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Effect of Leaf Shape on Boll Rot Incidence in Upland Cotton (*Gossypium hirsutum*)

¹R.J. Andres, ¹D.T. Bowman, ²K.S. Lawrence, ³G. Myers, ⁴P.W. Chee, ⁴E. Lubbers and ¹V. Kuraparthi

¹Department of Crop Science, North Carolina State University, Raleigh, NC 27695, USA

²Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849, USA

³School of Plant, Environmental and Soil Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

⁴Department of Crop and Soil Sciences, University of Georgia, Tifton, GA, 31794-0748, USA

Corresponding Author: V. Kuraparthi, Department of Crop Science, North Carolina State University, Raleigh, NC 27695, USA

ABSTRACT

Cotton boll rot is a fungal and/or bacterial infection associated with high humidity and a lack of ventilation in the canopy during the flowering, boll development and dehiscence of cotton (*Gossypium* spp.). The severity of infestation can vary widely but the disease manifests itself through reduced yields and a deterioration of fiber quality. A wide variety of causal agents negates the possibility of developing direct genetic resistance but previous studies have shown that okra leaf cotton is an effective way of controlling boll rot. However, the reduced leaf area of these cultivars limits yield potential. Conversely, sub-okra cotton has minimal field resistance to boll rot but yields competitively with normal-leafed varieties. Here, an experimental open-pollination approach was used to develop hybrid lines containing both the sub-okra and okra alleles. The ability of these lines to counter boll rot was tested against parental lines, a hand-pollinated F₁ and a normal-leaf control at five different locations throughout the southeastern United States. Data from the one location with sufficient boll rot showed that an open-pollinated okra X sub-okra line was just as effective at reducing boll rot as the okra leaf parent. This suggests that open-pollinated lines may be an effective means of combating boll rot that should not result in reduced yields, although the latter conclusion has yet to be confirmed.

Key words: Cotton, boll rot, leaf shape, okra, sub-okra, breeding

INTRODUCTION

Cotton boll rot is a disease of increasing importance in the southeastern US Cotton Belt. Potentially affected areas range from Louisiana to North Carolina and incidence appears more severe with increasing proximity to the coast. Projected increases in temperature, humidity, precipitation and abnormal weather events brought about by global warming will likely only amplify the incidence of boll rot in this area. Severe boll rot can cause yield losses ranging from 50-80% in hard hit areas of the southeastern U.S. and was responsible for a 15-30% yield reduction in Brazil during the 2012 growing season (Daryl Bowman, Gerald Myers-personal communication).

Efforts to develop resistant cultivars and/or effective chemical control methods have not yielded the desired level of success against boll rot. Cauquil (1975) stated that the best way to counter boll rot was to alter the microclimate that spurs its growth by reducing planting density, limiting

mineral fertilizer application and moderating irrigation. Jones (1982) noted that the use of skip rows and bottom defoliation showed effectiveness in reducing boll rot. However, these practices may come at the expense of overall yield and would be undesirable if sufficient boll rot failed to develop.

Nearly all tetraploid cotton cultivars possess the normal or broad leaf type. Along with normal (l), numerous mutant leaf types make up an allelic series located on chromosome 15 of the cotton D sub-genome including okra (L^o), sub-okra (L^u), Sea-Island (L^s) and super-okra (L^s) (Jones, 1982; Jiang *et al.*, 2000). Okra leaf shape is characterized by reduced photosynthetic area per leaf, a more pronounced lobing pattern with deeper sinuses and ectopic outgrowths/abnormal leaf margins (Fig. 1). Sub-okra possesses a less pronounced lobing pattern with a more complete leaf margin exhibiting only the occasional abnormality (Fig. 1). Lobes of sub-okra leaf also tend to have a slight upward curl to them. Sea-Island is a third leaf shape mutant found commonly in cultivars of *Gossypium barbadense* and super-okra is a severe leaf shape mutant characterized by an almost compound leaf structure consisting of narrow strips of leaf emanating from the petiole. Another allelic series for leaf shape is found on chromosome 1 of the A sub-genome consisting of lacinate

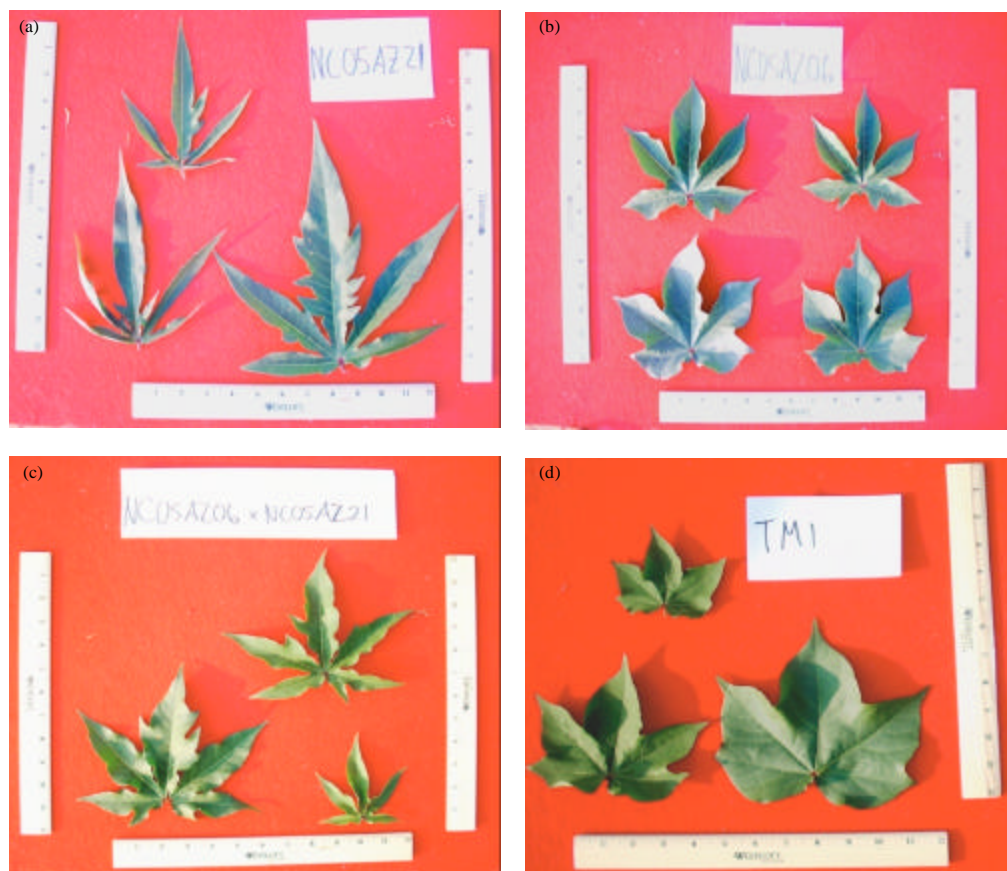


Fig. 1(a-d): Leaf Shape in Cotton (*Gossypium hirsutum*) (a) NC05AZ21-classical okra leaf shape, note increased lobing pattern with abundant ectopic outgrowths (b) NC05AZ06-sub-okra leaf shape with reduced lobing pattern relative to okra and rare ectopic outgrowths, (c) F_1 hybrid between NC05AZ06 and NC05AZ21 showing intermediate lobing but return of abundant ectopic outgrowths and (d) Texas Marker-1 (TM-1)-normal or broad leaf type characteristic of most cotton cultivars including NC10AZ10

(L^L), narrow (L), intermediate (L^h) and two alleles for broad leaf (L^B and l) Jones (1982). Laciniated leaf shape is similar in appearance to the okra leaf shape on chromosome 15 of the D sub-genome.

Jones (1975) compared leaf shape isolines in numerous varietal backgrounds and found that okra leaf shape can reduce the incidence of boll rot by 40%. Leaf shape mutants reduce the incidence of boll rot by providing a more open canopy structure that permits increased sunlight and air-flow to reach the interior of the cotton canopy. This alters the microclimate by reducing humidity, shade and moisture and increasing the temperature, creating a less hospitable environment for microbial growth. Prolonged periods of warm, cloudy and humid weather or extensive lodging can reduce the effectiveness of okra in combating boll rot (Jones, 1982).

The effect of okra leaf on yield has produced conflicting results. Some researchers have recorded a slightly positive effect on yield (Jones, 1982), while other studies showed that okra leaf had no effect on yield (Meredith, 1985) or a slight decrease in yield (Wilson, 1986). An increase in harvested yield for okra leaf lines is likely the result of reduced losses to boll rot rather than an increase in yield potential. On the other hand, the reduction in total leaf area seen in okra plants (60-65% of that produced by normal) may result in less than optimal light capture, reduced photosynthesis and by extension, reduced yield under optimal conditions (Jones, 1982; Wells and Meredith, 1986).

It should also be noted that okra leaf shapes show accelerated rates of flowering producing 50-100% more flowers than normal types. This increased rate of flowering does not contribute to any yield advantage as the mutants also show a significantly greater rate of boll shedding. However, this accelerated flowering contributes to early maturity and increased ability to compensate for losses induced by temporary biotic and abiotic stresses. Okra leaf shape lines have also shown increased resistance to the whitefly and pink bollworm, presumably through the lethal effects of greater canopy temperatures on larvae. On the other hand, okra lines appear more susceptible to plant bugs such as tarnished plant bugs and cotton flea hoppers and are also subject to greater weed pressure due to increased light penetration (Jones, 1982).

There appears to be no significant difference in yield between sub-okra and normal leaf cotton. However, the increase in air movement and light penetration is not great enough to produce a significant reduction in boll rot in the sub-okra genotypes. The more extreme leaf shape mutant okra displays a significant reduction in boll rot but the decrease in leaf area is likely intense enough to result in a substantial loss of yield under ideal conditions. This limits the application of okra lines to environments where boll rot is likely and severe enough to risk their reduction in agronomic potential.

The creation of a more open canopy structure as a means of disease control has been successfully deployed in other agricultural species such as common bean (*Phaseolus vulgaris* L.), grape (*Vitis vinifera*) and strawberry (*Fragaria xananassa*) (Blad *et al.*, 1978; Gubler *et al.*, 1987; Shuman, 2001). Similar to boll rot and leaf shape in cotton, the results of these studies showed that an open-canopy cultivar can out-yield a conventional type in environments characterized by high disease incidence. However, in years and locations where sufficient disease fails to develop, normal canopy cultivars have a significant yield advantage.

It would be interesting to determine the boll rot prevention ability of a line heterogeneous for both the sub-okra and the okra allele. F₁ hybrid plants of okra x sub-okra show intermediate leaf shape between their parents (Fig. 1). Due to its self-pollinating nature, hybrid seed production in

cotton is a difficult endeavor and commercially unviable. Therefore, along with hand pollinations, an experimental open-pollination nursery was used to develop two lines with various proportions of heterozygosity. The ability to produce a superior hybrid using an open-pollination approach is critical, as any advantages of a hand-pollinated F_1 would be negated by the trouble of producing seed.

Even though isogenic lines are not being used, any differences in boll rot resistance can be attributed to leaf shape since no other mechanism has yet been described that confers resistance to this disease. The objective of this experiment was to compare the ability of these hybrid lines to combat boll rot with that of their parents and a normal leaf variety in order to gauge the applicability of this approach.

MATERIALS AND METHODS

Development of hybrid lines: In order to carry out the open-pollination, ten plots each of the sub-okra line NC05AZ06 and the okra line NC05AZ21 were planted in alternating fashion in an isolation nursery in Clayton, NC during the summer of 2010. Pollination was allowed to occur naturally resulting in either self-pollination or cross-pollination by native insect pollinators. The two parents were harvested separately and the F_1 seed was bulked over plants belonging to the same line. This created two separate half-sib families that differed based on their female parent for use in the hybrid study. Hand-pollinations were carried out in a separate field in Clayton.

Evaluation of entries for boll rot resistance: In the summer of 2011, the hand-pollinated hybrid and both of the open-pollinated F_1 hybrids were tested against a normal leaf line (NC10AZ10) and both parental lines for boll rot incidence. NC10AZ10 was included as a check to gauge the level of boll rot in a normal-leaved variety relative to that seen in the experimental entries. The test covered five locations with four replicates per location. The locations were: Tifton, GA, Quincy, FL, Alexandria, LA and two tests at Fairhope, AL. All locations were chosen for their near annual incidence of boll rot. Boll rot at these locations is usually an annual occurrence, even without artificial promoters of boll rot such as excess irrigation or nitrogen fertilizer application. Each test was arranged in a 6×4 Randomized Complete Block Design (RCBD). Each plot was four rows wide and approximately 12.2 m in length with 3.6 m alleys. Quincy utilized six row plots in order to accommodate available planting equipment. Boll rot severity was measured by counting the ratio of rotted bolls to total bolls in a 1.8 m subsection of each of the two interior rows of each plot. Data was analyzed using SAS Proc GLM and Proc ANOVA (SAS, Cary, NC).

RESULTS

The four locations chosen for this study were all chosen because of their near annual incidence of boll rot. Their locations on or near the Gulf of Mexico place them in a region characterized by high temperatures as well as some of the highest annual rainfall totals in the contiguous United States. These conditions create an especially hot and humid environment during the summer months that is ideal for the development of boll rot, limiting the productivity of the region. However, the summer of 2011 was characterized by slightly elevated temperatures and a pronounced lack of precipitation, especially during the month of August. This prolonged period of hot and dry weather led to one of the least productive environments for the development of boll rot seen in some time. In addition to the lack of boll rot, overall poor growth due to late planting and excessively hot and dry weather led to the abandoning of the trial in Alexandria. Additionally,

Table 1: Effect of variety on boll rot incidence in Fairhope

Variety	Average No. of rotten bolls (%)
NC10AZ10 (normal)	29.20 ^a
Open-pollinated NC05AZ06×NC05AZ21 F ₁	21.79 ^{ab}
NC05AZ06 (sub-okra)	19.84 ^{ab}
Hand-pollinated F ₁	17.60 ^{ab}
Open-pollinated NC05AZ21×NC05AZ06* F ₁	14.21 ^b
NC05AZ21 (okra)	11.48 ^b
LSD	14.87

Means with the same letter are not significantly different at the $\alpha = 0.05$ significance level, LSD: Least significant difference

damage from Tropical Storm Lee led to abundant lodging and rank growth at both tests in Fairhope while the best growth occurred in Quincy. Across all locations, the simple ratio of rotted bolls to total bolls was 12%, well below the 20-30% that was hoped for and the 50-80% that can sometimes be seen in this region. Thus meaningful data was only collected from the one test at Fairhope, Alabama.

The first test location in Fairhope had the highest incidence of boll rot, affecting almost 20% of the total bolls. Tropical Storm Lee, through its sustained high winds and brief flooding, led to pronounced lodging of the test, a development known to offset the boll rot resistance of mutant leaf cultivars (Jones, 1982). Additionally, the fourth block was thrown out after being overrun by weeds which combined with the unfavorable weather to yield fewer than five total bolls in the two meter plots for some varieties. Nevertheless, at this location the okra leaf line NC05AZ21 had an average boll rot of 11.5% while its most phenotypically similar F₁ hybrid, the open-pollinated line NC05AZ21×NC05AZ06, had an average boll rot of 14.2%. Both of these entries showed significantly less boll rot than the normal leaf cultivar NC10AZ10 which had an average boll rot of 29.2% ($\alpha = 0.05$, Least Significant Difference (LSD) = 14.9%, Table 1). The okra leaf did outperform the normal leaf by almost 18% in terms of boll rot incidence. This result was encouraging and agreed with previous reports in the literature (Jones, 1975, 1982). The open-pollinated seed collected from NC05AZ21 also showed a highly significant 15% reduction in the incidence of boll rot compared to the normal leaf (Table 1).

DISCUSSION

The strategy used here may present a more practical approach to hybrid seed production in cotton. One of the open-pollinated lines used the sub-okra line (NC05AZ06) as the female parent and was biased to some extent towards the sub-okra allele. The other utilized an okra female parent (NC05AZ21) and was somewhat biased towards the okra leaf shape. Fiber quality is unlikely to show significant differences in the F₁ generation and thus was not collected. Since the lines under comparison were not isogenic, differences in yield cannot be solely attributed to leaf shape. It was expected that boll rot resistance will be negatively correlated to the varieties total leaf area. Okra leaf was expected to show the best ability to fight boll rot followed by the three hybrids which may not have been significantly different from each other or from okra. Ideally, one or both of the open-pollinated hybrids would have performed equally as well as the okra parent and/or the hand-pollinated hybrid in response to boll rot. Sub-okra and normal leaf were expected to show the lowest ability to cope with boll rot and were not expected to be significantly different from each other, although sub-okra may have had a slight advantage.

The data from Fairhope supported the hypothesis. Here, okra and its most similar F₁ hybrid, open-pollinated NC05AZ21×NC05AZ06, had significantly lower boll rot than the normal leaf

control cultivar. Okra showed an almost 18% reduction in boll rot compared to the normal leaf entry NC10AZ10 (Table 1). This agrees with previous reports in the literature that okra leaf cotton is an effective means of combating boll rot compared to normal leaf lines (Jones, 1975, 1982). To date, no other study has investigated the ability of hybrid lines segregating for leaf shape to reduce boll rot. Here, the open-pollinated entry NC05AZ21×NC05AZ06 also showed a significant reduction in boll rot compared to the normal leaf entry. This reduction was similar in nature to the abilities of its okra leaf parent observed both in this study and previously (Jones, 1975, 1982). Furthermore the reduction in leaf area of this open-pollinated line relative to both its sub-okra parent NC05AZ06 and the normal leaf control fits with the previously reported notion that anything that results in a more open canopy is likely to cause a reduction in boll rot (Cauquil, 1975; Jones, 1982).

While no other pair-wise differences were significant, the relative order of the boll rot incidence correlated roughly with expected leaf area. That this was observed in the environment showing the highest level of boll rot and in spite of extensive lodging, indicates at least the potential for deployment of an open-pollinated cultivar as a means to combat boll rot. Differences in the incidence of boll rot may have been even stronger had environmental conditions been more conducive to the development of disease and/or had lodging not occurred. Since no measurements were taken on the amount of out-crossing that occurred in the open-pollination nursery in Clayton in the summer of 2010, no estimates are available on the frequency of the sub-okra allele in the seed harvested from the okra parent. Measurements of outcrossing in the past at this location have indicated that rates may exceed 50% (Calhoun and Bowman, 1999; Josh Lee-personal communication). Therefore, it is expected that half of the resulting F_1 seed would have no copy of the sub-okra allele. This could explain the similarity of the performance of NC05AZ21 and Open-Pollinated (OP) NC05AZ21×NC05AZ06 at the Fairhope test location in the summer of 2011. The data from Fairhope 1 indicates that whatever increase in leaf area caused by the presence of these sub-okra alleles did not significantly alter the resistance to boll rot. Any increase in the yield potential brought about by the increase in leaf area would be independent of boll rot resistance and may make the use of an open-pollinated hybrid a feasible alternative to normal leaf cultivars in regions conducive to boll rot.

The inability to detect significant differences in the boll rot performance of the leaf shape varieties across the other experiments is mostly explained by a lack of disease development. All locations selected in this study were chosen due to a near annual incidence of boll rot. However, elevated temperatures and a lack of rainfall during the flowering and boll development periods throughout the region led to rotted boll ratios below the 20-30% minimum desired. Mean boll rot indices across all tests did fall into an order that fits somewhat with the hypothetical results but differences were not nearly significant enough to rule out their occurrence by chance. A larger sample size may or may not have been more effective at obtaining statistically significant results; an overall lack of growth due to the poor environmental conditions would likely have hindered the collection of additional data. The results from the one test (Fairhope 1 at 19.0%) show that the methods used are sufficient when boll rot incidence develops to an acceptable level. That the OP NC05AZ21 x NC05AZ06 (biased towards okra) was more resistant than the check provides some incentive to further investigate the ability of an open-pollinated hybrid as a means to combat boll rot.

CONCLUSION

This hybrid may be more resistant to boll rot than normal leafed lines but should not suffer as severe a yield potential penalty as a pure okra leaf cultivar.

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