Viability of seed-borne fungi *Alternaria alternata*, *Bipolaris sorokiniana* and *Drechslera teres* in barley seeds in the south of Brazil

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ABSTRACT

Agostinetto, L.; Casa, R.T.; Bogo, A.; Alves Neto, L.; Vieira Junior, J.A.L.; Fingstag, M.D.; Rosa, J.M. Viability of seed-borne fungi *Alternaria alternata*, *Bipolaris sorokiniana* and *Drechslera teres* in barley seeds in the south of Brazil. ***Summa Phytopathologica,*** v.46, n.1, p.26-30, 2020.

Some seed-borne fungi can survive in barley seeds; nevertheless, their survival period is unknown in barley seeds under storage conditions in Brazil. The aim of this study was to quantify the viability of the fungi *Alternaria alternata, Bipolaris sorokiniana* and *Drechslera teres* in barley seeds stored for 315 days during the off-season in the south region of Brazil. Each cultivar had 400 seeds disinfested and subjected to seed health testing in potato-dextrose-agar (PDA) from December 2011 to September 2012 at 35-day intervals. Data on fungal incidence and viability as a function of the storage time underwent regression analysis. All three fungi were detected in the five cultivars in all evaluated periods, and their incidence and viability significantly reduced with increasing storage periods. Monthly average reduction in the viability of *A. alternata* was 8%, while that of *B. sorokiniana* and *D. teres* was 10%. At the end of the off-season, viability of *A. alternata, B. sorokiniana* and *D. teres*, considering the average of cultivars, remained 49.8, 29.6 and 31.0%, respectively.

Keywords: Phytopathogenic fungi; *Hordeum vulgare*; seed health; survival.

RESUMO

Agostinetto, L.; Casa, R.T.; Bogo, A.; Alves Neto, L.; Vieira Junior, J.A.L.; Fingstag, M.D.; Rosa, J.M. Viabilidade de *Alternaria alternata*, *Bipolaris sorokiniana* and *Drechslera teres* transmitidos por sementes de cevada no sul do Brasil. ***Summa Phytopathologica,*** v.46, n.1, p.26-30, 2020.

Alguns fungos fitogênicos sobrevivem nas sementes de cevada, no entanto, o seu período de sobrevivência é desconhecido em condições de armazenamento de sementes cevada no Brasil. O objetivo do trabalho foi quantificar a viabilidade dos fungos *Alternaria alternata, Bipolaris sorokiniana* e *Drechslera teres* em sementes de cevada, armazenadas por 315 dias durante o período da entressafra na Região Sul do Brasil. Quatrocentas sementes de cada cultivar foram desinfestadas e submetidas ao teste de sanidade de sementes em meio de cultura batata-dextrose-ágar (BDA), com intervalo de tempo entre avaliação de 35 dias, desde dezembro de 2011 a setembro de 2012. Os dados de incidência e viabilidade dos fungos em função do tempo de armazenamento foram submetidos à análise de regressão. Os três fungos foram detectados nas cinco cultivares em todas as épocas avaliadas, com redução significativa negativa da incidência e viabilidade com o aumento do período de armazenamento. A redução média mensal de viabilidade de *A. alternata* foi de 8% enquanto de *B. sorokiniana* e *D. teres* foi de 10%. No final do período de entressafra a viabilidade dos fungos *A. alternata, B. sorokiniana* e *D. teres,* na média das cultivares, manteve-se em 49,8%, 29,6% e 31,0%, respectivamente.

Palavras-chave: Fungos fitopatogênicos, *Hordeum vulgare*, sanidade de semente, sobrevivência.

Barley (*Hordeum vulgare* L.) is recognized as the fourth most important crop in the world in terms of economic and social impact. Worldwide annual production of barley is approximately 170 million tons, cultivated in an area of 530 thousand ha, where the three major producing countries are Russia, Canada and Ukraine (10). In 2017/2018, Brazilian barley production areas consisted of approximately 108.4 thousand ha, with productivity estimated at 2.602 kg ha^{-1} (10). Barley production in Brazil is mainly for the brewing industry, which annually utilizes approximately 20 million tons (10). Consequently, Brazil is one of the largest barley-importing countries, producing only 30% of the national brewing industry's demand.

Barley is predominantly cultivated in the southern Brazilian states of Rio Grande do Sul and Paraná and relies on treated seeds planted under a no-till system with crop rotation (17, 25). Despite these management strategies, necrotrophic fungi can be found on aerial plant parts, which can lead to foliar disease epidemics and reduced yield (2).

The two most important barley foliar diseases in southern Brazil are brown spot and spot blotch, caused by *B. sorokiniana* and *D. teres*, respectively (20). These fungi can infect and grow in roots, coleoptiles or plumules, producing foliar spots and root diseases (20). *B. sorokiniana* transmission from seeds to the mesocotyl, coleoptile and plumule was quantified by Barba *et al*. (3) as 28.1%, 40.0% and 8.1%, respectively.

In the case of *D. Teres*, transmission from the seed to the plumule was 18%, according to Reis et al. (22), and 21%, according to Carmona et al. (8). Another important disease is caused by *A. alternata*, which develops 'black spot' symptoms in barley seeds, together with *B. sorokiniana* and *D. teres* (31). However, within-plant transmission of *A. alternata* has not been studied yet.

Host genetic resistance would be an ideal management strategy; however, most commercially available barley cultivars currently used in southern Brazil do not provide satisfactory resistance (17). Thus, use of healthy seeds is the second best option, which has the potential to prevent the introduction of these fungi into new growing areas. However, healthy seed production is not always possible due to factors linked to the seed infection mechanism (14).

Chemical treatment of seeds is one of the strategies used by barley seed companies to control seed-borne fungi. Nevertheless, seed health testing has revealed that a large number of treated seeds are infected by *A. alternata*, *B. sorokiniana* and *D. teres* (4). Moreover, incidence of seed-borne fungi in harvested seeds is high, further compromising the treatment effectiveness (6, 13, 23). Incorrect or inefficient fungicide treatment may allow the survival of necrotrophic fungi and the spread of diseases to other uninfested sites (6, 7).

Seed-borne transmission can be influenced by different factors, such as seed inoculum viability (1), which has not been quantified in barley seeds. Thus, the objective of this study was to quantify the viability of the seed-borne fungi *A. alternata, B. sorokiniana* and *D. teres* in cultivars of barley seeds stored during ten months of off-season between 2011 and 2012 in southern Brazil. Sowing of barley seeds in southern Brazil occurs between May and June in the states of Rio Grande do Sul and Paraná and from May to August in the state of Santa Catarina (17). Therefore, the seeds are stored for at least eight months (December to August) until sowing. During this storage period, the studied pathogens may survive in the seeds, the primary inoculum may be available and transmission to the seedlings may occur (8).

MATERIAL AND METHODS

Untreated barley seeds of five cultivars, MN 743, MN 6021, BRS Cauê, BRS Brau and BRS Elis, were obtained from the American Beverage Company (AmBev Company). Random samples of 400 seeds of each cultivar were subjected to seed health testing every 35 days, during 315 days, from December 2011 (processing of seeds) to September 2012 (end of the sowing season in southern Brazil). There were 10 samples of 400 seeds, which were examined for each of the five cultivars. Seeds were stored according to the Seed Analysis Rules of the Brazilian Ministry of Agriculture (5). Untreated barley seeds provided by AmBev Company were stored in 480 propylene seed bags (50 Kg each) at 4.5 m high, on 1.5 x 1.2 m wooden pallets, inside an 80 x 30 m masonry warehouse, where air temperature varied between 18 and 22 °C and relative humidity was around 60% under natural ventilation.

Seed health testing was carried out based on the protocol Potato-dextrose-agar media + antibiotic (streptomycin sulfate 0.05%) (PDA+A), according to Reis *et al*. (24) and Toledo *et al*. (30). Briefly, seed samples were disinfested with sodium hypochlorite (NaOCl 4%) and sterile water at the proportion of 1:1 (v/v) for 3 min, washed twice in sterile distilled water and dried with sterile filter paper. Experimental design was completely randomized with sub-samples of 25 seeds per sterile acrylic box, which were sown in PDA+A and incubated in a Biochemical Oxygen Demand (BOD), totaling 16 technical replicates randomly distributed inside the BOD. The acrylic germ-boxes were kept at 25°C and 12h photoperiod for seven to ten days until *A. alternata, B. sorokiniana* and *D. teres* growth was observed. *A. alternata*, *B. sorokiniana* and *D. teres* were identified under a stereoscopic microscope (40x) with microscopic slides for morphological identification of reproductive structures, according to Sivanesan (27), Mathre (15) and Simmons (26).

Pathogen viability was calculated as the inverse of the ratio of the observed incidence divided by the maximum incidence and expressed as percentage, according to the mathematical formula:

$$V\ (\%) = \left(\frac{i \times 100}{I} \right) - 100$$

where: V: fungal viability (%); i: fungal incidence per cultivar and evaluation time, and I: the highest fungal incidence per cultivar and evaluation time. In every case, maximum incidence was observed at day 0 and was considered 100% viability.

Data on incidence and viability were evaluated according to analysis of variance ($\alpha = 0.01$) and linear regression analysis. For *A. alternata*, *D. teres* and *B. sorokiniana*, these data were related to the storage period of barley seeds, yielding equations of estimated incidence reduction and viability of the pathogens over the storage period.

RESULTS AND DISCUSSION

A. alternata, *B. sorokiniana* and *D. teres* were detected in all evaluated barley cultivars and storage periods. Linear regression showed a significant negative relationship of both incidence and viability with storage time for *A. alternata*, *B. sorokiniana* and *D. teres* (Figures 1, 2 and 3). Similar results were obtained for *Bipolaris maydis* and *A. alternata* in maize by Tanaka *et al*. (29), for *Pyricularia grisea*, *B. sorokiniana* and *A. alternata* in triticale by Medina *et al*. (16) and for *A. alternata* in wheat by Casa *et al*. (9).

A. alternata, *B. sorokiniana* and *D. teres* remained viable in all evaluated cultivars during the 10-month storage period. There was a reduction in the disease incidence during the storage period, but 0% viability was not found for any cultivar. Similarly, Gwary *et al*. (11) showed that *Dreschslera* sp. was viable in sorghum seeds for at least twenty months, presenting 36% viability after this period. Casa et al. (9) demonstrated that *A. alternata* had 49.5% viability in weed seeds after 12-month storage. However, Sultana *et al*. (28) showed that *Alternaria* spp. were completely eliminated from cucumber seeds after eight months. Reis *et al*. (23) showed that storage of wheat seeds for long periods (19 and 22 months) reduced the viability of *Pyricularia oryzae* to 0%.

Considering the average of cultivars in the first evaluation, incidence of *B. sorokiniana* and *D. teres* was 37.8% and 12.4%, respectively, reducing to 3.6% and 1.6% after ten-month storage (Figure 1). At the end of the storage period, viability of *B. sorokiniana* and *D. teres* was 7.4% and 12.4%, respectively (Figures 2B and 2C). These results agree with those of Medina *et al*. (16) and Previero *et al*. (19), who found 5% and 40.6% viability for *B. sorokiniana* and *Drechslera* spp., respectively, after twelve-month storage of triticale and brachiaria.

Based on the average of cultivars, viability of *A. alternata* in seeds in the first evaluation reached 55.6%, reducing to 15% after ten-month storage (Figure 1A), which was 26% (Insert Figure 2) greater than that of *B. sorokiniana* and *D. teres*. Malaker *et al*. (14) found that *A. alternata*

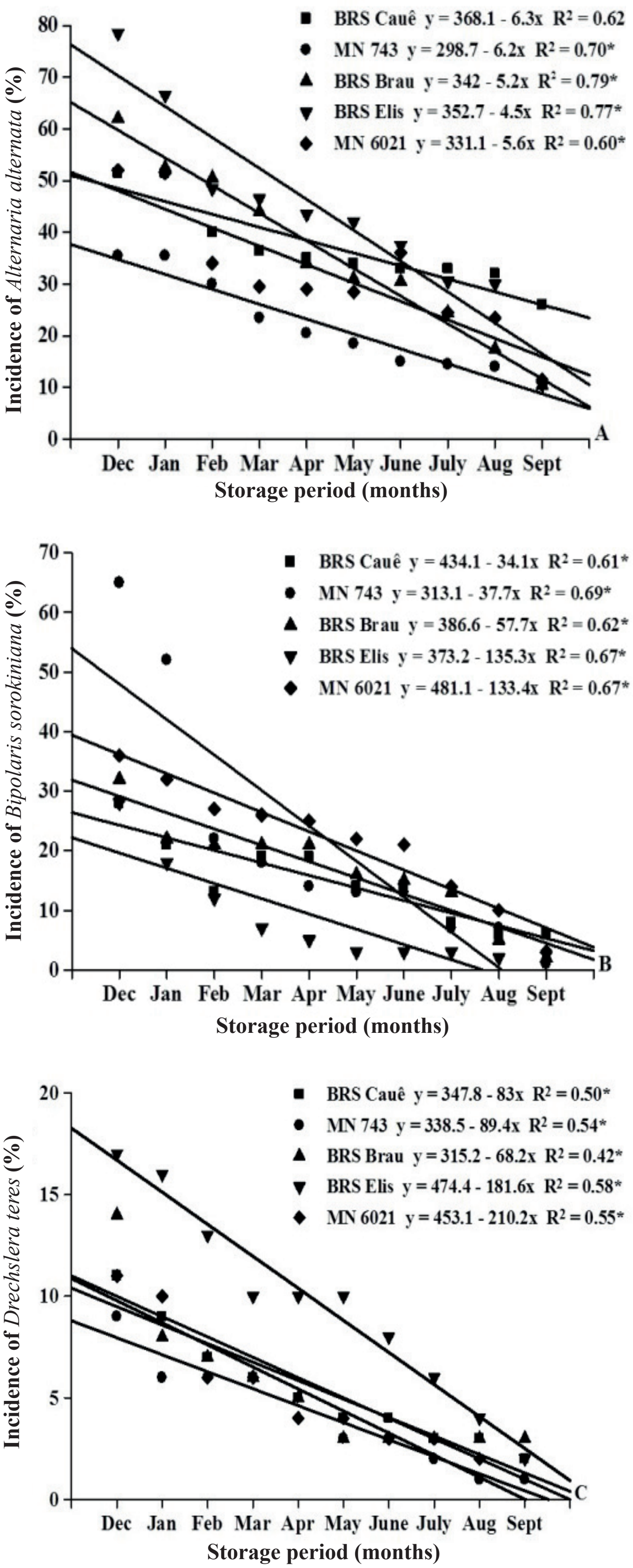

Figure 1. Relationship between the incidence (%) of *Alternaria alternata* (A), *Bipolaris sorokiniana* (B) and *Drechslera teres* (C) and the storage period (months) of barley seeds of cultivars BRS Cauê, MN 743, BRS Brau, BRS Elis and MN 6021. * Significant at p=0.01.

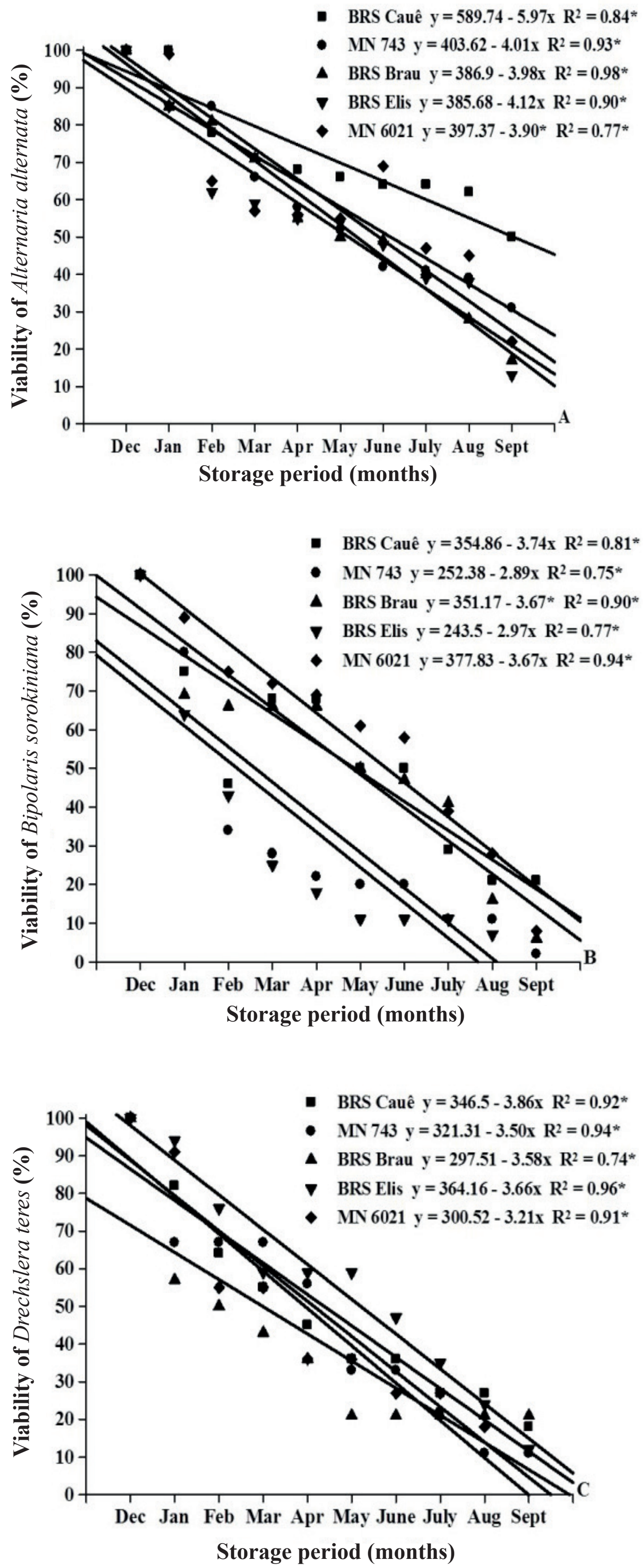

Figure 2. Relationship between the viability (%) of *Alternaria alternata* (A), *Bipolaris sorokiniana* (B) and *Drechslera teres* (C) and the storage period (months) of barley seeds of cultivars BRS Cauê, MN 743, BRS Brau, BRS Elis and MN 6021. * Significant at p=0.01.

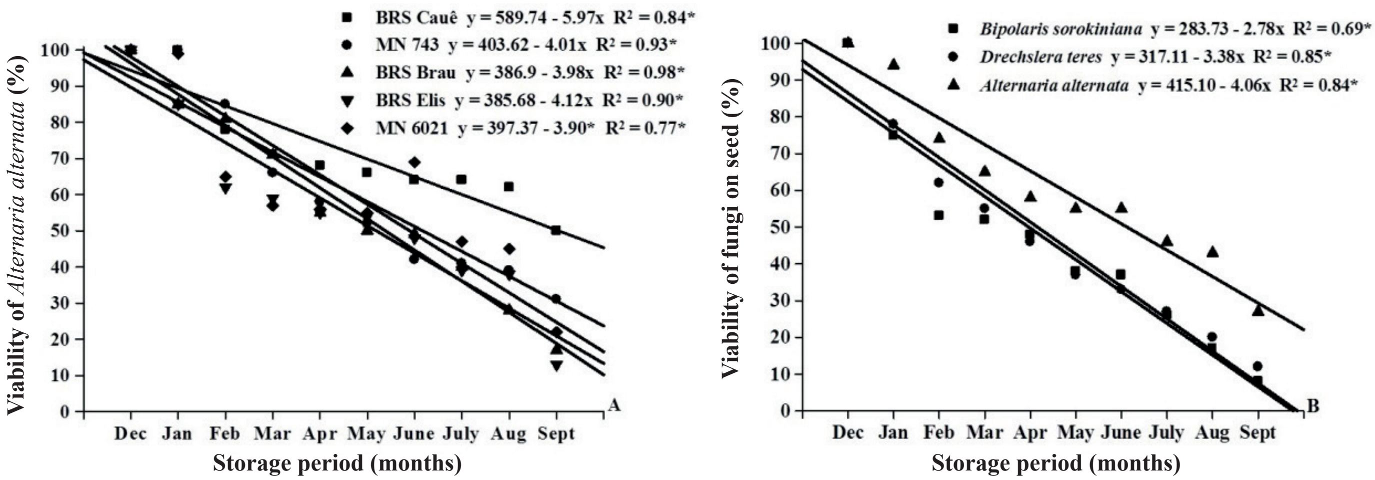

Figure 3. Relationship of the storage period (months) of barley seeds with the disease incidence (A) and the viability (B) of *Alternaria alternata*, *Bipolaris sorokiniana* and *Drechslera teres*. * Significant at p=0.01.

had its incidence reduced from 30% to 14.6%, while its viability reached 51.2% in wheat seeds after nine-month storage. Similarly, Casa *et al*. (9) demonstrated that *A. alternata* had initial incidence of 44.4%, which reduced to 22.4%, and viability of 49.5%, considering the average of 75 samples analyzed after six-month storage. Medina *et al*. (16) reported that *A. alternata* incidence reduced from 35% to 5% and its viability kept at 12.5% in triticale seeds stored for twelve months. Nascimento *et al*. (18) observed that the initial incidence of *A. alternata* was 95%, which decreased to 31%, while its viability of 52.9% in coriander seeds was maintained after six months of storage.

In Southern Brazil, barley seed sowing occurs during May and June in Rio Grande do Sul and Paraná States and during May and August in Santa Catarina State (17); therefore, seeds have to be stored for at least eight months (December - August). After storage for 175 - 280 days, which corresponds to the period when barley seeds are removed from the storage for sowing, the average incidence of *A. alternata, B. sorokiniana* and *D. teres* was 27.3, 10.4 and 3.7%, respectively (Figures 1, 2 and 3), and their average viability was 49.8, 29.6 and 31.0%, respectively.

These data demonstrate that the studied seed-borne fungi can survive in barley seeds from harvest to the following sowing season, ensuring primary inoculum availability, which is a probable mechanism for seed-borne fungal dissemination and introduction into new barley crop areas (8), resulting in new hotspots of barley brown spot and blotch spot diseases.

Spot blotch and net blotch are the major foliar diseases caused by fungi in barley in southern Brazil and are difficult to control. In the south region of Brazil, barley seeds are generally commercialized after chemical treatment. However, most of these chemical treatments have not eradicated *A. alternata, B. sorokiniana* and *D. teres* (12, 13, 23). In the present study, barley seeds were demonstrated to present a viable inoculum of *A. alternata*, *B. sorokiniana* and *D. teres* during storage, since incidence and viability of these fungi were maintained in the seeds. In addition, considering the capacity of these seed-borne fungi to be transmitted from infected seeds to other plant parts, seed health testing and efficient chemical eradication treatments are necessary to prevent diseases.

In the present study, the seed-borne fungi *A. alternata*, *B. sorokiniana* and *D. teres* were demonstrated to remain viable in barley seeds during the off-season in southern Brazil. Even ten months under typical storage conditions were not sufficient to eliminate these fungi. In particular, seed-borne *A. alternata* showed the highest overall incidence and viability among all tested cultivars. Further studies to determine storage conditions and effective chemical treatment of seeds will be necessary for proper recommendations for the control of the transmission of these diseases.

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