which highlights the advantages of modeling variance and error structures (Gilmour et al., 2006).

Phenotypic and genotypic correlations are of the same sign and similar magnitude most of the time (Lynch and Walsh, 1998). This seemed to be the case in the present study. The fact that

genetic correlations were higher than the phenotypic ones can be explained because the variance and covariance components used in the calculations come from a well specified mixed model analysis, thus only containing genotypic effects from which experimental noise was removed (i.e. scale effects as the season progresses). Consequently, they are a much more precise estimation of the genetic cause of similarity among the spotted wilt ratings that a genotype will receive at different assessment dates (Holland et al., 2003).

The high genetic correlation among assessment dates indicated a high consistency in the rankings obtained by the genotypes across the assessment dates. This finding coincides with those reported by Culbreath et al. (1997) and Murakami at al. (2006), who reported reduced cross-over of genotypic rankings at different assessment dates. Consequently, the differences among genotypes in their performance against TSWV should be perceived no matter the moment they are compared.

Despite the marked differences between the disease intensity between locations, there was a marked consistency of phenotypic performance across locations, PDs and their combinations, as indicated by the high Type B genetic correlation coefficients.

This suggests a scenario similar to that presented by McKeown et al. (2001) and different to the ones described frequently in published research where GxE interaction seemed to be the norm (Culbreath et al., 1997, 2005; Tillman et al., 2007). This apparent discrepancy could be caused by the use of ANOVA in those references, coupled with scale effects. The fact that Culbreath et al. (2005) reported that even though GxE interaction was present, the genotypic trends were consistent across locations and years seems to back up this explanation.

The high Type B genetic correlation suggests that there was no cross interaction among genotypes and environments. This finding could suggest that the wide array of resistance genes

present in the tested genotypes performed consistently when faced with the viral populations present at both locations, which could arise from similarities in the viral consensus sequence between locations (i.e. they are essentially the same) or the fact that both consensus sequences induce similar rankings on the tested genotypes in much the same way Mandal et al. (2006) reported for Georgia isolates.

Genotypic variances were the most important random variation source in this study. By having a wide range of resistances in the tested genotypes the genotypic variance is expected to be increased (Betrán et al., 2006), thus increasing the repeatability.

Another cause of high genetic variances relative to the error term was probably the correct modeling of the experimental data (Gilmour et al., 2006), which was accomplished throughout the present study.

Additionally, genetic causes (biochemical pathways) could predominantly have established the performance of the genotypes (Lynch and Walsh, 1998). This last explanation seemed to be supported by the high genetic correlation coefficients obtained here.

Conclusions

The modeling of correct variance and covariance structures of the tests provided a good estimation of variances and covariances which in turn allowed determination that location was a significant factor in establishing the spotted wilt ratings observed among genotypes. Meanwhile, planting date was only a significant factor under light epidemics or late in the season under heavy epidemics.

The high correlation among assessment dates indicated that the relative performance of genotypes can be perceived early in the season and the genotypic differences tend to persist until harvest time.

The high values of both Type B genetic correlation coefficients and repeatability estimates suggested a strong genetic determination of the observed genotypic differences in spotted wilt intensity ratings. This emphasizes the importance of resistant cultivars in the management of spotted wilt.

Table 1-1. Layout of data collection for planting date studies of peanut in Marianna and Citra, Florida in 2005.

Location	Planting date	Assessment Dates (in Days After Planting)			
		70 DAP	90 DAP	112 DAP	132 DAP
Citra	1[a]		X		
	2	X			X
	3		X	X	X[b]
	4	X		X	
Marianna	1			X	
	2		X	X	X[c]
	3		X	X	X[c]
	4			X[d]	X[cd]

The X indicates the cells in which spotted wilt damage was assessed. [a] Genotypes F435HO, NemaTAM and NC94002 were not planted. [b] F435HO was already dug. [c] F435HO and SunOleic 97R were already dug. [d] Replication one was discarded.

Table 1-2. Means and standard errors for tomato spotted wilt disease intensity ratings at different planting dates at Citra and Marianna, Florida in 2005.

Location		AD 70		AD 90		AD 112		AD132	
	Planting date	Mean (n)	S.E.	Mean (n)	S.E.	Mean (n)	S.E.	Mean (n)	S.E.
Citra	1[a]			0.066 (21)	0.010				
	2	0.057 (30)	0.009					0.157 (30)	0.018
	3			0.048 (30)	0.008	0.123 (30)	0.016	0.179 (27)[b]	0.019
	4	0.097 (30)	0.014			0.191 (30)	0.026		
Marianna	1					0.545 (30)	0.054		
	2			0.514 (30)	0.054	0.480 (30)	0.056	0.442 (24)[c]	0.049
	3			0.640 (30)	0.051	0.670 (30)	0.050	0.673 (24)[c]	0.047
	4					0.632 (20)[d]	0.063	0.548 (14)[cd]	0.070

[a] Genotypes F435HO, NemaTAM and NC94002 were not planted. [b] F435HO was already dug. [c] F435HO and SunOleic 97R were already dug. [d] Replication one was discarded.

Table 1-3. Location effect and variance ratios for bivariate analysis performed on transformed spotted wilt disease intensity ratings at three planting dates assessed in different times at Citra and Marianna, Florida in 2005.

Planting Date	*Assessment date*	P-value>F for location	$\sigma^2_{eM}/\sigma^2_{eC}$	$\sigma^2_{bM}/\sigma^2_{bC}$	$\sigma^2_{bM}/\sigma^2_{eM}$	$\sigma^2_{lg>}/\sigma^2_{e>}$
2	*132*	0.004	1.4	147712.9	0.7	5.1
3	*90*	<0.001	3.3	10.5	0.0	4.9
	112	0.004	4.5	1137.2	0.7	2.5
	132	<0.001	1.3	10.5	0.0	5.6
4	*112*	<0.001	0.7	0.7	0.2	20.2

σ^2_{eM}: Marianna error variance. σ^2_{eC} : Citra error variance. σ^2_{bM}: Marianna block variance. σ^2_{bC} : Citra block variance. $\sigma^2_{lg>}$: biggest location*genotype variance. $\sigma^2_{e>}$: biggest location error variance.

Table 1-4. Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for planting dates assessed at different times at Citra and Marianna, Florida in 2005.

Planting Date	*Assessment date*	Location	Predicted Value	SE	SED
2	*132*	Citra	0.3881	0.0391	0.0786
		Marianna	0.7858	0.1011	
	90	Citra	0.1879	0.0339	0.0875
		Marianna	0.9461	0.1045	
3	*112*	Citra	0.3349	0.0391	0.1193
		Marianna	1.0253	0.1400	
	132	Citra	0.4247	0.0331	0.0698
		Marianna	1.0097	0.0923	
4	*112*	Citra	0.4337	0.0552	0.0899
		Marianna	0.9532	0.1108	

Table 1-5. Planting date effect and variance ratios for univariate analysis performed on transformed spotted wilt disease intensity ratings at four planting dates assessed in different times at Citra and Marianna, Florida in 2005.

Location	*Assessment date*	P-value>F for planting date	$\sigma^2_{e>}/\sigma^2_{e<}$	$\sigma^2_{pld.g}/\sigma^2_{e>}$	$\sigma^2_{g}/\sigma^2_{e>}$
Citra	*70*	0.006	1.1	0.1	1.1
	90	0.023	1.0	0.1	0.4
	112	0.054	1.1	0.1	1.7
	132	0.031	1.4	0.0	0.9
Marianna	*90*	0.092	1.5	0.4	4.4
	112	0.096	7.6	0.1	2.4
	132	0.045	3.5	0.8	3.8

$\sigma^2_{e>}$: biggest error variance. $\sigma^2_{e<}$: smallest error variance. $\sigma^2_{pld.g}$: planting date by genotype interaction variance. σ^2_{g} : genotypic variance.

Table 1-6. Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for at four planting dates assessed in different times at Citra and Marianna, Florida in 2005.

Location	*Assessment date*	Planting Date	Predicted Value	SE	SED
Citra	*70*	2	0.2199	0.0304	0.0223
		4	0.2996	0.0300	
	90	1	0.2425	0.0298	0.0304
		3	0.1502	0.0301	
	132	2	0.3692	0.0355	0.0249
		3	0.4247	0.0370	
Marianna	*132*	2	0.6755	0.1067	0.0899
		3	0.9298	0.1057	
		4	0.8387	0.1091	

Table 1-7. Location by planting date combination (cell) effect and variance ratios for multivariate analysis performed on transformed spotted wilt disease intensity ratings at different planting dates assessed at different times at Citra and Marianna, Florida in 2005.

Assessment date	P-value>F for Cell	$\sigma^2_{e>}/\sigma^2_{e<}$	$\sigma^2_{b>}/\sigma^2_{b<}$	$\sigma^2_{b>}/\sigma^2_{ec}$	$\sigma^2_{cg<}/\sigma^2_{e<}$	$\sigma^2_{cg>}/\sigma^2_{e>}$
112	<0.001	6.9	285267592	0.7	1.6	20.2
132	<0.001	1.6	36619522	0.8	0.7	7.1

$\sigma^2_{e>}$: biggest error variance. $\sigma^2_{e<}$: smallest error variance. $\sigma^2_{b>}$: biggest block variance. $\sigma^2_{b<}$: smallest block variance. σ^2_{cg} : cell by genotype interaction variance. σ^2_{g} : genotypic variance.

Table 1-8. Transformed spotted wilt disease intensity rating predicted values, standard errors (SE) and standard errors of mean differences (SED) for location by planting date combinations (cells) assessed at different times in Citra and Marianna, Florida in 2005. Each assessment date was analyzed separately.

Cell	*Assessment date*	Predicted Value	SE	SED
1 (Citra, P.D.3)	*112*	0.3349	0.0394	0.0981
2 (Citra, P.D.4)		0.4337	0.0552	
3 (Marianna, P.D.3)		1.0253	0.1397	
4 (Marianna, P.D.4)		0.9532	0.1108	
1 (Citra, P.D.2)	*132*	0.3881	0.0401	0.0681
2 (Citra, P.D.3)		0.4358	0.0325	
3 (Marianna, P.D.2)		0.7863	0.1014	
4 (Marianna, P.D.3)		1.0599	0.0955	

P.D. Planting Date.

Table 1-9. Phenotypic and genetic correlations among transformed spotted wilt disease intensity ratings at four planting dates assessed in different times at Citra and Marianna, Florida in 2005. Values in brackets are standard errors.

	Phenotypic Correlation [SE]			Genetic Correlation [SE]		
Location	*Assessment Date*	*112*	*132*	*Assessment Date*	*112*	*132*
Citra	*70*	0.83 [0.08]	0.84 [0.07]	*70*	0.95 [0.06]	1 [0.08] [a]
	90	0.43 [0.19]	0.75 [0.15]	*90*	.085 [0.22]	1 [0.21] [a]
	112		0.71 [0.08]	*112*		0.99 [0.01]
Marianna	*90*	0.85 [0.06]	0.87 [0.06]	*90*	1 [0.02] [a]	1 [0.03] [a]
	112		0.89 [0.09]	*112*		1 [0.01] [a]

[a] Genetic correlations were kept in the theoretical range by constraining the covariance matrix (Gilmour et al. 2006)

Table 1-10. Type B genetic correlations and [standard errors] for transformed spotted wilt disease intensity ratings at four planting dates assessed in different times in Citra and Marianna, Florida in 2005. [a]

Coefficients obtained from Univariate Analysis		
Location	*Assessment Date*	Genetic Correlation
Citra	*70*	0.91[0.17]
	90	0.83[0.32]
	112	0.95[0.11]
	132	1[0.01]
Marianna	*90*	0.91[0.08]
	112	0.97[0.03]
	132	0.87[0.09]

Coefficients obtained from Bivariate Analysis			
Planting	*Assessment Date*		
Date	*90*	*112*	*132*
2			1[0.09]
3	0.71[0.22]	1[0.13]	1[0.25]
4		0.73[0.18]	

Coefficients obtained from Multivariate Analysis			
	Assessment Date		
	112		
Cell	Citra-PD4	Marianna-PD3	Marianna-PD4
Citra-PD3 [c]	1 [0.11][b]	1 [0.13][b]	1 [0.09][b]
Citra-PD4		0.91 [0.12]	0.73 [0.18]
Marianna-PD3			0.94 [0.08]
	AD132		
Cell	Citra-PD3	Marianna-PD2	Marianna-PD3
Citra-PD2	1 (0.24)[b]	1 [0.08][b]	1 [0.07][b]
Citra-PD3		1 [0.20][b]	1 [0.20][b]
Marianna-PD2			1 [0.04][b]

[a] Correlations were calculated among planting dates (across locations), between locations (across planting dates) and among cells (location by planting date combination) by applying different mixed models. [b] Variance components and genetic correlations were kept in the theoretical range by constraining the covariance matrix (Gilmour et al. 2006). [c] PD: Planting Date.

Table 1-11. Entry-mean repeatability estimates and their [standard errors] for transformed spotted wilt intensity ratings at three planting dates assessed three times in Citra and Marianna, Florida in 2005. Values for each location are separated by a slash, Citra being on the left and Marianna on the right.

	Assessment Date (days after planting)		
Planting Date	*90*	*112*	*132*
2			0.81 [0.11] / 0.94 [0.04]
3	0.82 [0.10] / 0.94 [0.04]	0.76 [0.14] / 0.86 [0.08]	0.57 [0.26] / 0.94 [0.04]
4		0.90 [0.06] / 0.98 [0.01]	

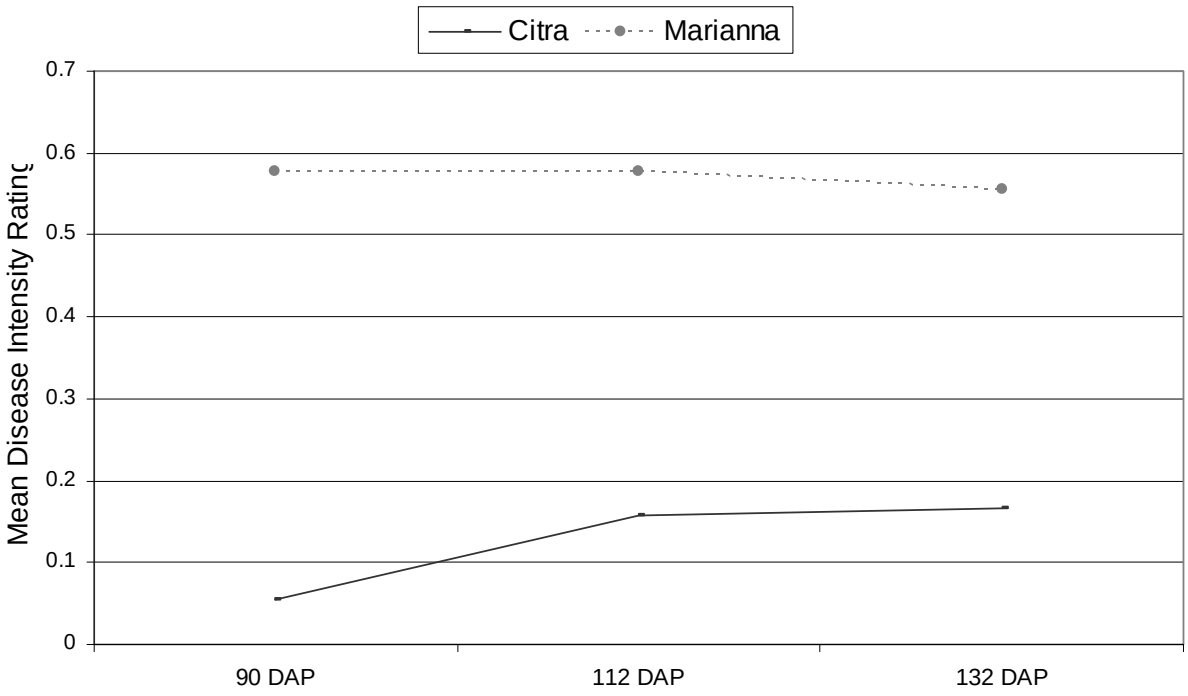

Figure 1-1. Change of spotted wilt disease intensity ratings over time at Citra and Marianna, Florida in 2005 (averaged over all planting dates and genotypes)

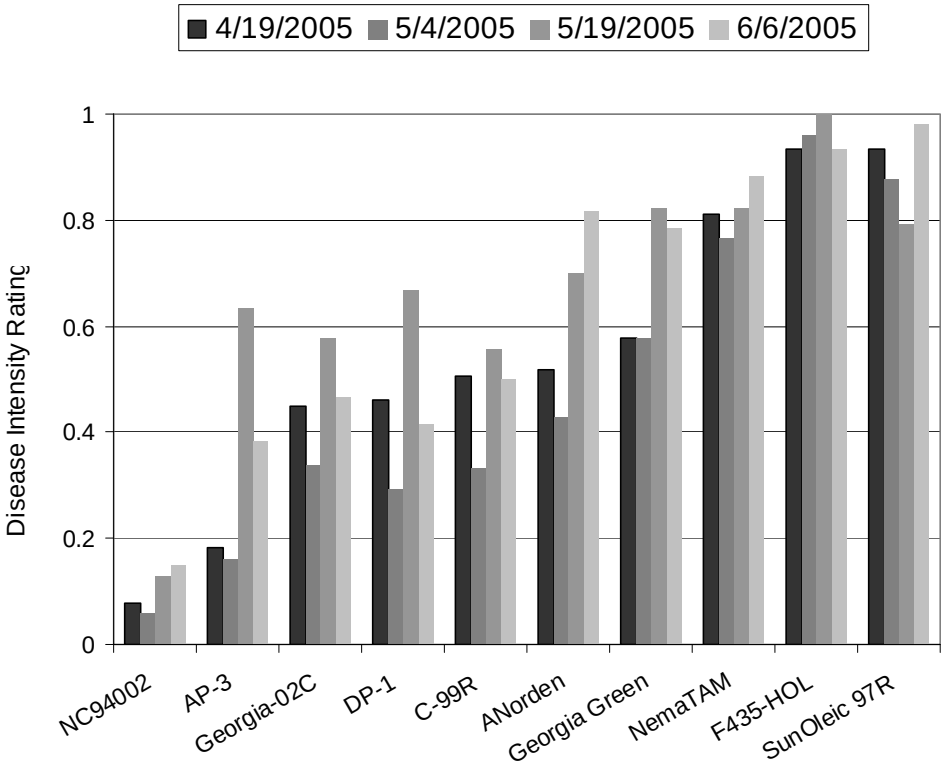

Figure 1-2. Spotted wilt disease intensity ratings at 112 days after planting for ten genotypes planted at different dates at Marianna, Florida in 2005.

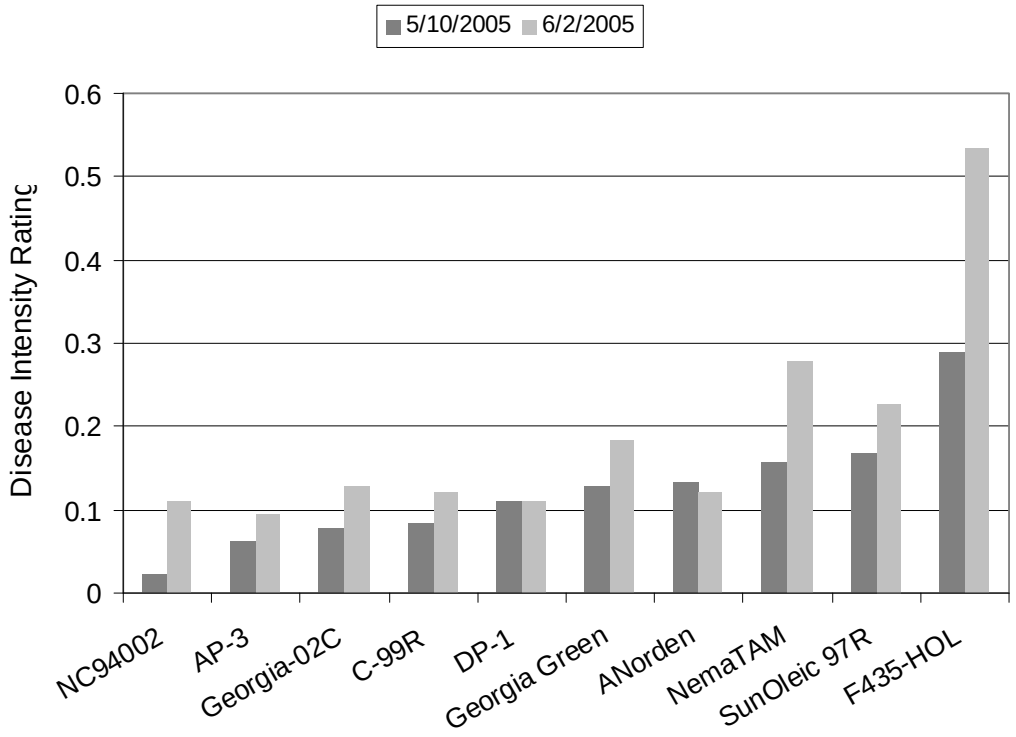

Figure 1-3. Spotted wilt disease intensity ratings at 112 days after planting for ten genotypes planted at different dates at Citra, Florida in 2005.

CHAPTER 2
HERITABILITY AND BREEDING VALUES OF TSWV RESISTANCE IN POPULATIONS FROM PEANUT (ARACHIS HYPOGAEA L.) CROSSES

Introduction

Tomato spotted wilt virus (*Bunyaviridae: Tospovirus* - TSWV) is a worldwide problem in both greenhouse and field crops (German et al., 1992). Since the first report of TSWV affecting peanut in Texas in the early '70s, it has become a major limiting factor to peanut production in the US (Culbreath et al., 2003).

Spotted wilt disease symptoms develop at least a week after inoculation (Hoffman et al., 1998) and can be seen quite early in the season (Chapter 1 of this dissertation). Typical aboveground symptoms include concentric ringspots, mosaic patterns, stunting, varied degrees of apical and leaf necroses and general chlorosis, also known as yellowing (Mitchell, 1996, Culbreath et al., 2003, Demsky & Reddy, 2004,). The presence of some of these symptoms, like stunting and yellowing, has been linked to the action of specific viral proteins (Prins et al 1997, Koll & Bütner, 2000). However, the nature and severity of symptoms depends on the consensus (predominant) RNA sequence in the virus population (Nagata et al., 1993; Mandal et al., 2006).

Generally, epidemic patterns vary significantly across locations, particularly if the locations are distant and contain dissimilar agroecosystems (Culbreath, 2003; Groves et al., 2003) with different crop species that react dissimilarly to TSWV (Kucharek et al., 2000) or different weeds that influence thrips dynamics (Northfield, 2005). Whatever the environment, the rate of progress of spotted wilt epidemics has been shown to be cultivar dependent in peanut (Culbreath et al., 1997; Murakami et al., 2006; Chapter 1 of this dissertation). Resistant cultivars are the most important factor in the management of the disease (Brown et al., 2007).

Spotted wilt resistance is an important goal for peanut breeders in Southeastern USA (Gorbet, 1999, Tillman et al., 2007). At present there are several resistant peanut cultivars and

breeding lines available. Most of them trace their resistance back to two unrelated sources, PI 203395/6 (both PIs come from the same original accession) and PI 576638. The former, typical hypogaea botanical variety members, are the spotted wilt resistance source of Georgia Green (Branch 1996), Georgia 01R (2002), DP-1 (Gorbet and Tillman, 2008, in press) and many others. Meanwhile, PI 576638 (a hirsuta botanical variety member) is the source of the resistance of several breeding lines that had shown remarkable field resistance to spotted wilt (Culbreath et al., 2005, D. Gorbet, pers. comm.).

The existence of a different mechanism of resistance between these two PIs has been hypothesized (Culbreath et al., 2005). Nonetheless, it has been shown that resistance is unrelated to vector non-preference or reproduction (Culbreath et al., 1996, 1997, 2000).

A third source of TSWV resistance has been observed in sisterline cultivars AP-3 (Gorbet, 2007) and Carver (Gorbet, 2006). In this case, the origin of the resistance is uncertain as neither of the parents of these two cultivars is resistant (D. Gorbet, pers. comm.)

In tomato and pepper, major genes for resistance to TSWV have been described, having different modes of action and penetrance (Rosello et al., 2001, Soler et al., 1998 Moury et al., 1998).

In species where inherited resistance is conditional or ambiguous, with no clear or consistent phenotypes, traditional genetic analyses become difficult (Lynch and Walsh, 1998; Bruening, 2006) and the heritability becomes the most important information for the plant breeder (Simmonds, 1979). The heritability (in its narrow sense) expresses which proportion of the phenotypic variability can be transmitted from parent to offspring (Falconer & MacKay, 1996) and it is the main determinant of the expected response to selection (Hallauer & Miranda, 1988).

Among the methods used for heritability estimation, the most used is the variance component method because of its adaptability to different situations (Nyquist, 1991). Heritability estimates depend not only on the genetic factors in the populations being analyzed but also on the environment in which they are tested (Falconer & MacKay, 1996). In most situations, better discrimination among genotypes is feasible by testing the populations in certain types of environments (Hall and Van Sanford, 2003, Venuprasad et al., 2007). These environments can be laboratory settings (Rapp & Juntila, 2001); locations (Finne et al., 2000) or even planting dates (Chapter 1 of this dissertation).

The expression of disease resistance is usually scored as a polychotomous variable, commonly called "ordered scale" (Connover, 1998). For this kind of trait, the unit of analysis can be the individual observations in the native scale (Huber et al., 1994) or plot means combined with a transformation such as arcsine or logistic (Holland et al., 1998). The use of individual values is known to provide better heritability estimates than plot means under most conditions when a REML approach is used in the linear mixed model context (Huber et al., 1994). Although the typical analytical approach is to adjust a threshold model, this usually yields similar results to REML variance estimation under the native scale under a wide range of conditions (Banks et al., 1985; Westfall 1987). Inaccurate heritability estimates are sometimes obtained when the frequency of a category in a polychotomous variable is very high across the whole population of individuals being tested (Yang et al., 1998). Under these conditions, the threshold model is only superior to estimates obtained from REML on the native scale if incidences are extreme and heritabilities are low to medium (Lopes et al., 2000).

Variance components for heritability estimation today are mostly obtained through Mixed Linear Model approaches. They offer the flexibility of analyzing various types of unbalanced data coming from non-traditional mating designs with good precision (Holland et al., 2003).

Using this approach, random effects such as breeding values can also be obtained (Lynch and Walsh, 1998). Breeding value is the sum of the additive effects of an individual's genes (Lynch and Walsh. 1998).

Recently, the use of mixed models coupled with REML has allowed the accurate estimation of additive variance and consequently of the breeding values. A common linear mixed model used mostly by animal breeders is the Animal Model (Mrode, 2005). It utilizes all genetic relationships among the individuals being analyzed, in order to obtain a more accurate estimation of the additive variance than traditional methods (Henderson, 1976; Lynch and Walsh, 1998). Breeding value estimates (BLUPs), obtained through the Animal Model are accurate because they take into consideration not only the performance of the individual but also that of its relatives (Mrode, 2005).

Breeding values are used to choose individuals in a population that are superior for a trait and that will provide a better progeny. They can also be an integral part of a selective index to choose individuals based on several traits (Mrode, 2005).

To gain insight into the genetics of resistance to TSWV in peanut, the objectives of this study were: 1) to provide heritability estimates from crosses involving different sources of resistance while assessing their potential to generate superior progenies; and 2) to explore the relationship among different symptoms of infection by TSWV.

Material and Methods

To study the inheritance of resistance to TSWV in peanut, three resistant genotypes (AP-3, DP-1 and NC94002) and a susceptible genotype (NemaTAM) were mated.

The source of resistance for each resistant parent was believed to be unique (D. Gorbet, pers. comm.). AP-3 and DP-1 are related through a common ancestor, their grandparent Florunner, which is extremely susceptible to TSWV (Culbreath et al., 1997; Tillman et al., 2007).

AP-3 is a runner-type cultivar whose parents display no noticeable spotted wilt resistance (Gorbet, 2007). DP-1 is also a runner-type cultivar and traces its resistance back to its grandparent PI 203396, which has produced numerous lines with good resistance (D. Gorbet, pers. comm.). This PI is a typical member of the hypogaea botanical variety. NC94002, traces its resistance back to its parent PI 576638, which is an accession belonging to the hirsuta botanical variety (D. Gorbet and T. Isleib, pers. comm.).

The resistant and susceptible parents were mated in the following combinations AP-3/ NemaTAM, NemaTAM/AP-3, NemaTAM/DP-1, NemaTAM/NC94002 and DP-1/NC94002. The resulting F_1, Backcross, F_2 and F_3 populations, together with their parents were field tested. For each cross, 25 $F_{2:3}$ (F_2-derived in F_3) families with enough seed were randomly selected and included in each test in at least two replications.

Field tests were conducted at the University of Florida Plant Science Research and Education Unit in Citra, Florida on a Candler Sand (Hyperthermic, uncoated Typic Quartzipsamments) during 2005 and 2006, at the North Florida Research and Education Center near Marianna, Florida on a Chipola loamy sand (Loamy, kaolinitic, thermic Arenic Kanhapludults) during 2006 and 2007 and at the North Florida Research and Education Center in Quincy, Florida on an Orangeburg fine sandy loam (fine loamy, siliceous, thermic, Typic Paleudults) during 2007. The tests details are shown in table 2-1.

The different crosses were randomized in each block, and the different generations of each cross were randomized within the cross sub-plots. The number of replications for each generation was variable within (by cross) and among tests, as can be seen in table 2-1.

At Citra, plots were one row, 0.9 m wide and 4.5 m long, planted with 6 seeds/m in 2005 and 3 seeds/m in 2006. At Marianna and Quincy, plots were two rows, 1.8 m wide and 4.5 m long, and planted with 3 seeds/m.

Tests were subjected to cultivation practices such as early planting, low plant population, no phorate (insecticide) application and single row planting pattern that are known to favor the development of spotted wilt epidemics (Culbreath et al., 2003). Natural TSWV-infected thrips populations were relied upon to cause spotted wilt epidemics. Samples were taken from symptomatic plants and tested by DAS-ELISA (SRA 30400/0096 kit, AGDIA, Elkhart, IN) to confirm TSWV infection.

With the exception of phorate at planting, plots were maintained according commercial peanut production practices for the region with fertilizer, herbicide, fungicide, insecticide and irrigation applied as recommended by the University of Florida Extension Service guidelines.

Every other plant was marked with a flag and each individual was assessed for five typical foliar symptoms of spotted wilt, which are mentioned in the literature (Mitchell, 1996, Culbreath et al., 2003; Demsky & Reddy, 2004): stunting, foliar symptoms (spots and/or mosaic), tip death, leaf necrosis and yellowing (chlorosis). In total, about 36,300 individual plants were evaluated. The following ad-hoc scales were used to score each symptom.

Stunting was defined as a reduction in plant size in at least one dimension (height, width). The degree of stunting was then assigned to a degree in the following ordered scale:

1. Light shortening of internodes, rendering the plant size about 80% of a normal plant (or reducing one of the plant's dimensions to about 80% of a normal one).

2. Noticeable shortening of internodes, plant size about 60% of a healthy one.

3. Marked shortening of internodes, plant size about 40% of a healthy one, leaflets showing signs of poor expansion.

4. Very marked shortening of internodes, plant size about 30% of a healthy one, leaflets poorly unfolded.

5. Extreme shortening of internodes, plant size about a 20% of a healthy one, plant shows unfolded leaves in crowded limb tips. No leaf has been unfolded recently.

Foliar symptoms were related to damage in photosynthetic pigments as shown by the presence of spots or mosaic patterns. Their incidence and severity were jointly assessed according to the following ordered scale:

1. A few hardly noticeable (faint) foliar symptoms in a few leaves.
2. Noticeable (easily observable) foliar symptoms in a few branches.
3. Marked (very evident) foliar symptoms in most braches.
4. Very marked (covering most leaves) foliar symptoms in most branches.
5. Very marked foliar symptoms in all braches.

Tip death was assessed according to the symptom incidence on the plant, as follows:

1. One stem tip dead
2. Up to 25% of the tips dead
3. Up to 50% of the tips dead
4. Up to 75% of the tips dead
5. All tips dead

Leaf necrosis was assessed where necrotic lesions tend to coalesce forming patches of dead tissue on the leaf. Its degree was scored as follows:

1. A few leaves with less than 1/10 of their surface necrotic.
2. Noticeable necrosis in a few branches.
3. Extended necrosis (up to 50% of the leaves affected) in most braches.
4. Very extended necrosis (up to 75% of the leaves affected) in most branches
5. Most leaves necrotic in all branches.

Yellowing was assessed as follows:

1. Leaves turn slightly yellow, no or very little leaf folding.
2. Leaves noticeably yellow, some leaf folding especially in the afternoon.
3. Leaves very yellow, noticeable leaf folding especially in the afternoon.
4. Grayish yellow leaves drooping in the afternoon, some reddish hue appears in the stems.

5. Always droopy, yellow leaves, reddish slightly dehydrated stems.

In all variables a score of 0 was assigned if no symptoms were apparent. The plants were scored at three points in time: 30, 60 and 120 days after planting (DAP), except at Citra in 2006 and Quincy in 2007 where no scoring was done 30 DAP.

Traditional mating designs used to estimate genetic variance components are applicable only when parental components are unrelated (Hallauer and Miranda, 1988). By using REML to estimate genetic variance components in a mixed model approach, it is possible to account for the relationship among individuals. A mixed model approach using a single trait Animal Model (Mrode, 2005) was employed to estimate genetic variance components from populations derived from the crosses. Since $F_{2:3}$ families made up most of the data and they mostly changed among environments, the analyses were performed by year and location according to the following model:

$$y = X\beta + Z_B u_B + Z_P u_P + Z_I u_I + Z_{NA} u_{NA} + e \quad \text{(Eq. 2-1)}$$

where **y** is the vector of observation for each individual; $\mathbf{Z}_B$ and $\mathbf{u}_B$ are the incidence matrix and vector of random block effects B~NID(0, σ_B^2), with $2 \leq B \leq 3$. $\mathbf{Z}_P$ and $\mathbf{u}_P$ are the incidence matrix and vector of random plot effects P~NID(0, σ_P^2), with $40 \leq P \leq 429$. $\mathbf{Z}_I$ and $\mathbf{u}_I$ are the incidence matrix and vector of random additive effects I~N((0, $A\sigma_A^2$). $\mathbf{Z}_{NA}$ and $\mathbf{u}_{NA}$ are the incidence matrix and vector of random non-additive genetic effects NA~ NID(0, σ_{NA}^2). **e** are the errors, which are NID(0, σ_e^2).

The additive matrix **A** (Henderson, 1976) has dimensions 37013x37013 with diagonal elements equal to 1 and off-diagonal elements equal to two times coancestry coefficient ($2r_{xy}$) between the individuals in the study. The diagonal non-additive matrix NA has dimensions 37013x37013 and was expected to account for each variation not accounted for the **A** matrix.

The vector of observations **y** is assumed to be univariate normal with mean E(y)= Xβ and variance-covariance Var(**y**)= $Z_B \sigma_B^2 Z'_B + Z_P \sigma_P^2 Z'_P + Z_I A \sigma_A^2 Z'_I + Z_{NA} Z'_{NA} + I\sigma_e^2$.

The REML method was used to estimate additive and non-additive variance components by using the ASREML software (Gilmour et al., 2006). Individual predicted breeding values (BLUPs) were obtained from the solutions for the individual effects in the above mentioned Animal Model. Mean F_2 and $F_{2:3}$ BLUPs (breeding values) were obtained by averaging the BLUPs of all the individuals in the corresponding population. BLUP Reliabilities (a measure of their accuracy) for parents were obtained by using the formula:

$$r = 1 - \frac{\sigma^2_{PREDICTION}}{\sigma^2_A} \qquad \text{(Eq. 2-2)}$$

where $\sigma^2_{PREDICTION}$ is the variance of the predicted breeding value and σ^2_A is the additive variance estimation from the corresponding model.

Mean F_2 and $F_{2:3}$ BLUPs were obtained by averaging the squared prediction standard errors for the BLUPs of all the individuals in the corresponding population and dividing them by the square of n (number of squared BLUPs used in the average).

Narrow-sense heritability was estimated on individual values, as

$$h_i^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{NA}^2 + \sigma_P^2 + \sigma_e^2} \qquad \text{(Eq. 2-3)}$$

where σ_A^2 is the additive variance, σ_{NA}^2 is the non-additive variance, σ_P^2 is the plot variance and σ_e^2 is the residual variance. The variance components were directly provided from the model fitting. The heritability estimates are probably upwardly biased because they may contain genotype-by-environment interaction variation not accounted for, because calculations were performed for each single environment (Nyquist, 1991).

Standard error of heritability was calculated using the Taylor Series Expansion method (Gilmour et al., 2006).

Genetic and phenotypic correlation coefficients were calculated as

$$r_g = \frac{\mathrm{cov}_{a_{XY}}}{\sqrt{\sigma^2_{a_X}\sigma^2_{a_Y}}} \qquad \text{(Eq. 2-4)}$$

where $\mathrm{cov}_{a_{XY}}$ is the additive covariance between trait X (stunting) and Y (spots); $\sigma^2_{a_X}$ and $\sigma^2_{a_Y}$ are the additive variances for traits X and Y respectively.

$$r_{ph} = \frac{\mathrm{cov}_{a_{xy}} + \mathrm{cov}_{NA_{xy}} + \mathrm{cov}_{P_{xy}} + \mathrm{cov}_{e_{xy}}}{\sqrt{\sigma^2_{ph_x}\sigma^2_{ph_y}}} \qquad \text{(Eq. 2-5)}$$

where $\sigma^2_{ph_x} = \sigma^2_{a_x} + \sigma^2_{NA_x} + \sigma^2_{P_x} + \sigma^2_{e_x}$ being $\sigma^2_{ph_x}$ the phenotypic variance, $\sigma^2_{a_x}$ is the additive variance, $\sigma^2_{NA_x}$ is the non-additive genetic variance, $\sigma^2_{P_x}$ is the plot variance and $\sigma^2_{e_x}$ is the residual variance for the trait X. Similar variances apply also for trait Y.

Standard errors of correlations were calculated using the Taylor Series Expansion method (Gilmour et al., 2006).

Results

The predominant spotted wilt symptoms in every test were stunting and foliar symptoms. Tip death, leaf necrosis and yellowing incidences never reached more than 5% in any test (Fig 2-1). Thus, no analysis was performed on them.

Epidemics varied considerably in their intensity among tests, ranging from light at Citra 2005 and 2006 to severe at Marianna and Quincy in 2007 (Table 2-2).

Locations with heavier epidemics, like Marianna, showed greater variability between years than locations with lighter epidemics (Citra).

Geographical area seemed important as Citra (North-Central Florida) and Marianna (Florida Panhandle) in 2006 were widely different in their epidemics whereas Marianna and Quincy, only 50 miles apart, displayed similar epidemics in 2007.

The epidemic progression was also very different among tests. As opposing examples, at Citra 2005 the epidemic changed very little after the first assessment date both in incidence (Fig 2-2) and severity (Fig. 2-3) while at Marianna 2007 there was a steady increase in both.

In general, the epidemic progression was rather different among genotypes, both in incidence and severity. Susceptible genotypes (like NemaTAM) exhibited a faster rate of increase in the percentage of symptomatic plants, especially in tests with the more severe epidemics (Fig. 2-4, 2-5). Symptom severity progression was also different among genotypes (Fig. 2-6). Most of the genotypes showed a similar average score for both stunting and foliar symptoms (data not shown). In most of the genotypes a range of severity from zero to five was observed at 30 DAP for both symptoms although the frequencies among genotypes were different.

The scores for stunting or foliar symptoms showed unimodal and multimodal distributions with varying degree of dispersion according to the environment and genotype. Even homogeneous homozygous genotypes like the parents (Fig. 2-7) showed dispersion. As no obvious dominant/susceptible threshold was observed among classes (Fig. 2-8), the study of inheritance of resistance as a quantitative trait was pursued.

Observed Variance Components

Mixed models were fitted for each assessment date for each test, provided there was enough variation in the data. In the case of Marianna 2006, low spotted wilt incidence (5% stunted plants) would not allow data analysis for the first assessment date.

In both tests at Citra and in Marianna 2006, singularity in the data matrix forced fit to a model without a non-additive variance.

The relative importance of each variance component in the model observed in each test was similar for both variables analyzed (Table 2-3).

The most important variance component in most cases was the residual variance. The second most important variance source was the additive variance, which ranged from slightly superior to the residual variance (in Citra 2005) to a rather small relative size in Citra 2006 and the first two assessment date in Marianna 2006. Non-additive variance, where calculated, was never different from zero. Block variance was negligible most of the time. Plot variance was in some cases rather small, as in Citra 2005 whereas in other cases it was more important than the additive variance, as in Marianna 2006.

The proportion of variation accounted for by the additive and plot sources tended to increase with passing time, with the former increasing more noticeably. Changes in the relative importance of the variance estimates in each year varied between locations. While at Marianna plot and additive variances were rather small in both years, at Citra, their relative importance changed with the year. This was probably due to the different generations tested in both years (only F2s and AP-3 in Citra ’05).

The adjustment provided by the model caused a change in the average stunting and foliar symptoms in some tests (Fig. 2-9), suggesting that the performance of relatives provided an important adjustment on the predicted performance of individuals. This was especially noticeable

in Marianna 2006 and Quincy 2007 where the estimated average values for both spotted wilt symptoms were smaller than the observed ones. However, in Citra 2006 and Marianna 2007 the agreement between observed and estimated averages was very good.

Heritability

Heritability estimates varied widely among tests (Table 2-4). The highest heritability was observed at Citra 2005 where only F_2 populations and AP-3 were grown. In the 2006 tests, the heritability was extremely low whereas in the 2007 tests the estimates were close to 0.3.

Heritability among assessment dates within a test tended to increase with time, but the extent of increase depended on the test. The only exception to this trend was the heritability of foliar symptoms at Citra 2005 which diminished as the season progressed.

Heritability estimates were quite precise as suggested by the small standard errors.

Phenotypic and Genetic Correlations

In some assessment dates the calculation of correlations was hampered by estimation problems such as lack of parameter convergence in the REML process or singularity in the data matrix. In other cases the REML estimate was calculated by bounding it within its theoretical space so no standard error was available.

Correlations between stunting and foliar symptoms were high in value and with small standard errors in every test and assessment date (Table 2-5). Phenotypic correlations ranged from 0.80 to 0.93 with the test at Citra 2005 showing the lowest values in each assessment date. Correlation coefficients from tests in 2007 were very similar. Genotypic correlations were higher than the phenotypic ones, ranging from 0.88 to 0.99. In the second and third assessment dates, three tests (Citra 2005, and Marianna and Quincy 2007) showed almost perfect genetic correlation.

As the genetic correlation between stunting and foliar symptoms was very high, the analysis of breeding values (BLUPs) is presented only for stunting.

Breeding Values (BLUPs)

The BLUPs obtained from each test showed a clear distinction among the susceptible (NemaTAM) and the resistant parents (AP-3, DP-1 and NC94002) (Fig. 2-10). The only exception was Citra 2005, where the only resistant parent grown was AP-3. In most tests NC94002 displayed the best (smallest) breeding value for stunting in every test it was in, whereas DP-1 was better than AP-3 in 3 out of 4 tests. A similar pattern was also observed for BLUPs for foliar symptoms (data not shown). There was, however, an important variation in the BLUPs of each parent among tests. This is somewhat expected because, although most of the individuals in each test were genetically related, they were not identical.

The reliability of the breeding values (which provides a measure of their accuracy) was intermediate for all tests, except for the ones in 2006 (Fig. 2-11) in which the average reliability for individuals in segregating generations was zero. The parents in each test had intermediate to high reliability because of their great number of relatives included in each test, as each parent was grown alongside with its F_1, F_2, $F_{2:3}$ and even backcross individuals.

Both tests in 2007 showed the most damaging epidemics and the best breeding value reliabilities. Consequently, their comparison follows. The rank correlation among the generation-mean BLUPs at both 2007 tests was highly significant ($r=0.93$, $p<0.0001$) suggesting a very similar breeding value of each generation irrespective of the location in that year.

Generation BLUPs

When average BLUPs were calculated for the different generations in both 2007 tests, the best ones belonged to the RxR (resistant with resistant) cross (Figs. 2-12 and 2-13). Among the

SxR (susceptible with resistant) crosses, those involving the most resistant parent (NC94002) were the best at Marianna.

Reciprocal crosses between AP-3 and NemaTAM had very similar average F_2 BLUPs in both locations, implying no maternal effect.

The average BLUPs for all F_3 populations were similar to the ones for F_2 populations at both locations. This suggests that the 25 F_2 plants sampled in 2006 in each F_2 population were able to capture most of the variability present in that population.

The average BLUP for F_2 and F_3 populations were usually intermediate to their parents, suggesting additivity. The only exception was the F_3 population for the RxR cross at Quincy 2007, which had a higher (worse) BLUP than its most susceptible parent (DP-1). However, this could have been caused by the fact that the sampling of the F_2 plants in the previous year didn't reflect correctly the true genetic composition for that population.

Individual BLUPs

The percentage of individuals with BLUPs better than their best parent varied widely depending on the cross and the test. In general and as expected (because of increasing additive variance due to selfing), the comparison between F_2 and $F_{2:3}$ populations showed the latter having both higher individual BLUP variability (Figs. 2-14 and 2-15) and higher percentage of individuals with BLUP superior to their best parent (Fig. 2-16).

Comparing both tests grown in 2007, it can be seen that in Quincy 2007, 30% and 48% of F_2 and F_3 individuals (respectively) had better breeding values than NC94002 in the cross between this line and DP-1 (Fig. 2-16). Surprisingly, the same cross in Marianna 2007 only showed 6% and 3% (F_2 and F_3) of individuals better than NC94002 and it failed to produce an individual with breeding value better than NC94002 in the other three tests (data not shown). The remaining cross involving NC94002 as a parent also showed a difference in the percentage of

individuals with better (smaller) BLUP than NC94002 observed between tests in 2007. In this case, however, the difference between tests grown in 2007 was less drastic than in the cross DP-1 x NC94002. While the crosses between AP-3 and NemaTAM showed small percentages of good individuals in Marianna, they failed to deliver better BLUPs than AP-3 in Quincy. Meanwhile, the cross between NemaTAM and DP-1 didn't produce any individual with better breeding value than DP-1.

Family BLUPs

In four out of five crosses there were individuals with better BLUPs than their best parent. However, the proportion of families with average BLUPs for stunting better than their best parent was rather small (Fig. 2-16). Most crosses produced one or two superior families in Marianna 2007. The only exception to this general trend was the cross between two resistant parents (DP-1/NC94002), which produced 13 out of 25 $F_{2:3}$ families with better (smaller) average BLUPs than the most resistant parent in Quincy 2007. However, the same cross only produced one superior family in Marianna 2007.

Discussion

Locations have generally different epidemic patterns, particularly if they are significantly distant and contain different agroecosystems (Culbreath et al., 2003; Groves et al., 2003).

Among the locations tested in this study, Citra typically does not have serious spotted wilt epidemics even under agronomic practices that increase the likelihood of strong epidemics (Tillman, pers. comm., Chapter 1 of this dissertation). One likely explanation is that the agroecosystem of Marion County (where Citra is located) is dominated by warm-season grass pastures while crops that are TSWV host species are a small minority (2002 Census of Agriculture).

Meanwhile, both Marianna and Quincy have routinely severe spotted wilt epidemics (T. Momol, pers. comm., Tillman et al., 2007, Culbreath et al., 2005). The important proportion of farmland devoted to susceptible crops, like peanut, tobacco, tomato and vegetables (2002 Census of Agriculture) and/or the presence of weed species that are better hosts of thrips during early spring could explain the chronically strong epidemics in these two locations (Kucharek et al., 1990; 2002 Census of Agriculture, Northfield, 2005).

In the present study, there were different epidemic patterns among genotypes with the most resistant genotypes showing a slower progression, most noticeably under severe epidemics. Similar findings have been reported previously (Culbreath et al.,, 1997; Murakami et al.,, 2006; Chapter 1 of this dissertation).

Five typical spotted wilt symptoms were selected from the literature to be assessed (Demsky & Reddy, 2004, Culbreath et al., 2003), but only stunting and foliar symptoms reached significant incidences. Yellowing has been reported in Texas as a very important symptom of TSWV (Mitchell, 1996). Yellowing (chlorosis) and stunting in tobacco are caused by TSWV NSM protein (Prins et al., 1997) which increases the molecular exclusion limit of plasmodesmata causing traffic disruption of substances across them.

Thus, it would be expected that both symptoms would be present frequently when a plant displays spotted wilt symptoms. Nonetheless, in the present study stunting was common but chlorosis was observed only occasionally. This could be due to the fact that the predominant viral sequence present in the tests mainly causes stunting and foliar symptoms (Nagata et al., 1993; Mandal et al., 2006)

The high genetic correlation between stunting and foliar symptoms suggests either pleiotropy acting on the same direction or gametic phase disequilibrium (Lynch and Walsh,

1998). If the latter were the case, the genetic determinants of both types of symptoms would have to be tightly linked, as thousands of F_3 individuals were assessed and some recombinants should have occurred thus reducing the correlation. Similar incidences among genotypes at 30 DAP suggested that thrips feeding preference was not an issue, in agreement with previous findings (Culbreath et al., 1996; 1997).

In spite of the fact that the parents are inbred there was still a wide range of severity in the symptoms suggesting either different inoculation times or differential progression of the disease among plants within a genotype. Taking into account that at least a week is required to develop symptoms (Hoffman et al., 1998) it seems clear that a potential period of three weeks of inoculum exposure could cause a wide range of symptom severities very early in the season.

Incomplete penetrance of resistance to TSWV has been reported in tomato when inoculated by thrips (Rosello et al., 2001). This could also explain the variable symptom severity observed in the present study, particularly in the resistant parents. Whichever the case, the score distribution didn't suggest the use of traditional Mendelian segregation analysis (Lynch and Walsh, 1998) so a quantitative approach to analyze the symptoms scores as polychotomous variables was necessary.

The heritability values varied noticeably among tests, which is usually the case when calculating estimates even from similar populations (Nyquist, 1991; Lynch and Walsh, 1998). However, the small standard errors suggested the estimates were rather precise.

In the present study three unrelated resistant genotypes, which represent the different sources of resistance to spotted wilt known to date (D. Gorbet, pers. comm.) were used. By crossing them to a susceptible parent, segregating populations with wide variability in spotted wilt resistance were obtained.

The Animal Model utilizes all relationships among individuals in a test by using a numerator relationship matrix (Mrode, 2005) thus accounting for most of the additive variance which results in more accurate estimations of variance components and breeding values. The fact that most of the individuals on each test were related to some extent, even when belonging to different crosses, provided better estimates of the additive covariances than the use of traditional heritability estimation methods (Henderson, 1976; Lynch and Walsh, 1998). Although similar kinds of populations were used each year, the variance components were quite variable among tests. This is sometimes the case, even when repeating tests few days apart (Chapter 1 of this dissertation), doing them in a laboratory setting (Rapp & Juntila, 2001) or in the field (Finne et al., 2000).

Spotted wilt epidemics are highly variable among locations and even from year to year at a single location (Culbreath et al., 2003). The range of intensity of the epidemic observed among the tests was certainly wide. Apparently the geographical location was more important than the year, in accordance with the results described in the Chapter 1 of this dissertation.

Heritability estimates are influenced by the relative amount of total variation due to genetic causes (Lynch and Walsh, 1998). The wide range of epidemic intensity probably accounted for a large part of the variability in heritability estimates. Inaccurate heritability estimates are often obtained when the frequency of a category in a polychotomous variable is very high across the whole population of individuals being tested (Yang et al., 1998). In the present study most of the individuals showed a reasonable dispersion in the score frequencies. The use of individual values is known to provide better heritability estimates than plot means under most conditions when a REML approach is used (Huber et al., 1994). Consequently, even when the heritability estimates obtained here were quite variable, they are expected to be accurate. Heritability estimates in the

present study were usually in the medium to low range, with the values increasing as the season progressed. This was expected as the difference in symptom severity in genotypes with different resistance tends to increase with time (Culbreath et at., 1997; Murakami et al., 2006) unless the epidemic reaches a final intensity very early in the season (Chapter 1 of this dissertation). If the additive variance and consequently the heritability tend to increase toward harvest time, selection for resistant genotypes would be more effective when conducted closer to harvest (Hallauer & Miranda, 1988). Heritability estimates for a trait is at the core of any individual multi-trait selection index. Its magnitude determines the importance (weight) that's assigned to the trait while selecting individuals based on that index (Hallauer and Miranda, 1988).

In the University of Florida Peanut Breeding Program (UFPBP), performance of segregating populations against TSWV has been assessed based on a "holistic" score assigned to plots or plants in which all spotted wilt symptoms are considered (Gorbet, 1999, B. Tillman, pers. comm.). Simmons (1979) pointed out that every breeder has in his/her mind a multi-trait selective index but it is usually not put into writing. Taking into account the high genetic correlation between the most frequent spotted wilt symptoms and the "workable" value of heritability estimates here reported, it seems reasonable that the inclusion of spotted wilt resistance as a part of this "unwritten" selective index could be the cause of the observable improvement in the overall level of spotted wilt resistance in the breeding populations observed in the UFPBP compared to older, but good performing genotypes from the pre-TSWV era like Florunner, Sunrunner or SunOleic 97R (Culbreath et al., 2005; Tillman et al., 2007).

The variable reliability observed among and within tests could only be explained by a variable importance of the environment, as the genetic structure and the type of genetic relationships among individuals were quite similar in each test. When using full sib records (as is

our case in the $F_{2:3}$ families), reliability always increases as the number of tested sibs increases but with decreasing relative gain (Mrode, 2005). The increase in reliability from increasing the number of sibs is larger when heritability is lower. Although in this study the number of sibs tested varied among tests, this doesn't seem to account for the observed variation in the BLUP estimate reliabilities. Even within each test and among $F_{2:3}$ families of the same cross, the reliabilities had important variation. This seems to point to experimental noise as the most probable cause of this fact (Mrode, 2005).

As expected under the assumption of additive mode of action for spotted wilt resistance, the cross between resistant parents produced the population with best BLUPs. Culbreath et al. (2005) reported that genotypes that inherited spotted wilt resistance from PI 203395 were less resistant than genotypes derived from another resistance source (PI 576638). They suggested the possibility that both sources could provide different resistance genes.

In the present study, both resistance sources were represented by DP-1 and NC94002, respectively. This cross provided few or no individuals with better breeding value than NC94002 in four tests but it provided a high percentage of superior individuals in Quincy 2007. This could point to different resistance mechanisms for genes coming from these two resistance sources, as suggested by Culbreath et al. (2005). The reason these different mechanisms would only be appreciated in one out of five tests is unknown. Perhaps Quincy provided a better discriminating environment for such effect. This ability of some environments to be better at discriminating has also been cited for other traits and species (Hall and Van Sanford, 2003; Venuprasad et al., 2007).

All the SxR crosses produced populations with similar breeding values. Most populations had a rather small proportion of individuals with better BLUP than their resistant parent. In fact,

the average breeding value of the F_2 and F_3 populations were usually intermediate to their parents' breeding values. Both facts seem consistent with an additive mode of action (Falconer & MacKay, 1996).

As additive variance increases with selfing (Nyquist, 1991), individual BLUP tend to increase so more individuals in each distributional extreme can be found in F_3 than in F_2. Although further selfing continues to increase the additive variance, resource limitations always force some type of selection in early generations (Simmonds, 1979).

The low individual heritability but good reliability of family BLUP suggests that taking into consideration the family performance for spotted wilt resistance when selecting individuals among $F_{2:3}$ families, as is frequently practiced in peanut breeding programs in Southeastern USA, is a safe breeding strategy (Falconer and Mackay, 1996; Hallauer and Miranda, 1988). When selecting in populations derived from the resistant parents used here, inclusion of spotted wilt resistance a part of a selective individual multi-trait index is acceptable. However, it should be given a moderate weight because of its modest heritability.

It seems clear that RxR crosses would provide better populations to select for spotted wilt resistance and those having NC94002 as a parent would display the best response to selection.

Conclusion

The use of the Animal Model provided accurate and precise heritability estimates. They ranged from 0.01 to 0.71, but were most frequently in the low to medium range. The estimates increased as the epidemics progressed.

The almost exclusive spotted wilt symptoms detected in each test were stunting and foliar symptoms (spots or mosaics). Tip death, leaf necrosis and yellowing (chlorosis) were rare. There was high phenotypic and genotypic correlation among stunting and foliar symptoms suggesting either pleiotropy or a very strong coupling linkage among their genetic determinants.

Breeding values for the different generations of the five crosses tested seemed to suggest additivity as the main mode of action in the determination of the resistance to spotted wilt. The resistant parents produced populations with similar breeding values when crossed to the susceptible parent. The population from a cross between resistant parents exhibited the best breeding values for resistance to spotted wilt.

Based on the calculated heritability estimates, pedigree selection within the populations used in this study should not put too much weight on individual selection in early generations based on resistance to TSWV. More emphasis on including resistance as a part of a multi-trait individual selective index with a corresponding moderate weighting seems recommendable. Additionally, familial performance can provide surrogate estimations of an individual's real resistance.

Table 2-1. Sowing date, replication number and design of tests assessing performance against spotted wilt in five peanut crosses in Florida.

Test	Plots in each generation[a]					Design and block number [d]	Sowing date
	Parents	F_1	F_2	F_3[b]	BC		
Citra '05	13[c]	1	2-5	NA	NA	CR w/variable replications	5/30
Citra '06	4-8	1-2	1-9	2	NA	RCB, 2 blocks	5/24
Marianna '06	6-12	1	6[d]	2-3	NA	RCB, 3 blocks	4/28
Marianna '07	6-12	1-2	9	2	0-2	RCB, 3 blocks	4/24
Quincy '07	6-12	0-2	9	2	0-2	RCB, 3 blocks	4/25

[a] Plot number in each generation varied depending on seed availability. [b] All $F_{2:3}$ families changed from year to year and some changed from test to test within years. [c] Only AP-3 grown. [d] F_2 DP-1 / NC94002 not grown

Table 2-2. Mean (S.D.) score for each spotted wilt symptom at 30, 60 and 120 days after planting, at each of five field tests in which five peanut populations were evaluated in Florida.

	2005	2006		2007	
	Citra	Citra	Marianna	Marianna	Quincy
	30 Days After Planting				
n	1073	N/A	10713	10669	N/A
Stunting	0.66 (1.58)	N/A	0.17 (0.79)	0.32 (0.99)	N/A
Spots and Mosaic	1.15 (1.92)	N/A	0.17 (0.69)	0.33 (1.02)	N/A
Tip Death	0.08 (0.54)	N/A	0 (0)	0.01 (0.14)	N/A
Leaf Necrosis	0.08 (0.51)	N/A	0 (0.02)	0 (0.07)	N/A
Yellowing	0 (0)	N/A	0 (0)	0 (0)	N/A
	60 Days After Planting				
n	1073	3867	10646	10538	10032
Stunting	0.69 (1.63)	0.36 (1.06)	0.99 (1.73)	1.27 (1.81)	1.24 (1.91)
Spots and Mosaic	1.24 (1.98)	0.46 (1.22)	1.24 (1.92)	0.15 (2.01)	1.43 (2.13)
Tip Death	0.09 (0.58)	0.01 (0.15)	0.01 (0.16)	0.02 (0.2)	0.09 (0.47)
Leaf Necrosis	0.09 (0.54)	0 (0.03)	0.01 (0.13)	0 (0.1)	0 (0.08)
Yellowing	0 (0)	0.01 (0.16)	0.00 (0.04)	0.10 (0.5)	0.06 (0.39)
	120 Days After Planting				
n	1071	3809	10246	10028	9825
Stunting	0.68 (1.48)	0.56 (1.29)	2.34 (1.85)	3.27 (1.69)	3.21 (1.53)
Spots and Mosaic	1.23 (1.77)	0.75 (1.51)	3.13 (1.76)	3.63 (1.7)	3.66 (1.59)
Tip Death	0.07 (0.49)	0.01 (0.15)	0.01 (0.11)	0.03 (0.27)	0 (0.07)
Leaf Necrosis	0.03 (0.28)	0 (0.03)	0 (0.03)	0 (0.02)	0 (0.02)
Yellowing	0 (0)	0.01 (0.12)	0.04 (0.12)	0.05 (0.39)	0.04 (0.34)

N/A: Not Available

Table 2-3. REML variance estimates for stunting and foliar symptoms caused by TSWV in populations derived from five peanut crosses tested at Citra, Florida in 2005 and 2006, Marianna, Florida in 2006 and 2007 and Quincy, Florida in 2007.

	Stunting					Foliar symptoms				
	Citra 2005									
Assessment date	σ^2_{Block}	σ^2_{Plot}	σ^2_A	σ^2_{NA}	σ^2_e	σ^2_{Block}	σ^2_{Plot}	σ^2_A	σ^2_{NA}	σ^2_e
30 DAP	a	0.040	2.539		1.699	a	0.155	4.096	a	2.050
60 DAP	a	0.040	2.539	a	1.699	a	0.198	2.315	a	2.889
120 DAP	a	0.042	2.827	a	1.114	a	0.278	1.048	a	2.455
	Citra 2006									
30 DAP	c	c	c	c	c	C	c	c	c	c
60 DAP	0.001	0.017	0.021	a	1.070	0.002	0.018	0.030	a	1.423
120 DAP	0	0.054	0.036	a	1.539	0.000	0.083	0.065	a	2.110
	Marianna 2006									
30 DAP	b	b	b	b	B	B	b	b	b	b
60 DAP	0	0.106	0.087	a	2.774	0	0.149	0.133	a	3.403
120 DAP	0.004	0.382	0.305	a	2.641	0.012	0.358	0.358	a	2.298
	Marianna 2007									
30 DAP	0	0.014	0.013	a	0.946	0.001	0.011	0.017	a	1.012
60 DAP	0.015	0.102	0.235	0	2.955	0.034	0.140	0.320	0	3.578
120 DAP	0.095	0.250	0.698	0	1.739	0.056	0.211	0.790	0	1.783

Table 2-3. Continued

	Stunting					Foliar symptoms				
	Quincy 2007									
Assessment date	σ^2_{Block}	σ^2_{Plot}	σ^2_A	σ^2_{NA}	σ^2_e	σ^2_{Block}	σ^2_{Plot}	σ^2_A	σ^2_{NA}	σ^2_e
30 DAP	b	b	b	b	b	b	b	b	b	b
60 DAP	0.003	0.197	0.197	0	3.227	0.004	0.248	0.268	0	3.983
120 DAP	0.008	0.243	0.640	0	1.396	0.019	0.274	0.738	0	1.394

σ^2_{Block}: Block variance; σ^2_{Plot}: Plot variance; σ^2_A: Additive variance; σ^2_{NA}: Non-additive variance. a: term not included in the model. b: date not assessed. c: incidence too low to allow analysis

Table 2-4. Heritability (S.E.) estimates for stunting and foliar symptoms caused by TSWV on peanut populations from five crosses at different assessment dates in five tests in Floirida. Estimates were calculated using univariate Animal Models.

Test	Variable	30 DAP	60 DAP	120 DAP
Citra 2005	stunting	0.59 (0.05)	0.59 (0.05)	0.71 (0.04)
	foliar symptoms	0.65 (0.04)	0.43 (0.08)	0.28 (0.11)
Citra 2006	stunting	a	0.02 (0.01)	0.02 (0.01)
	foliar symptoms	a	0.02 (0.01)	0.03 (0.01)
Marianna 2006	stunting	b	0.03 (0.01)	0.09 (0.02)
	foliar symptoms	b	0.04 (0.01)	0.12 (0.03)
Marianna 2007	stunting	0.01 (0.01)	0.07 (0.01)	0.26 (0.03)
	foliar symptoms	0.01 (0.01)	0.08 (0.02)	0.28 (0.03)
Quincy 2007	stunting	b	0.05 (0.02)	0.28(0.03)
	foliar symptoms	b	0.06 (0.02)	0.31(0.03)

a: incidence too low to allow analysis. b: date not assessed.

Table 2-5. Phenotypic and genetic correlation (S.E.) estimates between stunting and foliar symptoms caused by TSWV on peanut populations from five crosses at different assessment dates in five tests in Florida. Estimates were calculated using univariate Animal Models.

	30 DAP	60 DAP	120 DAP
Citra 2005	0.80 (0.02) / 0.88 (0.03)	0.82 (0.02) / 0.99 (0.01)	0.83 / 0.99 e
Citra 2006	a	b	0.91 (0.1) / 0.95 (0.03)
Marianna 2006	c	0.93 (0.01) / 0.95 (0.03)	0.84 (0.01) / 0.95 (0.02)
Marianna 2007	d	0.90 / 0.99 e	0.92 (0.01) / 0.99 (0.0.1)
Quincy 2007	c	0.91 / 0.99 e	0.92 (0.01) / 0.99 (0.01)

a: incidence too low to allow analysis. b: Singularity in datamatrix c: date not assessed. d: parameters didn't converge. e: Standard Error not available because REML estimate was bounded.

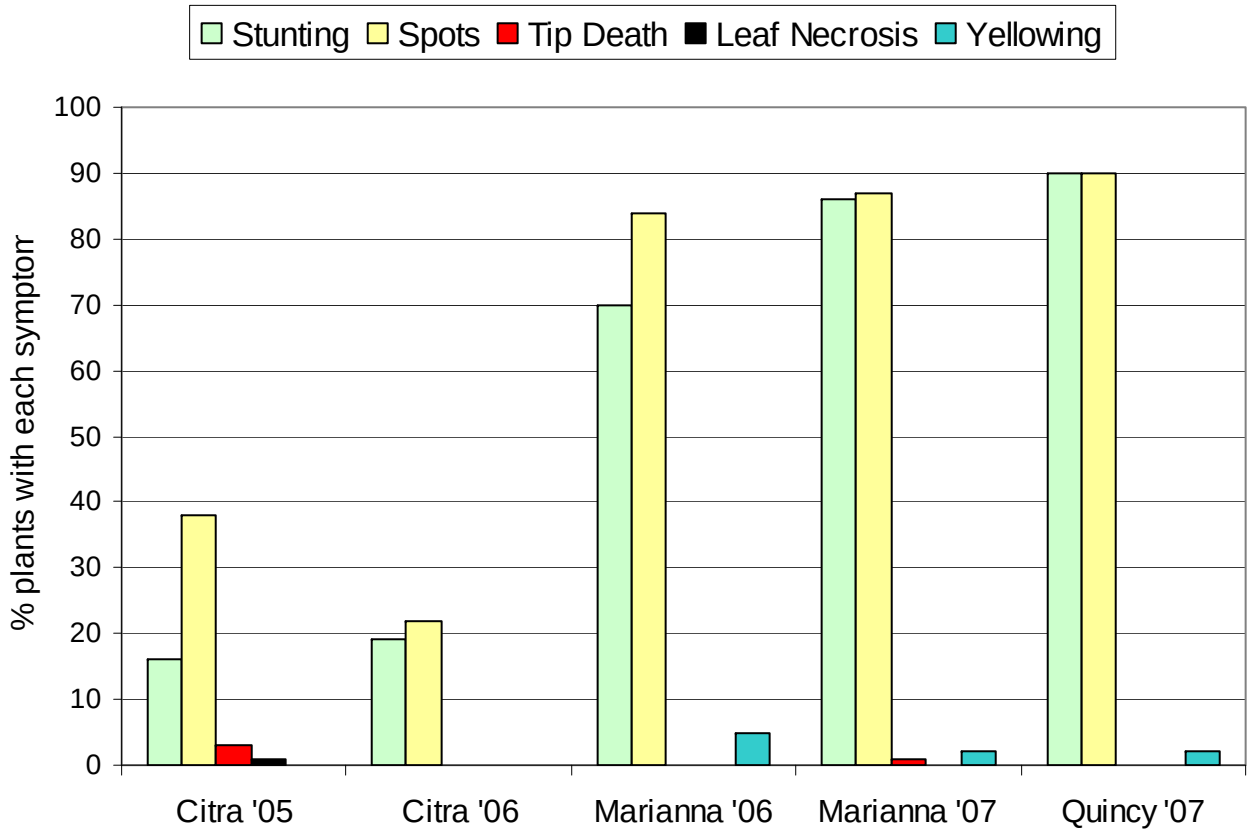

Figure 2-1. Incidence of spotted wilt symptoms at 120 days after planting in populations from five peanut crosses tested in five Florida environments.

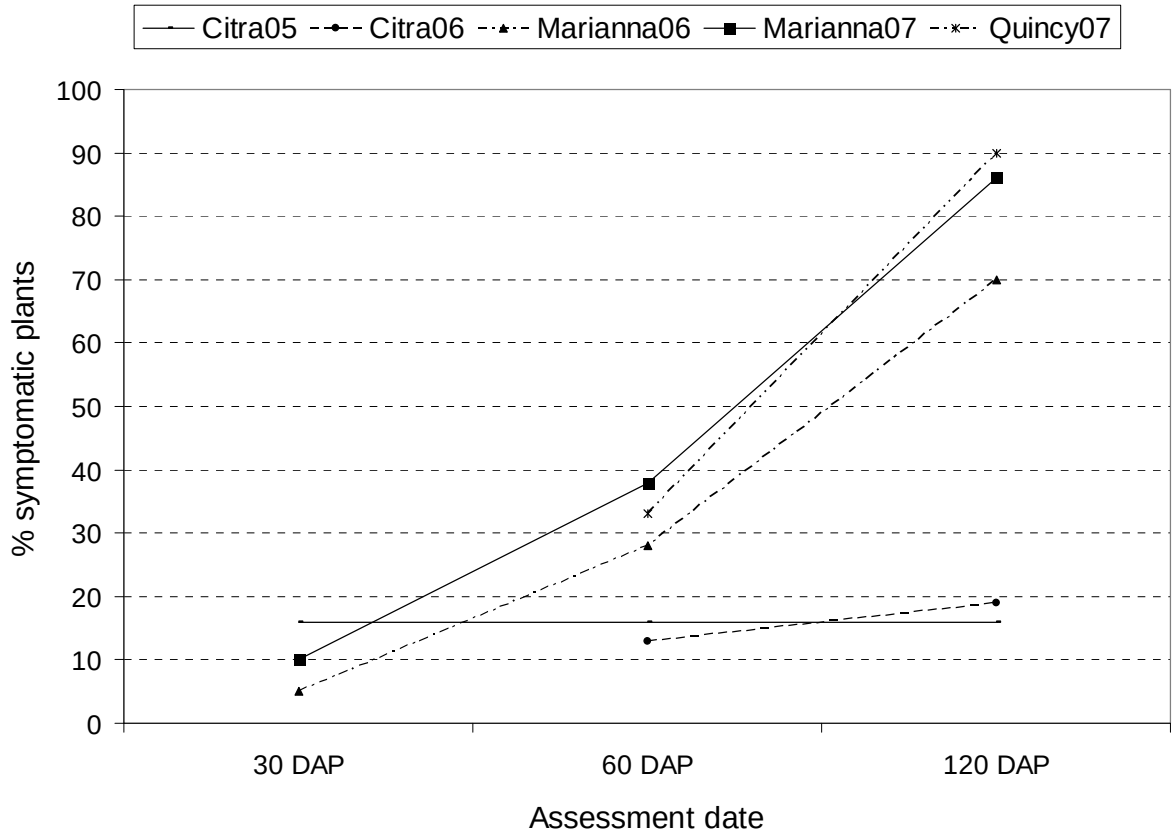

Figure 2-2. Percentage of plants displaying spotted wilt symptoms in five peanut crosses in five field tests assessed at three dates in five Florida environments.

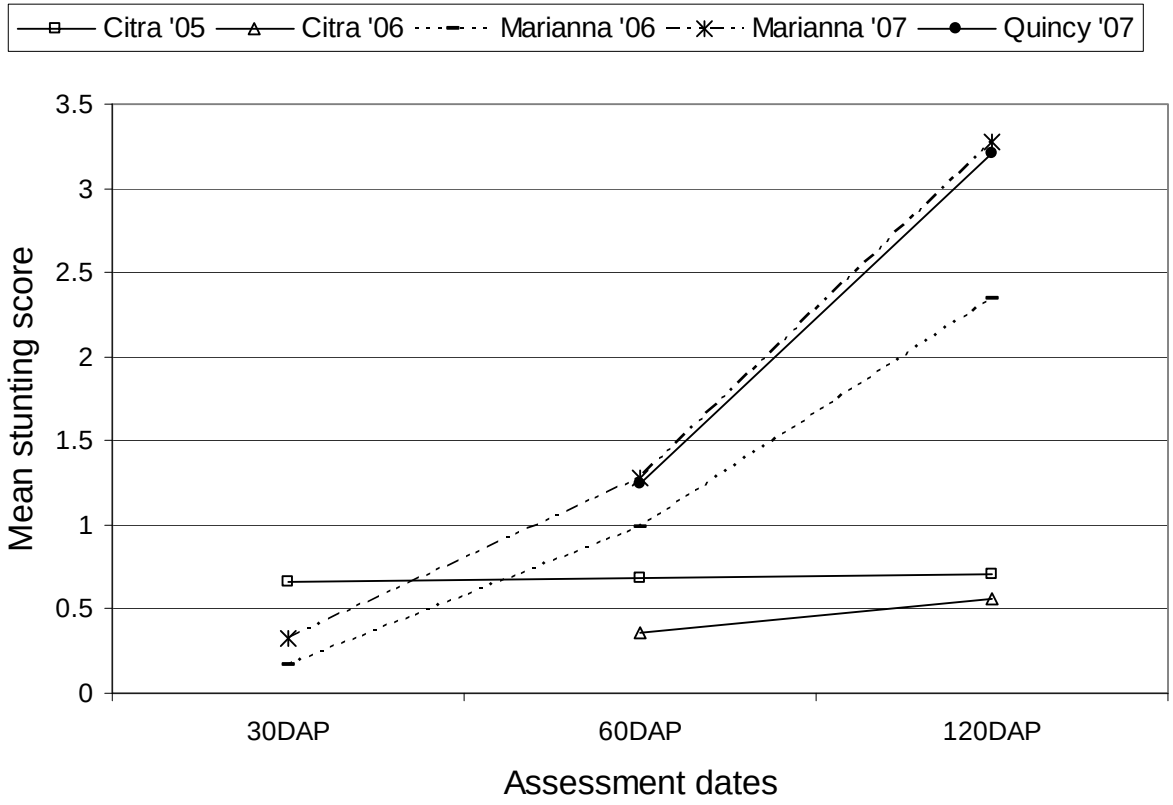

Figure 2-3. Stunting severity progression in five peanut crosses, in five field tests assessed at three dates in three Florida locations.

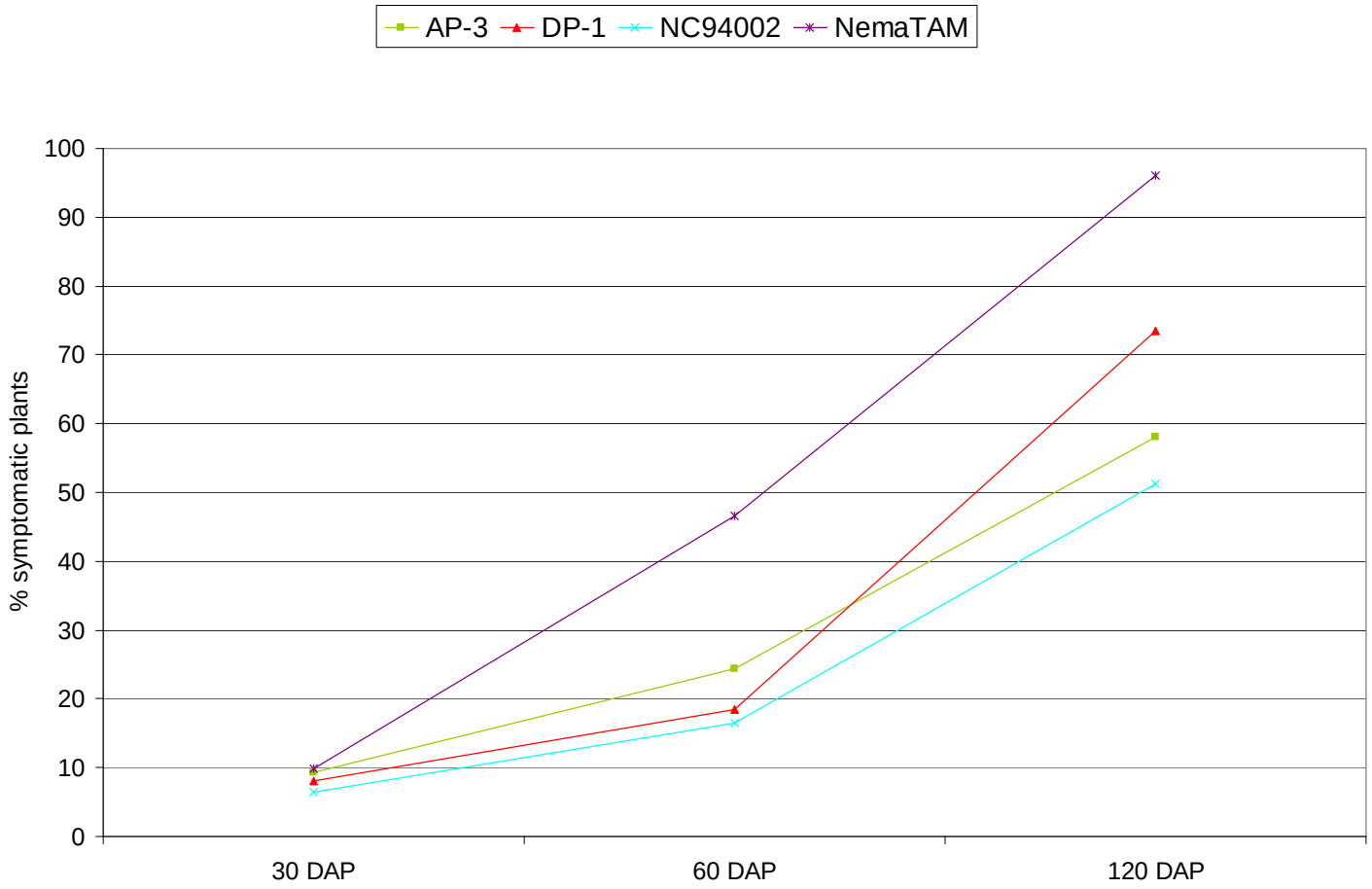

Figure 2-4. Spotted wilt incidence in four peanut genotypes at three assessment dates at Marianna, Florida in 2006.

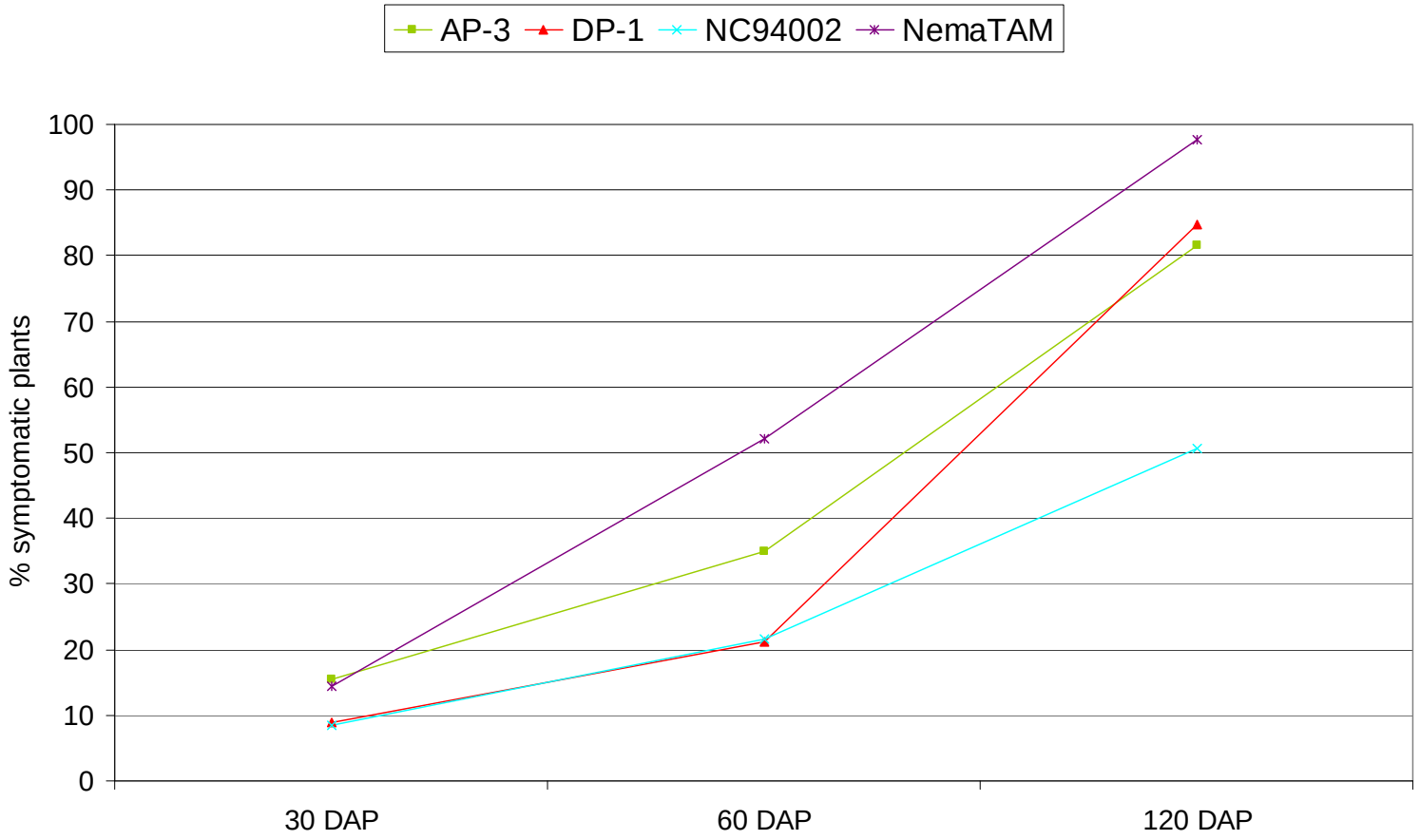

Figure 2-5. Spotted wilt incidence in four peanut genotypes at three assessment dates at Marianna, Florida in 2007.

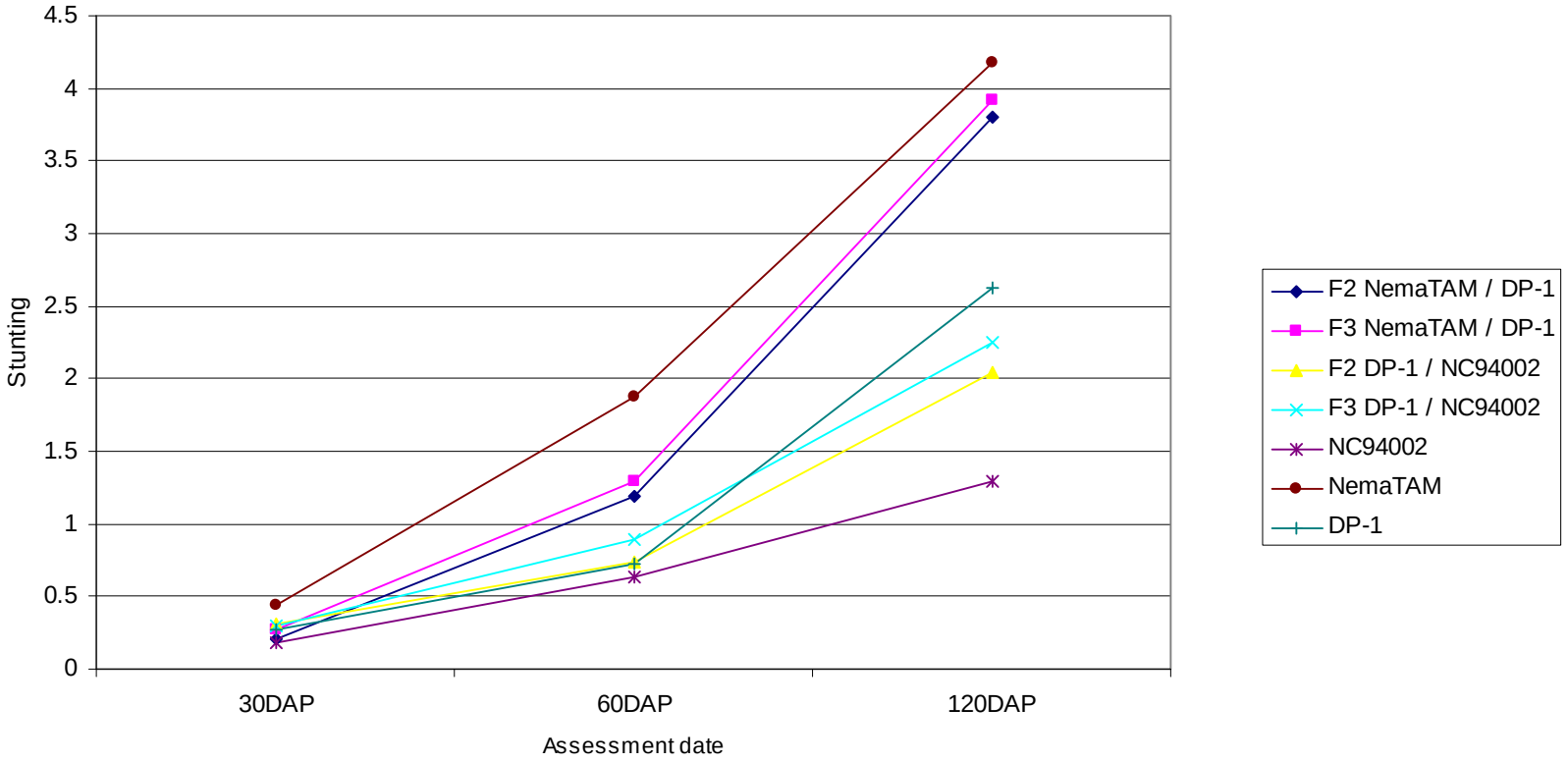

Figure 2-6. Observed stunting severity among different populations from five peanut crosses, at three different dates at Marianna, Florida in 2007.

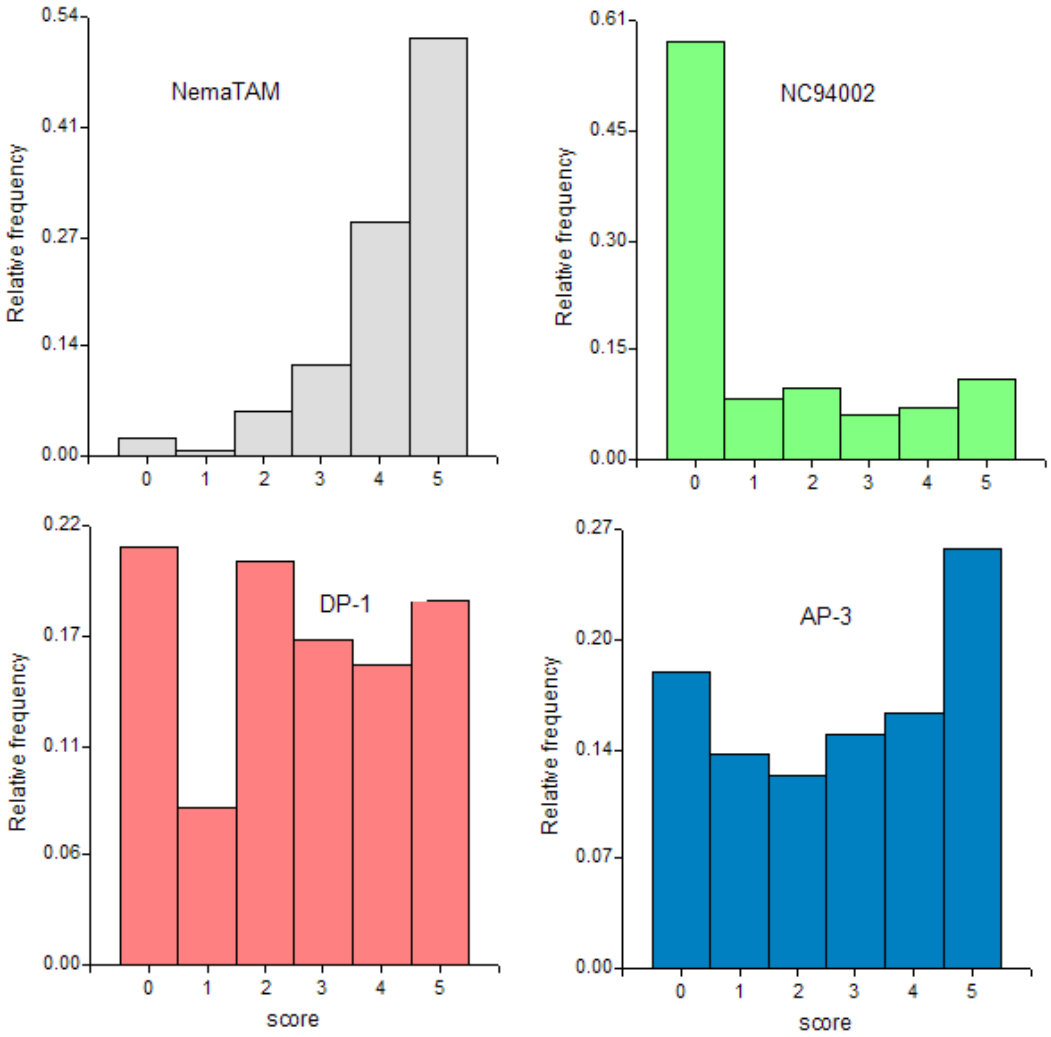

Figure 2-7. Frequency distributions for TSWV-induced stunting scores among parents of five peanut crosses field tested at Marianna, Florida in 2007.

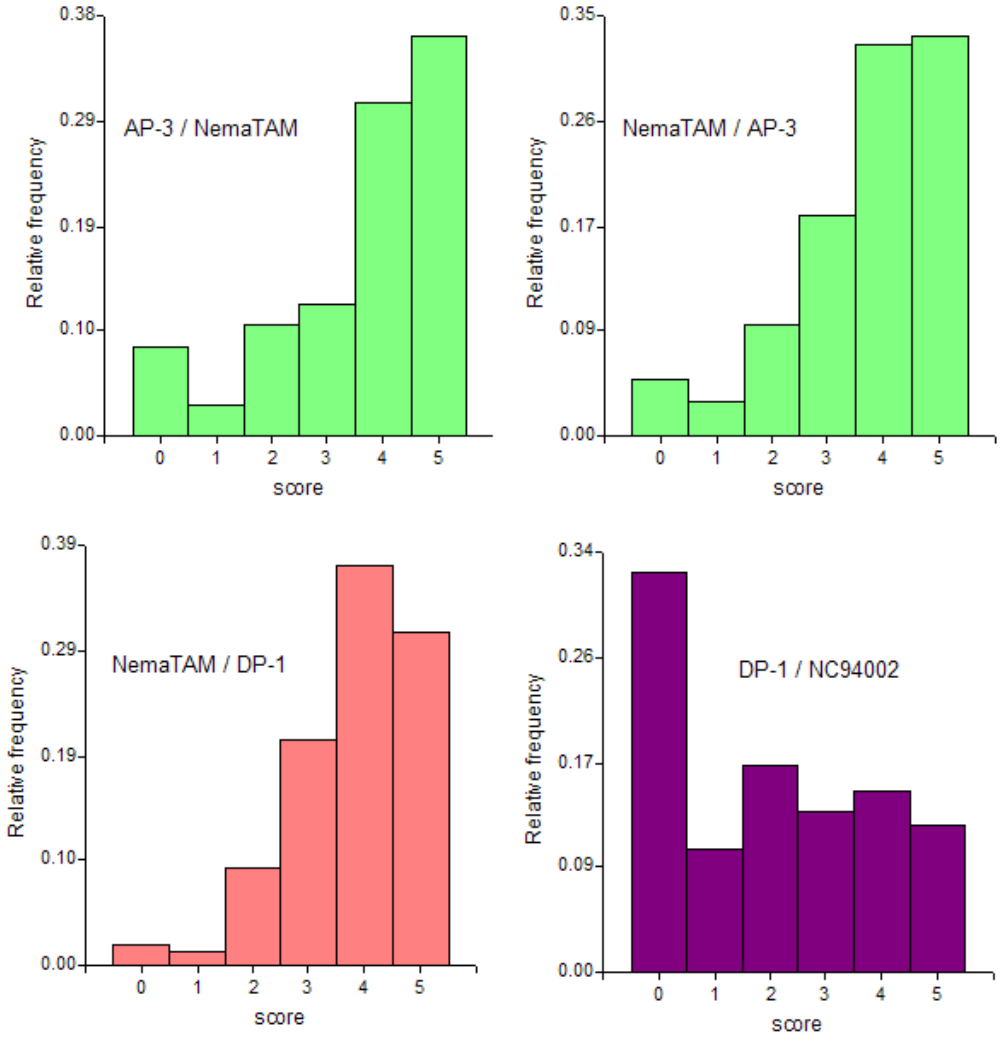

Figure 2-8. Frequency distributions for TSWV-induced stunting scores among F2 populations from four peanut crosses field tested at Marianna, Florida in 2007.

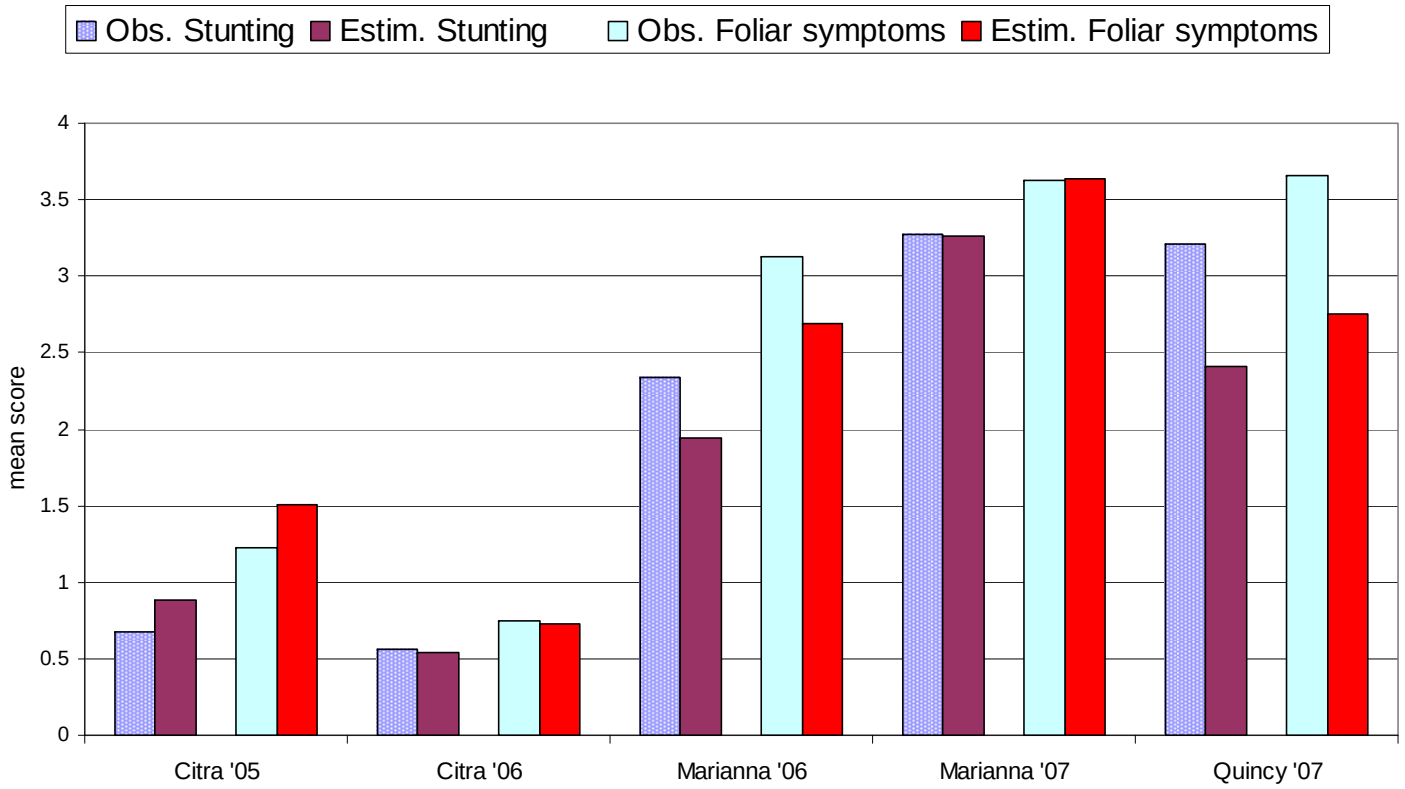

Figure 2-9. Observed vs. estimated overall severity in stunting and foliar symptoms caused by TSWV on five peanut crosses in five field tests in Florida.

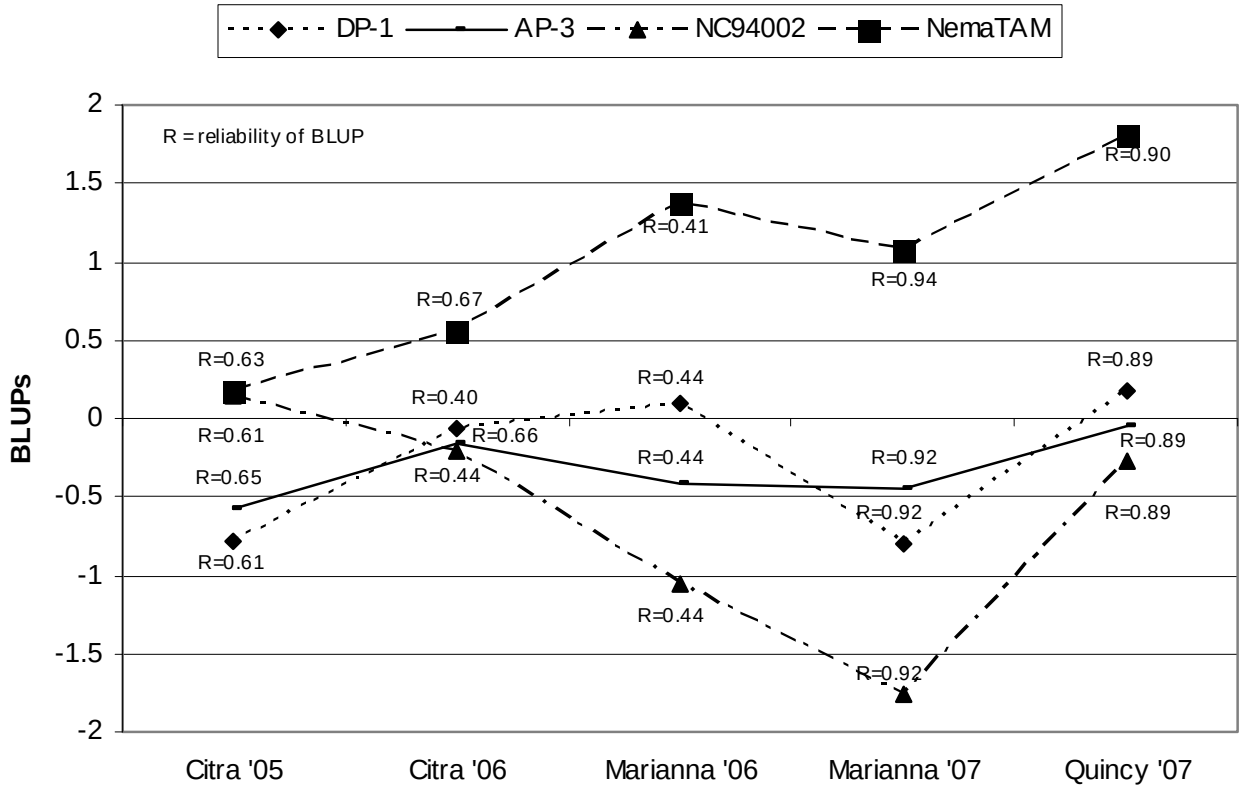

Figure 2-10. Best linear unbiased predictors (relative to test average) for TSWV-induced stunting in parents of five peanut crosses assessed in five field tests in Florida.

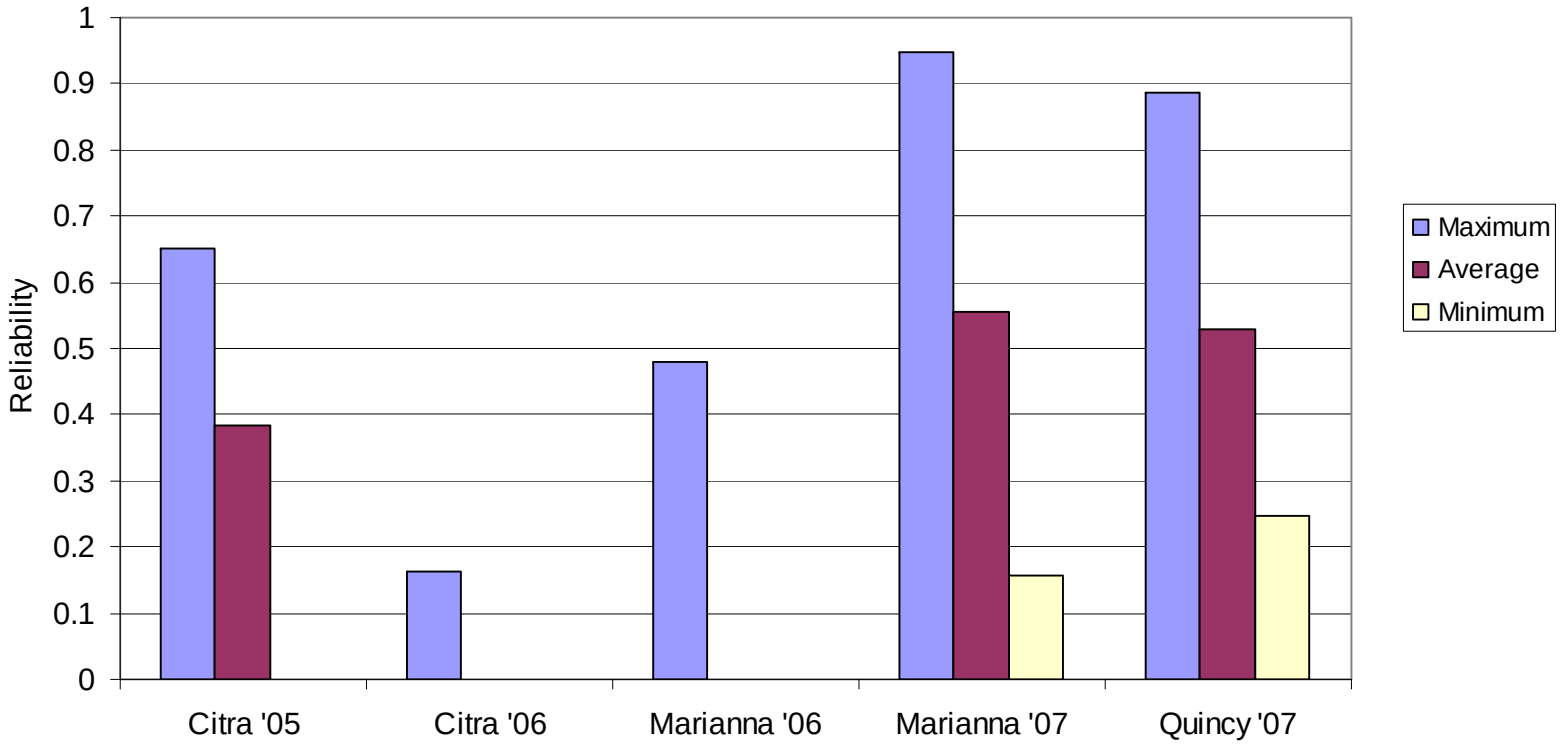

Figure 2-11. Breeding values' reliability for TSWV-induced stunting among individuals in segregating populations derived from five peanut crosses evaluated in five field tests.

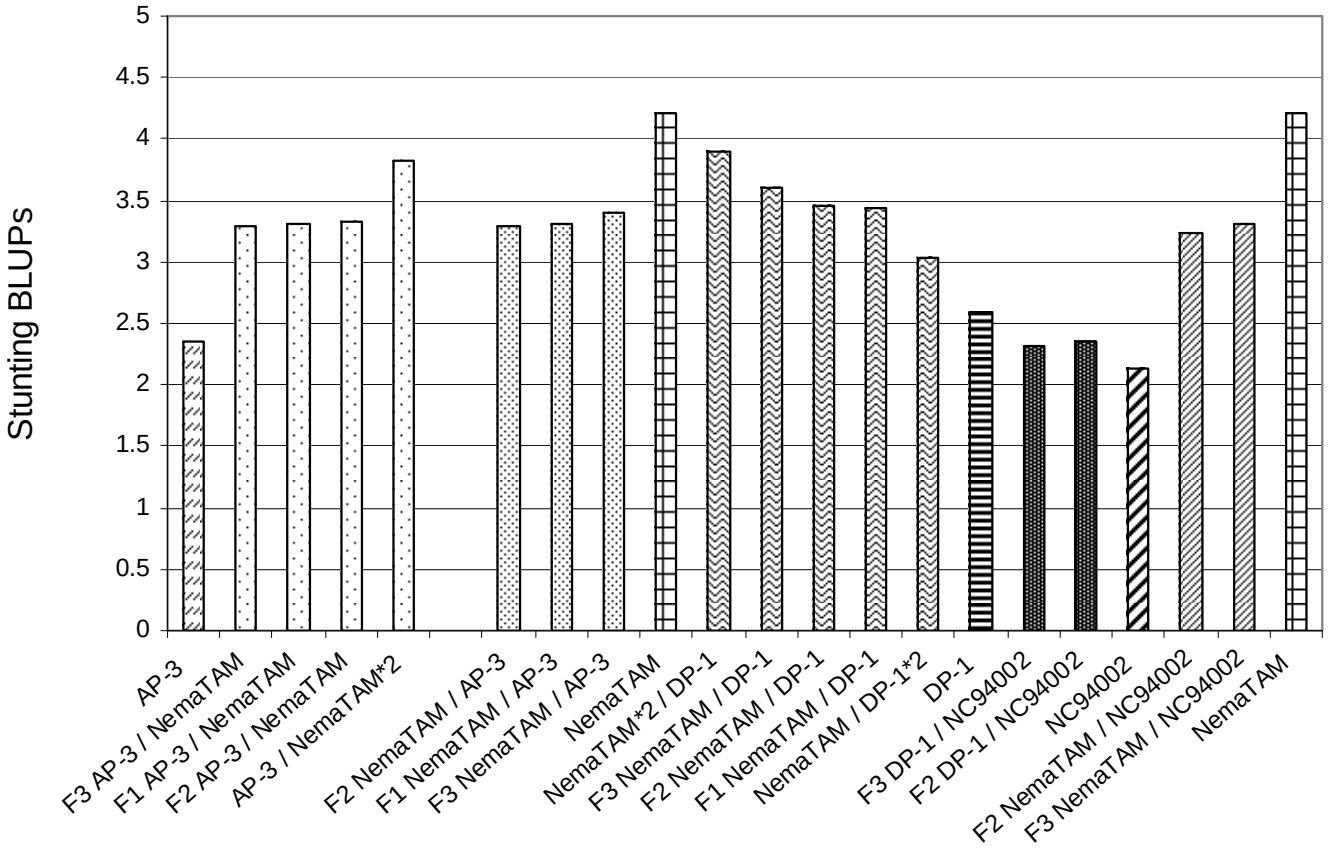

Figure 2-12. Generation-mean best linear unbiased predictors for TSWV-induced stunting in populations from five peanut crosses tested at Quincy, Florida in 2007 (all reliabilities were above 0.9).

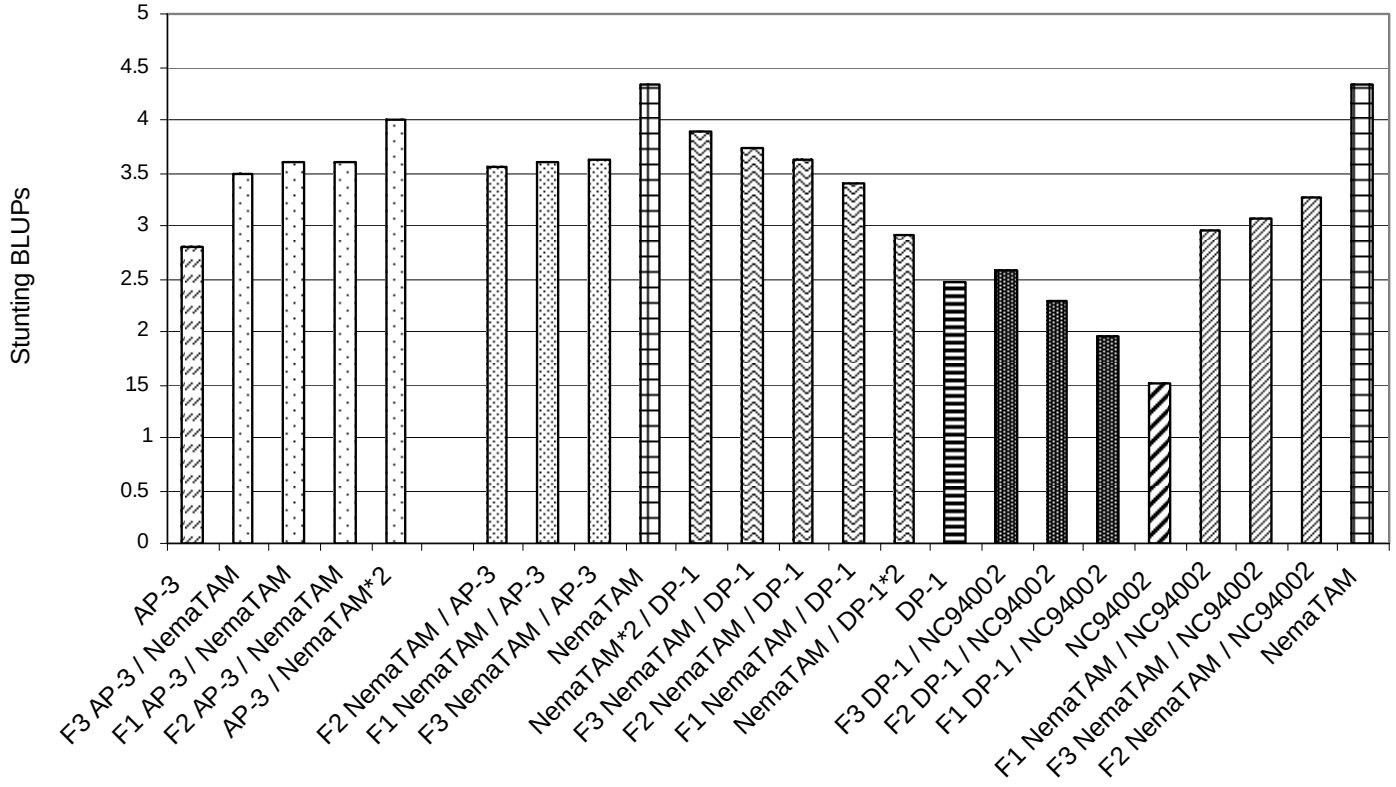

Figure 2-13. Generation-mean best linear unbiased predictors for TSWV-induced stunting in populations from five peanut crosses tested at Marianna, Florida in 2007 (all reliabilities were above 0.9).

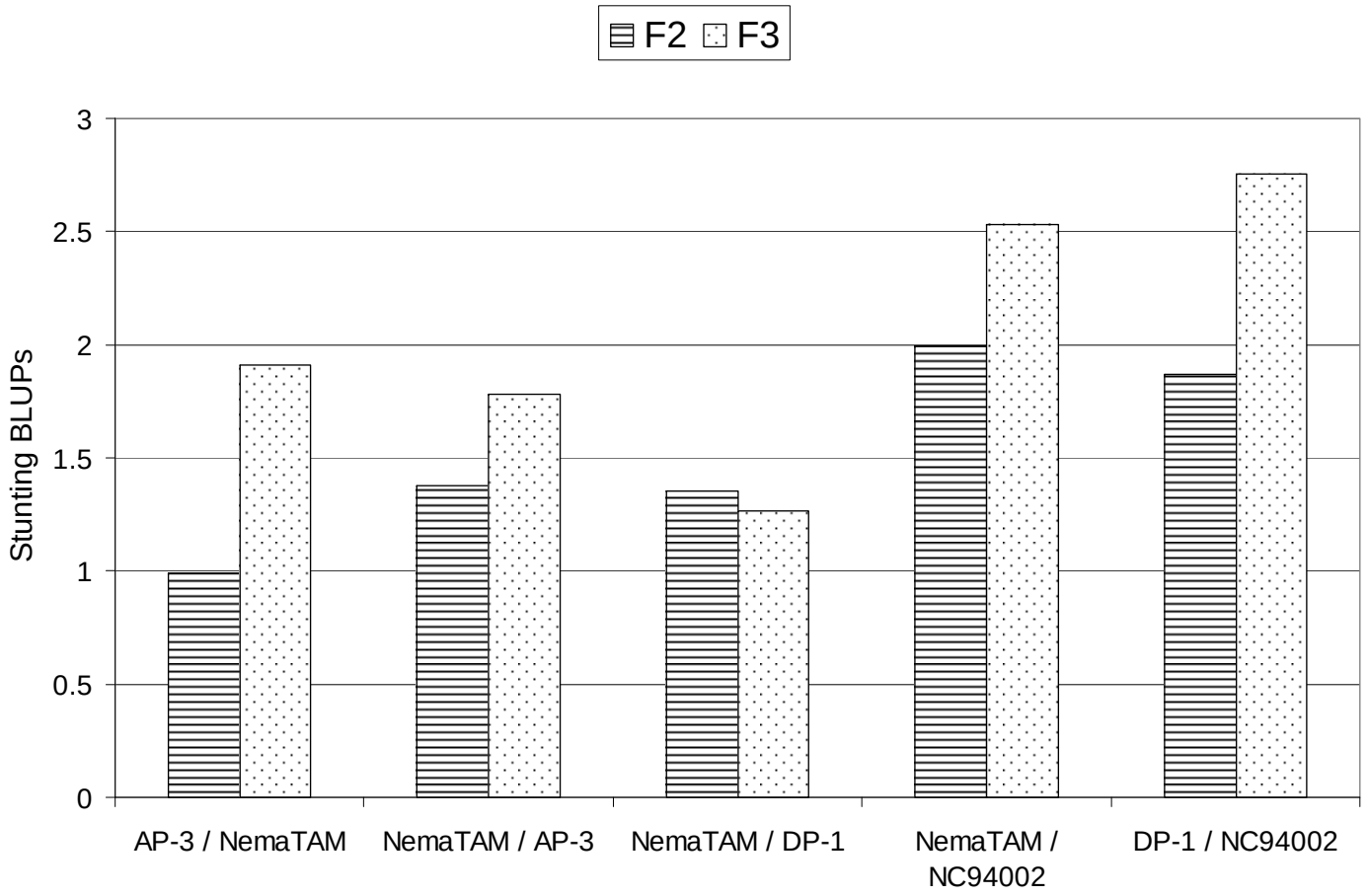

Figure 2-14. Variability of best linear unbiased predictors for TSWV-induced stunting in the F_2 and F_3 generations of five peanut crosses tested at Marianna, Florida in 2007.

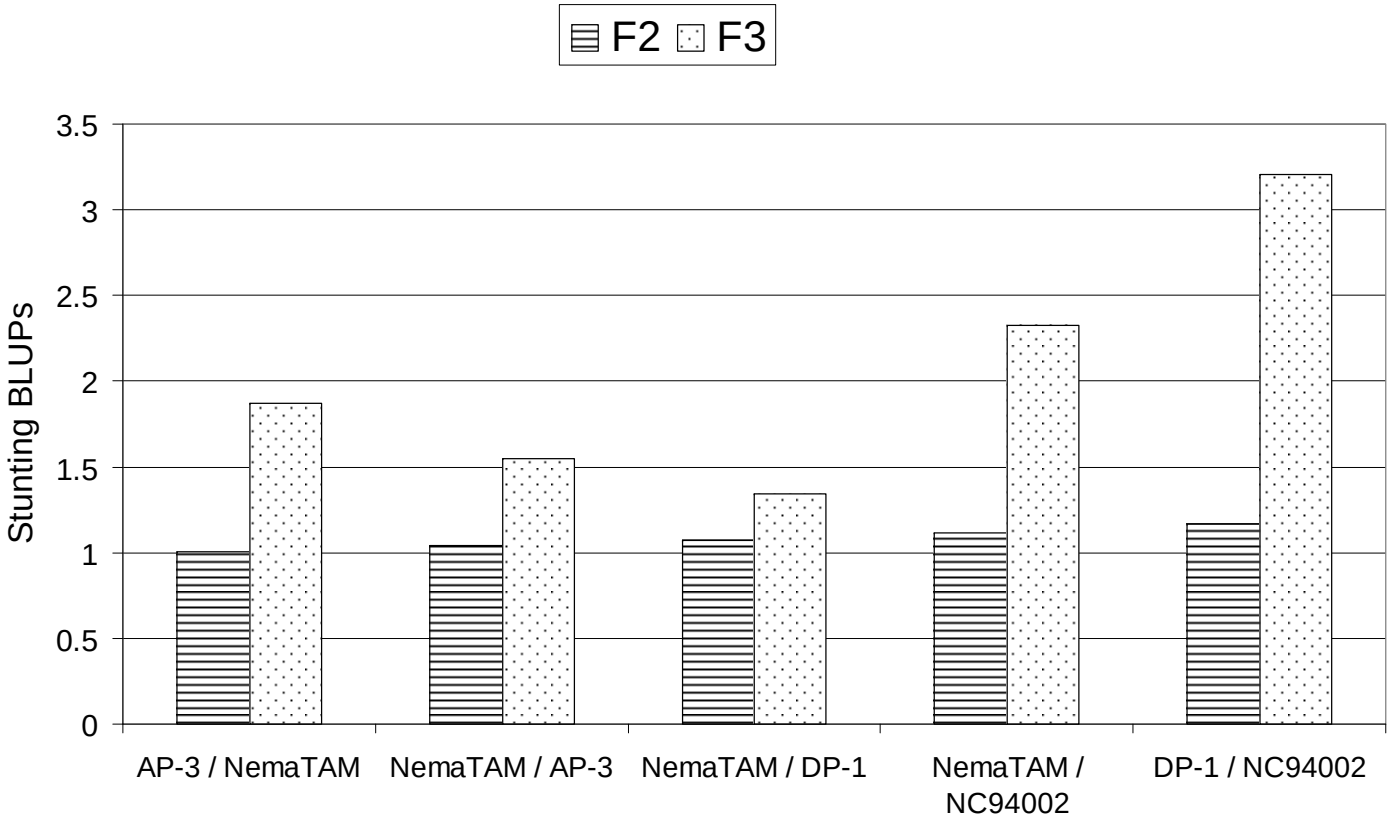

Figure 2-15. Variability of best linear unbiased predictors for TSWV-induced stunting in the F_2 and F_3 generations of five peanut crosses tested at Quincy, Florida in 2007.

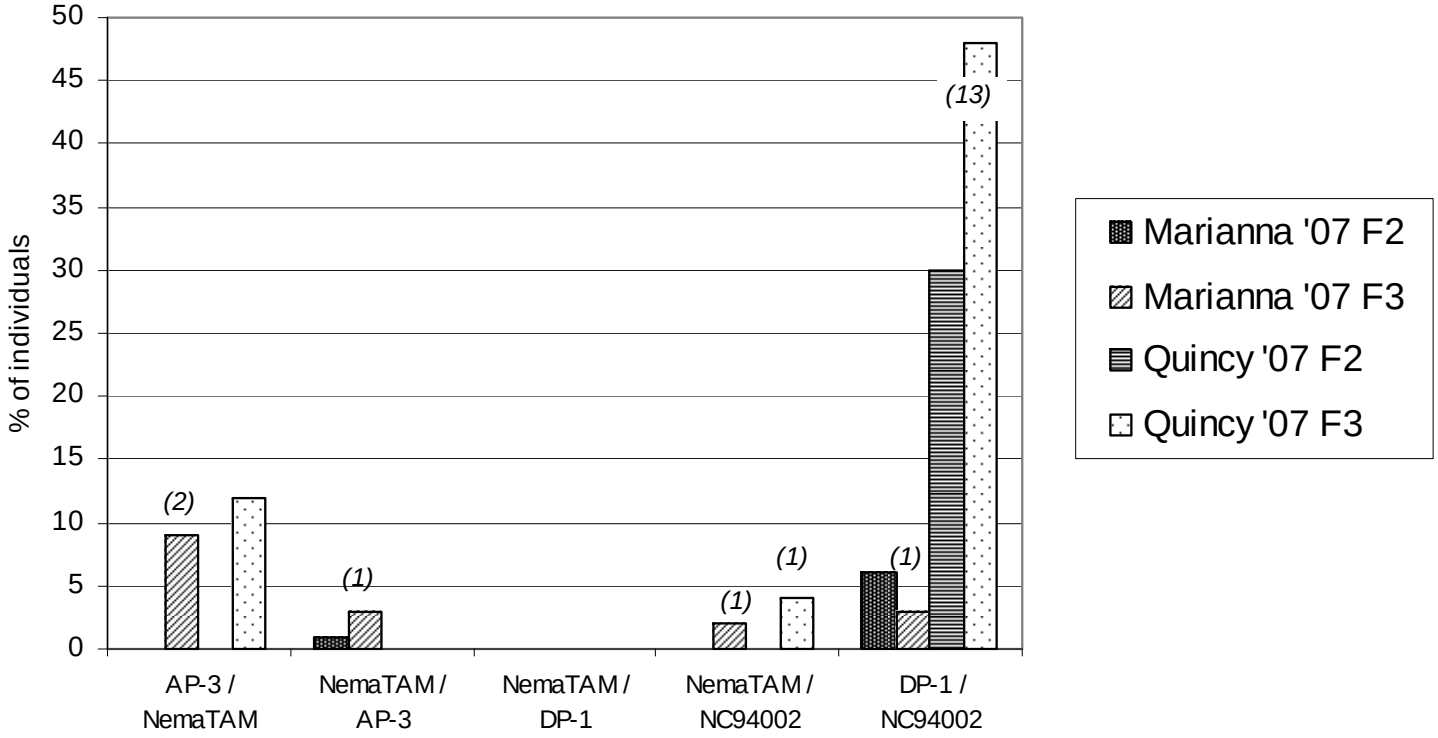

Figure 2-16. Percentage of individuals and *(number of F_3 families)* displaying BLUPs for TSWV-induced stunting above their best parent, in each of five peanut crosses tested at Marianna and Quincy, Florida in 2007.

CHAPTER 3
ARTIFICIAL INOCULATION STUDIES IN THE TSWV-PEANUT PATHOSYSTEM

Introduction

Tomato spotted wilt virus (*Bunyaviridae:Tospovirus*) is a species of quasi-spherical enveloped particles containing three single-stranded RNA (Kormelink et al., 1992). It is a cosmopolitan pathogen of 1090 plant species in 85 families (Parrella et al., 2003). It is vectored in a propagative and circulative manner exclusively by thrips of the genera *Thrips* and *Frankliniella* (Ullman et al., 2002). Wherever TSWV incidence has increased enough to cause economic losses, it has remained a chronic problem in many economically important crops. This is the case in the Southeastern USA, where the peanut (*Arachis hypogaea* L.) crop has suffered intermittent heavy losses since 1993. The initial increasing trend in TSWV incidence has now been reversed (Brown et al., 2007) by a combination of factors including planting date, stand density, row pattern, insecticide use and resistant cultivars (Culbreath et al., 2003). The resistance level of the cultivar is the most important tool in the management of this disease (Brown et al., 2007). So far most resistance assessments have been conducted in the field and relied on natural epidemics. This means that a valid characterization of each genotype has been resource-consuming involving several seasons and locations. As an alternative approach, artificial inoculation methods have been developed to reduce the time needed to assess a genotype targeting its commercial release as a cultivar and also to diminish the overall resource requirements.

Several artificial inoculation methods have been described for the TSWV-peanut pathosystem (Halliwell and Philley, 1974; Clemente et al., 1990; Pereira, 1993; Hoffman et al., 1998). According to published results the most consistent one was developed by Mandal and coworkers (2001). In this method important factors are the antioxidants in the extraction buffer

and the type and amount of abrasives used in the rubbing. Oxidation, which reduces the life of TSWV outside the cell, seems to be a common remark among the bibliographical sources and thus different concentrations of several reducing chemicals have been evaluated (Halliwell and Philley, 1974; Clemente et al., 1990; Pereira, 1993; Mandal et al., 2001). With reference to abrasives, Clemente et al. (1990) found no differences in the rates of TSWV artificial inoculation using various grit sizes of Carborundum whereas Mandal and coworkers (2001) reported that the type of added abrasive in the inoculum was very important. Some other factors have been cited in the literature as influencing the outcome on inoculation experiments. Ng et al. (2004) reported that the concentration of virus that effectively reached the target tissues was very important in the transmission efficiency of *Lettuce infectious yellows closterovirus* to lettuce. Even with standardized conditions, different factors can produce varied actual damage after inoculation, which could lead to an inconsistent number of viral particles entering the leaf (Pereira, 1993). Consequently a better assessment of the actual damage inflicted while rubbing could be extremely useful in ruling out this factor as a cause of variability.

A fact that has hampered the resistance assessment in the TSWV-peanut pathosystem is that external symptoms do not always reflect the concentration of TSWV in the plant (Resende et al., 2000; Mandal et al., 2001; Lyerly et al., 2007). Additionally, asymptomatic TSWV infections in peanut have been reported (Culbreath et al., 1992). Nonetheless, the frequency with which plants within a cultivar express symptoms early after inoculation has been suggested as a good indicator of viral titer in the tissue (Kresta et al., 1995) and also of resistance (Culbreath at al., 2003).

While an artificial inoculation method should accelerate the screening process leading to the detection of resistant genotypes, it should also reduce the environmental variation of plant

reaction through the standardization of several factors. This should allow a better estimation of the genotype's true reaction to the virus. Consequently, the objective of this series of studies was to determine the relative importance of age of inoculum, virus concentration in the inoculum, and amount of rubbing during inoculation on the frequency of infection. A secondary objective was to determine if there was an association between ELISA values and symptom expression. To address these objectives three studies were conducted during the spring of 2005.

Materials and Methods

Plant Culture

Georgia Green, a widely grown runner market-type cultivar was used in all tests. This cultivar displays some field resistance to TSWV (Culbreath et al., 1996) but it is susceptible under artificial inoculation (Mandal et al., 2001).

One seed was planted in each 164 ml plastic container (Cone-tainer C10, Stuewe & Sons, Corvallis, Oregon) containing all purpose professional growing mix consisting of Canadian sphagnum peat moss 75 to 85%, perlite 15 to 20%, and vermiculite 5 to 10% (Berger Peat Moss, Saint-Modeste, Quebec, Canada) and irrigated every other day with distilled water. No fertilizer was added to the mix. Test plants were grown until inoculation was performed in a chamber made of a shelf with fluorescent lights (Gro-Lux, Osram Sylvania, Danvers, Massachusetts) all surrounded by a transparent plastic sheet. Conditions inside this chamber were 12-h light period (12 klx intensity) and 23°C min. and 34°C max. Those seedlings with uniform size and vigor were used for inoculation 12-14 days after planting. The average seedling height was variable among tests ranging from five to seven cm, whereas the average number of fully expanded leaves ranged from two to five.

Inoculum Preparation

Infected leaf tissue from greenhouse grown peanut plants (cv. Georgia Green) were collected and pre-chilled in a refrigerator and ground (1:10 [wt/vol] tissue:buffer unless otherwise stated) with freshly prepared ice-cold 0.01 M potassium phosphate buffer, pH 7.0, containing 0.2% sodium sulfite and 0.01 M 2-mercaptoethanol using a chilled pestle and mortar as described by Mandal et al. (2001). Debris was removed by squeezing the ground extract through a pad of nonabsorbent cotton. To this homogenate, Celite 545 (Fisher Scientific, Fair Lawn, NJ) and Carborundum 320 grit (Fisher Scientific) were each added to a final concentration of 1% each. The inoculum was kept on ice until the inoculation process was completed.

Sap Inoculation

Test plants were dusted with Carborundum on the youngest fully expanded leaf 12-14 days after sowing. Two leaflets (one basal and one apical) were inoculated by rubbing them four times (unless otherwise stated) with a cotton swab (Johnson & Johnson, Skillman, NJ) dipped in the inoculum. After inoculation the plants were sprayed with distilled water and placed in a growth room at 25/19°C, 50% RH, 12-h light period and 15 klx of light intensity and were irrigated every two days using distilled water.

Description of Tests

Test 1: Effect of elapsed time from preparation to inoculation on infection frequency

Three time lapses from the inoculum preparation (zero, ten and twenty minutes) were compared. At each time, 10 plants were inoculated using the same inoculum batch and these 30 plants (10x3) were considered a block within a randomized complete block design. There were three blocks totaling 90 inoculated plants. Additionally as controls, six plants were rubbed with buffer only plus abrasives and six more plants were left untreated.

Test 2: Determining the importance of amount of rubbing on infection rate

Leaves were rubbed four, six or eight times using a cotton swab. Once prepared the inoculum was used immediately. Ten plants were inoculated per treatment using the same inoculum batch and these 30 plants (10x3) were considered a block within the RCBD. There were four blocks totaling 120 plants. As controls, eight plants were rubbed with buffer only plus abrasives and eight more plants were left untreated.

Test 3: Evaluating the influence of inoculum concentration on infection rate

Two inocula, each using a different tissue:buffer ratio, were compared (1:10 and 1:20). After obtaining the usual 1:10 inoculum, half of it was allocated in another mortar and a similar volume of buffer was added. Once prepared both inocula were used immediately. Different swabs were used for each level of the dilution factor. Ten plants were inoculated per treatment and these 20 plants (10x2) were considered a block within a RCBD. There were four blocks totaling 80 plants. As controls, eight plants were rubbed with buffer only plus abrasives and eight more plants were left untreated.

Imposing Treatments

In Test 1 plants were inoculated by rubbing them four times with a cotton swab. The time to inoculate each plant was about ten seconds so for every treatment the real inoculation time between the first and the last plant was almost two minutes. In the Test 2 plants were inoculated according to the layout L1-L2-L3-L3-L2-L1-L1-L2-L3-L3-L2-L1 (L means “Factor Level”)and so on. In Test 3 plants were inoculated according to the layout L1-L2-L2-L1-L1-L2-L2-L1 and so on.

Evaluation of Inoculated Plants and Analysis of Data

The measured variables in each experiment were as follows:

Appearance of systemic symptoms: as viral lesions on inoculated leaves were not observed, the plants were considered as "infected" when chlorotic spots followed by mosaic rings and necrotic spots developed in the newly emerging leaves (systemic symptoms). In their absence the plants were considered "healthy".

Serological detection of TSWV by ELISA: optical density (OD) values greater than the average value plus 3 times the standard deviation (cut-off value) of the two negative control wells, belonging to healthy plants of C11-2-39 peanut line, were considered positive for the presence of TSWV. Due to contamination of the negative controls in Test 1, a cut-off value was set taking into account the usual values obtained for this type of control. As the highest value ever obtained for this negative control has been 0.006 in several previous ELISA (data not shown) it was considered reasonably conservative to use 0.06 as a cut-off value.

TSWV infection was confirmed by alkaline phosphatase labeled DAS-ELISA according to manufacturer's instructions (Agdia Inc., Elkhart, Indiana). Absorbance was measured at 405 nm with an automated microplate reader (Model 680, Bio-RAD, Hercules, CA,USA). Two replications were made on each sample, and averages were used for evaluation.

Recording of symptomatic plants and ELISA were done 3 weeks post-inoculation. Two apical leaflets in the youngest leaf plus one apical leaflet on the youngest fully expanded leaf and young secondary roots were used for ELISA. If new leaves were observed as symptomatic they were used for ELISA instead of using random leaves. Since the tested tissue was not weighted and the obtained macerate volume was variable, the ELISA values were used only to categorize the plants as infected or not.

In all the three tests, treatments were established by the combination of inoculum batch and the level of the factor being tested (elapsed time, # of rubbings or inoculum dilution). Each treatment was represented by 10 inoculated plants.

Both binary variables (appearance of systemic symptoms and TSWV ELISA detection) were analyzed by Multiple Logistic Regression with a binomial distribution and logit link function using SAS (SAS Institute, 2000). The applied model in Test 1 was

$$\text{logit}(\pi)=\alpha+\beta_1^3\ Time+\beta_1^3\ Batch \qquad \text{(Eq. 3-1)}$$

where π is the probability of the plant being symptomatic or being ELISA positive depending on the response variable being analyzed. The parameter β_i refers to the effect of the "i" level of a factor (say Time) on the log odds that the dependent variable equals one of the two possible outcomes, say "infected", controlling the levels of Batch (Agresti, 1996). "Time" denotes the amount of elapsed time from inoculum preparation (0', 10' and 20') while "Batch" denotes the inoculum batch used.

The adjusted models for Test 2 were:

$$logit(\pi)=\alpha+\beta_1^3\ Rubbings+\beta_1^4\ Batch; \qquad \text{(Eq. 3-2)}$$

and

$$logit(\pi)=\alpha+\beta_1^4\ Batch; \qquad \text{(Eq. 3-3)}$$

where "rubbings" denotes the number of rubbings applied during inoculation, being the rest of the terms as described in Test 1.

The applied model for Test 3 was

$$logit(\pi)=\alpha+\beta_1^2\ \text{Dilution}+\beta_1^4\ Batch \qquad \text{(Eq. 3-4)}$$

where "Dilution" denotes the inoculum dilutions tested, being the rest of the terms as described in Test 1.

The logits of the unknown binomial probabilities (i.e., the logarithms of the odds) are modeled as a linear function of the X_i. The unknown parameters β_j are usually estimated by maximum likelihood.

The full model containing both elapsed time and inoculum batch as factors was used and in the event of a factor being found non-significant, it was removed from the model (Agresti, 1996). Fisher's Exact Test was used to detect association between "Appearance of systemic symptoms" and "ELISA status".

Results

Test 1

The overall percentage of inoculated plants showing visual symptoms of systemic infection was 18% (most of them with mild severity, Table 3-1) while no localized symptoms were observed in the inoculated leaves. According to the ELISA test, 43% of the plants were systemically infected. Four out of 16 plants showing systemic symptoms failed to be detected by ELISA. Nonetheless, the association between systemic symptoms and ELISA status was statistically significant (p=0.0289) according to Fisher's Exact Test.

Neither the elapsed time from inoculum preparation nor inoculum batch were significant (p=0.09 and 0.13 respectively) in the logistic regression for systemic symptoms. In the case of the serological status, both factors were found significant (p=0.02 and 0.05).

The obtained Maximum Likelihood Estimates can be seen in Table 3-2

The Prediction Equations obtained for the logit of the probability of a Positive ELISA result under each treatment were then:

For Time 0' and Batch 3, logit($\hat{\pi}$)=-0.3336+0 Time+0 Batch

For Time 0' and Batch 1, logit($\hat{\pi}$)=-0.3336+0 Time+(-0.7978 Batch)

For Time 0’ and Batch 2, logit($\hat{\pi}$)=-0.3336+0 Time+(0.6253 Batch)

For Time 10’ and Batch 3, logit($\hat{\pi}$)=-0.3336+(0.7680 Time)+0 Batch

For Time 10’ and Batch 1, logit($\hat{\pi}$)=-0.3336+(0.7680 Time)+(-0.7978 Batch)

For Time 10’ and Batch 2, logit($\hat{\pi}$)=-0.3336+(0.7680 Time)+(0.6253 Batch)

For Time 20’ and Batch 3, logit($\hat{\pi}$)=-0.3336+(-0.9494 Time)+0 Batch

For Time 20’ and Batch 1, logit($\hat{\pi}$)=-0.3336+(-0.9494 Time)+(-0.7978 Batch)

For Time 20’ and Batch 2, logit($\hat{\pi}$)=-0.3336+(-0.9494 Time)+(0.6253 Batch)

These prediction equations can be expressed as the predicted probability of a plant being infected. For example, using the third inoculum batch twenty minutes after it was prepared, the predicted probability of a plant to be ELISA positive would be:

{exp[-0.3336+(-0.9494 Time)+(0.6253 Batch)}/{1+ exp[-0.3336+(-0.9494 Time)+(0.6253 Batch)}= 0.34

Similarly, the predicted probabilities for the other treatments were:

For Time 0’ and Batch 3,

{exp[-0.3336+(0 Time)+(0 Batch)}/{1+ exp[-0.3336+(0 Time)+(0 Batch)}= 0.42

For Time 0’ and Batch 1,

{exp[-0.3336+(0 Time)+(-0.7978 Batch)}/{1+ exp[-0.3336+(0 Time)+(-0.7978 Batch)}= 0.24

For Time 0’ and Batch 2,

{exp[-0.3336+(0 Time)+(0.6253 Batch)}/{1+ exp[-0.3336+(0 Time)+(0.6253 Batch)}= 0.57

For Time 10’ and Batch 3,

{exp[-0.3336+(0.7680 Time)+(0 Batch)}/{1+ exp[-0.3336+(0.7680 Time)+(0 Batch)}= 0.61

For Time 10' and Batch 1,

{exp[-0.3336+(0.7680 Time)+(-0.7978 Batch)}/{1+ exp[-0.3336+(0.7680 Time)+(-0.7978 Batch)}= 0.41

For Time 10' and Batch 2,

{exp[-0.3336+(0.7680 Time)+(0.6253 Batch)}/{1+ exp[-0.3336+(0.7680 Time)+(0.6253 Batch)}= 0.74

For Time 20' and Batch 3,

{exp[-0.3336+(-0.9494 Time)+(0 Batch)}/{1+ exp[-0.3336+(-0.9494 Time)+(0 Batch)}= 0.22

For Time 20' and Batch 1,

{exp[-0.3336+(-0.9494 Time)+(-0.7978 Batch)}/{1+ exp[-0.3336+(-0.9494 Time)+(-0.7978 Batch)}= 0.11

For Time 20' and Batch 2,

{exp[-0.3336+(-0.9494 Time)+(0.6253 Batch)}/{1+ exp[-0.3336+(-0.9494 Time)+(0.6253 Batch)}= 0.34

As can be seen from the parameter estimates for each factor level, the probability of obtaining a positive ELISA increased by using the second inoculum batch or by using the inoculum 10 minutes after its preparation while that probability decreased by using the first inoculum batch or by using the inoculum 20 minutes after its preparation.

Test 2

The OD cut-off value for this test was set at 0.004. Thirty nine percent of the plants were declared positive by ELISA while only 13% displayed visual symptoms. Despite this difference

between plants declared infected by visual or serological means, Systemic symptoms and ELISA status were highly significantly associated ($p<0.0001$).

As in the previous test, neither number of rubbings nor inoculum batch were found significant in determining the appearance of systemic symptoms after the inoculations ($p=0.93$ and $p=0.65$ respectively).

In the case of response variable "ELISA Status", the factor "number of rubbings" was non-significant. Consequently, a model containing only "Batch" was adjusted. Under this model, "Batch" was found significant ($p=0.0357$) as a factor determining the "ELISA Status" after the artificial inoculations. The difference of infectivity between the most and the least infective batches was very noticeable (53% vs 20% of infected plants after inoculation). The respective predicted probabilities of a plant being infected after being inoculated with these extreme batches were:

$\{\exp[-0.4865+(0.4865\ \text{Batch})\}/\{1+\exp[-0.4865+(0.4865\ \text{Batch})\}= 0.5$, for the most infective Batch.

$\{\exp[-0.4865+(-0.8998\ \text{Batch})\}/\{1+\exp[-0.4865+(-0.8998\ \text{Batch})\}= 0.2$, for the least infective Batch.

Test 3

The plants used in this study although of similar age to those of the previous tests in this project were slightly more developed, being 7 cm high and having an average of two more fully expanded leaves than those used previously (2-3 vs. 5). The cause of this was a slightly higher average temperature in the growth chamber, probably caused by the air conditioning being shut-off in the building during most of this period. The OD cut-off value for this test was set at 0.005. Seventy nine percent of the plants were declared positive by ELISA, while no plant displayed visual symptoms.

Neither “Inoculum Dilution” (p=0.4136) nor “Inoculum Batch” (p=0.9734) were found significant in determining the “ELISA Status” after the inoculation. The infectivity among batches was much less variable than in the previous two tests (Table 3-3).

Discussion

Test 1

Very low infection levels were attained compared with data from literature using this method (Mandal et al., 2001). Although low infection rates are not uncommon, no single factor has been detected as the cause (N. Martinez-Ochoa, pers. comm.).

The marked impact of plant age at inoculation on infection success has been demonstrated by several authors. Mandal and co-workers (2001) and Hoffman et al. (1998) obtained high percentages of symptomatic plants (75% and 90% respectively) for plants at 14 DAP. In spite of using the technique of Mandal et al. (2001), the percentages obtained in this experiment were smaller and similar to those reported by Pereira (1993). According to Noordam (1973), Branch et al. (2003) and S. Mullis (pers. comm.) well-irrigated non-stressed plants are more prone to become infected or to develop symptoms. As the small volume of substrate in which the plants were raised in the present work tended to dry very easily, the plants were subjected to short but frequent periods of water stress which could have contributed to the lower-than-expected number of infected plants. The irrigation problem was solved in the subsequent tests by increasing the applied water volume.

Similar to Culbreath et al. (1992), almost half of the ELISA positive plants in this study were asymptomatic. Hoffmann et al. (1998) reported that symptomatic TSWV infected leaves were readily detected by ELISA although asymptomatic leaves of infected plants did not always give positive ELISA readings. Similarly, four plants visually scored in our experiment as infected were not detected as such by ELISA. Kresta et al. (1995) in peanut and Canady et al.

(2001) in tomato also observed a similar phenomenon. A possible explanation could be that several plant parts were used for ELISA and that this pooling of tissues displaying mild symptoms and no symptoms could have lowered the absorbance value for the pooled sample.

The effect of time between inoculum preparation and inoculation on the frequency of symptomatic plants was important although the observed trend seemed illogical. Tomato spotted wilt virus is usually described as a short living virus outside the cell even in reducing solutions (Halliwell & Philley, 1974; Hoffman et al., 1998). In the present experiment, a declining percentage of infected plants was expected as the time from inoculum preparation increased. Nonetheless, the inoculum seemed to be less infective immediately after preparation than 10 min. later. The most plausible explanation for this result could be a sampling induced statistical bias artifact due to the small number of blocks (batches) used. The inherent viral load variability among batches has been addressed by some authors, who suggested the use of samples from similar tissues and showing similar symptoms (Hoffman et al., 1998; Mandal et al., 2001). Nonetheless, "Inoculum Batch" reached statistical significance in our study, indicating that even when similar tissue (age and position) was extracted from the donor plants and pooled to prepare inoculum, viral concentration was still highly variable.

Test 2

As in Test 1, the percentage of infected plants was far lower than that reported by Mandal and coworkers (2001), even though the inoculation protocol was very similar. Two thirds of the ELISA plants were asymptomatic. In contrast to Test 1, every plant displaying symptoms was confirmed by ELISA in Test 2.

With reference to the number of rubbings during inoculation, the factor was statistically non-significant. The range used here (4-8 rubbings) is typical of what has been used while inoculating (N. Ochoa, pers. comm.). Its non-significance could explain why this detail is not

found in the literature even though the actual damage inflicted to the leaf directly influences the number of virions that reach the target parenchymatous tissue (Ng et al., 2004). Damage also impacts the degree and speed of development of necrosis caused by abrasion (Hoffman et al., 1998). No necrosis developed after inoculation in the abrasion area so apparently the number of rubbings applied was rather mild. Peanut is known for its strong load of waxes in its leaves (Samdur et al., 2003) so it's possible that the abrasion didn't always provide enough injury to serve as entry points for the virions.

As in Test 1, the factor Inoculum Batches was statistically significant. It is evident that even pooling tissue with similar symptoms was not enough to avoid important variability in the infectious capacity of the inoculum batches.

Test 3

The percentage of infected plants in this test was greater than in the previous two tests and approached the levels reported by other authors (Hoffman et al., 1998; Mandal et al., 2001). Surprisingly, no symptomatic plant was observed. Lyerly et al. (2002) reported that there were instances where peanut plants infected with TSWV exhibited no visible symptoms and some plants even recovered from initial infection and appeared normal. Krista et al. (1995) found that peanut leaves with very low ELISA titer exhibited extremely mild symptoms. However, the ELISA titers obtained in this test are in the usual range obtained while using similar conditions (S. Mullis, pers. comm.) and overall are similar to those obtained in Tests 1 and 2 described in this chapter (data not shown). Mandal et al. (2001) observed a delay in symptom expression associated with plant age. Since age is associated with plant size, it could be possible that the older plants used in the present test were displaying this kind of delay, when compared to the smaller ones used in test 1 and 2.

Both tested factors (inoculum dilution and inoculum batch) were statistically non-significant, probably due to a similar number of viable virions reaching the target tissues between both treatments. The smallest dilution used here (10:1) is certainly weaker than that reported by some researchers (Hoffman et al., 1998; Pereira, 1993; Mandal et al., 2001). This suggests that even a 20:1 buffer:tissue ratio can provide similar infection rates than higher ratios allowing a more efficient use of tissue donor plants. This factor can be especially important while preparing batches for a large number of plants from limited amounts of infected tissue from donor plants.

Conclusions

Following the protocol suggested by Mandal et al. (2001), this study determined that both number of rubbings and inoculum dilution had no effect on the outcome of artificial inoculations. The elapsed time from inoculum preparation showed an unexpected trend as the infectivity did not fall with time as suggested by some authors (Halliwell and Philley, 1974; Clemente et al., 1990; Mandal et al., 2001). A new test using more replications per treatment could provide further insight on this issue.

Inoculum batch was an important factor, probably highlighting the fact that viral titer is highly variable even when using infected tissue with similar characteristics (age, plant position). This stresses the importance of standardizing the inoculation process.

In two of the three tests the difference between the percentages of symptomatic and ELISA-detected plants was similar to that reported by Culbreath et al. (1992) but this was not the case in Test 3. Other factors could exist that influence the visual symptom development that was not controlled in this test.

The overall low infection rates obtained in comparison with other reports using similar techniques clearly suggest that additional work is necessary to detect which factors caused the

observed outcomes in the present work while using the method described by Mandal et al. (2001).

Table 3-1. Effect of elapsed time after preparation of Tomato spotted wilt virus inoculum on the number of peanut plants declared infected by visual examination or serological means.

Treatment	Symptomatic plants [a]	ELISA-positive plants
0’ – batch 1	1	2
10’ – batch 1	6	5
20’ – batch 1	1	1
0’ – batch 2	4	3
10’ – batch 2	2	9
20’ – batch 2	0	5
0’ – batch 3	2	9
10’ – batch 3	0	4
20’ – batch 3	0	1

[a] Number of plants for each treatment=10

Table 3-2. Maximum Likelihood Estimates for time and inoculum batch effects on artificial inoculation of Georgia Green peanut.

Parameter	*Estimate*	*S.E.*
Intercept	-0.3336	0.2354
Time (10')	0.768	0.3274
Time (20')	-0.9494	0.3504
Batch (1)	-0.7978	0.3442
Batch (2)	0.6253	0.3274

Table 3-3. Effect of inoculum dilution on the number of peanut plants declared infected by ELISA.

Treatment	*ELISA-positive plants* [a]
10:1 - batch 1	8
20:1 - batch 1	8
10:1 - batch 2	7
20:1 - batch 2	8
10:1 - batch 3	10
20:1 - batch 3	6
10:1 - batch 4	8
20:1 - batch 4	8

[a] Number of plants for each treatment=10

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BIOGRAPHICAL SKETCH

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