The long-term sustainability of the U.S. Southwest cotton industry is at risk from the threat of global climate change and heightened competition for diminishing water resources. With the availability of high-throughput tools to phenotype informative traits under relevant growing conditions, it may be possible to more efficiently develop cotton cultivars that are resilient to environmental stress. In that light, we developed and evaluated a novel tractor-based phenotyping system that deployed sets of sensors to simultaneously measure canopy reflectance and temperature on a cotton recombinant inbred line mapping population. The population was evaluated under well-watered and water-limited conditions in a replicated field trial at Maricopa, Arizona, with trait measurements taken at different times on multiple days in July and August. Measurements of canopy normalized difference vegetation index (NDVI) and temperature were moderately to highly heritable and showed expected interactions of genotype with water regime and time of day. Through a quantitative trait locus (QTL) analysis of these time-related phenotypic data, identified QTLs were shown to have an expression pattern that varied by water regime and time of day. The phenotyping system enabled repeated measurement of traits throughout the growing season, facilitating the identification of differentially expressed QTLs for stress-adaptive traits.