Survey, survival and control of *Alternaria alternata* in wheat seeds [1]

Ricardo Trezzi Casa[2], Paulo Roberto Kuhnem Junior[2]*, Amauri Bogo[2], Ana Maria Munerati Belani[2], Jonatha Marcel Bolzan[2], Filipe Souza Oliveira[2], Marta Maria Casa Blum[3]

ABSTRACT **-** The fungus *Alternaria alternata* was quantified in 75 wheat seed samples collected from three different regions of southern Brazil for Cropping and Use Value (CUV) I, II and III. Fungal presence was evaluated in two hundred disinfested seeds per sample before sowing in a potato-dextrose-agar medium + antibiotic (PDA+A). Fungus survival was evaluated every 45 days for 180 days for three seed batches from six wheat cultivars stored in propylene bags in a storehouse, with air temperature varying between 18 to 22 °C and relative air humidity around 60%. The efficacy of carboxin+thiram, difenoconazol, thiram, triadimenol, triticonazol and triticonazol + iprodione fungicides to control *A. alternata* was determined. *A. alternata* was detected in all the samples with an incidences of 39.6 %, 38.8% and 35.9% for the CUV I, CUV II and CUV III regions, respectively. The highest mean incidence of the fungus was found in the CUV I region, the coolest and most humid, and was significantly different from the other two regions. The average reduction in *A. alternata* viability in the wheat cultivar seeds was 49.5% during the 180 days of storage (inter-harvest period), demonstrating that infected seeds are the primary inoculum source for the fungus. The triticonazol + iprodione fungicide mixture efficiently controls *A. alternata*.

Index terms: fungi, seed health, seed treatment, *Triticum aestivum*.

Levantamento, sobrevivência e controle de *Alternaria alternata* em sementes de trigo

RESUMO – O fungo *Alternaria alternata* foi quantificado em 75 amostras de sementes de trigo produzidas na região Sul do Brasil, em três Regiões de Valor de Cultivo e Uso (VCU) I, II e III. Duzentas sementes por amostra foram desinfestadas antes de serem semeadas em meio de batata-dextrose-ágar + antibiótico (BDA+A). A sobrevivência do fungo foi determinada em sementes armazenadas em sacos de propileno em armazém de alvenaria durante 180 dias com temperatura do ar entre 18 e 22 ºC e umidade relativa próxima de 60%, com avaliações em intervalos de 45 dias. Foram analisados três lotes de sementes de seis cultivares de trigo. A eficácia de fungicidas carboxim+thiram, difenoconazole, thiram, triadimenol, triticonazole e triticonazole + iprodiona no controle de *A. alternata* foi determinada. *A. alternata* foi detectado em todas as amostras analisadas, com incidência média de 39,6%, sendo 48,0%, 34,8% e 35,9%, respectivamente para regiões de VCU1, VCU2 e VCU3. Na região VCU1, considerada mais fria e úmida, houve maior incidência média do fungo, diferindo significativamente das demais. Houve redução média de 49,5% na viabilidade de *A. alternata* na média das cultivares de trigo durante o período de entressafra, comprovando que as sementes infectadas são fonte de inóculo primário para o fungo. O fungicida triticonazole + iprodiona é eficiente no controle de *A. alternata*.

Termos para indexação: *Triticum aestivum*, fungo, sanidade de semente, tratamento de semente.

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[2]Departamento de Agronomia, (CAV/UDESC) Universidade Estadual de Santa Catarina, 88520-000-Lages, RS, Brasil.

[3]Universidade Regional Integrada do Alto Uruguai e das Missões, Caixa Postal 743, 99700-000-Erechim, RS, Brasil.

*Corresponding author <prkuhnem@gmail.com>

Introduction

Wheat leaf spot epidemics are frequent in monocultures and no-till fields and fungi, such as *Drechslera tritici-repentis* (Died.) Shoem. (yellow spot), *Bipolaris sorokiniana* (Sacc.) Shoem. (spot blotch) and *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano (septoria leaf blotch) predominate in this system. The disease occurs mainly on cloudy or rainy days, which are very common in South Brazil (Reis and Casa, 2007). Other fungi, such as *Fusarium nivale* (Fr.) Ces. and *Pyricularia grisea* (Cooke) Sacc., can also cause leaf spot but they are sporadic and of secondary importance.

In general, high leaf spot severity in monoculture and no-till systems can cause an increase in pathogenic fungal incidence in harvested seeds (Prestes et al., 2002) and these fungi can use the seeds as a survival and dissemination mechanism (Reis and Casa, 1998).

The infected seeds are an important source of primary inocula of necrotrophic fungi in winter cereals (Mathur and Cunfer, 1993; Reis et al., 1999). The fungi survive mainly as a mycelium in the endosperm, tegument or embryo of the cereal seeds (Reis and Casa, 1998). During the storage of the infected seeds, fungal viability can be maintained during the inter-harvest period until the next sowing season (Reis et al., 1995; Telles Neto et al., 2007). After sowing, the necrotrophic fungi may grow on the seedling coleoptiles and reach the soil surface. The mycelium that grows superficially on the coleoptiles may also invade it and colonize the interior plumule, which emerges with leaf spots. The fungi produce the inoculum in moist and light environments and they are disseminated by wind or rain-splashes on to the plant's own leaves or on to neighboring plants (Casa et al., 2005).

The fungus *Alternaria alternata* (Fr.FR.) Keissler is considered one of the causal agents of black point in wheat seeds (Bhowmik, 1969; Mathur and Cunfer, 1993). This fungus is usually detected on wheat seeds in Brazil (Reis and Casa, 1998), but there are no reports about its transmission and control. Species of saprophytic or pathogenic *Alternaria* sp. can be found on wheat leaf spots (Zillinsky, 1984) and the presence of *A. triticina* Prasada and Prabhu, causing wheat leaf spot in Argentina (Perelló and Sisterna, 2006), raises the hypothesis that the pathogen may be present in seeds and grains imported from Argentina but have remained undetected as yet, since it is classified as an Absent Quarentenary Pest (A1) (Brasil, 2008).

Wheat seeds are generally commercialized without any fungicidal treatment. Tests to verify seed health have shown significant amounts of *Alternaria*, which can be easily identified by the large conidia, which are usually ovoid or ellipsoid, ranging light brown to brown in color, multi-cellular, with longitudinal, transverse and, sometimes, oblique septa. The colonies are generally scattered, gray to dark brown colored, with an olive green tonality, with dark conidiophores- different from the hyphae- solitary or arranged in progression (Wiese, 1977; Rotem, 1994; Simmons, 2007).

The use of healthy seeds and/or their treatment with fungicides are control strategies to reduce or eliminate the inoculi from the seed (Reis and Casa, 2007). The complete elimination of seed pathogens is difficult and the choice of fungicide and the rate must be made according to fungus species incidence.

The objectives of this study were: to evaluate the presence of *A. alternata* in 75 wheat seed samples produced in three different south Brazil Cropping and Use Value (CUV I, II and III) regions during the 2007 crop season; to quantify fungal viability in six wheat cultivars stored during the inter-harvest period of 180 days and, to evaluate the efficacy of the fungicides recommended by the Wheat Research Committee for seed treatment.

Material and Methods

The tests on seed health were conducted at the Plant Pathology Laboratory of the Santa Catarina State University (Lages, SC, Brazil), between 2007 and 2009.

Experiment I: Incidence of *A. alternata* in wheat seeds

A. alternata was detected in wheat seeds produced in Rio Grande do Sul, Santa Catarina and Paraná states during the 2007 crop season. Fungal incidence was quantified on 75 wheat seed samples harvested at producer Seed Processing Units (SPU) of cooperative and research institutes. Seed sampling was done according to the Seed Analysis Rules (SAR) of the Ministry of Agriculture (Brasil, 1992). The samples were collected in Rio Grande do Sul, Santa Catarina and Paraná states in 2007, which constitute the regions designated for wheat and triticale Cropping and Use Value (CUV). Due to Normative Instruction nº 058 of November 19th 2008, these regions were regrouped for purposes of analysis into regions of wheat CUV experiments (Salvadori et al., 2008) as: Region CUV I- cold, humid and high altitude (30 samples collected); Region CUV II- relatively hot, humid and low altitude (30 samples collected); and Region CUV III- hot, relatively dry and low altitude (15 samples collected) (Table 1).

Table 1. Incidence of *Alternaria alternata* in wheat seeds collected from different counties that compose regions with different wheat and triticale cropping and use values (CUV).

CUV 1		CUV 2		CUV 3	
County	I[1] (%)	County	I (%)	County	I (%)
1. Campos Novos (1)	13	Abelardo Luz (1)	29	Londrina (1)	51
2. Campos Novos (2)	39	Abelardo Luz (2)	33	Londrina (2)	50
3. Campos Novos (3)	35	Abelardo Luz (3)	25	Londrina (3)	53
4. Campos Novos (4)	36	Abelardo Luz (4)	31	Londrina (4)	16
5. Campos Novos (5)	06	Abelardo Luz (5)	45	Londrina (5)	39
6. Canoinhas (1)	43	Abelardo Luz (6)	41	Londrina (6)	46
7. Canoinhas (2)	53	Abelardo Luz (7)	25	Londrina (7)	24
8. Canoinhas (3)	44	Cafelândia (1)	29	Londrina (8)	29
9. Canoinhas (4)	45	Cafelândia (2)	42	Londrina (9)	12
10. Castro (1)	83	Campo Mourão (1)	39	Londrina (10)	51
11. Castro (2)	83	Campo Mourão (2)	43	Nova Aurora (1)	51
12. Castro (3)	78	Cascavel	19	Nova Aurora (2)	44
13. Castro (4)	81	Dois Vizinhos (1)	78	Palotina (1)	32
14. Castro (5)	81	Dois Vizinhos (2)	56	Palotina (2)	16
15. Castro (6)	88	Giruá (1)	58	Palotina (3)	24
16. Lages	08	Giruá (2)	34		
17. Não-Me-Toque	36	Giruá (3)	38		
18. Passo Fundo	20	Giruá (4)	19		
19. Ponta Grossa (1)	71	Realeza	46		
20. Ponta Grossa (2)	93	Santo Augusto (1)	36		
21. Ponta Grossa (3)	65	Santo Augusto (2)	32		
22. Ponta Grossa (4)	79	São Borja (1)	34		
23. Tupanciretã (1)	37	São Borja (2)	23		
24. Tupanciretã (2)	19	São Borja (3)	28		
25. Tupanciretã (3)	54	São Borja (4)	35		
26. Tupanciretã (4)	29	Três de Maio (1)	29		
27. Vacaria (1)	38	Três de Maio (2)	26		
28. Vacaria (2)	34	Xanxerê (1)	25		
29. Vacaria (3)	38	Xanxerê (2)	17		
30. Vacaria (4)	10	Xanxerê (3)	26		
Means	48.0 a		35.4 b		35.9 b
C.V. (%)			44.7		

Region CUV I- cold, humid and high land; Region CUV II- relatively hot, humid and low land; and Region CUV III- hot, relatively dry and low land.

[1]I-Incidence of *Alternaria alternata*

Means followed by same letter did not differ significantly by the Tukey test ($p \leq 0.01$).

The seeds from each sample were sown in potato-dextrose-agar media + antibiotic (streptomycin sulfate 0.05%) (PDA+A) in acrylic boxes. Two hundred seeds, disinfested with sodium hypochlorite solution (2%), were distributed in groups of 25 seeds per acrylic box and sown in PDA+A and incubated in BOD (Biochemical Oxygen Demand) at a temperature of 25 ºC with 12 hours of photoperiod for seven days. Colonies were quantified and analyzed using a stereo microscope and *A. alternata* was identified from the spore morphology.

The *A. alternata* incidence data were transformed into $\sqrt{x+1}$ and submitted to an analysis of variance ($p \leq 0.01$). The means were compared using the Tukey test ($p \leq 0.01$) and the results expressed as *A. alternata* incidence per sample, state and CUV Region.

Experiment II: Survival of *A. alternata* in stored wheat seeds

The survival of *A. alternata* was determined for the wheat seed cultivars Abalone, BRS Pardela, Fundacep Cristalino, Onix, Safira, Quartzo, harvested from the Santa Catarina upland region from commercial crops between the end of November and beginning of December 2008 and stored during the inter-harvest period of 180 days (six months).

Three lots per cultivar were analyzed. Each lot consisted of 270 propylene seed bags (50 Kg each) in piles around 4.5 m high, on 1.5 x 1.2 m palettes over a wood platform inside an 80 x 30 m storehouse, with the air temperature varying between 18 to 22 °C and relative air humidity around 60%. Samples from the stored seeds were collected every 45 days during the 180 days of storage, beginning in December 18th, 2008, according to the wheat sowing dates of the MAPA Agricultural Zoning (Salvadori et al., 2008). The seed health test followed the same methodology as described for Experiment I.

The *A. alternata* incidence data were transformed into $\sqrt{x+1}$ and submitted to an analysis of variance ($p \leq 0.01$). The results obtained for incidence and storage time were submitted to a regression analysis.

Experiment III: Chemical control of *A. alternata*

The seed treatment was done with fungicides recommended by the Brazilian Commission for Wheat and Triticale Research (Salvadori et al, 2008), to control *B. sorokiniana*, *D. tritici-repentis*, *S. nodorum*, *Ustilago tritici* (Pers.) and *P. grisea*. Seeds from the Fundacep Cristalino and Onix cultivars harvested in 2008 in the Santa Catarina upland region, were analyzed.

One kg of seed per cultivar was mixed with each fungicide in a plastic bag with 2% water and hand shaken until seed coverage was homogeneous. The fungicides and rates are shown in Table 2.

Table 2. Fungicides recommended for wheat seed treatment by the Brazilian Commission for Wheat and Triticale Research.

Technical name	Commercial name	Formulation	Concentration	Rate	
				g a.i.[1]/100 kg of seed	L or Kg c.n.[1]/ 100 kg of seed
Carboxin + thiram	Vitavax-Thiram	SC	200+200	50+50	0.25
Difenoconazole	Spectro	SC	150	30	0.20
Thiram	Thiram	SC	480	210	0.44
Triadimenol	Baytan	SC	150	40.5	0.27
Triticonazol	Premis	SC	200	45	0.23
Triticonazol + Iprodione	Premis+Rovral	SC+SC	200+500	30+50	0.15+0.10

[1]a.i. active ingredient; c.n. commercial name

The treated seeds were sown in acrylic boxes with PDA+A as already described in Experiment I. The seeds were incubated in BOD at 25 °C with a 12 hour photoperiod for seven days. The number of the seeds colonized by *A. alternata* was counted using a stereo microscope (Zeiss 40X) and considered infected with the presence of at least one conidiophore bearing conidia and/or the presence of a colony on the culture media.

Data were submitted to an analysis of variance and the means compared by the Tukey test ($p \leq 0.01$). The results were expressed as fungus incidence.

Results and Discussion

Experiment I: Incidence of *A. alternata* on wheat seeds

A. alternata was detected with a 39.8% of incidence in all the 75 samples analyzed (Table 1). The mean incidence for the CUV I, CUV II and CUV III regions was 48.0%, 35.4% and 35.9%, respectively. The coldest and most humid CUV I region, had the highest mean incidence of *A. alternata* compared with the CUV II and CUV III regions (Table 1). The latter two regions (CUV II and CUV III) did not show any significant differences in mean fungus incidence (Table 1). The high mean incidence of *A. alternata* in the coldest region of wheat production is noteworthy because there is no information in the literature about climate conditions favorable to *Alternaria* infection in wheat seeds. The 39.8% mean incidence can be considered high because official information from the Agriculture Ministry, classifies *Alternaria* as an absent quarentenary pest (Brasil, 2009). This classification would be unacceptable for risk analysis if the transmission of the fungus from seed to seedling has been here quantified.

The detection and identification of *Alternaria* species is common in various crop seeds (Rotem, 1994; Simmons, 2007), including wheat (Bhowmik, 1969; Wiese, 1977; Reis and Casa, 1998). *A. alternata* has also been detected in seeds from other crops, including rye (Bollen et al., 1983), soybean (Baird et al., 1997), cotton (Pizzinatto et al., 2005), beans (Moraes and Menten, 2006), coriander (Reis et al., 2006) and barley (Roháčik and Hudec, 2007).

Experiment II: The survival of *A. alternata* in wheat seeds

There was a significant difference between cultivars, seed lots, evaluation time and their interactions, including the triple interaction of cultivars, seeds lots and evaluation time according to the *F* test at the 1% probability level (Table 3). Thus each lot for each cultivar was analyzed separately in time (Figure1).

Table 3. Analysis of variance for incidence of *Alternaria alternata* in seeds of six wheat cultivars, in three lots, evaluated after processing and at 45, 90, 135 and 180 days of storage.

Source	D.F.	M.S. *Alternaria alternata* (%)
Cultivar (C)	5	1,213.62*
Lot (L)	2	13.58*
Time (T)	4	6,657.41*
C x L	10	30.44*
C x T	20	115.28*
L x T	8	17.78*
C x L x T	40	21.27*
Block	3	6.08
Error	267	3.91
Total	359	-
C.V.	-	5.91

*Significant according to the F test ($p \leq 0.01$)

From the regression analysis data, a linear model was adjusted for all the cultivars independent of the seed lot and a gradual reduction in *A. alternata* incidence with increased storage period was observed.

The incidence of *A. alternata* in the six wheat cultivars decreased by 22% during the 180 days of the inter-harvest storage period (Figure 1). This reduction ranged from 50.3% to 25.2% for Abalone, 47.8% to 20.5% for BRS Pardela, 40.6% to 20.0% for Fundacep Cristalino, 43.5% to 22.7% for Onix, 34.1% to 19.9% for Safira and from 50.2% to 26.0% for Quartzo, beginning with 44.4% for the first evaluation (18th December, 2008) and finishing with 22.4% for the last evaluation (16th June, 2009), respectively.

Considering 100% of fungus viability in the first evaluation, a mean reduction of 49.5% of *A. alternata* viability by the end of the storage period could be estimated (Figure 1). Reductions of 41.6%, 47.8%, 48.2%, 49.9%, 50.7% and 57.1% can be verified separately in the Safira, Onix, Quartzo, Abalone, Fundacep Cristalino and the BRS Pardela cultivars, respectively (Figure 1). The results show that *A. alternata* can survive by infecting wheat seeds from seed processing (December) until sowing time (June) in the upland region of Santa Catarina, Brazil.

A similar result of 58.1% reduction in viability was found for *Fusarium graminearum* Schwabe on wheat seeds after 6 months of storage (Telles Neto et al., 2007). Reis et al. (1995) also detected the presence of *Pyricularia grisea* Cooke (Sacc.) on Anahuac and Tapejara wheat seeds stored for 19 and 22 months, respectively and the reductions were estimated at between 50 and 60% after 7 to 8 months of storage. In the triticale seed of the IAC-2 cultivar stored under natural ambient conditions, the fungus *A. alternata* decreased 5 to 10% over 6 months (Medina et al., 2009).

In general, seed health tests are made close to the sowing date and there has already been a reduction in the incidence of *A. alternata* and other fungi in stored wheat seeds. However, this shows that infected seeds keep the fungal inoculum viable during the inter-harvest period and a fungicide seed treatment is necessary to control the fungus.

Experiment III: Chemical control of *A. alternata* in wheat seeds

There was no significant difference in *A. alternata* incidence between the Onix and the Fundacep Cristalino cultivars. However, there was a significant difference between the fungicide treatments and the interaction between fungicides and cultivars (Table 4).

The highest and most significant reduction of *A. alternata* incidence on the Onix cultivar was obtained with triticonazol + iprodione and difenoconazol fungicides with 55.1 and 44.9% of control, respectively (Table 5), values which can be considered low. With the Fundacep Cristalino cultivar, a significant reduction was obtained with triticonazol + iprodione, difenoconazol, carboxim + thiram and thiram fungicides, with 83.3%, 62.3%, 61.7% and 43.3% of control, respectively (Table 5). The fungicides triadimenol and triticonazol were not efficient in controlling *A. alternata* for either cultivar (Table 5). The iprodione active ingredient was responsible for the control of *A. alternata* in the Onix and Fundacep Cristalino cultivars.

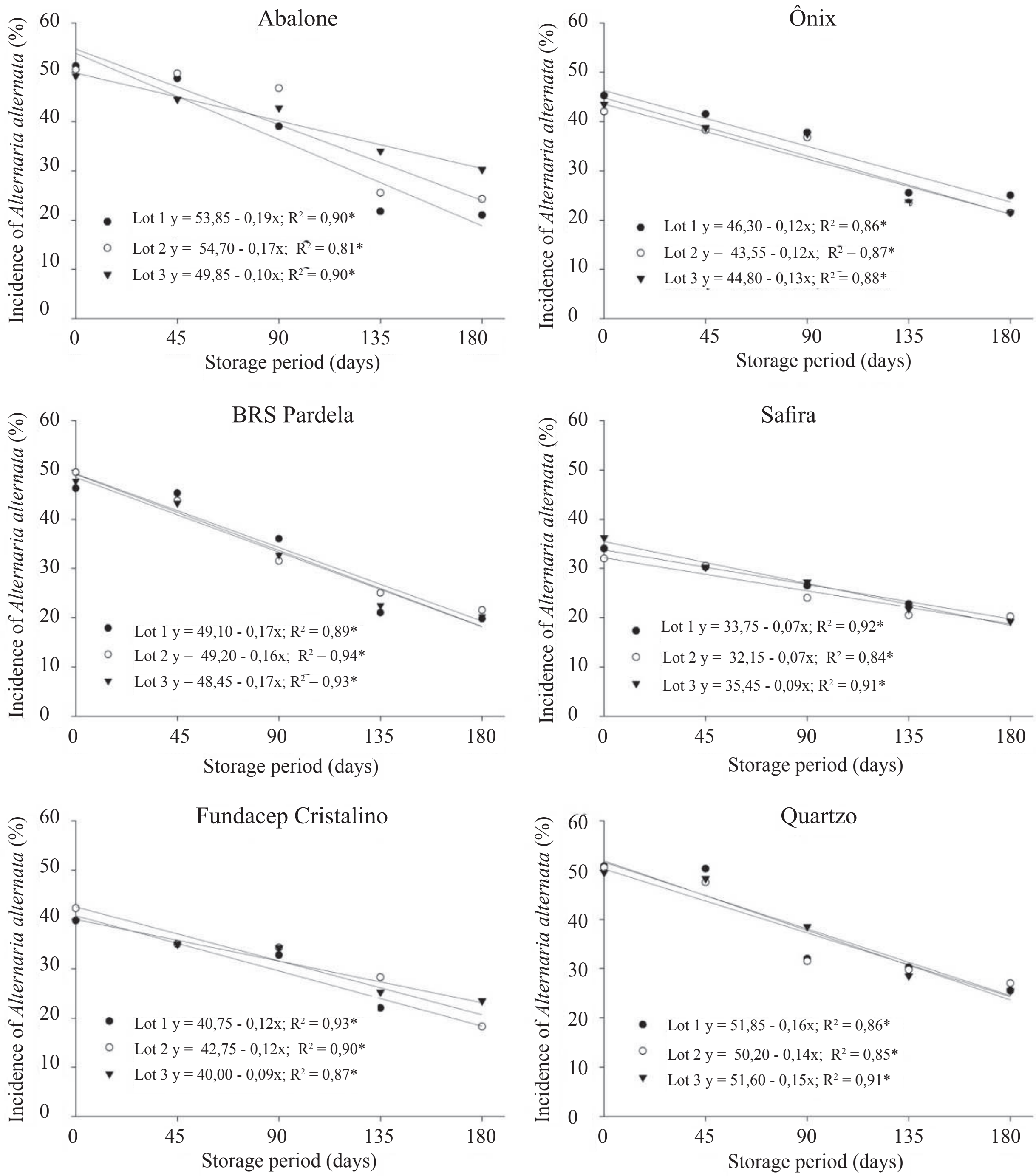

Figure 1. Relationship between the incidence of *Alternaria alternata* (%) and the storage period (days) for three lots of six wheat cultivars.

Table 4. Analysis of variance of chemical control of *Alternaria alternata* in seed treated with six fungicides from two wheat cultivars.

Source	D.F.	M.S. *Alternaria alternata* (%)
Cultivar (C)	1	6.64
Fungicide (F)	6	8.72*
C x F	6	1.44*
Block	3	0.40
Error	39	0.26
Total	55	-
C.V.	-	11.44

* Significant according to the F test ($p \leq 0.01$)

The mixture triticonazol + iprodione is recommended for controlling *D. tritici-repentis* and *B. sorokiniana* associated with wheat seeds (Salvadori et al., 2008). These two fungi, like *Alternaria,* belong to the Dematiaceae family, demonstrating the fungicidal efficiency of iprodione against pathogens of this family. The mixture iprodione + thiram is efficient for controlling *B. sorokiniana* "in vitro" and "in vivo" in wheat (Diehl, 1987; Forcelini and Reis, 1987; Goulart and Paiva, 1993). This mixture was also the best fungicide mixture to control *A. dauci* (Kuhn) Groves and Skolko and *A. alternata* on the seeds of the coriander Verdão cultivar, both for the filter paper test and the transmission test to seedlings (Reis et al., 2006).

Table 5. Incidence (%) of *Alternaria alternata* in seeds of Onix and Fundacep Cristalino cultivars treated with fungicides recommended by the Wheat Research Commission**.**

Treat.	Technical name	Rate[1]	*Alternaria alternata* (%)			
			Fundacep Cristalino		Onix	
			Incidence	Control	Incidence	Control
1	Carboxin + thiram	50+50	20.0 a*	18.4	11.5 bc	61.7
2	Difenoconazol	30	13.5 b	44.9	11.3 bc	62.3
3	Thiram	210	16.5 ab	32.6	17.0 b	43.3
4	Triadimenol	40.5	24.0 a*	2.0	35.0 a	0
5	Triticonazol	45	26.5 a*	0	37.0 a	0
6	Triticonazol + iprodione	30+50	11.0 b*	55.1	5.0 c	83.3
7	Control	---	24.5 a	---	30.0 a	---
C.V.			11.55		12.17	

[1](g a.i./100 kg of seed); Means followed by same letter in the column did not differ significantly by the Tukey test ($p \leq 0.01$).

Conclusions

The frequency and incidence of *A. alternata* in wheat seeds produced in southern Brazil is high.

A. alternata keeps its viability in the inter-harvest period and considering that seeds are stored for a period of six or seven months, it may be concluded that infected seeds are the primary inoculum source of *A. alternata.*

The triticonazol + iprodione fungicide mixture gives efficient control of *A. alternata*.

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