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Comparison of inoculation methods to assess the aggressiveness of *Diaporthe longicolla* isolates, causing stem blight of soybean

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Diaporthe longicolla (Hobbs) Santos, Vrandecic and Phillips, causes Phomopsis seed decay and stem blight of soybean (*Glycine max* L.). The two diseases have caused a yield loss of 0.6 million metric ton in soybean production in 2014 in the United States. In this study, we compared four inoculation methods to study the aggressiveness of *D. longicolla* isolates on soybean as a stem pathogen in the greenhouse. The methods (stem wound; toothpick; mycelium contact; and spore injection) were tested on soybean cv. Williams 82 under greenhouse conditions ($26\pm 2^{\circ}\text{C}$, >85% relative humidity and 14 hours of alternating day and light conditions). For each of the four methods, five isolates (DIA-076 = Clay County SD; DIA-017 = Ballard County KY; DIA-056 = Knox County, IN; DIA-063 = Wabash County; IN; DIA-071 = Tippecanoe County, IN) were used to inoculate 10 plants at V3 (third trifoliolate) growth stage per isolate-inoculation method in each of the two experimental repeats. For the stem wound inoculation method, mycelium contact inoculation method and toothpick inoculation method, *Diaporthe*-infested mycelial plug was used as the inoculum source. For the spore injection inoculation method, a suspension of spore (α -conidia) and mycelia was used as the source of inoculum. At 21 days after inoculation, length of the lesion caused by *D. longicolla* on the stem of the soybean plants was measured. No significant interaction of *D. longicolla* isolates and inoculation methods ($P > 0.05$) was observed. Among inoculation methods, significant differences were observed (LSD=0.29; $P < 0.001$) and stem wound method showed the largest lesion length on soybean plants. Among isolates, significant differences were observed (LSD= 0.36; $P = 0.005$) and isolate DIA-056 was the most aggressive. The results from this study will be helpful in the identification of an inoculation method and a *D. longicolla* isolate to screen soybean germplasm resistance for *D. longicolla* in future.