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COMMERCIAL AND IMPROVED GERMPLASM EVALUATIONS FOR FUSARIUM WILT, FOV RACE 1 WITH ROOT-KNOT NEMATODES AND RACE 4

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Abstract

Host plant resistance is the most economic and effective strategy for Fusarium wilt control. To implement steps to develop resistant germplasm to this pathogen, existing commercial Acala, non-Acala Upland (*Gossypium hirsutum* L.) and Pima (*G. barbadense*) cultivars, as well as improved germplasm were subjected to FOV race 1 (R1) with root-knot nematodes and race 4 (R4) field disease pressures, and non-infested field. All cotton entries tested were infected by R1 and R4, and variability in symptoms for FOV R1 and R4 occurred in both *G. hirsutum* and *G. barbadense* cottons. Significant differences were observed for plant survival within each race and between races of FOVs. Disease severity was significantly greater for FOV R4 than for FOV R1 as expressed by foliar symptoms and vascular root staining ratings. Plants infected by FOV tended to be smaller with a larger number of nodes than those grown in non-infested soils. In host plant resistance in Pima cottons, known resistant (Phy-800) and susceptible (DP-744) commercial cultivars under R4 pressure were observed to respond significantly different under R1 pressure. DP7-44 was more resistant than Phy-800 under R1 pressure based on plant survival and vascular staining. The host plant resistance observations may suggest that two independent genes and/or an additional gene may control the R1 and/or R4 resistance. Additional research is needed to address host plant interactions between races. Certain forms of this pathogen can survive for a long time in soils even if the host plant is not present. This research is providing information about the susceptibility of commercial and improved germplasm cottons, allowing growers to make informed decisions regarding the choice of cotton cultivar to plant in fields infested with FOV R1 and/or R4. In addition, this research identified potentially improved resistant germplasm and raises the need to breed cotton in California with resistance to existing FOV races.

Introduction

Fusarium wilt (*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans) in cotton was first described by Atkinson (1892) in the USA. FOV is a soil-inhabiting organism and can survive for long periods in soils, even in the absence of cotton, making it nearly impossible to eradicate from a field. Host plant resistance is the most economic and effective strategy for Fusarium wilt control. Eight genotypes of FOV, called races, have been described throughout the world. Until recently, only race 1 and race 2 were known to occur in the United States (DeVay, 1986; Smith et al., 1981). In 2005, four of the races were identified in California (Kim et al., 2005). These races were originally classified based on pathogenicity tests on different cotton species, *G. hirsutum*, *G. barbadense*,

and *G. arboretum* L. (Armstrong and Armstrong, 1958, 1960, 1978; Ibrahim, 1966), and by their pathogenicity on alfalfa, soybean, and tobacco (Armstrong and Armstrong, 1978). Today, these races are categorized according to a number of genetic markers. Based on sequence differences in EF-1α, PHO, and BT genes and intergenic spacer (IGS) restriction enzyme digests, worldwide strains of FOV can be classified into five major lineages (Kim et al., 2005). In Pima cottons a single dominant major gene and one or more minor genes that may provide trangressive segregation have been reported for FOV resistance (Fahmy, 1927; Mohamed, 1963; Ulloa et al., 2006, in press) as well as two major dominant genes with inter locus additivity that provide a high degree of resistance to Sea Island cotton (Smith and Dick, 1960). However, conflicting results have been reported for Acala and non-Acala Upland cottons resistance to the Fusarium wilt disease (Smith and Dick, 1960; Kappelman, 1971). It has been difficult to identify/develop highly FOV resistant Acala/Upland cottons, which suggests that resistance in the *G. hirsutum* gene pool may be more complex than in Pima (*G. barbadense*) cottons (Ulloa et al., 2006, in press).

Fusarium wilt was first noted in California in 1959 (Gaber and Paxman, 1963) where the number of infested sites remained relatively few until the mid 1970s, when the number of sites increased substantially (Hillocks, 1992). The Fusarium wilt evaluated in California and the western states during studies conducted in the 1960s and 1970s was caused by FOV races 1 or 2, and was typically found in sandy or sandy-loam soils with significant root-knot nematodes (*Meloidogyne incognita*) populations (Veech, 1984; Bell, 1984). Susceptibility to Fusarium was substantially increased in the presence of the root-knot nematode (Garber et al., 1979). Cottons developed for resistance to FOV in soils infested with *M. incognita* usually maintained their resistance when simultaneously challenged by both organisms (Sasser, 1972; Heal and Orr, 1982). Recently, race 4 of FOV has been identified in cotton plants grown in California fields (Kim et al., 2005). This race, first identified in India on Asiatic cottons, had not previously been identified in the U.S.

In the San Joaquin Valley most cotton crop loss to Fusarium wilt was thought to all be associated with races 1 or 2, and in these fields the worst damage was observed when there was also significant nematode damage from root knot nematode (Veech, 1984; Bell, 1984). However, in the past few years, race 4 FOV caused extensive symptoms in cotton plants grown in clay loam and loam soils in which root knot nematode populations and root damage from nematodes were nonexistent or extremely low. In these field evaluations in California, disease expression of race 4 has been most severe in Pima cotton fields, but the fungus also has the capability to infect and cause disease in Acala and Upland cottons (Kim et al., 2005; Hutmacher et al., 2005; Ulloa et al., 2005b).

Herein, we present the first field evaluations for commercial and improved germplasm of Acala, non-Acala Upland cottons (*Gossypium hirsutum* L.) and Pima (*G. barbadense*) cultivars subjected to FOV race 1 in typical sandy soil with interaction with root-knot nematodes, race 4, and non-infested soils in order to implement steps to develop resistant germplasm to this pathogen and to provide information about the susceptibility of commercial and improved germplasm cottons, helping growers to make informed decisions regarding the choice of cotton cultivar to plant in fields infested with FOV R1 and R4.

Material and Methods

Pathogen

Field investigations were conducted in a commercial field and at the Research and Extension Center UC Shafter CA naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 1 and 4. For Fusarium wilt race 1, the field was a typically sandy soil field with presence of root-knot nematodes. For race 4, the field consisted of clay loam and loam soil in which root knot nematode populations and root damage from nematodes in previous cotton crops were extremely low or nonexistent. The identity of FOV races 1 and 4 in plants grown in these fields was confirmed (Kim et al., 2005) before the trials were initiated and during the growing season for this research.

Plant Material

In this study, for Acala/Upland (*Gossypium hirsutum*) cottons, 15 entries were included: cvs Ultima EF, Riatta_RR, acc. C704_RF, accs. C103, and C702 (CPCSD Co), Phyto-72, Phyto-710_RR, and Phyto-715_RF (Phytogen Co), DP444_BR, DP-6222_R, and DP6207 (Delta and Pine Land Co), FM960_B2R (FiberMax – Bayer Corp.), ST4575_BR (Stoneville Pedigreed Seed Co), OA 265 (Olvey & Associates Ltd), and SJ-Upland_Suceptible_Check (USDA-ARS WICS Res. Unit, Cotton Enhancement Program). For Pima (*G. barbadense*) cottons, 11 entries were included: Phyto-800 and Phyto-810R (Phytogen Co), DP-744 and DP-340 (Delta and Pine Land Co), OA-353 and OA-357 (Olvey & Associates Ltd), HA-195 (Hazera Seed Ltd.), E-303 and E-503 (Cobalt Co.), and PS_6 and P_73 (USDA-ARS).

Field Evaluations

On 8 April 2005, 60 seeds were planted from the above entries in a commercial field to be known to be infested with FOV race 4, and plants were grown in one-row plots 3 m long with 1m row spacing in a randomized complete block design with three replications.

On 28 April 2005, 100 seeds were planted from the above entries in a field known to be infested known field with FOV race 1 with root-knot nematodes, and plants were grown in one-row plots 10 m long with 1m row spacing in a randomized complete block design with three replications.

On 4 May 2005, 100 seeds were planted from the above entries in a field known to be non-infested with FOV race 1, root-knot nematodes, and/ or race 4, and plants were grown in one-row plots 10 m long with 1m row spacing in a randomized complete block design with three replications.

In order to determine the level of tolerance /resistance for each entry, five plants were assayed from each replication for foliar symptoms (F_Damage), vascular stain (Vascular_S), number of nodes (Node_C), plant height in centimeters (Plant_Ht), presence/damage of root-knot nematodes (Root-knot), and plant population counts/survival (Stand). Data collection was performed for each plot-entry at three different stages of plant development: 1) plant establishment, 2) five weeks after, and 3) at crop maturity in order to monitor and determine the level of tolerance/resistance to FOV. The above variable-entry data grown in infested field were identified herein by adding the following letters at the end of the variable: for race 4 assigned R4, for race 1 with nematodes assigned R1, and for non-infested assigned F16.

Evaluations of individual plants were rated for disease severity based on the following scale for F_Damage: 0 = no foliar symptoms, 1 = chlorosis and/or wilt restricted to cotyledons or first leaf and/or stunting, 2= similar symptoms extended beyond the first leaf, 3 = moderate to severe foliar symptoms usually with leaf lost, 4 = severe symptoms with the whole plant affected, 5 = plant dead; Vascular_S 0 = no vascular staining evident, 1 = light vascular staining evident as spotty areas, 2 = more continuous color staining - covering area equal to between one quarter and one half of stem cross-section, 3 = moderate vascular staining (intensity of the brown/black color) evident in a band most of the way around the stem cross section, 4 = vascular staining darker, and 5 = plant dead. Figure 1 shows typical symptoms of FOV R1 under infested field conditions.

Results and Discussion

Under infested field conditions, seed germination was always hard to determine with certainty. However, Acala/Upland (Phy-72 and SJ-Suc-Check) and Pima (DP-744 and Phy-810R) entries susceptible to FOV R4 were observed to have the higher number of plants per plot under FOV R1 disease pressure based on plant survival at crop maturity (Fig. 2). Based on foliar symptoms and vascular staining, disease severity was significantly greater for FOV R4 than for FOV R1, and Pima cultivars grown in California were more susceptible to FOV R1 and R4 than any Acala and non-Acala Upland cottons (Figs. 3 and 4). For FOV R4, there was a wide range of foliar symptoms and levels of aggressiveness observed across tested entries, and the diagnosis of this disease was easily scored, with symptoms that included cotyledon pigmentation and drop, individual or multiple leaf chlorosis and necrosis, plant wilt, and death of plants during, a longer period. On the other hand the entries tested under FOV R1 in general presented mild to non-existed foliar symptoms even though vascular staining and root-knot were visible, except for the susceptible FOV upland control (Fig. 3). No distinct differences were observed between R1 and R4 FOV symptoms. On older plants, foliar symptoms usually began on the margins of lower leaves (Fig 1). Plants started to die within a few days after emergence. Even though Pima cultivars were more susceptible to FOV, Pima germplasm highly resistant to FOV race 4 has been identified both in the field and greenhouse studies (Ulloa et al., 2006, in press). Plants infected by FOV tended to be smaller with a greater number of nodes than those grown in soils not-infested with FOVs (Figs. 5 and 6).

Recently, Ulloa et al. (2006, in press) reported positive correlations between foliar damage and vascular staining which indicated that the two traits correspond in measuring disease severity for Pima entries. However, for Acala and non-Acala Upland cottons, the lack of correlation among evaluation sites for foliar damage or vascular stain suggested that the variation measured by the disease severity scales within the evaluation sites did not capture the true variation of this disease. Significant differences were difficult to detect using the disease severity scales because of asymptomatic foliage and plant performance in most cases.

Known Pima resistant (Phy-800) and susceptible (DP-744) commercial cultivars under R4 pressure were observed to respond significantly different under R1 pressure, DP7-44 being more resistant than Phy-800 under R1 pressure based on plant survival and vascular staining (Figs. 1 and 4). In Pima cottons a single dominant major gene and one or more minor genes that may provide trangressive segregation have been reported for FOV resistance (Fahmy, 1927; Mohamed, 1963; Ulloa et al., 2006, in press) as well as two major dominant genes with inter locus additivity

that provide a high degree of resistance to Sea Island cotton (Smith and Dick, 1960). However, conflicting results have been reported for Acala and non-Acala Upland cottons' resistance to the Fusarium wilt disease (Smith and Dick, 1960; Kappelman, 1971). The inheritance of the resistance of Upland cottons to Fusarium wilt has been reported from dominant gene types with minor modifying genes (Smith and Dick, 1960) to quantitatively to be inherited and controlled by several major genes and minor modifying genes (Kappelman, 1971). The host plant resistance observations in this study may suggest that two independent genes and/or an additional gene may control the R1 and/or R4 resistance in Pima cottons. Additional research is needed to address host plant interactions between races. Our research continues to provide a great deal of information about the status of commercial and improved germplasm of cottons and their level of resistance to FOV R1 and/or R4, allowing growers to make informed decisions regarding the choice of cotton cultivar to plant. In addition, this research identified potentially new improved resistant germplasm and raises the need to breed cotton in California with resistance to existing FOV races.

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Figure 1. Image of foliar symptoms (Foliar_D) and root vascular staining (Vascular_S) of disease expression for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 with presence of root-knot nematode symptoms.

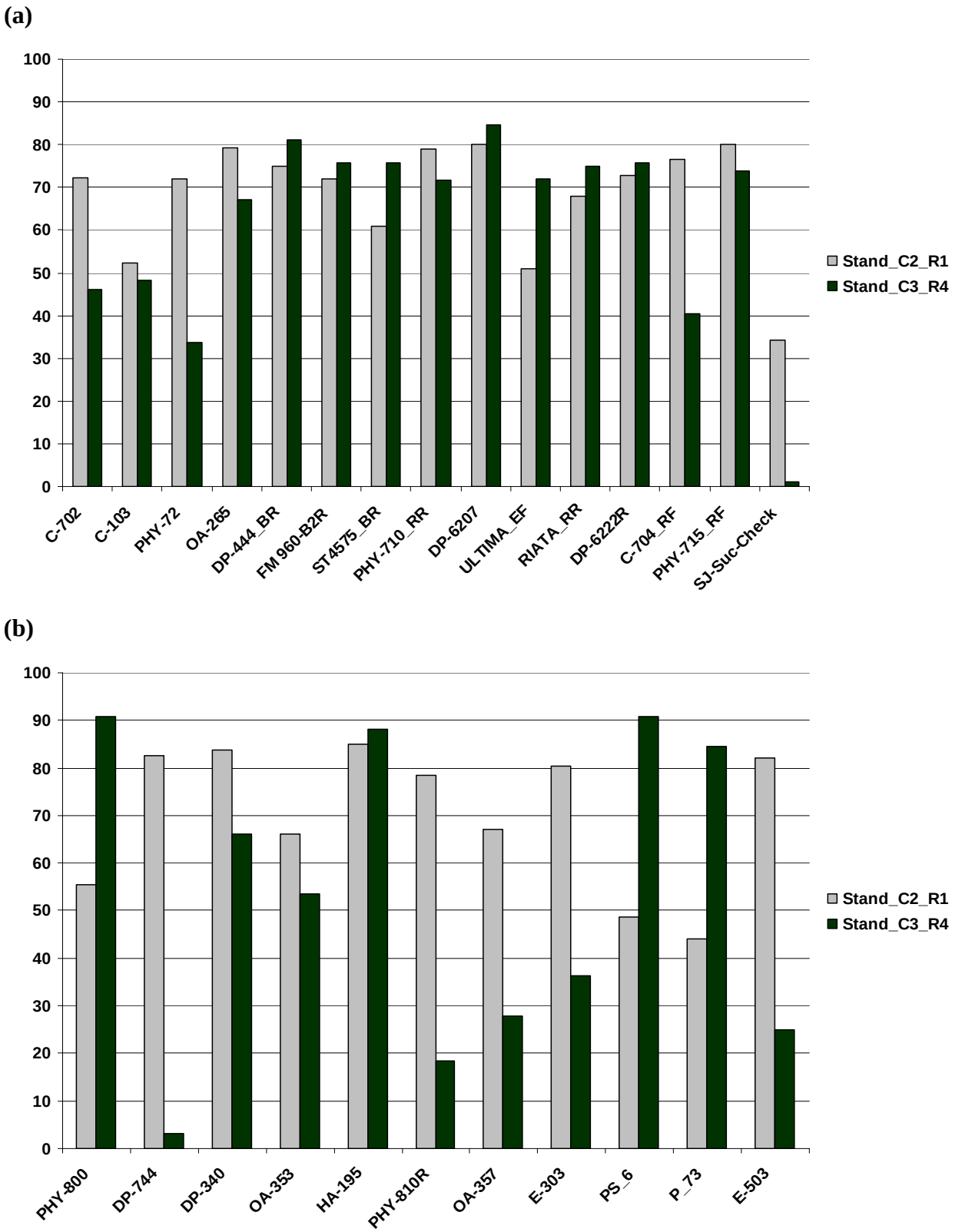
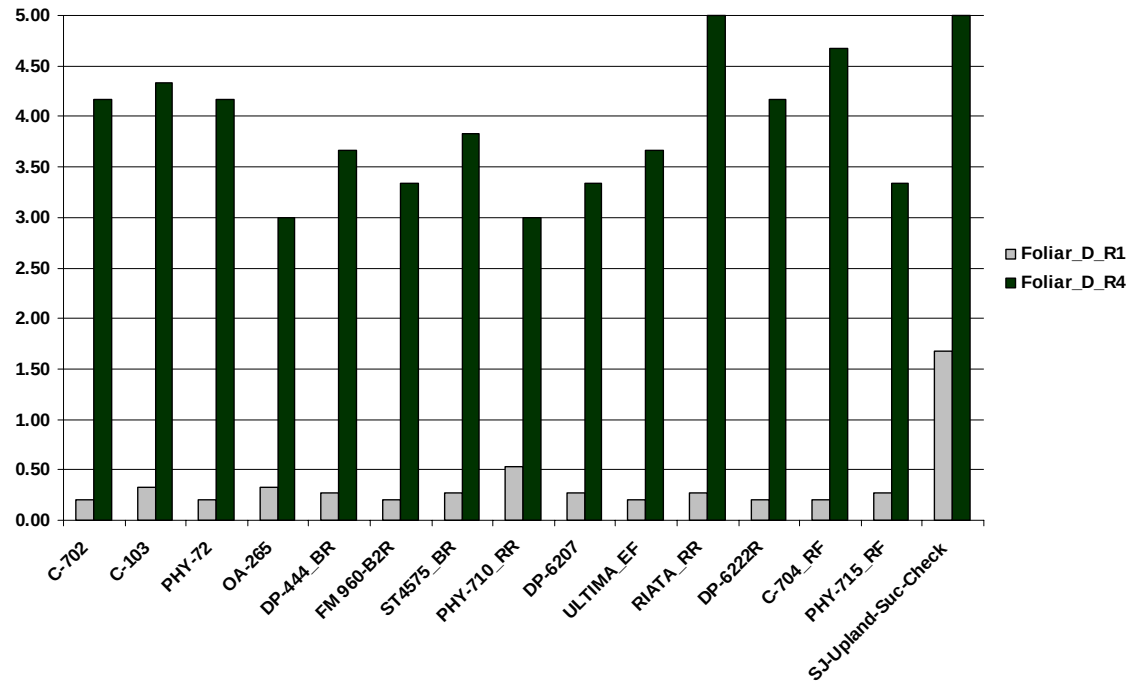


Figure 2. percentage of plant survival (Stand_C) at crop maturity of infected plants for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 (R1) and 4 (R4) for **(a)** Acala and non-Acala Upland (*Gossypium hirsutum* L.) and **(b)** Pima cottons (*G. barbadense* L.).

(a)



(b)

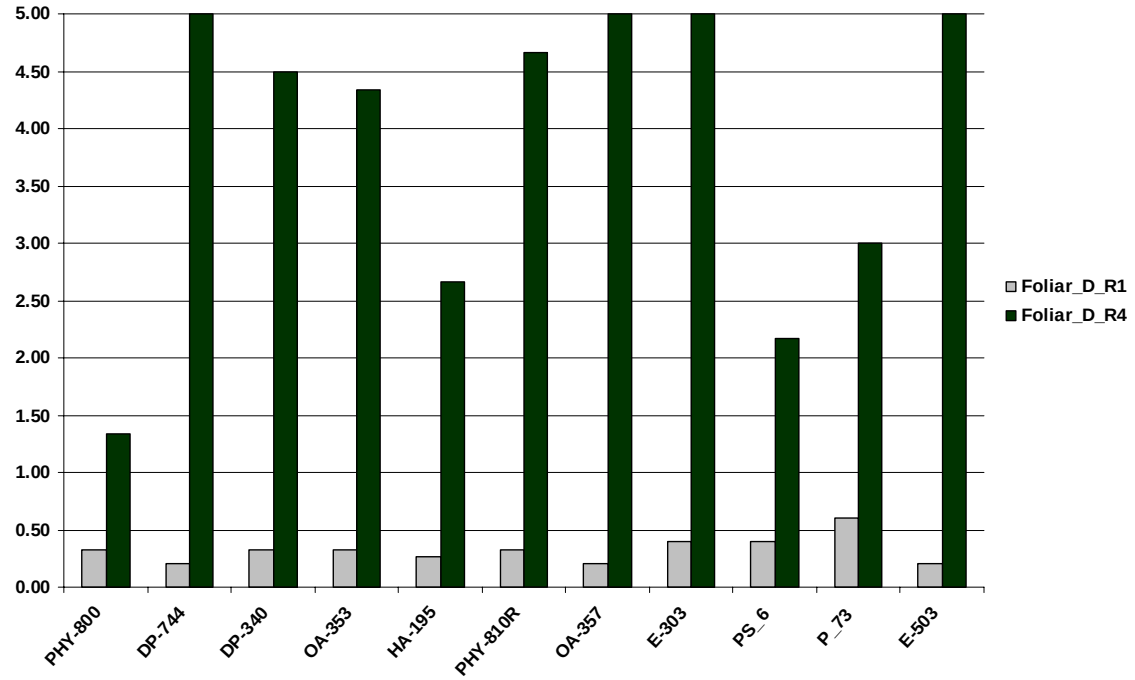
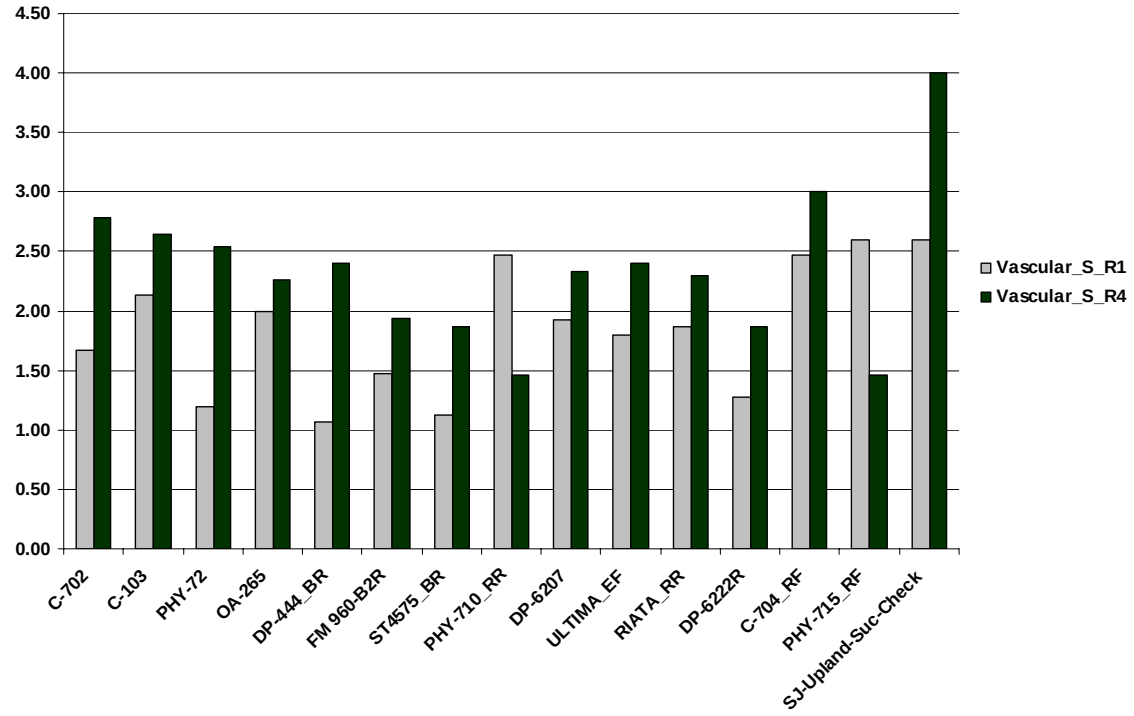


Figure 3. Average mean values of foliar symptoms (Foliar_D) of infected plants for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 (R1) and 4 (R4) for **(a)** Acala and non-Acala Upland (*Gossypium hirsutum* L.) and **(b)** Pima cottons (*G. barbadense* L.).

(a)



(b)

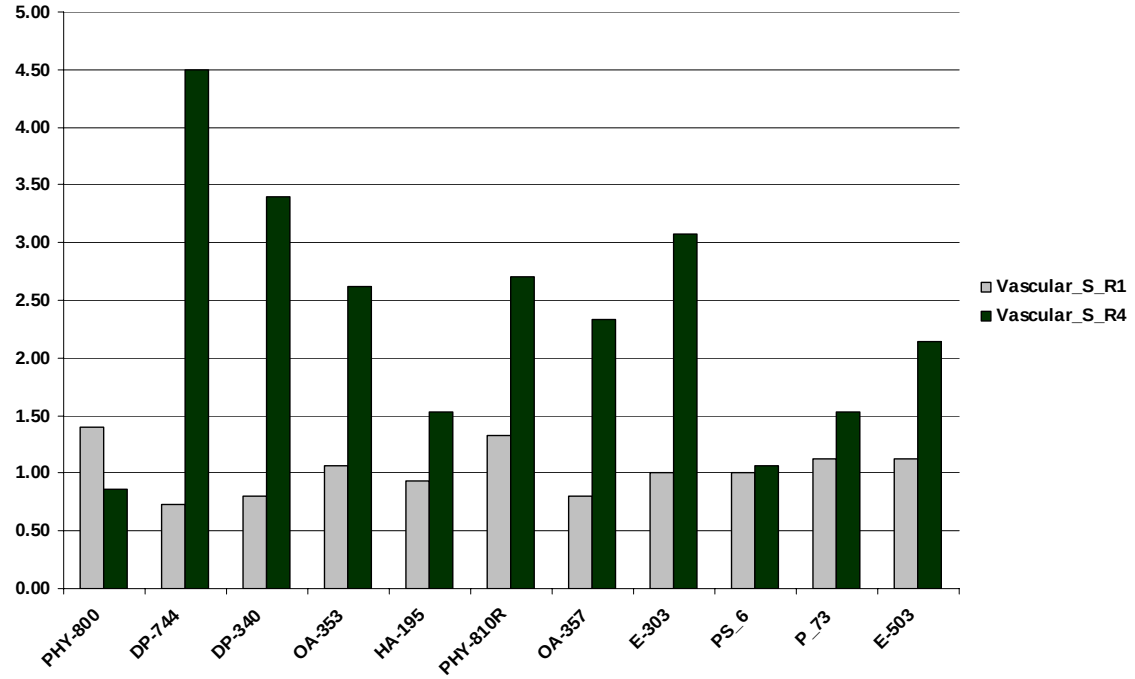


Figure 4. Average mean values of root vascular staining symptoms (Vascular_S) of infected plants for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 (R1) and 4 (R4) for **(a)** Acala and non-Acala Upland (*Gossypium hirsutum* L.) and **(b)** Pima cottons (*G. barbadense* L.).

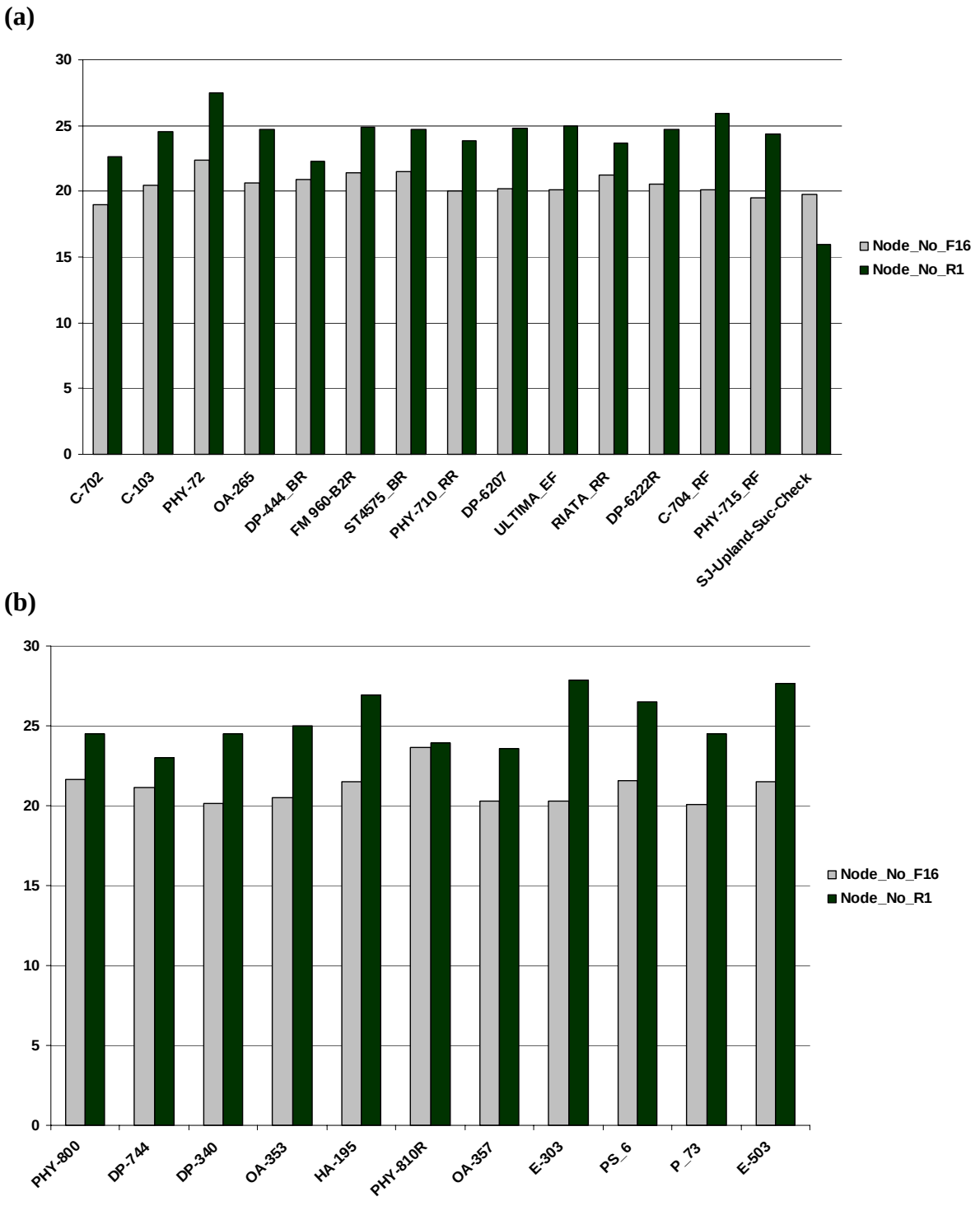
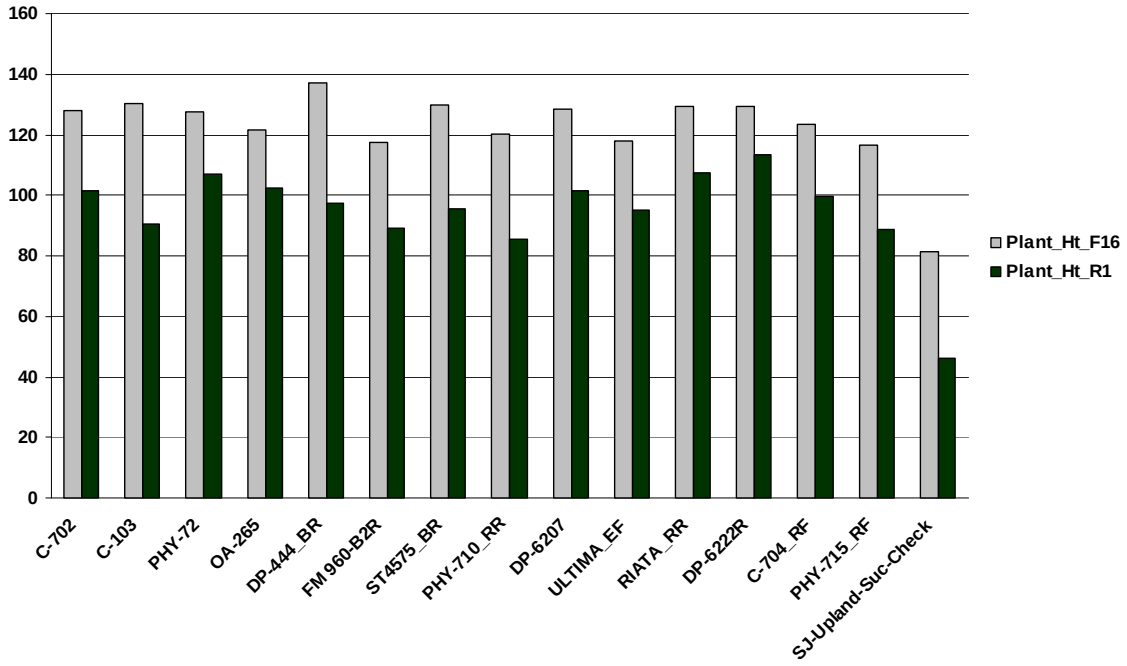


Figure 5. Average mean values of number of plant nodes (Node_No) of infected plants for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 (R1) and non-infected plants grown in non-infested field (F16) for **(a)** Acala and non-Acala Upland (*Gossypium hirsutum* L.) and **(b)** Pima cottons (*G. barbadense* L.).

(a)



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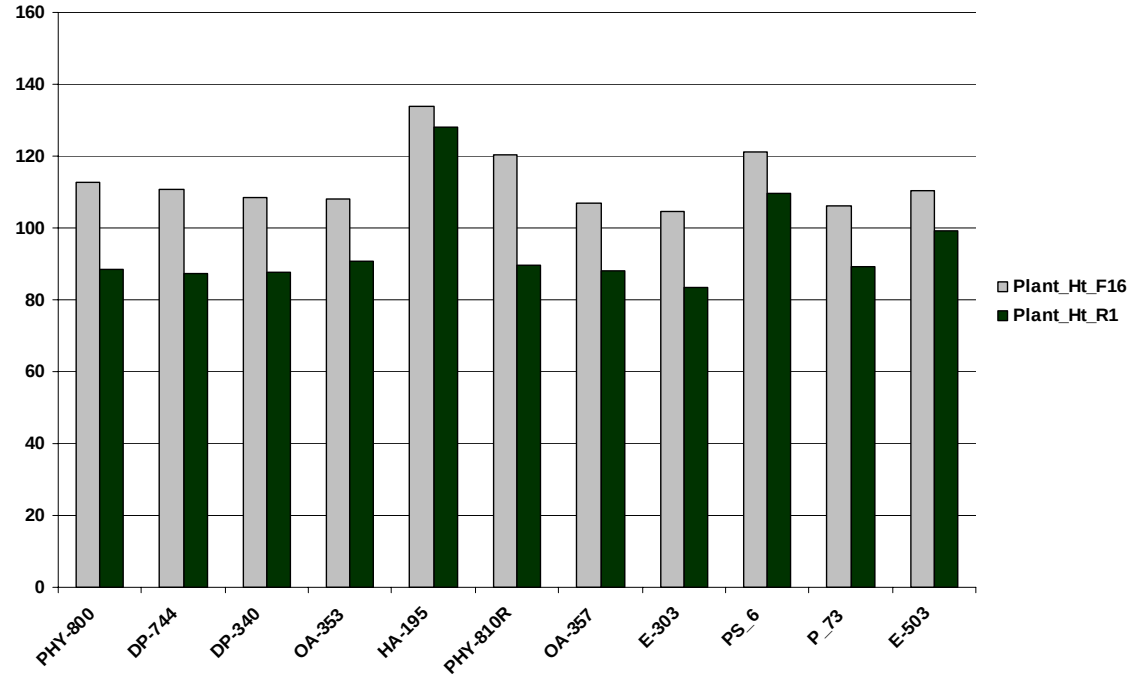

Figure 6. Average mean values of plant height in centimeters (Plant_Ht) of infected plants for *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 1 (R1) and non-infected plants grown in non-infested field (F16) for **(a)** Acala and non-Acala Upland (*Gossypium hirsutum* L.) and **(b)** Pima cottons (*G. barbadense* L.).