

**USE OF DIGITAL IMAGE ANALYSIS, VIABILITY STAINS, AND GERMINATION
ASSAYS TO ASSESS COTTON POLLEN VIABILITY**

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Abstract

Pollen viability assays differ in their ability to distinguish viable from nonviable pollen. Four cotton pollen viability assays were compared to determine their relative accuracy as determined by the correlation to seed set and ease of use. Brewbaker & Kwack (B & K) media, B & K media plus aniline blue, the fluorochromatic reaction (FCR), and Alexander's stain were compared. Digital images of viable pollen grains germinated in B & K media containing different sucrose concentrations were analyzed using morphometry software to quantify pollen grains and pollen tube growth to optimize the B & K media viability test. Pollen from conventional, nontreated Roundup Ready (RR), and Roundup-treated RR cotton plants grown in a phytotron was used to compare the four methods using pollen samples with known high and low viability. Roundup treatments to RR cotton reduced pollen viability in all methods tested. Correspondingly, the number of seeds per boll was also reduced. Field studies conducting reciprocal crosses between Roundup-treated and non-treated RR cotton demonstrated that only male organs (anthers and pollen), and not female organs (stigma and ovaries), were sensitive to Roundup as measured by seed set from bolls where either the male or female, or both parents were treated with glyphosate. Pollen tube formation as measured by the B & K method was most closely related to seed set per boll as measured by the decline in pollen viability and seed set in Roundup-treated versus nontreated RR varieties. Alexander's stain, gave the highest viability estimates of all methods. The FCR method indicated that many pollen grains from Roundup-treated RR cotton were irregularly shaped and only partially fluorescein diacetate (FD) stained. Ten to 20% of these irregularly shaped grains, although not morphologically normal, were viable according to the B & K method. Morphometric analysis of digital images of pollen germinated in B & K media with differing sucrose concentrations found the greatest pollen tube area per pollen grain ratio with B & K media + 1% w/v sucrose, although pollen tubes were formed at all sucrose concentrations. Each method tested gave different estimates of pollen viability, however, the B & K method which measures pollen tube emergence was the most closely related to seed set as well as being one of the simplest and least equipment-demanding methods.