Use of the conductivity test to estimate soybean seed emergence and resistance to infection by *Fusarium oxysporum* in the southern United States

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Summary
This study was designed to evaluate the conductivity test as a tool to predict soybean susceptibility to *Fusarium oxysporum* and the effectiveness of a single fungicide seed treatment for arresting disease symptoms. The effects of cultivar, seed storage, and seed treatment on soybean emergence in inoculated media under controlled environmental conditions were investigated, as well as emergence in field soils. Fungicide treatments significantly improved emergence in controlled environment studies compared to an untreated control. Seedling emergence in infested medium in a controlled environment, or cool saturated field soil, was significantly negatively correlated (-0.92 – -0.97) with conductivity. Based on this correlation, it was concluded that the conductivity test could be developed as a tool to estimate soybean seed susceptibility to invasion by *F. oxysporum*.

Introduction
Seed vigour describes the potential of a seed lot for rapid emergence and development of healthy seedlings under a wide range of field conditions (McDonald 1980). Numerous vigour tests have been developed to estimate seed performance, specific to, or with application across multiple plant genera. The cold test and accelerated aging test are commonly used to evaluate soybean seed vigour. The conductivity test is accepted as an accurate method to determine seed vigour as well as estimate field performance (Vieira *et al.* 1992, Abdul-Baki and Anderson 1973, Matthews and Bradnock 1967). The conductivity test measures the flow of an electric current through seed leachate to estimate electrolytes released from seed during early stages of germination. Electrolytes are leached from seed in the form of organic acids, amino acids and proteins, carbohydrates, and inorganic ions through cell membranes (Bewley and Black 1994). Potassium (K^+) is the primary ion leached from seed and has been used as an indicator of seed membrane integrity and, thus, seed deterioration (Custodio and Marcos-Filho 1997). Quantification of these collective electrolytes constitutes the basis for the conductivity test. Yaklich *et al.* (1979) reported a significant correlation between seed vigour, physiology, and biochemical processes during imbibition and early stages of soybean germination. High levels of electrolyte leakage have been associated with greater infection of pea (*Pisum sativum* L.) seeds by *Fusarium* sp. (Kraft 1986). In soybean, *Fusarium* spp. have been shown to cause both pre-emergence and post emergence losses. Disease symptoms in soybean include root rot (French 1962), wilt (Leath and Carroll 1982) and hypocotyl and root lesions on germinating seed (Farias and Griffin 1989), resulting in delayed or reduced emergence. Matthews and Bradnock (1968) reported that seeds with low vigour were more susceptible to infection by soil-borne fungi, and increased severity of infection was correlated with electrolyte leakage. Electrolytes leached from common bean (*Phaseolus vulgaris* L.) stimulated germination of chlamydospores of *F. solani* (Schroth and Cook 1964). Similar results were reported in pea by Kraft (1986).

For simplicity, 'conductivity test' will be used to describe electrolyte leakage as a measure of cell membrane integrity in this study. The purpose of this study was to investigate the conductivity test as a tool to estimate the susceptibility of some soybean seed lots to pathogen invasion by *Fusarium oxysporum* and the effectiveness of single seed fungicide treatments against disease symptoms.

Materials and methods
Soybean was planted in *Fusarium oxysporum* infested soil media in a controlled environment study to estimate the effect of storage length and seed treatment on susceptibility to *F. oxysporum* invasion. A growth chamber (Conviron® CMP 3246, Conviron, 222 South 5th Street, PO Box 347, Pembina, ND 58271, USA) was used to approximate ambient field temperature conditions (saturated soil and 20°C for 10 hours increasing to 27°C for 14 hours in 1998; 18°C for 10 hours increasing to 22°C for 14 hours in 1999). Sterile soil media infested with *F. oxysporum* was used for all controlled environment studies. *Fusarium oxysporum* was cultured on potato dextrose agar, and conidia and mycelium were scraped from the agar into distilled water. The resultant suspension was filtered through two layers of cheesecloth and adjusted to 10^6 spores mL^{-1}. Sterile soil media was placed in flats with 22 cm^3 cells, and each cell was infested with 2 mL of the *F. oxysporum* suspension immediately prior to planting soybean seed. Six replications of forty plants per treatment were planted as a randomized complete block design. Three cultivars (Hutcheson, Hartz 5999, and Deltapine 5354) under three storage conditions (8 months, 20 months and artificially aged stress), and two single application seed treatments or an untreated control, were planted in all treatment combinations in each replication. The artificially aged stress treatment involved exposing seed to 48 hours of 45°C and 100% relative humidity (RH), redrying at 30°C back to 12% seed moisture and storage. All soybean seed storage environments consisted of ambient warehouse conditions. Seed treatments included either Vitavax® 200 (5,6 dihydro-2-methyl-*N*-phenyl-1, 4-oxathiin-3-carboxamide), or Apron® FL (2,6-dimethylphenylmetoxyacteyl-amino-propionic acid methyl ester), at the rate of 0.54 mL kg. Loss to *F. oxysporum* was calculated as a percentage of seed exhibiting symptoms similar to those observed in laboratory pathogenicity tests from the total number of seed planted per treatment at 8 and 15 days after planting (DAP) and expressed as emergence of healthy seedlings. Seed vigour was estimated from each storage treatment by the conductivity test using a Wavefront, Inc. G-2000 (Wavefront, Inc., 912 N. Main Street, Ann Arbor, MI 48104, USA) seed analyzer. Four replications of 25 seeds each were soaked for 24 hours in 2 mL deionized water $seed^{-1}$ in individual seed cells and conductivity of the seed leachate was measured in microSiemens (µS) $seed^{-1}$.

Seed fungicide treatments were not included in the conductivity test evaluation. Seeds were planted in flats containing soil media infested with *F. oxysporum*, as described above, immediately following the conductivity test and placed in a growth chamber in a randomized complete block design with four replications. Temperature within the growth chamber was identical to those specified previously for 1998 and 1999 to mimic ambient field conditions each year. Loss to *F. oxysporum* was evaluated in a manner similar to that used for the other growth chamber studies.

Using treatment combinations similar to those in the growth chamber study, field experiments were conducted on a Marietta fine sandy loam (a fine-loamy,

mixed, thermic, siliceous Aquic Fluventic Eutrochrepts) located at the Rodney R. Foil Plant Science Research Center (Starkville, MS) and on a Brooksville Demopolis silty clay loam (a loamy, carbonatic, themric, shallow, Typic Udorthents) located at the Black Belt Branch Experiment Station (Brooksville, MS) during 1998 and 1999. Field plots consisted of 4 rows, 6.1 m long with 0.97 m intra-row spacing in 1998 and were reduced to two rows of the same dimensions in 1999. Field experiments were replicated three times and analyzed as a randomized complete block with split-split restrictions. Trifluralin (α,α,α-trifluor,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) was applied pre-plant at the rate of 1.12 kg a.i. ha^{-1} to control weeds. Germination tests were conducted prior to planting each year and pure live seed (PLS) was used to establish a population of 63 456 plants ha^{-1} in each plot. Seedling percent emergence was calculated at 8 and 15 days after planting (DAP) pure live seed (PLS). Seed loss to seed rot was determined in a manner similar to evaluations in the growth chamber studies.

Statistical analysis was conducted using the Statistical Analysis System (SAS Institute 1994). Analysis of variance was accomplished using the General Linear Models (GLM) procedure, and means were separated by the least significant difference (LSD) test. Pearson's correlation coefficients were calculated to evaluate relationships between the data.

Results and discussion

Growth chamber studies utilizing a known *F. oxysporum* infestation level in the soil media showed that seed treatment with either Vitavax 200 or Apron FL was effective in reducing induced seed rot over the untreated control (Table 1). Protection provided by Apron FL was not significantly better than Vitavax 200. Seed exposed to accelerated aging during storage performed poorly independent of cultivar; similar results have been reported in pea by Short and Lacy (1976) and Harman *et al.* (1978). In the field studies, application of either seed treatment did not result in consistent improved emergence (Table 1). The conductivity test differed significantly among cultivars for 1998 but not in 1999 (Table 2). Yaklich *et al.* (1979) and Vieira *et al.* (1992) reported similar cultivar differences for seed conductivity in soybean. Seed stored 20 months showed greater conductivity than seed stored only 8 months, however, emergence in 1998 did not differ in the growth chamber (Table 2). In 1999, seed did not differ in the conductivity test when stored for 20 or 8 months. Under ideal warehouse conditions, storing soybean seeds longer than one year typically leads to a decline in field performance, particularly in the southern United States, unless storage is in a controlled environment (Delouche 1977). However, seed stored for 20 months actually increased in growth chamber emergence over 8 months storage in 1999 (Table 1). Reasons for this increase were not apparent. In both years, accelerated aging resulted in significantly reduced emergence (Table 1) and elevated seed conductivity (Table 2).

In the field, seedling emergence was very low for each year of the experiment although all seed lots prior to planting were above 80% minimum germination required for commercial sale. In 1998 low emergence was attributed, in part, to poor seed to soil contact. Prior to planting in 1999, a second set of press wheels were added to the planter. Seedling emergence was significantly influenced by a cultivar × storage interaction in 1998 but not in 1999 (data not shown). Neither cultivar nor storage treatment resulted in consistent field emergence from year to year; positions for these variables changed in rank from location to location in 1998 (Table 1). In 1998 field soil temperatures ranged from 23 to 25°C at planting and remained optimum throughout germination and emergence. In 1999, soil temperature at planting averaged 24°C over both sites. At both locations in 1999, ambient temperature dropped to 10°C, accompanied by rainfall saturating the soil to field capacity immediately after planting and persisting for 7 days. The drop in air temperature lowered soil temperatures to 14–16°C for 7 days. These conditions in 1999 contributed to increased seed rot and delayed emergence. Seedling emergence did not occur until 8 days after planting at both locations. This time period for emergence is similar that reported for soybean exposed to *F. oxysporum* (Farias and Griffin 1989). Tyagi and Tripathi (1983) reported that temperatures below 20°C delayed germination up to twice as long as that at 30°C, and the lag in time to complete germination was likely sufficient to predispose the seed to *Fusarium* sp., as well as other pathogens in the field. Single fungicide seed treatments did not improve field emergence compared with the control except at Starkville in 1999 (Table 1).

The conductivity test was not significantly correlated with seedling emergence after 15 days at both field locations in 1998; however, it was significantly correlated with emergence in the growth chamber under temperature conditions mimicking ambient field conditions that year (Table 3). No loss to soybean seed rot was observed in any of the field plots in 1998. Under growth chamber conditions (20°C for 10 hours increasing to 27°C for 14 hours and saturated soil) in 1998, seed rot occurred when seed was planted in the presence of a known level of *F. oxysporum*. In 1999, the conductivity test was significantly negatively correlated with seedling emergence when cool, saturated soil conditions persisted for 7 days immediately after planting (Table 3). Keeling (1974) reported that high soil-moisture levels immediately after planting increased seed exudate and incidence of seed rot. In 1999, a high incidence of seed rot was observed in the field at both locations as well as in the growth chamber, which contributed to reduced emergence and stand establishment. The negative relationship between electrolyte leakage and emergence observed in this study is similar to that reported in pea (Short and Lacy 1976, Kraft 1986) and common bean (Schroth and Cook 1964). Seed exhibited overall greater seed leachate conductivity in 1999 (Table 2). This combined with cool, saturated soils, was associated with increased seed rot and decreased seedling emergence in the field and growth chamber (Table 1).

Use of a seed treatment prior to planting soybean is a recommended practice,

Table 1. Per cent emergence for soybean cultivars under different storage conditions and seed treatments in the growth chamber and in the field at Brooksville and Starkville, MS in 1998 and 1999.

	Per cent emergence					
Treatment	Growth chamber		Brooksville		Starkville	
Cultivar	1998	1999	1998	1999	1998	1999
Hartz 5999	57.6a[A]	24.8a	31.7a	13.2a	44.1a	32.9b
Hutcheson	46.9b	25.6a	33.1a	9.8 a	40.5a	32.2b
Deltapine 5354	59.3a	21.1b	37.2a	14.1a	44.4a	36.2a
Storage						
8 months	61.3a	32.6b	38.8a	16.7a	49.8a	48.1b
20 months	59.1a	36.3a	25.3b	20.4a	34.9c	51.4c
Aged	43.3b	2.6c	37.8a	0.0b	44.3b	1.4c
Seed treatment						
Control	58.9b	19.6b	34.4ab	13.1a	41.4a	30.9b
Apron® FL	73.8a	27.0a	32.0b	10.7a	44.3a	33.2a
Vitavax® 200	71.3a	24.8a	35.6a	13.3a	43.3a	37.2a

[A] Means within a column for cultivar, storage, or seed treatment followed by the same letter were not significantly different (LSD=0.05).

Table 2. Mean conductivity values for three soybean cultivars and three storage treatments in 1998 and 1999.

Treatment	Conductivity (μS) seed^{-1}	
Cultivar	1998	1999
Hartz 5999	513.41a[A]	661.68a
Hutcheson	498.43a	680.53a
Deltapine 5354	425.31b	621.93a
Storage		
8 months	290.64c	481.15b
20 months	335.76b	533.87b
Aged	810.74a	917.17a

[A] Means within a column for cultivar or storage followed by the same letter were not significantly different (LSD=0.05).

Table 3. Correlation coefficients comparing soybean seed for conductivity (μS seed^{-1}) with growth chamber emergence or field emergence at 15 days after planting in 1998 and 1999.

Location	Year	Correlation coefficient
Growth chamber	1998	-0.64*
Brooksville	1998	-0.15
Starkville	1998	-0.10
Growth chamber	1999	-0.92**
Brooksville	1999	-0.93**
Starkville	1999	-0.97**

*,** Significant at 0.10 and 0.01 level of probability, respectively.

particularly when planting into cool, saturated soils in the early spring. These conditions prevailed in the field during this study in 1999. In the growth chamber where a known quantity of *F. oxysporum* was present, use of either Vitavax 200 or Apron FL proved effective in reducing the incidence of seed rot and improving emergence. Although field results in this study were not consistent, application of a seed fungicide treatment remains a recommended practice where fungal pathogens are present or when planting into potential stress environments conducive to their proliferation.

The conductivity test has been shown to accurately estimate seed performance in the field (Abdul-Baki and Anderson 1973, Custdio and Marcos-Filho 1997). The conductivity test did not consistently predict seed performance in either the growth chamber or the field based on length of storage, and storage of soybean seed for 20 months is not recommended due to decline in vigour. Only when seed was artificially aged at high temperature and relative humidity did the conductivity test consistently show seed vigour had declined. Based on correlation analysis there was a relationship between loss due to seed rot and the conductivity test in the growth chamber where a known quantity of *F. oxysporum* was present. This relationship was documented in 1999 under cool, saturated soil conditions. Under those conditions, germination was slowed and seeds were more subject to *F. oxysporum* invasion. Consequently, emergence of healthy seedlings was reduced. Based on results of this research, the conductivity test has potential as a predictive tool for soybean susceptibility to pathogen invasion when inoculum is present under cool, saturated soil conditions.

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