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Seed-borne pathogens and electrical conductivity of soybean seeds

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ABSTRACT: Adequate procedures to evaluate seed vigor are important. Regarding the electrical conductivity test (EC), the interference in the test results caused by seed-borne pathogens has not been clarified. This research was carried out to study the influence of *Phomopsis sojae* (Leh.) and *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx. fungi on EC results. Soybean seeds (*Glycine max* L.) were inoculated with those fungi using potato, agar and dextrose (PDA) medium with manitol (-1.0 MPa) and incubated for 20 h at 25 °C. The colony diameter, index of mycelial growth, seed water content, occurrence of seed-borne pathogens, physiological potential of the seeds, measured by germination and vigor tests (seed germination index, cold test, accelerated aging and electrical conductivity), and seedling field emergence were determined. The contents of K^+, Ca^{2+}, and Mg^{2+} in the seed and in the soaking solution were also determined. A complete 2 × 4 factorial design with two seed sizes (5.5 and 6.5 mm) and four treatments (control, seeds incubated without fungi, seeds incubated with *Phomopsis* and seeds incubated with *Colletotrichum*) were used with eight (5.5 mm large seeds) and six (6.5 mm large seeds) replications. All seeds submitted to PDA medium had their germination reduced in comparison to the control seeds. This reduction was also observed when seed vigor and leached ions were considered. The presence of *Phomopsis sojae* fungus in soybean seed samples submitted to the EC test may be the cause of misleading results.
Keywords: *Glycine max*, *Phomopsis sojae*, *Colletotrichum dematium* var. *truncata*, vigor

Introduction

Tests used for assessing seed vigor are intended to identify basic differences in physiological potential between lots with similar germination (AOSA, 2002; Vieira et al., 1994; Vieira and Krzyzanowski, 1999). The most studied quick tests are related to the initial events of the seed deterioration process such as the loss of cell membrane integrity and reduction of respiratory and biosynthetic processes (Tokushisa et al., 2009).

The electrical conductivity (EC) test is one of those used to evaluate soybean seed vigor (Colete et al., 2004; Panobianco et al., 2007). The relation between water content, organizational level of seed cellular membranes and quantity of leachates in the soaking solution is the theoretic base of the EC test. Thus, the higher the speed of the restoring of cell membrane integrity which occurs at the onset of the germination process, the lower the amount of leachates released to the soaking solution, indicating high seed vigor (Carvalho et al., 2009).

Several factors affect EC test results, such as seed size, temperature, imbibition period, initial seed water content and storage temperature (Loeffler et al., 1988; Panobianco and Vieira, 1996; Tao, 1978; Vieira et al., 2001; Vieira et al., 2002; Panobianco et al., 2007). However, the influence of pathogens present on the seeds under analysis has not been sufficiently clarified. Seed-borne pathogen reduces the physiological potential of soybean seeds (Galli et al., 2007).

Colletotrichum dematium (Pers. ex Fr.) Grove var. *truncate* (Schw.) Arx. and *Phomopsis sojae* (Leh.) are reported as important fungi associated to soybean seeds. They can cause severe yield reduction and deterioration of seeds, leading to decreased physiological potential, field seedling emergence and storage period (Sinclair and Backman, 1989).

Effects of seed pathogens on EC results were observed by Panizzi and co-workers[¶]. They found a positive correlation between EC and accelerated aging results, that is, seeds with low germination after accelerated aging showed low EC results, or, in other words, the results of one test contradicted the results of the other. Based on these observations a hypothesis was formulated stating that fungi developing in the seed would utilize the nutrients leached from the seed and this would produce EC results that do not measure the real amount of ions and organic substances leached from the seeds.

Considering that the EC results may be influenced by seed-borne pathogens, the effect of *Phomopsis sojae* and *Colletotrichum dematium* var. *truncata* on electrical conductivity test results in soybean seeds was evaluated.

Materials and Methods

The study was conducted in Jaboticabal, state of São Paulo, Brazil (21°15′ S, 48°18′ W; altitude 613.38 m), using two seed sizes of the soybean cultivar Embrapa 48 (5.5 and 6.5 mm round hole sieves with 92 and 95 % germination, respectively). *Phomopsis sojae* and *Colletotrichum dematium* var. *truncata* fungi were isolated from soybean seeds, multiplied using PDA culture

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medium (200 g of potato extract, 18 g of agar, 20 g of dextrose, and 1000 mL of distilled water), conserved in test tubes containing the fungal colonies of the culture medium, and covered with sterile mineral oil. The fungi were transferred into the Petri dishes containing PDA culture medium or PDA culture medium plus manitol, at a water potential of -1.0 MPa. Solution concentrations were prepared using Van't Hoff's formula (Salisbury and Ross, 1991).

A 40 mm disk of each pure fungal colony was chopped and transferred to individual Petri dishes, which were randomly distributed in a growth chamber at 25 °C, for 24 days. A preliminary evaluation was conducted, comparing fungal development of the two culture media with and without water restriction (0.0 and -1.0 MPa). The restriction of water potential has the objective to prolong the contact of the seed with the pathogen, favoring greater fungal incidence without seed germination (Machado et al., 2001). Ten dishes were used for each fungus species and concentration. Evaluations were made daily, measuring mycelial growth of each fungus through the underside of the plaques until one of the treatments covered the entire plaque. Colony diameter and mycelial growth index (MGI) were determined using the formula proposed by Oliveira as reported by Machado et al. (2001).

For fungal inoculation, seeds without cracks were visually selected and then disinfected with sodium hypochlorite + distilled water (1:1) for 30 s, rinsed with autoclaved distilled water, and dried at room temperature on sterilized sheets of filter paper. These seeds were then distributed in a single layer over colonies of each fungus, developed on Petri dishes with PDA culture medium (-1.0 MPa), and slightly pressed into the medium. After 20 h, seeds were removed and kept in a dry chamber with a forced air flow, at an initial temperature of 25 °C, which was raised first to 27 °C, then 29 °C, reaching a maximum of 32 °C, until seeds returned to their initial weight. Next, seeds were maintained in a cold chamber (10 °C) for seven days until all seeds of the treatments control (pure seeds), control in PDA culture medium (-1.0 MPa) without fungus, seeds inoculated with the *Phomopsis sojae* fungus (-1.0 MPa) and those inoculated with the *C. dermatium* var. *truncata* fungus (-1.0 MPa) reached hygroscopic equilibrium. Following that, the seeds were submitted to field and laboratory tests as described:

Seed water content (SWC): two 20-seed samples per treatment were used to determine SWC by means of the oven method at 105 ± 3 °C for 24 h (ISTA, 1999). SWC was determined before and after they were exposed to accelerated aging.

Germination: eight 5.5-mm seed samples and six 6.5-mm seed samples (for both sizes seed samples were formed by 50 seeds), disinfected and non-disinfected, were distributed in plastic boxes (26 × 16 × 8.5 cm) with sand at room temperature (25-30 °C) for the germination test. Normal seedlings were counted on the eighth day after sowing, according to procedures in ISTA (1999).

Vigor – Speed of germination index (SGI)*:* was calculated applying the germination test in sand. Emerged seedlings were counted daily until the number of seedlings became stable. At the end of the test, the SGI was calculated based on the daily seedling emergence data (Maguire, 1962).

Vigor – Electrical conductivity (EC): was calculated using eight 5.5-mm 50 seed samples and six 6.5-mm 50 seed samples. All seeds were weighed to one hundredth of a gram. Then, seeds were soaked in 75 mL deionized water at 25 °C for 24 h. After this period, EC readings were made with the help of a conductivity meter. The results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds (Hampton and TeKrony, 1995; Vieira and Krzyzanowski, 1999).

Vigor – Accelerated Aging (AA): was calculated using eight 5.5-mm 50 seed samples and six 6.5-mm 50 seed samples taken from a 42 g seed sample spread in a single layer on a stainless steel screen in plastic germination boxes (11.0 × 11.0 × 3.5 cm). Distilled water (40 mL) was added to each box and maintained at 41 °C for 48 h (Marcos Filho, 1999). Seeds were submitted to the germination test after the aging period (ISTA, 1999).

Vigor – Cold Test: was calculated using the plastic box with soil method; a 2/3 sand and 1/3 soil mixture was used as a substrate. Soil was obtained from an area cultivated with soybean. Eight 50 5.5-mm seed samples and six 50 6.5-mm seed samples were used. The substrate was placed in plastic boxes (26 × 16 × 8.5 cm), sown with seeds and covered with an approximately 3 cm layer of the same substrate and moistened to 70 % of its water holding capacity. The boxes were covered and conditioned in a cold room at 10 °C for seven days. After this period, the boxes were removed from the cold chamber and taken to a room kept at alternating temperatures of 25 and 30 °C for five days. At the end of this period, the number of normal seedlings was counted (AOSA, 2002; Barros et al., 1999).

Seedling emergence in the field (SE): was conducted using eight fifty 5.5 mm seed samples and six fifty 6.5 mm seed samples. Seeds were manually distributed in 1.5 m long and 0.25 m apart rows at 2-3 cm depth. Emerged seedling evaluation was made 14 days after sowing (Nakagawa, 1999).

Seed health: Each treatment consisted of 200 disinfected soybean seeds [seeds were exposed to a sodium hypochlorite + distilled water (1:3) solution for three minutes] and 200 non disinfected seeds, in accordance with procedures recommended by the filter paper method (Limonard, 1966). Seeds were evenly placed on Petri dishes (10 seeds/dish) on three sheets of filter paper soaked in distilled water and maintained in an incubation chamber

for seven days at a temperature of 20 ± 2 °C and 12 h of white light alternating with 12 h of darkness. The incidence of fungus on the seeds was evaluated at the end of this period with the help of a stereoscopic microscope.

Chemical composition of the soaking solution: After the electrical conductivity reading, seeds along with the solution were poured into a container using a funnel and Whatman Grade nº 1 Filter Paper in order to separate the soaking solution. In the soaking solution, potassium levels were determined by the flame photometric method and magnesium and sodium levels by the atomic absorption spectrometry method (Bataglia et al., 1983). Values were expressed in mg of ion kg^{-1} of seeds.

Chemical composition of seeds: Eight and six repetitions of 200 seeds each were used for 5.5 and 6.5 mm seed sizes, respectively. Seeds were maintained in an oven at 60 °C for 12 h. Afterwards, seeds were ground in an industrial micro mill, digested and analyzed as described above for chemical composition of the soaking solution (Bataglia et al., 1983).

Statistical analysis: A completely random design in a 2 × 4 factorial arrangement with two seed sizes - 5.5 mm and 6.5 mm - and four treatments (control, seeds in PDA culture without fungi, seeds inoculated with *P. sojae* fungus and seeds inoculated with *C. dermatium* var. *truncata*) were used with eight and six replications respectively for 5.5 and 6.5 mm seed sizes. Also a completely random design with ten replications was used for fungal colony growth with means compared by the Tukey test ($p < 0.05$) for each fungus. None of the data were transformed for the statistical analysis.

Results and Discussion

Seed water contents varied initially between 8.9 and 9.2 %, reached values between 28 and 33.8 % after PDA incubation and returned to 8.5 to 9.4 % following drying after inoculation. These variations, according to Marcos Filho (1999), were within acceptable levels. Seed water content (SWC) after accelerated aging was also within acceptable levels, i.e., between 27 and 29.5 %. Similar SWC values for soybean accelerate aged seeds were reported by Colete et al. (2004) - 27.2 to 28.4 % and Vanzolini and Carvalho (2002) - 27.2 to 29.0 %.

Water restriction promoted by manitol did not inhibit mycelial growth of *P. sojae* and *C dematium* var. *truncata* fungi. On the contrary, mycelial growth of these organisms increased compared to that on PDA without water restriction. Machado et al. (2001) reported similar results with the same pathogens (Table 1).

Control (pure seeds) and control in PDA culture medium soybean seeds initially presented low incidence of *P. sojae* and *C. dematium* var. *truncata* fungi, the values observed varying between 0.0 and 1.5 % (Table 2). After artificial inoculation of both pathogens, the incidence of these fungi increased. The greatest incidence was detected in the 5.5 mm seeds, in which *P. sojae* infection was of 72.5 % in non-disinfected seeds and of 25 % in disinfected seeds, whereas *C. dematium* var. *truncata* infection was of 79.2 % in non-disinfected seeds and of 3 % in disinfected seeds. Cultivar Embrapa 48 is considered to be moderately resistant to *Phomopsis sp* according to cultivars reaction (Embrapa Soja, 2006). Seeds after inoculation with *Phomopis* and *Colletotrichum* showed 0 to 1.5 % incidence of four other fungi including saprophytic and storage fungi (*Cladosporium* sp., *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp.).

Variation in germination of the soybean seeds infected with *Phomopsis* sp. was observed in the same lot at distinct storage periods (Henning, 1996). In other words, the fungus in seeds stored under favorable conditions rapidly lost its viability and this brought about improved laboratory germination results. Increased germination depends on the initial physiological potential of the seed (Henning, 1996); however, seedlings that achieve emergence are weak and, or have infected cotyledons, resulting in systematically infected plants whose symptoms only appear near maturity.

Table 1 – Diameter of colony and mycelial growth index of *Phomopsis sojae* and *Colletotrichum dematium* var. *truncata* fungi, using medium culture of PDA and PDA plus manitol (-1.0 MPa).

Fungus	PDA	PDA + Manitol (-1.0 MPa)
	Diameter of colony	
	cm	
Phomopsis sojae	6.89 b	8.60 a
Colletotrichum dermatium var. truncata	5.80 b	8.46 a
	Mycelial growth index	
Phomopsis sojae	1.4 b	1.9 a
Colletotrichum dermatium var. truncata	0.9 b	0.5 a

Means in the line, followed by the same letter, are not different (Tukey test, $p \geq 0.05$)

Table 2 – Incidence of *P. sojae* and *C. dematium* var. *truncata* fungi in soybean seed lots as function of seed treatments: Control (pure seeds, without inoculation), PDA (seeds without inoculation, kept on a PDA culture medium for 20 hours) and Inoculated Seeds (either with *P. sojae* or *C. dematium* var. *truncata* fungi, for 20 hours).

Control				PDA (-1.0 MPa)				Inoculated Seeds (-1.0 MPa)			
ND[1]		WD		ND		WD		ND		WD	
5.5[2]	6.5	5.5	6.5	5.5	6.5	5.5	6.5	5.5	6.5	5.5	6.5
Incidence of *Phomopsis sojae* %											
0.5	1.0	0.5	0.0	0.0	1.5	0.0	0.5	72.5	64.0	25.0	8.5
Incidence of Colletotrichum *dermatium* var. *truncate* %											
1.0	0.5	0.0	0.0	0.5	1.0	0.5	1.0	79.5	75.0	3.0	2.0

[1]Disinfection with sodium hypochlorite: seeds without disinfection (ND) and with disinfection (WD); [2]Sieves 5.5 and 6.5 mm.

Germination results in sand of the control seeds in PDA culture medium (STB), seeds inoculated with *P. sojae* (SIP) and *C. dematium* var. *truncata* (SIC) showed no differences ($p \geq 0.05$). On the other hand, the germinative performance of the control (ST) was very high: 92 %, the 5.5-mm seeds and 95 %, the 6.5-mm ones ($p < 0.05$). The drop in seed germination observed for the STB treatment is supposed to be due to the high susceptibility of the Embrapa 48 cultivar seeds to imbibing damage (França Neto et al., 1998).

The 6.5-mm seeds had higher germination in sand and higher vigor evaluated by the speed of germination and cold test than the remaining treatments. This may be explained by the fact that the 6.5-mm seeds were of better sanitary and physiological quality (Tables 2 and 3). This can be associated with inoculated tissue in the seed, being better when located only on the tegument (Zorrila et al., 1994). However, if the inoculation is located on the cotyledons, the correlation is present under all environmental conditions (Dhingra and Acuña, 1997).

Vigor (Table 4) evaluated by the speed of germination index (SGI) of seedling emergence in sand, cold test (CT) and seedling emergence in the field (SE) showed no differences among the treatments control seeds in PDA culture medium (STB), seeds inoculated with *Phomopsis sojae* (SIP) and seeds inoculated with *C. dematium* var. *truncata* (SIC). There were differences only between these treatments and the control (ST).

Environmental conditions during field experiment were characterized by rainy days favoring seedling field emergence. On the other hand, seed deterioration after sowing was more pronounced and consequently, the establishment of a plant population was less than if seeds were sown in soil with low moisture level, which does not allow rapid germination and seedling emergence; given that *Phomopsis* spp. fungus grows quickly in soil with low moisture (Gleason and Ferris, 1985), thereby reducing the number of emerging seedlings. An inverse correlation between percent of seeds infected by *Phomopsis* spp. and percent germination in a seed lot (Henning and França Neto, 1980) is not always confirmed under field conditions, where there is also a large variability between germination test results in the laboratory and seedling emergence in the field (Dhingra and Acuña, 1997).

Table 3 – Germination of soybean seeds using sand as substrate, as function of seed sizes and treatments: control (ST), seed control in PDA culture medium (STB), seeds inoculated with *P. sojae* (SIP) and seeds inoculated with *C. dematium* var. *truncata* (SIC) fungi.

Sieves	ST	STB	SIP	SIC	Means
			%		
5.5 mm	92	66	69	68	74 B
6.5 mm	95	77	77	70	80 A
Means	94 a	72 b	73 b	69 b	
CV (%)	7.5				

Means followed by the same capital letter in the column and small letter in the line are not different (Tukey test, $p \geq 0.05$).

There was an interaction between seed size and seed treatment (Table 4). The small seeds (5.5 mm), inoculated with *C. dematium*, had the lowest germination after aging. This treatment, which had the greatest incidence of this fungus (Table 2), with values varying from 79.5 % for non-disinfected seeds to 3 % for disinfected seeds, differed only from the control. When the 6.5 mm large seeds are examined, those inoculated with *P. sojae* show the lowest percentage of normal seedlings (56.7 %) for a fungal incidence of 64 % in the non-disinfected seeds and of 8.5 % in the disinfected seeds (Table 2), indicating less vigor compared to STB and SIC .

Damage caused by these pathogens may be directly related to their inoculum potential and the seed locus where fungal penetration takes place (Machado, 1988). The extent of their damage depends also on seed physiological quality – the higher the seed vigor, the more resistant it is to fungal penetration (Mycock and Berjak, 1995).

Table 4 – Vigor evaluated by speed of germination index, cold, accelerated aging and electrical conductivity tests and seedling field emergence as function of seed treatments: control (ST), seed control in PDA culture medium (STB), seeds inoculated with *P. sojae* (SIP) and seeds inoculated with *C. dematium* var. *truncata* (SIC) fungi.

Sieves	ST	STB	SIP	SIC	Means
			Speed of Germination Index		
5.5 mm	13.5	10.3	9.6	9.5	10.7 B
6.5 mm	14.3	11.3	10.8	11.2	11.9 A
Means	13.9 a	10.8 b	10.2 b	10.3 b	
CV (%)	6.7				
			Cold Test		
			%		
5.5 mm	84.8	62.3	59.8	59.5	73.8 B
6.5 mm	87.7	70.8	72.8	70.0	79.9 A
Means	86.2 a	66.5 b	66.3 b	64.8 b	
CV (%)	7.8				
			Accelerated Aging		
			%		
5.5 mm	92.3 Aa	52.0 Bb	52.6 Ab	49.5 Bb	-
6.5 mm	94.3 Aa	60.3 Abc	56.7 Ac	66.3 Ab	-
CV (%)	8.6				
			Electrical Conductivity		
			$\mu S\ cm^{-1}\ g^{-1}$		
5.5 mm	98.3	86.3	76.7	88.9	87.5 A
6.5 mm	89.9	78.6	73.4	86.4	82.1 B
Means	94.1 a	82.4 b	75.1 c	87.6 b	
CV (%)	6.2				
			Seedling emergence		
			%		
5.5 mm	91.8	69.0	71.8	75.3	79.6 A
6.5 mm	92.3	76.3	76.8	73.0	76.9 B
Means	92.0 a	74.2 b	74.1 b	72.7 b	
CV (%)	11.6				

Means followed by the same capital letter in the column and small letter in the line are not different according (Tukey test, $p \geq 0.05$); CV (%) = Coefficient of Variation.

A difference between the control and control in PDA was observed in all vigor tests (Table 4). Seeds were placed in PDA culture medium and allowed to imbibe for 20 h until SWC reached 28 % for both seed sizes.

EC results (Table 4) showed differences between the treatments STB, SIP, and SIC and the control (ST). The seeds exposed to contamination by *Phomopsis* (SIP) were, apparently, of quality superior to that of the control (ST) as well as to those of the STB and SIC treatments. These results indicate that soybean seeds contaminated with *Phomopsis sojae* may show a lower EC result (higher vigor) although they may have similar performances in other seed vigor tests such as speed of germination index and cold test. SIC treatment presented results similar to those of AA. Considering that the SWC reached by seeds after inoculation and EC data (Table 4), it was found that seeds inoculated with *P. sojae* fungus presented values different from those of the control seeds in PDA culture. However, SWC of the latter reached the greatest percentages of 33.8 and 31.0 % respectively for seed sizes 5.5 and 6.5 mm.

Seeds of different sizes as well as seeds submitted to the different treatments differed ($p \leq 0.01$) as to amounts of leached potassium and magnesium (Table 5). On the other hand, only seed treatments had an influence on the amount of leached calcium since there was an interaction between seed size and treatment for this mineral.

Potassium was verified to be the cell component which leached most in comparison with the other elements studied (Table 5). This fact has prompted several researchers to pay special attention to potassium, suggesting the quantity of leached potassium in the soaking solution as a means to measure soybean seed vigor (Custódio and Marcos Filho, 1997; Dias et al., 1995, 1997). Inoculating the seeds with *P. sojae* resulted in amounts of leached potassium, calcium, and magnesium lower than those resulting from the inoculation of the seeds with *C. dematium* var. *truncata*.

Table 5 – Potassium, calcium, and magnesium leachates in soybean seeds soaking solution as functions of seed sizes and treatments: control (ST), seed control in PDA culture medium (STB), seeds inoculated with *P. sojae* (SIP) and seeds inoculated with *C. dematium* var. *truncata* (SIC).

Sieves	ST	STB	SIP	SIC	Means
			Potassium		
			mg kg^{-1}		
5.5 mm	2736.6	2341.6	1986.8	2319.0	2346.0 A
6.5 mm	2447.0	2091.7	1897.0	2296.0	2183.0 B
Means	2591.8 a	2216.7 b	1941.8 c	2307.5 b	
CV (%)	6.3				
			Calcium		
			mg kg^{-1}		
5.5 mm	74.5 Aa	58.3 Ab	47.5 Ac	52.6 Bbc	58.2 A
6.5 mm	74.5 Aa	53.5 Abc	51.6 Ac	59.2Ab	59.7 A
Means	74.5 a	55.9 b	49.5 c	55.9 b	
CV (%)	8.1				
			Magnesium		
			mg kg^{-1}		
5.5 mm	87.4	60.4	53.1	56.9	64.5 B
6.5 mm	92.0	63.2	59.7	74.0	72.2 A
Means	89.7 a	61.8 bc	56.4 c	65.5 b	
CV (%)	11.0				

Means followed by the same capital letter in the column and small letter in the line are not different (Tukey test, $p \geq 0.05$); CV (%) = Coefficient of Variation.

Regarding the calcium ion, there was an interaction ($p \leq 0.05$) between seed size and treatment. For 5.5 mm seed size, SIP was different from STB, and for 6.5 mm seed size, SIP and SIC were different. Variation (Table 5), particularly that of seeds inoculated with *P. sojae* (SIP), explains the low EC values obtained (Table 4).

There was an interaction for calcium between seed size and treatment. There was a difference between STB and SIC for K^+ (seed sizes 5.5 and 6.5 mm), Ca^{2+} (seed sizes 5.5 and 6.5) and Mg^{2+} (seed size 5.5). The latter was different for STB and SIC treatments, but was not different for treatments with 6.5 size seeds.

The question that still remains to be answered is why seeds infected with *P. sojae* present in the soaking solution lower contents of K^+, Ca^{2+} and Mg^{2+} and consequently lower EC than non-inoculated seeds in PDA culture medium (STB). Assuming that the PDA medium had a priming effect on the seeds, a consequent increment in their physiological performance was to be expected, as reported by some authors (Nascimento, 2003; Nascimento and Aragão, 2004) for melon seeds. These authors emphasize that these results are much clearer when these seeds are placed to germinate under conditions of thermal stress, but the results here reported show that it did not happen, and all the seeds submitted to PDA displayed a poorer performance than the control seeds.

The best performance in the EC test was displayed by the seeds inoculated with *P. sojae* (SIP treatment) (Table 4). This result suggests that the fungus consumed potassium ions from the seed during the first 20 h after inoculation. This observation may be explained by the lower value of the leached ion (Table 5) and by the chemical composition of the seed (Table 6), that did not differ from those of STB treatments, indicating that the potassium ion present in the fungus was not liberated during the soaking period.

Observing the EC and leached ions results (Tables 4 and 5), it is still difficult to say that the seed infected by *Phomopsis* fungi can affect the usefulness of EC as a vigor test. It demands more research, especially using seeds infected with *Phomopsis* during seed production in the field at different levels. Thus, if seeds from the field were taken it is possible to avoid the effect of the conditioning period during artificially seed infection.

To explain the reduction of EC as well as the leaching of potassium, calcium and magnesium into the soaked seed solution due the presence of the seed-borne pathogen, especially *Phomopsis sojae* it can be hypothe-

Table 6 – Potassium, calcium and magnesium contents of soybean seeds, cultivar Embrapa 48, as functions of seed size and the treatments: control (ST), seed control in PDA culture medium (STB), seeds inoculated with *P. sojae* (SIP) and seeds inoculated with *C. dematium* var. *truncata* (SIC) fungi.

Sieves	ST	STB	SIP	SIC	Means
			Potassium		
			mg kg^{-1}		
5.5 mm	17.4	17.2	16.9	16.6	17.0 A
6.5 mm	16.6	16.3	16.2	16.1	16.3 B
Means	17.0 a	16.8 ab	16.5 bc	16.3 c	
CV (%)	2.0				
			Calcium		
			mg kg^{-1}		
5.5 mm	1.4	1.5	1.4	1.4	1.41 A
6.5 mm	1.4	1.4	1.4	1.3	1.38 B
Means	1.41 ab	1.42 a	1.37 ab	1.35 b	
CV (%)	3.8				
			Magnesium		
			mg kg^{-1}		
5.5 mm	1.95 Bbc	1.90 Bc	1.99 Bab	2.01 Ba	1.96 B
6.5 mm	2.10 Aa	2.10 Aa	2.10 Aa	2.08 Aa	2.10 A
CV (%)	1.83				

Means followed by the same capital letter in the column and small letter in the line are not different (Tukey test, $p \geq 0.05$); CV (%) = Coefficient of Variation.

sized for those seeds submitted to PDA medium that the fungus promoted some consumption of the ions, because the reduction on the EC results was followed by reductions on K^{+}, Ca^{2+} and Mg^{2+}.

Conclusion

Soybean seeds infected with *P. sojae* may produce misleading positive EC results.

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References

Association of Official Seed Analiysts [AOSA]. 2002. Seed Vigor Testing Handbook. AOSA, Lincoln, NE, USA.

Barros, A.S.R.; Lima Dias, M.C.L.; Cicero, S.M.; Krzyzanowski, F.C. 1999. Cold test. chp. 5, p. 1-15. In: Krzyzanowski, F.C.; Vieira, R.D.; França Neto, J.B., eds. Seed vigor: concepts and tests. Abrates, Londrina, PR, Brazil (in Portuguese).

Bataglia, O.C.; Furlani, A.M.C.; Teixeira, J.P.F.; Furlani, P.R.; Gallo, J.R. 1983. Methods of chemical analysis of plants. Instituto Agronômico de Campinas, Campinas, SP, Brazil (in Portuguese).

Carvalho, L.F.; Sediyama, C.S.; Reis, M.S.; Dias, D.C.F.S.; Moreira, M.A. 2009. Influence of soaking temperature of soybean seeds in the electrical conductivity test to evaluate physiological quality. Revista Brasileira de Sementes 31: 9–17 (in Portuguese, with abstract in English).

Colete, J.C.F.; Vieira, R.D.; Dutra, A.S. 2004. Electrical conductivity and soybean seedling emergence. Scientia Agricola 61: 386-391.

Custódio, C.C.; Marcos Filho, J. 1997. Potassium leakage test for the evaluation of soybean seeds physiological quality. Seed Science and Technology 25: 549–564.

Dias, D.C.F.S.; Marcos Filho, J.; Carmelo, Q.A.C. 1995. Potassium leaching test for the evaluation of soybean seed vigor (*Glycine max* (L.) Merril). Scientia Agricola 52: 444–451 (in Portuguese, with abstract in English).

Dias, D.C.F.S.; Marcos-Filho, J.; Carmelo, Q.A.C. 1997. Potassium leakage test for the evaluation of vigor in soybean seeds. Seed Science and Technology 25: 7–18.

Dhingra, O.D.; Acuña, R.S. 1997. Pathology of soybean seeds. UFV, Viçosa, MG, Brazil (in Portuguese).

Embrapa Soja. 2006. Technology for Soybean Production, Central Region of Brazil. 2006. Embrapa-Soja, Londrina, PR, Brazil (in Portuguese).

França Neto, J.B.; Krzyzanowski, F.C.; Henning, A.A.; Costa, N.P. 1998. Imbibing Damage of Brazilian Soybean Cultivars During Germination Test. Embrapa Soja, Londrina, PR, Brazil (in Portuguese).

Galli, J.A.; Panizzi, R.C.; Vieira, R.D. 2007. Effect of *Colletotrichum dermatium* var. *truncate* and *Phomopsis sojae* in sanitary and physiological quality of soybean seeds. Summa Phytopathologica 33: 40–46 (in Portuguese, with abstract in English).

Gleason, M.L.; Ferris, R.S. 1985. Influence of water potential on de performance of soybean seeds infected by *Phomopsis* sp. Phytopathology 75: 1236–1241.

Hampton, J.G.; TeKrony, D.M. 1995. Handbook of Vigor Test Methods. ISTA, Zurich, Switzerland.

Henning, A.A. 1996. Seed Pathology. Embrapa-CNPSo, Londrina, PR, Brazil (in Portuguese).

Henning, A.A.; França Neto, J.B. 1980. Evaluation problems in the germination of soybean seed lots with high incidence of *Phomopsis* sp. Revista Brasileira de Sementes 2: 9–22 (in Portuguese, with abstract in English).

International Seed Testing Association [ISTA]. 1999. International rules for seed testing. Seed Science and Technology 27: 1–333 (supplement).

Limonard, T. 1966. A modified blotter test for seed health. Netherlands Journal of Plant Pathology 72: 319–321.

Loeffler, T.M.; TeKrony, D.M.; Egli, D.B. 1988. The bulk conductivity test as an indicator of soybean seed quality. Journal of Seed Technology 12: 37–53.

Machado, J.C. 1988. Seed Pathology: Basis and Applications. MEC/ESAL/FAEPE, Brasilia, DF, Brazil (in Portuguese).

Machado, J.C.; Oliveira, J.A.; Vieira, M.G.G.C.; Alves, M.C. 2001. Artificial inoculation of fungi in soybean seeds using manitol solution. Revista Brasileira de Sementes 23: 95–101 (in Portuguese, with abstract in English).

Maguire, J.D. 1962. Speeds of germination-aid selection and evaluation for seedling emergence and vigor. Crop Science 2: 176–177.

Marcos-Filho, J. 1999. Accelerated aging test. chp. 3, p. 1–24. In: Krzyzanowski, F.C.; Vieira, R.D.; França Neto, J.B., eds. Seed vigor: concepts and tests. Abrates, Londrina, PR, Brazil (in Portuguese).

Mycock, D. J.; Berjak, P. 1995. The implications of seed associated mycoflora during store. p. 747–766. In: Kigel, J.; Galili, G., eds. Seed development and germination. Marcel Dekker, New York, NY, USA.

Nakagawa, J. 1999. Vigor tests based on seedling development. chp. 2, p. 1–24. In: Krzyzanowski, F.C.; Vieira, R.D.; França Neto, J.B., eds. Seed vigor: concepts and tests. Abrates, Londrina, PR, Brazil (in Portuguese).

Nascimento, W.M. 2003. Muskmelon seed germination and seedling development in response to seed priming. Scientia Agricola 60:71–75.

Nascimento, W.M.; Aragão, F.S.S. 2004. Muskmelon seed priming in relation to seed vigor. Scientia Agricola 61:114-117.

Panobianco, M.; Vieira, R.D. 1996. Electrical conductivity of soybean soaked seeds. I. Effect of genotype. Pesquisa Agropecuária Brasileira 31: 621-627.

Panobianco, M.; Vieira, R.D.; Perecin, D. 2007. Electrical conductivity as an indicator of pea seed aging of stored at different temperatures. Scientia Agricola 64: 119–124.

Salisbury, F.B.; Ross, C.W. 1991. Plant Physiology. 4ed. Wadsworth, Belmont, CA, USA

Sinclair, J.B.; Backman, P.A. 1989. Compendium of Soybean Diseases. 3ed. APS Press, St. Paul, MN, USA.

Tao, K.L.J. 1978. Factors causing variations in the conductivity test for soybean. Journal of Seed Technology 3: 10–18.

Tokushisa, D.; Sediyama, C.A.Z.; Hilst, P.C.; Dias, D.C.F.S. 2009. Electrical conductivity test for physiological quality evaluation of papaya seeds (*Carica papaya* L.). Revista Brasileira de Sementes 31: 137–145 (in Portuguese, with abstract in English).

Vanzolini, S.; Carvalho, N.M. 2002. Effect of soybean seed vigor on its field performance. Revista Brasileira de Sementes 24: 33–41 (in Portuguese, with abstract in English).

Vieira, R.D.; Carvalho, N.M.; Sader, R. 1994. Vigor tests and its possibility of use. p. 31–47. In: Vieira, R.D.; Carvalho, N.M., eds. Seed vigor tests. Funep, Jaboticabal, SP, Brazil (in Portuguese).

Vieira, R.D.; Krzyzanowski, F.C. 1999. Electrical conductivity test. chp. 4, p. 1–26. In: Krzyzanowski, F.C.; Vieira, R.D.; França Neto, J.B., eds. Seed vigor: concepts and tests. Abrates, Londrina, PR, Brazil (in Portuguese).

Vieira, R.D.; Penariol, A.L.; Perecin, D.; Panobianco, M. 2002. Electrical conductivity and initial moisture content of soybean seeds. Pesquisa Agropecuária Brasileira 37: 1333–1338.

Vieira, D.R.; TeKrony, D.M.; Egli, D.B.; Rucher, M. 2001. Electrical conductivity of soybean seeds after storage in several environments. Seed Science and Technology 9: 599–608.

Zorrila, G.; Knapp, A.D.; McGee, D.C. 1994. Severity of *Phomopsis* seed decay, seed quality evaluation and field performance of soybean. Crop Science 34: 172–177.