VERSION: 1.0	**DATE:** 2001
PATHOGEN: Phomopsis/Diaporthe complex	
HOST: Soybean (Glycine max)	
COMMON NAME: pod and stem blight; seed decay; stem canker	
METHOD: Sb 2.1 Culture plate (McGee, 1986) (formerly Sf 2.1)	
METHOD CLASS: STANDARD (A)	
SAMPLE: 400 seeds	

PROCEDURE:

1. Seeds are surface sterilized in 0.5% NaOCl for 1 minute and then rinsed in sterile water.

2. Plate seeds on potato dextrose agar (PDA) adjusted to pH 4.5.

3. Seed incubation at 25°C for 7 days under continuous light.

4. Seeds are evaluated for presence of the three fungi associated with the Phomopsis/Diaporthe complex in soybeans according to the following descriptions. Cultures on PDA are floccose and rope-like, turning tan to brown with age. In reverse, the colonies are tan to dark brown with black, pulvinate stromata. Pycnidia are black, stromatic, solitary or aggregated, and usually unilocular.

> **Note:** There is considerable controversy regarding the taxonomy of this group. These descriptions should therefore, only be used to identify the Diaporthe/Phomopsis complex and not the individual pathogens.

Phomopsis longicolla, previously known as Phomopsis sp., are floccose, dense and white, with occasional greenish yellow areas on PDA. In reverse, colonies are colorless, with black, spreading stromata. Pycnidia are black, stromatic, solitary or aggregated, unilocular or multilocular, with prominent necks more than 200µm long. Alpha conidia are hyaline, ellipsoid to fusiform, guttulate, and measure 5-9 x 1.5-3.5µm. Beta conidia, which are rarely formed, are hyaline, filiform, and hamate. No perithecia are formed on PDA.

Diaporthe phaseolorum var. caulivora cultures on PDA are white, closely appressed, with tufted aerial mycelium. In reverse, colonies are colorless, with black circular stromata, less than 2mm in diameter. Perithecia are black and globose, measuring 165-340 x 282-412µm, and have a long protruding beak. Asci measure 30-40 x 4-7µm. Ascospores are hyaline, elongate-ellipsoidal, two-celled, slightly constricted at the septum, biguttulate in each cell, and measure 8-12 x 3-4µm. Pycnidia rarely occur on PDA.

Diaporthe phaseolorum var. meridionales cultures on PDA are white even colonies with brown chlamydospores. Stromata are irregular in shape (2-10mm long). Perithecia are similar to those of D. phaseolorum var. caulivora, but they have wider neck width, i.e. 100 compared to 55µm.

Diaporthe phaseolorum var. sojae presents with no beak, or a beak less than 200µm long. Alpha conidia are hyaline, usually fusiform, guttulate, and measure 5.5-10.5 x 1.3-3.5µm. Beta conidia, which are more commonly produced, are hyaline, filiform, and hamate. Perithecia are nearly spherical and measure 148-282 x 185-346µm. They have long, tapered beaks, measuring 60-100 x 60-150µm, and usually are solitary, not clustered. Asci are elongate and clavate, measuring 35- 51 x 3.3-10µm. Ascospores are similar in shape to alpha conidia, but are larger, 9-13 x 2-6µm, and bicellular. They are biguttulate in both cells.

REFERENCES:

McGee, D. C. 1986. Prediction of Phomopsis seed decay by measuring soybean pod infection. Plant Disease. 70:329-333.