Understanding the nature of fiber properties variation in cotton (*Gossypium hirsutum* L.) canopies is critical for designing production systems that will provide more uniform fiber to textile mills. Our objective was to compare first branch node position bolls (FP1) and second branch node position bolls (FP2) for boll weight and AFIS fiber properties. In 2004 and 2005, a 1-m row section of four replicates of Stoneville 1218 Bt/RR and DPL 555 Bt/RR were hand harvested and sympodial and branch node positions and flowering date of each boll were recorded. These bolls were weighed, hand-ginned, and fiber was subjected to advanced fiber information system (AFIS) analysis. Averaged over both years and both cultivars, FP1 bolls were about 12% heavier than FP2 bolls. Differences between the two sympodial positions were similar for all flowering dates. Differences also occurred between FP1 and FP2 bolls for fiber length, fiber fineness, and immature fiber content. However, there was little difference between FP1 and FP2 bolls for these fiber properties when comparisons were made at individual flowering dates. This suggests that environmental conditions during boll development have a more significant role in determining individual boll fiber properties than does the sympodial position on a branch. In addition, the distribution of fiber length by weight was also determined on a subset of bolls. Inspection of these data suggests that within a cultivar, FP1 and FP2 bolls are similar for within boll fiber length distribution, but there are cultivar differences for within-boll fiber length distribution.