THE USE OF DISEASE SEVERITY VARIABLES IN PREDICTING EFFICACY OF FOV4 RESISTANCE SELECTION

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Abstract

In 2015, 85 Upland (*Gossypium hirsutum* L.) accessions from the USDA-ARS Cotton Collection and 126 F_6 Pima-S6 x Pima-S7 (*Gossypium barbadense* L.) recombinant inbred lines were evaluated for disease performance under pressure of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (FOV4) in a replicated field trial naturally infested with FOV4 in the central San Joaquin Valley of California. Statistical analyses were performed to test the efficacy and biological importance of several variables with respect to each one's ability to aid the selection of FOV4 resistant lines. Disease severity was measured by foliar severity (FS), vascular root staining (VRS), and plant survival (PS). Plant vegetative growth was measured as emergence, plant height, and number of main-stem nodes at specific times after emergence. We analyzed the relationships of these variables to evaluate the predictability of one variable response from the other variable observation. Selected results from this research demonstrated that FS was a reliable predictor of VRS in Pima cotton (r = 0.80), but less so within the *G. hirsutum* accessions from the collection (r = 0.54). In Pima, VRS was highly negatively correlated with PS eight weeks after emergence (r = -0.73), but in Upland the correlation was much lower (r=-0.28). Number of nodes and plant height were highly correlated in each type of cotton (r = 0.84 and 0.73 for Pima and Upland types, respectively). Finally, analyses showed a statistically significant tendency toward increased variability in the analyzed factors (FS, VRS, and PS) as symptom severity decreased. Using the described disease severity indices, this analysis suggests that the resistance/tolerance screening method utilized is highly reliable for use in identifying and eliminating poor-performing lines.

Introduction

FOV4 wilt in cotton has been recognized as disease with potential for economic injury in California since 2003. FOV4 has been found to be present in fields even in the absence of root knot nematode (*Meloidogyne incognita*) (RKN), a distinguishing characteristic from other races of FOV identified previously in California. Yield depression observed in FOV4-affected fields has largely been caused by stand loss and stunting, with fields exhibiting stand losses ranging from as little as 10% to nearly 100%.

Today, fields infested with FOV4 have been identified in all cotton growing counties in the San Joaquin Valley of California, but not in southern desert or Sacramento Valley production areas. No chemical control measures for FOV4 tested to date have given adequate control to effectively reduce economic damage in highly-infested fields, and cultural practices such as modified tillage or equipment cleaning have only served to reduce the spread of the potentially long-surviving FOV4 spores, chlamydospores. So far, the spread of FOV4 outside of California to other cotton producing regions within the US has not been confirmed. Thus, continued efforts to breed for FOV4 resistance in Pima and Upland cultivars remain critical as an approach to being prepared for potential spread of a long-lived soil borne pathogen.

In this study, data from one site-year at a field naturally-infested with FOV4 used for screening cotton cultivars for their FOV4 resistance was evaluated for relationships between commonly used disease severity and associated plant development and vigor under FOV4 pressure. The purpose of this evaluation was to elucidate the efficacy of what has become a traditional FOV4 performance rating system we have used in California and to identify current strengths and possibilities for improving future disease screening approaches used in breeding efforts.

Materials and Methods

This evaluation was conducted using data collected in 2015 from a San Joaquin Valley field naturally infested with FOV4. The field utilized was a loam soil, furrow irrigated using good quality (EC<0.6 dS/m) groundwater. Standard local grower practices were used for ground preparation, cultural practices in bed formation, irrigation and cultivation practices. Since most tested cultivars were conventional, without transgenic herbicide-resistance or insect-resistance, no over-the-top herbicide applications were used, and grower standard weed and insect control measures for conventional cultivars were employed.

Plant Populations and Sampling

Data from two plant populations planted contiguously were utilized. One population consisted of 126 generation F_6 Pima-S6 x Pima-S7 recombinant inbred lines (RILs), and the other consisted of 85 Upland accessions from the USDA-ARS Cotton Collection. Twenty foot plots on 30 inch beds were seeded at 60 seeds/plot (just over 52,000 seeds/acre) and were replicated three times. Commercial controls of known FOV4 susceptibility – Phytogen 802 RF and Deltapine 340 (Pimas), and Phytogen 725 RF and FM 2334 GLT (Uplands) – were planted in replication to check for disease pressure presence. Samples of five plants from each plot were removed from the soil keeping the taproot intact for full plant evaluation. Ratings and measurements utilized in correlation and regression analyses for this study were plot averages.

Disease Rating, Plant Vigor, and Growth and Development Measurements

Disease severity was measured by two variables – vascular root staining (VRS) and foliar disease symptom severity (FS). VRS was measured after cutting a longitudinal section from the cotyledonary node to the tip of the portion of the tap root extracted from the ground, exposing the core of the vascular cylinder. Scores from 0-5 were assigned to signify a range of no symptoms to complete death, respectively (Table 1). Plant survival (PS) was measured at four (PS_4) and eight (PS_8) weeks after planting as living plants divided by plants emerged. Longevity was measured as live plants at eight weeks divided by live plants at four weeks. Emergence was measured as a percentage of all plants, alive and dead, at four weeks after planting divided by the 60 seeds that were sown. Height was measured from the cotyledonary node to the growing tip of the plant. Total main-stem nodes were counted on the primary stem from the cotyledonary node (zero nodes) with the last node demarked by the latest emerged leaf no smaller than a quarter.

Table 1. Fusarium race 4 disease severity rating scale utilized in San Joaquin Valley field screening trials.

Score	Foliar symptom	Vascular symptom
0	No symptom	No symptom
1	Chlorosis, wilting	Light, discontinuous streaks
2	Necrotic lesion on 1 leaf	Continuous, light to dark streaks
3	Necrotic lesions on 2+ leaves	Vascular streaking + cortical stain
4	Dieback, defoliation	Root blackening and water soaking
5	Whole aerial death	Root death

Statistical Analyses

All statistical analyses were performed using the Analysis ToolPak add-in for Microsoft Excel 2010. Correlation and regression analyses were performed on plot averages on each population. Each population was analyzed separately. Regression analyses were performed on variable pairs which included at least one disease variable (VRS or FS) and had correlation coefficients of 0.71 or greater (only combinations with an R^2 of 0.5 or greater). To examine the relative precision of score assignment as a function of disease severity, average VRS and FS scores for each entry within a population were plotted against their respective coefficients of variation (CV) in a regression analysis.

Results and Discussion

Pima VRS by FS were positively correlated ($r = 0.80$) and VRS by PS_8 were negatively correlated (r = -0.73). Disease variables for the Upland population tested in this analysis were relatively less correlated with any other variable when compared with Pimas, however Upland VRS by FS were positively correlated (r = 0.54). Upland VRS by PS_8 correlation was notably low (r = -0.28). The relatively high positive correlation of VRS with FS in Pima represents what we have found to be a typical expression of FOV4 disease in Pima cultivars, making estimation of FOV4 severity relatively predictable without even examining roots, although VRS should not be foregone in field diagnostic protocols. It is a helpful relationship in recurrent selection of individuals within a screened population as plants exhibiting low FS scores can be expected to correspondingly exhibit low VRS scores. This relationship was less pronounced within the Upland population, but it was still positive. The apparent lack of a relationship between PS_8 and VRS in the Upland population illustrates the importance of utilizing variables beyond disease severity proper when evaluating FOV4 resistance as it is known that if soil inoculum levels reach relatively high levels, the disease has a profound effect on stand density even with many tested Upland cultivars.

The linear regression of Pima VRS by FS was significant ($p < 0.001$, $R^2 = 0.64$) (Figure 1). Linear regression of Pima VRS by PS_8 was also significant ($p < 0.001$, $R^2 = 0.53$) (Figure 2). The linear regression of Upland VRS by FS was also significant, although relatively less correlated than in the Pimas ($p < 0.001$, $R^2 = 0.29$). Similarly, linear regression of Upland VRS by PS_8 was significant, but even less correlated ($p < 0.001$, $R^2 = 0.08$).

The regression of the coefficients of variation for Pima and Upland VRS and FS with their respective disease severity scores were all significant ($p < 0.001$) and exhibited inverse relationships (Figure 3). The inverse relationships between disease severity scores and their respective coefficients of variation indicated that the scoring system used was particularly precise at identifying entries that were relatively susceptible. In other words, entries exhibiting severe FOV4 symptoms could reliably be rejected as unsuitable for recurrent selection. Alternatively, entries with low disease severity had less precise rating relative to the value of their respective scores. Considering the high level of precision on rating poor performing entries, this lack of precision for good performing entries indicates the possibility of escapes from disease pressure and reinforces the need for recurrent selection under more tightly controlled environments. As a part of our screening and selection procedures, these field-evaluated entries will also be evaluated under greenhouse conditions using a root-dip artificial inoculation process.

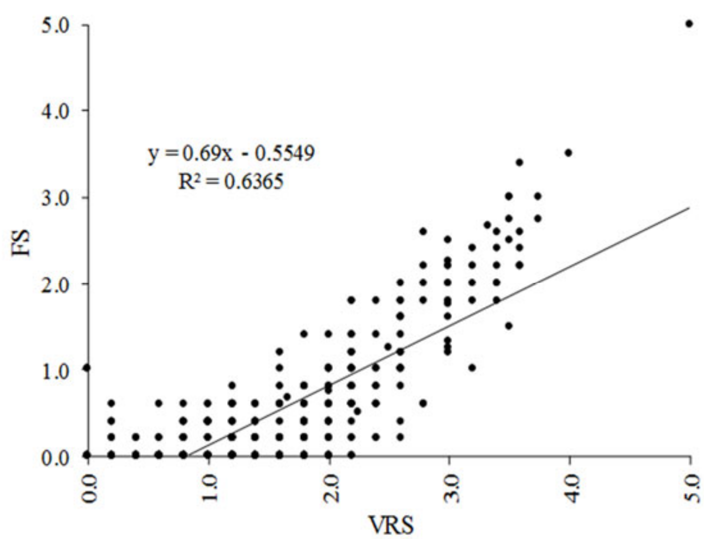

Figure 1. Linear regression of Pima VRS by FS.

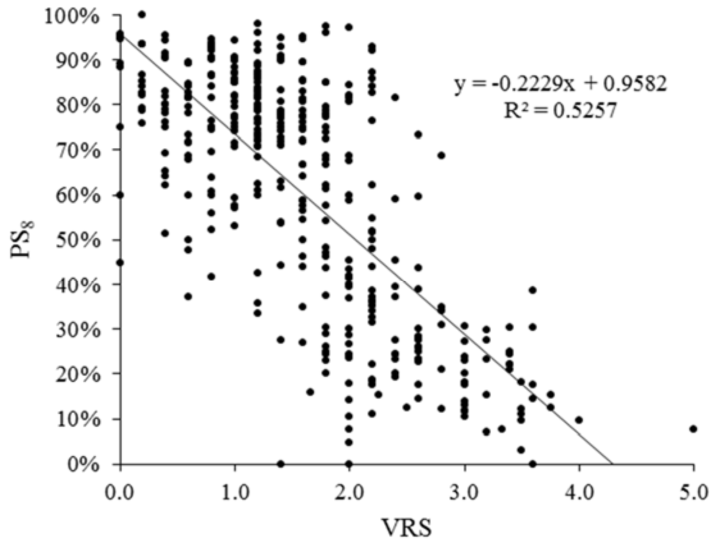

Figure 2. Pima VRS by PS_8.

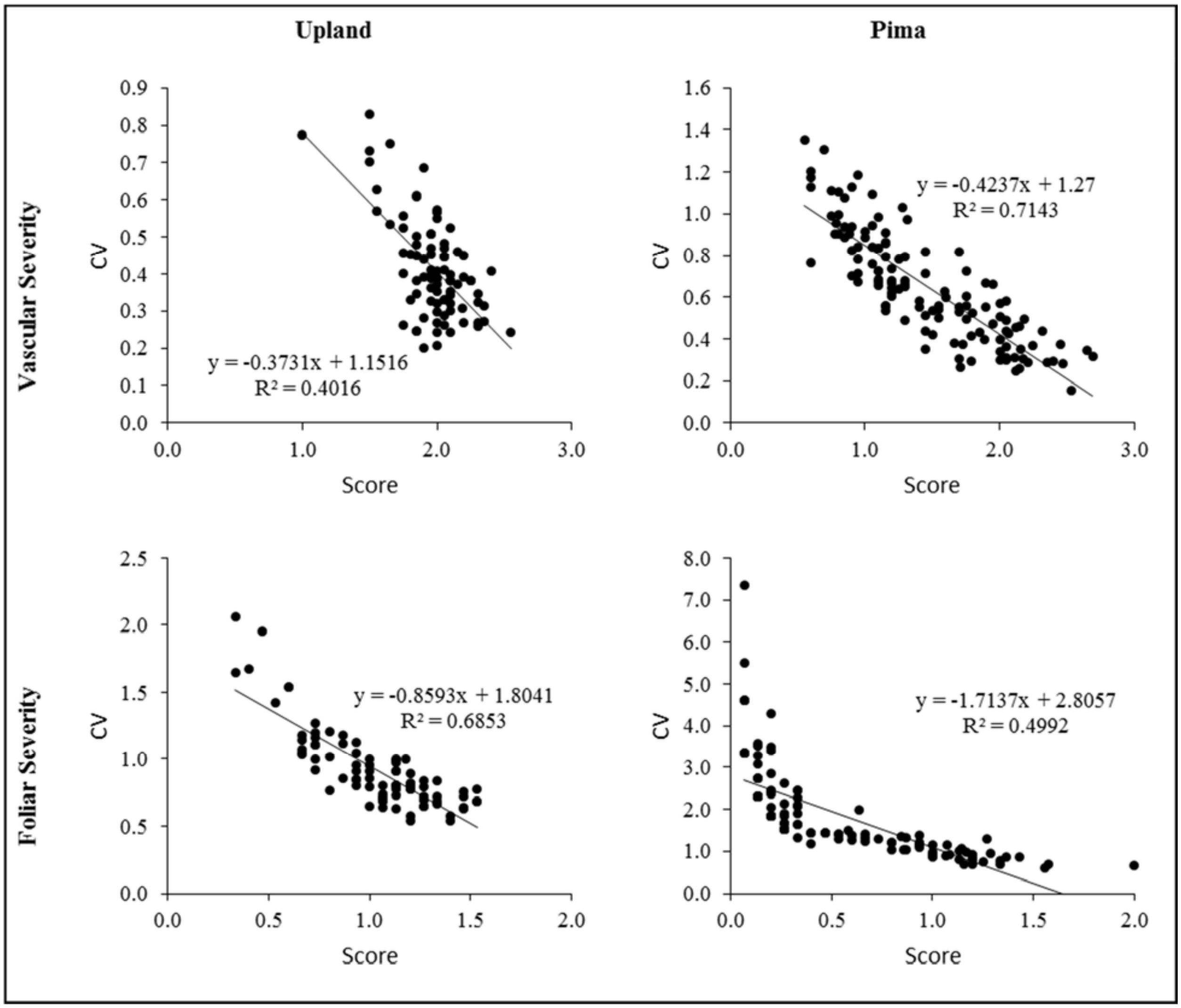

Figure 3. Linear regression of disease severity scores with their respective coefficients of variation for Pima and Upland populations.

Summary

Several FOV4 disease severity variables were evaluated in Upland and Pima populations for their correlative relationships and linear regression with one another and with other plant growth and development measurements. Additionally, disease severity variables were analyzed for their linear regression with their respective coefficients of variation to examine the precision of the disease rating system employed. No disease severity variable within the upland population was as highly correlated with any other variable as within the Pimas. Within the Pima population, VRS was positively and negatively correlated with FS and PS_8, respectively. The linear regressions of those latter correlated pairs were significant. The linear regressions of FS and VRS within the Pima and Upland populations were all significant and expressed inverse relationships. It was concluded that the data indicated the disease rating system employed is highly effective at rejecting poor performing entries while reinforcing the need for recurrent selection and multiple FOV4 resistance screening tactics.

Acknowledgements

We would like to thank the California Cotton Alliance, the California Cotton Growers Association Research Committee, USDA-ARS, Cotton Incorporated, the University of California Cooperative Extension (UC-ANR), and the Univ. CA Davis Plant Sciences Department for financial support of the field and greenhouse projects. We also acknowledge the support and efforts of the San Joaquin Valley Quality Cotton group, collaborators Bob Stipanovic and Phil Roberts, cooperating growers Don McMillan, Stanley Bonds and Greg Palla, technicians Raul Delgado, Wen Flores, Ramon Flores, Walter Martinez, Isaac Giron, Eddie Padilla, Sarah Parry, Laura Martinez, Jorge Garcia, and Charlie Garcia.