

Incidence of pathogens and field emergence of soybean seeds subjected to harvest delay¹

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ABSTRACT – This study aimed at evaluating seed health quality, as well as seedling emergence in the field, of eight soybean cultivars (UFV-16, Splendor, Vencedora, Confiança, UFV-18, UFV-TN 105, Garantia, and Celeste) subjected to different harvest periods. To this, seeds were harvested at the growth stages R8, R8+15 days, and R8+30 days, and then submitted to health test and to the test of seedling emergence in field. Results showed that although seeds harvested at 15 and 30 days after the stage R8 had shown a significant increase of fungi infection, especially by *Fusarium* spp., *Phomopsis* spp., and *Epicoccum* spp., the percent seedling emergence in the field was reduced only to seeds harvested 30 days after the reproductive stage R8. Cultivars have shown differentiated behavior between each other in relation to the percent incidence rate of fungi and seedling emergence in the field; and the seeds least infected by fungi were those allowing the highest percent emergence of seedlings in the field.

Index terms: *Glycine max*, seed health, harvest time.

Incidência de patógenos e emergência em campo de sementes de soja submetidas ao retardamento de colheita

RESUMO - O presente estudo foi realizado com o objetivo de avaliar a qualidade sanitária das sementes, bem como a emergência de plântulas em campo, de oito cultivares de soja (UFV-16, Splendor, Vencedora, Confiança, UFV-18, UFV-TN 105, Garantia e Celeste) submetidas a três diferentes épocas de colheita. Para isso, as sementes foram colhidas nos estádios reprodutivos R8, R8+15 dias e R8+30 dias, e então submetidas aos testes de sanidade e emergência de plântulas em campo. Os resultados mostraram que apesar das sementes colhidas aos 15 e 30 dias após o estágio R8 terem apresentado um aumento significativo da infecção por fungos, especialmente por *Fusarium* spp., *Phomopsis* spp. e *Epicoccum* spp., o percentual de emergência das plântulas em campo foi reduzido somente para as sementes colhidas aos 30 dias após o estágio reprodutivo R8. As cultivares apresentaram comportamento diferenciado entre si, em relação aos percentuais de incidência por fungos e emergência de plântulas em campo, sendo que as sementes menos infectadas por fungos foram as que proporcionaram maior porcentagem de emergência de plântulas no campo.

Termos para indexação: *Glycine max*, sanidade de sementes, época de colheita.

Introduction

The range of diseases affecting soybean crop is rather extensive, and those caused by fungi are considered as of major importance; not only by their higher number, but also by the losses caused to the yield and the quality of the seeds.

Once seeds highly colonized by pathogens, and with low vigor and low viability, usually have low germination rate; when some of these seeds germinate they will produce weak and diseased plants that are the carriers of initial inoculum

for the spread of such pathogens. The problem is exacerbated when the infected seeds do not show any external symptoms, thus being a risk for spreading disease through infected seeds (Krzyzanowski et al., 2008); once these seeds cannot be eliminated during processing, unless they are displaying deformities and/or with their specific mass reduced and which enable their elimination when passing through the density separator and by the gravity table (Dhingra and Acuña, 1997). Moreover, most of the economically important diseases occurring on the soybean crop is caused by seedborn

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pathogens (Henneberg et al., 2012). Therefore, in production systems of high quality seeds, the use of the health test is essential on determining the seed health condition of a given sample; and consequently the seed quality of the whole lot.

The soybean seed reaches its maximum dry mass accumulation and its maximum physiological quality at R7 stage (physiological maturation), when it should be harvested. However, due to the high moisture content present in the seeds at this stage, the recommended harvesting point is the R8 stage, when 95% of pods have the typical coloration of ripe pod, although the seed moisture remains high. Nevertheless, by several factors it is not always possible to harvest the seeds at the most proper time, and thereby delaying the harvest is a need.

When the harvest delay occurs under high temperature, as well as under high relative humidity conditions the amount of seeds infected by pathogenic microorganisms rapidly increases and infection of the seeds generally occurs after onset of pod maturation; although such infection might occur while the pods are still immature (Dhingra and Acuña, 1997). In this regard, some studies have already shown that there is an increase in the ratio of seeds infected by fungi when seeds remain in the field after the reproductive stage R8 (Braccini et al., 2003; Dias et al., 2005).

Soybean seeds subjected to high temperature and high RH throughout the maturation stage until harvest are exposed to an increased fungi incidence, especially from the genera *Fusarium* spp. and *Phomopsis* spp., which are considered the main responsible for reduction on percent germination of the freshly-harvested seeds when these are assessed by the health test in the laboratory (França-Neto and Henning, 1992). In a study carried out on the effect of fungicide application and of harvest time on soybean seeds, Barros et al. (2005) have observed a significant increase on fungi incidence, as well on reduction of percent germination of the seeds subjected to harvest delay. Therefore, when the seeds have high rates of infection, the germination test should be conducted in sand substrate; or else these seeds should be evaluated by seedling emergence test under field conditions.

It is possible that the extent of the percent infection by fungi and the percent germination of soybean seeds harvested after the recommended maturation stage is also influenced by the genotypes used. Furthermore, it is assumed that the field emergence test performed with the seeds harvested under such circumstances is more adequate than the germination test carried out under laboratory conditions. Therefore, the objective of this study was to evaluate the health quality of the seeds, as well as the emergence of the seedlings in field of eight different soybean cultivars harvested at three different harvesting periods.

Materials and Methods

The study was carried out at the Soybean Breeding Laboratory and at the Seed Research Laboratory of Department of Plant Science, Federal University of Viçosa (UFV), headquartered at Viçosa municipality, state of Minas Gerais. To this, seeds of eight soybean cultivar (UFV-16, Splendor, Vencedora, Confiança, UFV-18, UFV-TN 150, Garantia, and Celeste) were produced at the Experimental Field Professor Diogo Alves de Mello during the 2007/08 crop season. Harvesting was performed at three different harvest periods: reproductive R8 stage (when 95% of pods have the typical coloration of mature pods), and 15 days and 30 days after this maturation stage.

The experimental design used was a randomized block design, with four replications and with treatments arranged in a split-plot model, where the plots were represented by the eight cultivars, and the subplots represented by the three harvest dates. Each plot was consisted by eight 5 m long rows, with 0.5 m interspaces between each other, and with the subplots formed by two of those rows; and discarding the two side rows (plot's border rows).

Climatic conditions were monitored throughout the harvest period (from March 31 to May 28, 2008), and the meteorological data were provided by the Sector of Meteorology of the Department of Agricultural Engineering, Federal University of Viçosa.

Plants were hand harvested when the seeds were still presenting the moisture content between 14% and 20%, which was determined by the oven method at 105 ± 3 °C, for 24 h (Brasil, 2009). After harvesting, the plants with the pods were dried under shade, and when the seeds had moisture content of about 12%, the plants were threshed in a stationary thresher, and the seeds were then subjected to cleaning and sorted through circular screens with mesh diameter of 4.76 mm and 5.16 mm, to obtain a higher number of seeds. Afterwards, seeds were packed into cotton bags and kept under laboratory environmental conditions; and after this period the seeds were subjected to the health test, and subsequently to the seedling field emergence test.

Health test: for evaluating health quality of seeds the blotter test was used (Goulart, 2004; Henning, 2005). To that, four subsamples of 25 seeds each, to each treatment and replication were previously treated with 70% ethanol, for 1 min., and with 2% sodium hypochlorite, during the same period, and then washed with distilled-sterile water. Immediately after, seeds were placed into transparent plastic boxes (Gerbox® type), which had been washed and disinfected with 2% sodium hypochlorite, containing from six to eight previously autoclaved filter paper sheets moistened with distilled-sterile water and treated with streptomycin. The boxes containing seeds were then incubated

under laboratory environmental conditions, at approximately 25 °C, for seven days. After such incubation period, the fungi that developed hyphae or reproductive structures on the seeds, and which are characteristic to each fungus species were identified with the aid of stereomicroscope.

Seedling field emergence test: this test was carried out with two subsamples of 50 seeds each, to each treatment and replication, which were sown into 1.0 m long furrows at a depth of about 3.0 cm, and with 0.5 m interspaces between the furrows. The percent emergence of seedling was computed with the values obtained by the counting of the numbers of seedling with the first pair of leaflets fully developed, what occurred at the thirteenth day after sowing to all cultivars.

Prior to ANOVA computation, all data obtained in the experiments were subjected to normality tests (Lillifors test) and homogeneity of residual variances between treatments (Cochran test). Means were compared by Tukey test ($p < 0.05$); and except to mean data on percent emergence, mean data obtained to fungi incidence percent were transformed to $\arcsin \sqrt{x/100}$ to perform ANOVA.

Results and Discussion

Throughout the harvest period the relative humidity has remained between 70% and 92%, and the average temperature

has ranged between 15.5 and 23.1 °C; but rainfalls occurred in small volumes only in the last 25 harvesting days; however, the highest rainfall volume that occurred during such period was 11.6 mm.

The seeds of all cultivars assessed were infected by several pathogenic fungi genera such as: *Fusarium* spp., *Phomopsis* spp., *Epicoccum* spp., *Cercospora kikuchii*, *Colletrichum truncatum*, *Chaetomium* spp., and *Alternaria* spp.; however, the three first fungi cited have occurred with higher frequency.

On Table 1 is presented a summary of the ANOVA computed with mean data obtained for total percent fungi incidence (TIF), frequency of *Fusarium* spp (FF), *Phomopsis* spp. (FP), and *Epicoccum* spp. (FE), as well as the field emergence (E) of seeds of the soybean cultivars harvested in the three different harvest times studied (stage R8, R8 +15 days, and R8 +30 days). As it can be verified there has been a statistically significant effect of cultivars at levels of 1% and 5% probability of F test to all variables; except for frequency of *Fusarium* spp., which was not significantly influenced by the two sources of variation. The effect of harvesting time was statistically significant ($p < 0.01$) for the overall incidence of fungi, frequency of *Phomopsis* spp., and for seedling emergence in the field. It should also be stressed the lack of significant interaction of cultivar x harvesting time to all characteristics evaluated.

Table 1. Summary of analysis of variance of data obtained for the total percent incidence of fungi (TIF), frequency of the fungi *Fusarium* spp (FF), *Phomopsis* spp. (FP), and *Epicoccum* spp (FE), and seedling emergence in field (E), of seeds of eight soybean cultivars harvested at different harvesting times (stage R8, R8 +15 days, and R8 +30 days).

Source of variation	DF	Mean Square				
		TIF	FF	FP	FE	E
Bloc	3	226.3966	315.0911	725.5182	248.8121	150.2638
Cultivar (C)	7	1102.3062**	364.6095	678.1628*	351.0640*	524.2321**
Residue (a)	21	61.0844	192.2916	189.5898	105.7455	132.1686
Harvest time (E)	2	1677.2303**	413.2684	1387.8517**	23.8101	2147.5417**
E x C	14	31.7004	99.5027	101.0087	82.8843	38.7321
Residue (b)	48	37.9579	145.1384	129.5804	91.0270	28.3888
CV a (%)		23.0	34.2	40.4	57.2	14.6
CV b (%)		18.2	29.7	33.4	53.1	6.8

**Significant at 5% probability level by F test; * Significant at 1% probability level by F test; DF = degrees of freedom; CV = coefficient of variation

There has been an increase in the number of seeds infected by fungi insofar as harvest was delayed, i.e., a delay of 15 days in the harvesting time after reproductive stage R8 has caused a marked increase on the incidence of pathogenic fungi in the seeds, which was exacerbated when the seeds were harvested at 30 days after the ideal harvesting time to all the eight soybean cultivars studied (Table 2). The overall mean of fungi incidence in the seeds was 54.8%, and despite all cultivars have shown

a trend to increased infection levels with the delaying in the harvest time, some of those cultivars have shown lower values than the other cultivars; as for example the cultivars UFV-18, Celeste, and UFV TN-105, which have late or semi-late cycle, have shown 33.7%, 34.8%, and 49.6%, respectively of infection by fungi (Table 2). This behavior can be explained by the fact that harvesting of longer cycle cultivars does not coincide with the period of highest rainfall occurrence, which implies in a

lower fungi incidence in the seeds; while the early and mid-cycle cultivars may have higher infection levels, as they are subjected to high temperatures and high relative humidity at the end of their cropping cycles (Hamawaki et al., 2002).

Table 2. Mean percent values obtained for the overall incidence of fungi on seeds from eight soybean cultivars harvested at two harvest times after the maturation stage R8.

Cultivar	Harvesting time/ incidence (%)			Mean*
	Stage R8	Stage R8 + 15 days	Stage R8 + 30 days	
UFV-16	63.6	56.6	78.2	66.1 c
Splendor	51.9	61.4	72.5	61.9 bc
Vencedora	56.6	57.4	73.2	62.4 bc
Confiança	63.4	67.2	81.1	70.5 c
UFV-18	18.6	34.6	47.8	33.7 a
UFV-TN 105	37.8	46.6	64.4	49.6 ab
Garantia	49.4	60.6	69.0	59.7 bc
Celeste	29.2	33.1	42.0	34.8 a
Mean*	46.3 A	52.2 B	66.0 C	54.8

*Means followed by the same lowercase letter in the column and uppercase letter on the line do not significantly differ between each other by Tukey test ($p < 0.05$). Values transformed to arcsine $\sqrt{x/100}$ for ANOVA.

Differentiated behavior of soybean cultivars as to fungal incidence, as well as to their increase on seeds after the reproductive stage R8 were also found by Braccini et al. (2000). However, Minuzzi et al. (2010) have verified that as the harvest time was increased from the R7 reproductive stage, the percent fungal incidence has also linearly increased to all the soybean cultivars assessed, and that fungi *Aspergillus* spp., *Fusarium* spp., *Phomopsis* spp., and *Cercospora kikuchii* were the most frequent in the seeds.

Regarding to incidence of *Fusarium* spp., the high percent incidence of this fungus was similar in the seeds of all cultivars, with no distinction among harvest times,

i.e., there was no significant effect of cultivar and harvest time on incidence of this fungus in seeds (Table 3). Despite many researchers consider this fungus as a saprophyte or as a weak parasite; to Henning (2005), this fungus is included among the fungi pathogenic to soybean seeds, for causing germination problems similar to those caused by the fungus *Phomopsis* spp. in the tests performed in laboratory. Thus, corroborating what has already been published by Dhingra and Acuña (1997), where the authors claim that *Phomopsis* spp. and *Fusarium* spp. are the two most damaging fungi to the soybean seed production under the climatic conditions of Brazil.

Table 3. Mean percentage values obtained for incidence of *Fusarium* spp., in seeds of eight soybean cultivars harvested at two harvest times after the maturation stage R8.

Cultivar	Harvest time/ incidence (%)			Mean*
	Stage R8	Stage R8 + 15 days	Stage R8 + 30 days	
UFV-16	60.9**	68.9	45.2	58.3 a
Splendor	68.9	73.6	57.9	66.8 a
Vencedora	62.6	71.2	64.3	66.0 a
Confiança	70.4	68.1	66.7	68.4 a
UFV-18	67.1	67.0	70.1	68.1 a
UFV-TN 105	78.5	72.8	65.8	72.4 a
Garantia	43.1	53.5	49.0	48.5 a
Celeste	80.6	69.9	53.8	68.1 a
Mean*	66.5 A	68.1 A	59.1 A	64.6

*Means followed by the same lowercase letter in the column and uppercase letter on the line do not significantly differ between each other by Tukey test ($p < 0.05$). ** Values transformed to arcsine $\sqrt{x/100}$ for ANOVA.

It was also verified that when the harvest was carried out 30 days after stage R8 there was a statistically significant

increase in the number of seeds infected by *Phomopsis* spp. (Table 4); differently from what was observed for the

seeds harvested at 15 days after the R8 stage (Table 2) in which the 15-day delay in harvest time was sufficient to significantly increase the number of seeds infected by fungi. Nevertheless, among the different cultivars there was statistically significant difference only between the UFV-18 and Garantia cultivars. A general way, the soybean cultivars

can be sorted according to the levels of infection, and the UFV-18 (40.8%) and Garantia (72.5%) cultivars represent the extreme values and the other cultivars represent the intermediate values of infection. Hence, the cultivar UFV-18 deserves emphasis since it repeats the same behavior presented for the overall fungi incidence in the seeds.

Table 4. Mean percent values obtained to the incidence of the fungus *Phomopsis* spp. in seeds of eight soybean cultivars harvested in two harvest times after the R8 maturation stage.

Cultivar	Harvest time/Incidence (%)			Mean*
	Stage R8	Stage R8 + 15 days	Stage R8 + 30 days	
UFV-16	65.9**	62.0	76.2	68.0 ab
Splendor	46.4	38.3	61.6	48.8 ab
Vencedora	52.7	41.6	56.2	50.2 ab
Confiança	46.4	61.3	69.1	59.0 ab
UFV-18	25.4	46.3	50.6	40.8 a
UFV-TN 105	46.0	52.7	63.3	54.0 ab
Garantia	72.9	66.0	78.8	72.5 b
Celeste	38.7	36.1	72.5	49.1 ab
Mean*	49.3 A	50.5 A	66.0 B	55.3

*Means followed by the same lowercase letter in the column and uppercase letter on the line do not statistically differ between each other by Tukey test ($p < 0.05$).

** Values transformed to $\arcsin \sqrt{x/100}$ for ANOVA.

According to Henning (2005) *Phomopsis* spp. compromises the results of the germination test in the laboratory, and is responsible for reducing percent seed germination. Therefore, it has been the cause of the failure of many seed lots intended to planting, even those containing seeds with high physical and genetic purity levels. This occurs because in the germination process into paper roll there is no constant contact between the infected seed coat and the embryo axis and cotyledons, which does not occur when the germination test is conducted in soil or sand, once the infected seed coats are left on the substrate during emergence. In this sense, França-Neto and Henning (1992) developed a more efficient diagnosis to assess soybean seed

quality that surpasses the deleterious effects of fungi restricted to the seed coat and prevents the rejection of seed lots with good quality seeds.

The harvest time had no influence on incidence of *Epicoccum* spp., which is contrary to what was verified in relation to cultivars, where they have had a differentiated behavior (Table 5). As observed for the incidence of *Phomopsis* spp., cultivars can be sorted according to seed infection levels, with emphasis on the UFV-16 cultivar, which has had the lowest value for incidence (14.4%) differing only from the cultivars Splendor and Celeste, both with 40.2% incidence; whereas the other cultivars showed intermediate values.

Table 5. Mean percent values obtained to the incidence of the fungus *Epicoccum* spp. in seeds of eight soybean cultivars harvested in two harvest times after the R8 maturation stage.

Cultivar	Harvest time/Incidence (%)			Mean*
	Stage R8	Stage R8 + 15 days	Stage R8 + 30 days	
UFV-16	16.6**	5.5	21.0	14.4 a
Splendor	34.9	40.4	45.3	40.2 b
Vencedora	32.1	21.6	24.9	26.2 ab
Confiança	40.4	29.9	22.5	31.0 ab
UFV-18	27.1	45.9	37.4	36.8 ab
UFV-TN 105	36.3	32.4	32.4	33.7 ab
Garantia	19.9	32.1	17.6	23.2 ab
Celeste	40.0	47.7	32.6	40.2 b
Mean*	30.9 A	31.9 A	29.2 A	30.7

*Means followed by the same lowercase in the column and uppercase letter on the line do not statistically differ between each other by Tukey test ($p < 0.05$). ** Values transformed to $\arcsin \sqrt{x/100}$ for ANOVA.

In assessing health quality of soybean seeds, the fungus *Epicoccum* spp. has not been listed among the most frequent pathogens (Costa et al., 2003; Gomes et al., 2009; Minuzzi et al., 2010). It is possible that in studies on seed pathology its low incidence or the lack on its detection in soybean seeds is due to the possibility of this fungus to be mistaken with other pathogens at the evaluation time by the health test.

In relation to seedling emergence in the field, results have shown that the delay of 30 days in the harvest time, after the R8 stage, has significantly reduced the percentage

of emerged seedlings (Table 6). As far as the different cultivars, it was observed that percent values obtained for seedling emergence in the field have had in a stratified format, and at three different levels: one level with the percent emergence above 80%, represented by the cultivars Vencedora, UFV-18, UFV TN-105 and Celeste; another level with the percent emergence of 65%, represented only by the cultivar UFV-16, and the other cultivars have formed an intermediate group with germination percentage ranging between 76% and 80% (Table 6).

Table 6. Mean of percent values obtained for field emergence of soybean seedlings, originating from seeds of eight cultivars harvested in two harvest times after the R8 maturation stage.

Cultivar	Harvest time/Emergence (%)			Mean*
	Stage R8	Stage R8 + 15 days	Stage R8 + 30 days	
UFV-16	75	72	49	65 b
Splendor	83	79	69	77 ab
Vencedora	88	88	76	84 a
Confiança	82	78	67	76 ab
UFV-18	89	89	71	83 a
UFV-TN 105	90	88	75	84 a
Garantia	86	80	71	79 ab
Celeste	87	87	79	84 a
Mean*	85 A	82 A	70 B	79

*Means followed by the same lowercase letter in the column and uppercase letter on the line do not statistically differ between each other by Tukey test ($p < 0.05$).

Despite working with a single cultivar Pelúzio et al. (2008) concluded that the delay in harvest resulted in decrease in germination and vigor of soybean seeds. However Santos et al. (2005) in studies with different soybean genotypes have found that seed harvested at the physiological maturity showed no difference in the percent of seedling emergence in the field. Nevertheless, when subjected to a harvest delay from 15 to 30 days after the R8 stage different behaviors occurred among cultivars. Thus, the delay on harvesting is capable of signaling those genotypes that exhibit better seed quality or to better tolerate deterioration conditions imposed by delayed harvest (Lima et al., 2007).

Despite the delay on harvest for 15 days after the R8 stage have led to the increased percentage of the seedlings infected by fungi, that same period was not sufficient to cause reduction in the percentage of seedling emergence in the field, which occurred only after 30 days precisely when there was a higher incidence of fungi in the seeds. Albrecht et al. (2008) found that the highest fungi incidence may have been the cause of problems in the performance of the soybean seeds in terms of physiological quality. However, Henning et al. (2011) found a high incidence of fungi in corn seeds; however, without compromise physiological quality of the seeds harvested at

physiological maturity point. Thus, the increased incidence of fungi in the seeds do not necessarily implies on reduction of seedling emergence in the field. Evidently, the damage level caused to seedling emergence is also dependent on the genera of the fungi detected with higher frequency, once the seed germination in sand or soil is less affected by fungi, especially those confined to the seed coat.

The reduction in the seedling emergence percentage can not be attributed only to the effect of the fungi infection, but also to the reduction of the physiological quality of the seeds, caused for the harvest delay. The emergence in the field was little affected by the seeds infection, since the reduction of the emergence percentage occurred only in the seeds with higher infection level, harvested 30 days after the R8 stadium.

Conclusions

Soybean seeds harvested at 15 or 30 days after the reproductive stage R8 show significant increase on infection by fungi, especially from genera *Fusarium* spp., *Phomopsis* spp., and *Epicoccum* spp.

The percentage of seedling emergence under field conditions is negatively affected when the seeds used in the

sowing were harvested 30 days after reproductive stage R8.

The soybean cultivars had different performance in relation to fungi incidence and seedling emergence in the field; the cultivars less infected by fungi had the highest seedling emergence percentage.

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