

Physiological quality of corn seeds treated with insecticides and stored at different temperatures






Abstract – The objective of this work was to evaluate the effect of the industrial treatment with insecticides on the physiological quality of corn (*Zea mays*) seeds during storage at different temperatures. Seeds of the BM 950 PRO3 and BM 709 PRO2 hybrids were subjected to industrial treatment with insecticides based on chlorantraniliprole, cyantraniliprole, and clothianidin, as well as to a control, and stored at different temperatures (10, 20, and 30°C) for 0, 90, 180, 270, and 360 days, in a 4x3x5 factorial arrangement for each hybrid, in a completely randomized design. To evaluate the quality of the seeds, tests of moisture content, germination, seedling emergence, accelerated aging, and cold were performed. For the seeds of both hybrids, germination is preserved at minimum commercialization standards for up to 360 days of storage, regardless of the insecticide or storage temperature. The temperature of 10°C preserves seed vigor and minimizes the negative effects caused by the insecticides during storage. At 20 and 30°C, vigor is cumulatively impaired as storage is extended, mainly at 30°C. Chlorantraniliprole results in a greater preservation of seed vigor regardless of storage temperature, whereas clothianidin provides a greater loss of vigor in industrially treated corn seeds, especially when stored at 30°C.

Index terms: *Zea mays*, deterioration, industrial seed treatment, storability.

Qualidade fisiológica de sementes de milho tratadas com inseticidas e armazenadas em diferentes temperaturas

Resumo – O objetivo deste trabalho foi avaliar o efeito do tratamento industrial com inseticidas sobre a qualidade fisiológica de sementes de milho (*Zea mays*) ao longo do armazenamento, em diferentes temperaturas. Sementes dos híbridos BM 950 PRO3 e BM 709 PRO2 foram submetidas ao tratamento industrial com inseticidas à base de clorantraniliprole, ciantraniliprole e clotianidina, além de a um controle, e armazenadas em diferentes temperaturas (10, 20 e 30°C) por 0, 90, 180, 270 e 360 dias, em arranjo fatorial 4x3x5 para cada híbrido, em delineamento inteiramente casualizado. Para avaliar a qualidade das sementes, foram realizados testes de grau de umidade, germinação, emergência de plântulas, envelhecimento acelerado e de frio. Para as sementes dos dois híbridos, a germinação é preservada nos padrões mínimos de comercialização por até 360 dias de armazenamento, independentemente do inseticida ou da temperatura de armazenamento. A temperatura de 10°C preserva o vigor das sementes e minimiza os efeitos negativos causados pelos inseticidas durante o armazenamento. A 20 e 30°C, o vigor é cumulativamente prejudicado à medida que o armazenamento é prolongado, principalmente a 30°C. O clorantraniliprole resulta em maior preservação do vigor da semente, independentemente da temperatura de armazenamento, enquanto a clotianidina proporciona maior perda de vigor em sementes de milho tratadas industrialmente, especialmente quando armazenadas a 30°C.

Termos para indexação: *Zea mays*, deterioração, tratamento industrial de sementes, armazenabilidade.

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Introduction

The quality of seeds is expressed through their potential to perform vital functions and form normal seedlings, even under stressful conditions after sowing (Pereira et al., 2011). The satisfactory establishment of the seedling stand in the field depends directly on the quality of the used seeds, which favors the emergence of seedlings in less time and with greater uniformity (Marcos-Filho, 2015; Pereira et al., 2019).

To simulate the adverse conditions in the field and assist in the evaluation of seed lots, the concept of vigor emerged, together with several tests to quantify this attribute (Baldini et al., 2018). Due to the competitiveness of the market, the demand for high vigor seeds is increasing, and this quality should be verified and maintained in all stages of production and post-harvest.

One of the main factors influencing the quality of corn (*Zea mays* L.) seeds is their storage condition, with emphasis on the temperature and relative humidity in the warehouse, which are essential to preserve the viability of the lots (Timóteo & Marcos-Filho, 2013). Associated with other environmental factors, corn seeds treated and stored with insecticides may show a greater drop in vigor and germination during the storage period (Oliveira et al., 2020).

When seeds receive chemical treatment, phytosanitary products are applied to envelop the integument in a protective layer against organisms that cause damage, such as pests and pathogens (Vazquez et al., 2014). With the advancement in application technology and the development of less toxic molecules, seed-producing companies have been adopting techniques that seek to optimize the logistics of such treatment (Mariucci et al., 2018). One of these techniques is industrial seed treatment, in which the used products are applied in the processing line itself and the treated seeds are stored until sowing (Brzezinski et al., 2015).

However, industrial seed treatment has limitations, especially in relation to the possible phytotoxic effects of the used phytosanitary products on seeds during storage, such as loss of germination potential and vigor in some cases (Santos et al., 2018; Carvalho et al., 2020; Oliveira et al., 2020). Currently, for corn seed treatment, especially for industrial seed treatment, several insecticide molecules have been used, including chlorantraniliprole, cyantraniliprole, and clothianidin

(Zhang et al., 2019); however, studies related to their effects on seed quality during storage are still needed.

In researches on the storage of treated seeds, insecticides should be taken into account because they are widely adopted in industrial corn seed treatment and they are reported to reduce seed quality during storage (Salgado & Ximenes, 2013; Deuner et al., 2014; Fátima et al., 2014; Mariucci et al., 2018; Oliveira et al., 2020).

The objective of this work was to evaluate the effect of the industrial treatment with insecticides on the physiological quality of corn seeds during storage at different temperatures.

Materials and Methods

The experiment was carried out at the main seed analysis laboratory of Department of Agriculture of Universidade Federal de Lavras, located in the municipality of Lavras, in the state of Minas Gerais, Brazil. Corn seeds of the BM 950 PRO3 and BM 709 PRO2 single-cross hybrids were used, classified physically as C1M and R3M, respectively (Brasil, 2009). The lots were obtained from the 2019/2019 harvest, produced in the municipality of Paracatu, also in the state of Minas Gerais.

Both used hybrids were treated a priori with a standard spray solution composed of the deltamethrin (8.0 mL 100 kg⁻¹ of seeds) and pyrimphos-methyl (1.6 mL 100 kg⁻¹ of seeds) insecticides for storage pests and of the carbendazim + thiram (60 mL per 60,000 seeds) fungicides.

Then, the seeds were treated with additional insecticides using the industrial seed treatment technology. The following insecticides were applied: chlorantraniliprole (625 g L⁻¹ of active ingredient), cyantraniliprole (600 g L⁻¹ of active ingredient), and clothianidin (600 g L⁻¹ of active ingredient). For seed treatment, the solution applied consisted of: a polymer of the Biocroma line (BioGrow, Valinhos, SP, Brazil), with a density of 1.05 to 1.15 g mL⁻¹, viscosity of 300 to 1,000 cPs at 25°C, and pH of 6 to 8; and distilled water. Afterwards, the Biogloss liquid (BioGrow, Valinhos, SP, Brazil) drying powder was applied, with the chemical characteristic of a mixture of mineral charges (2.8 to 3.2 g mL⁻¹) insoluble in water. Table 1 details seed treatment; all final volumes of the spray solution were 1,500 mL 100 kg⁻¹ of seeds.

The Arktos Laboratório L5K laboratory machine (Momesso, Birigui, SP, Brazil) was used to carry out the treatments, with a 15 hertz calibration in the equipment's inverter, simulating the process of industrial seed treatment in batches. Treatments were applied by subdividing the lots into 2.5 kg of seeds per treatment, dividing the total volume of the spray solution into two applications of 20 s to ensure coverage uniformity, followed by 10 s to finish off with the drying powder.

The seeds were then placed in multifoliate paper bags and stored in chambers of the biochemical oxygen demand (BOD) type, with controlled temperature. The simulated constant temperatures throughout storage were 10, 20, and 30°C. Treatment and storage were carried out on 9/4/2019, and seed physiological quality was assessed at 0 day (right after treatment), 90, 180, 270, and 360 days after storage. Temperature and relative humidity conditions during the storage period are shown in Figure 1.

The physiological quality of the seeds was analyzed using the tests of moisture content, germination, seedling emergence, accelerated aging, and cold.

Seed moisture content (%) was evaluated by the forced-air convection oven method, at 105°C, for 24 hours, in four replicates, according to the rules for seed analyses in Brazil (Brasil, 2009).

In the germination evaluations, the seeds were distributed evenly between two sheets of germination test paper, using a volume of distilled water for imbibition 2.5 times the weight of the dry paper. Then, the rolls were conditioned in the EL006 Mangelsdorf-type germinator (Eletrolab Indústria e Comércio de Equipamentos para Laboratório Ltda., São Paulo, SP, Brazil), at a temperature of 25°C. Four replicates of 50 seeds per hybrid were used. The assessments were carried out seven days after sowing, and the results

were expressed as percentage following the rules for seed analyses (Brasil, 2009).

For the seedling emergence test, a substrate composed of a mixture of sand + soil at a ratio of 2:1, respectively, was placed in plastic trays. After sowing, the seeds were covered with a layer of 2.0 to 3.0 cm of the substrate, which was moistened to 60% of its retention capacity; irrigation was performed when necessary. The trays were kept in a plant growth chamber at 25°C and under a 12-hour photoperiod. Four replicates of 50 seeds per hybrid were used. The evaluations were done on the seventh day after sowing, by computing the number of emerged seedlings, and the results were expressed in percentage.

The cold test was carried out using a substrate consisting of 2/3 of sand and 1/3 of soil, deposited in plastic trays. The seeds were evenly distributed and covered with a layer of approximately 2.0 to 3.0 cm of the substrate, which was irrigated until reaching 70% of its retention capacity. Four replicates of 50 seeds per hybrid were used. The seeds remained in a cold chamber for seven days at 10°C. Then, they were kept, during seven days, in the plant growth chamber with a temperature of 25°C and a photoperiod of 12 hours; at the end of that period, normal seedlings that emerged were counted, and the results were expressed as percentage (Caseiro & Marcos Filho, 2002).

For vigor evaluations through accelerated aging, the used method was an adaptation of the clear polystyrene tray, containing 40 mL of distilled water and a single layer of seeds covering the entire suspended screen. Subsequently, the covered trays were kept in a BOD chamber at 42°C for 96 hours (Marcos-Filho, 2020). Then, four replicates of 50 seeds per hybrid were subjected to the methodology described for the germination test (Brasil, 2009), and the assessments were carried out four days after sowing, with results expressed in percentage of normal seedlings.

Table 1. Insecticides and rates used for the treatment of corn (*Zea mays*) seeds.

Insecticide	Active ingredient in spray solution (g 100 kg ⁻¹ of seeds)	Drying powder (mL 100 kg ⁻¹ of seeds)	Polymer (mL 100 kg ⁻¹ of seeds)	Control ⁽¹⁾ (mL 100 kg ⁻¹ of seeds)
Chlorantraniliprole	210	100	582	582
Cyantraniliprole	210	100	575	575
Clothianidin	210	100	575	575
-	0	100	750	750

⁽¹⁾Water, without insecticide.

The experimental design used was completely randomized, consisting of a 4x3x5 factorial arrangement, with four seed treatments using different active ingredients (Table 1), at three temperatures (10, 20, and 30°C) and during five periods (0, 90, 180, 270, and 360 days) of storage. Each corn hybrid was analyzed independently.

The analysis of variance was used for the statistical analyzes with the aid of the Sisvar software (Ferreira, 2014), at 5% probability, by the F-test, and Tukey's test, also at 5% probability, was used for the analysis of the means of the sources of qualitative variation. For quantitative sources, polynomial regression analyzes were performed, with the choice of significant mathematical models at 5%, with a higher coefficient of determination.

Results and Discussion

Through the analysis of variance, it was possible to observe significant differences ($p < 0.05$) between insecticides, temperatures, and storage periods, with or without interactions between these factors, depending on each physiological parameter analyzed.

In general, seed moisture content showed similar trends throughout storage, with values starting low,

varying up to 180 days, and later stabilizing as a function of storage temperatures (Figure 2). This can be attributed to two main factors: the hygroscopic balance between the seeds and the environment since the storage period began in September – early spring; and to the increase in the relative humidity of the environment due to the spring and summer seasons (Figure 1), which increased seed moisture content, especially in the first 90 days.

According to the obtained results (Figure 2), higher storage temperatures provided a lower relative humidity after 180 days, with an increase in the difference between treatments as the storage period was extended. This could be related to some factors involved in the hygroscopic balance of seeds, such as the temperature and relative humidity of the environment. Seed moisture content varies according to the storage environment, with higher temperatures favoring gradual drying (Santos et al., 2020). The increase in the moisture holding capacity of air at higher temperatures causes seeds to lose more moisture to the environment (Marcos-Filho, 2015).

Storage time and conditions are, therefore, important factors to be considered in seed production (Carvalho et al., 2014). Depending on the analysis of the data, the

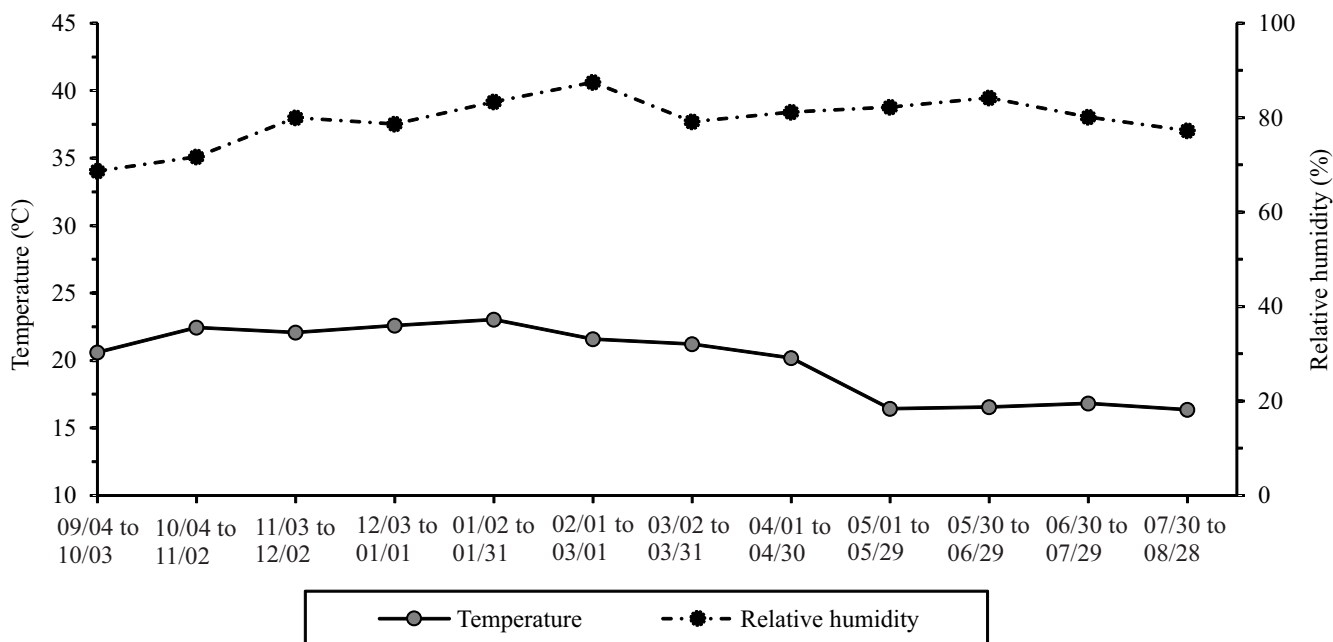


Figure 1. Average values of temperature and relative humidity during the storage period of treated corn (*Zea mays*) seeds. Data were obtained from the environmental station at Universidade Federal de Lavras, located in the municipality of Lavras, in the state of Minas Gerais, Brazil. Source: Inmet (2021).

isolated effect of the storage period on the germination response variable was significant (Figure 3).

The average germination of both assessed hybrids after the application of the used phytosanitary products and before storage was greater than 90%, which shows the high initial quality of the materials and their suitability for use in industrial seed treatment. The estimated drop in germination during storage was linear, as follows: 98.35, 97.53, 96.71, and 95.89% for the BM 950 PRO3 hybrid and 93.52, 92.39, 91.27, and 90.14% for BM 709 PRO2 at 90, 180, 270, and 360 days, respectively.

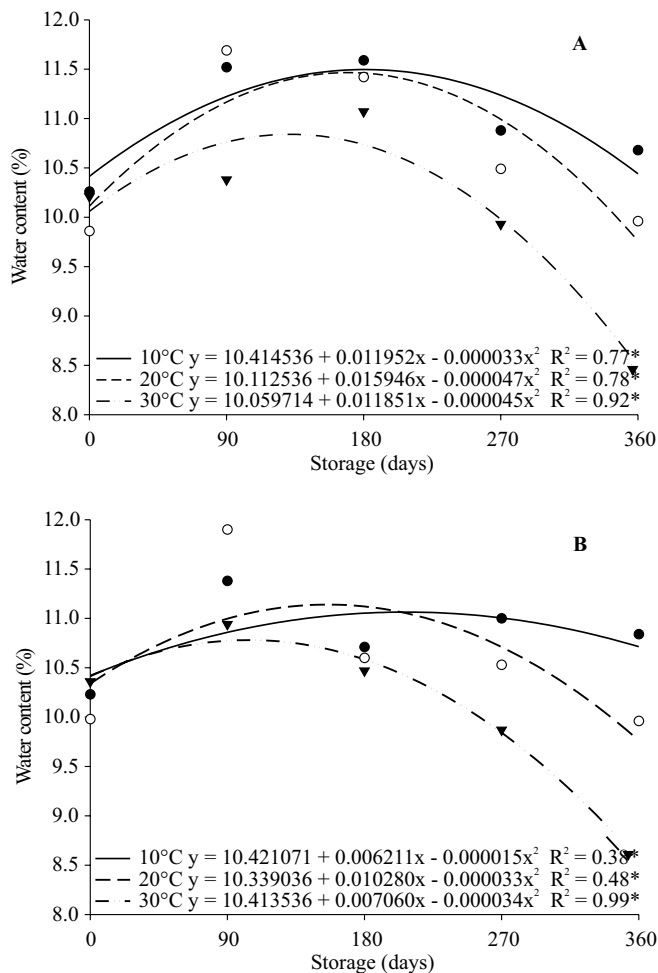


Figure 2. Moisture content of corn (*Zea mays*) seeds from the BM 950 PRO3 (A) and BM 709 PRO2 (B) hybrids as a function of storage temperatures of 10 (●), 20 (○), and 30°C (triangle) during storage periods of 0, 90, 180, 270, and 360 days. *Significant by the F-test, at 5% probability.

When assessing the germination potential of corn seeds during storage, Heberle et al. (2019) also found a drop in the trend curves of all lots after 90 days, regardless of the storage environment. Furthermore, the authors highlighted the importance of low temperatures and a lower relative humidity in the environment to maintain, for a longer period – up to 450 days after storage in a cold chamber –, a sufficient seed quality for marketing.

In the present study, even with a significant reduction in germination during the storage period, seed quality did not drop below 85% for both hybrids even after 360 days of storage; consequently, the used seeds could be marketed according to Brazilian legislation (Brasil, 2013).

Storage temperature, therefore, is an essential factor for maintaining the viability of corn seeds. In isolation, it affected the results obtained for germination, seedling emergence, and the cold test (Table 2).

Storage at 30°C was considered inadequate, with lower means in all tests compared with 10°C. This confirms that the use of lower temperatures – ideal range below 20°C – is crucial to preserve the quality of corn seeds during storage (Rahmawati & Aqil, 2020).

Seeds stored at 20°C had a higher germination than those stored at 30°C; however, in the other tests, no

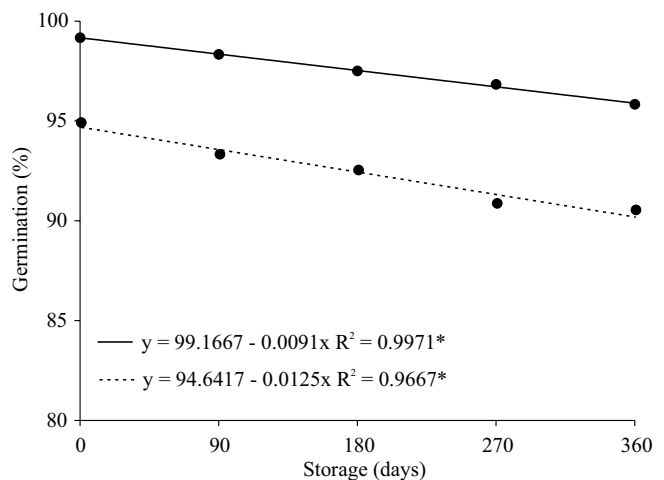


Figure 3. Germination of corn (*Zea mays*) seeds from the BM 950 PRO3 (continuous line) and BM 709 PRO2 (dotted line) hybrids as a function of storage periods of 0, 90, 180, 270 and 360 days. *Significant by the F-test, at 5% probability.

significant differences were observed for 30°C for the BM 950 PRO3 hybrid (Table 2). This is indicative of a drop in vigor before the loss of germination potential (Marcos-Filho, 2015), i.e., of a stress condition that may later affect germination if storage at 20°C is extended.

Higher storage temperatures are responsible for accelerating the metabolic rate and respiration of seeds, favoring biochemical reactions that degrade proteins and membranes, consume reserves, inhibit vigor and germination, and reduce storage viability time of seeds (Stefanello et al., 2015).

In the accelerated aging test, the interaction between temperatures and storage periods was significant for the vigor of seeds from both hybrids (Figure 4).

As the storage period is extended, there is cumulative stress on the metabolic apparatus of the seeds, especially at higher temperatures, with wearing of the membrane repair mechanisms and loss of vigor in the accelerated aging methodology, besides a drop in enzymatic activity, which acts to remove toxic compounds such as peroxides (Kurek et al., 2019). However, at 10°C, the decrease in metabolic activity preserves the enzymatic repair apparatus of the cells for a longer period (Marcos-Filho, 2015), resulting in a greater capacity of seeds to suppress deterioration (Timóteo & Marcos-Filho, 2013).

Predicting the safe storage period for the treated hybrid corn seeds is highly important since seeds can be stored for more than six months before sowing and

Table 2. Germination, seedling emergence (SE), and cold test of corn (*Zea mays*) seeds from the BM 950 PRO3 and BM 709 PRO2 hybrids as a function of storage temperatures⁽¹⁾.

Temperature (°C)	BM 950 PRO3			BM 709 PRO2		
	Germination ⁽²⁾ (%)	SE ⁽²⁾ (%)	Cold test ^(2,3) (%)	Germination ⁽²⁾ (%)	SE ⁽²⁾ (%)	Cold test ^(2,3) (%)
10	98.15a	98.60a	98.43a	93.65a	93.83a	93.80a
20	97.65a	98.00b	98.00ab	92.78a	92.58b	92.90b
30	96.80b	97.65b	97.38b	90.78b	91.45c	92.00c
CV (%) ⁽⁴⁾	1.50	1.51	1.83	2.57	1.80	1.74

⁽¹⁾Means followed by equal letters, in the columns, do not differ from each other by Tukey's test, at 5% probability. ⁽²⁾The values are the averages of four replicates of 50 seeds analyzed in different storage periods after industrial seed treatment. ⁽³⁾Percentage of normal seedlings after seed exposure to 10°C and high substrate humidity during seven days. ⁽⁴⁾Coefficient of variation.

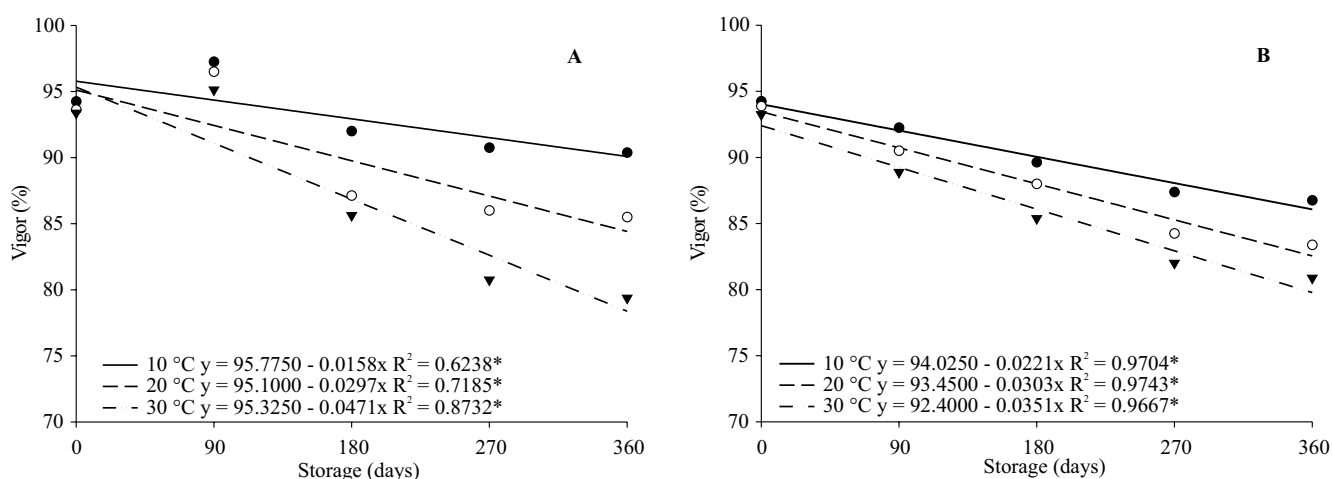


Figure 4. Vigor after accelerated aging of corn (*Zea mays*) seeds from the BM 950 PRO3 (A) and BM 709 PRO2 (B) hybrids as a function of storage temperatures of 10 (●), 20 (○), and 30°C (triangle) during storage periods of 0, 90, 180, 270, and 360 days. *Significant by the F-test, at 5% probability.

the latent effects that impair seedling development are intensified as this period is extended (Figure 4). The observed degenerative effects can be enhanced when seed treatment is associated with insecticide products (Deuner et al., 2014).

Regarding the insecticide products used in seed treatment, their active ingredients significantly affected germination, seedling emergence, cold test, and accelerated aging for the BM 709 PRO2 hybrid, as well as germination and vigor due to accelerated aging for BM 950 PRO3 (Table 3).

The effects of the insecticides on the seeds also differed (Table 3). Seeds of the BM 950 PRO3 hybrid showed a higher germination when treated with cyantraniliprole, compared with chlorantraniliprole. In addition, seeds of BM 709 PRO2 had better results when treated with chlorantraniliprole rather than with clothianidin.

Likewise, for both studied hybrids, vigor due to accelerated aging was higher in the control seeds and in those treated with chlorantraniliprole (Table 3). Oliveira et al. (2020) observed that some insecticide products can affect the vigor of corn seeds in the treatment followed by storage with clothianidin, thiamethoxam, and fipronil, compared with the control.

For the BM 950 PRO3 hybrid, no significant differences were found between treatments regarding the cold test and seedling emergence (Table 3). However,

for BM 709 PRO2, the values for these variables were higher when using the control and chlorantraniliprole and lower with clothianidin. Among the analyzed molecules, the active ingredient clothianidin is the only one belonging to the class of neonicotinoids; the others belong to the class of anthranilic diamides. When evaluating the phytotoxicity of insecticides on hybrid corn seeds after treatment and storage, Oliveira et al. (2020) reported a drop in germination after nine months in seeds treated with clothianidin, thiamethoxam, and fipronil; the first two ingredients are part of the chemical group of neonicotinoids and the last one of the pyrazole group.

The selectivity and low toxicity of the insecticides to the physiological quality of stored seeds is an important characteristic of the products applied for seed treatment, especially of those used in industrial seed treatment (Rocha et al., 2020). Therefore, before being commercially applied, both the effect of a single insecticide and the interaction of two or more products needs to be clarified (Delian et al., 2016; Mariucci et al., 2018). Interactions, mainly between insecticides, can intensify the negative effects of active ingredients on the viability of corn seeds during storage (Baldini et al., 2018).

The obtained results are indicative that the storage period with lower loss of seed quality is directly related to: the environment and period in which the seeds are stored; the phytosanitary products used;

Table 3. Germination, seedling emergence, cold test, and accelerated aging of corn (*Zea mays*) seeds from the BM 950 PRO3 and BM 709 PRO2 hybrids as a function of treatments with insecticides⁽¹⁾.

Hybrid	Insecticide ⁽²⁾	Germination ⁽³⁾ (%)	Seedling emergence ⁽³⁾ (%)	Cold test ^(3,4) (%)	Accelerated aging ⁽³⁾ (%)
BM 950 PRO3	Control (water)	97.47ab	98.37a	98.23a	90.43a
	Chlorantraniliprole	97.13b	98.13a	97.60a	90.73a
	Cyantraniliprole	98.03a	98.03a	98.33a	89.50ab
	Clothianidin	97.50ab	97.80a	97.57a	88.70b
	CV (%) ⁽⁵⁾	1.50	1.51	1.83	3.33
BM 709 PRO2	Control (water)	92.73ab	93.43a	93.67a	88.60a
	Chlorantraniliprole	93.13a	93.03a	93.27ab	88.73a
	Cyantraniliprole	92.10ab	92.03b	92.63bc	87.77ab
	Clothianidin	91.63b	91.97b	92.03c	87.07b
	CV (%) ⁽⁵⁾	2.57	1.80	1.74	2.41

⁽¹⁾Means followed by equal letters, in the columns, do not differ from each other by Tukey's test, at 5% probability. ⁽²⁾Insecticides applied in industrial seed treatment. ⁽³⁾The values are the averages of four replicates of 50 seeds analyzed in different storage periods (0, 90, 180, 270, and 360 days) at different temperatures (10, 20, and 30°C). ⁽⁴⁾Percentage of normal seedlings after seed exposure to 10°C and high substrate humidity during seven days. ⁽⁵⁾Coefficient of variation.

and the interaction between the active ingredients of the insecticides, which is important to consider since seed-producing companies apply treatments that may negatively interact with other treatments targeting pests and pathogens in the field.

Conclusions

1. For corn (*Zea mays*) seeds from hybrids BM 950 PRO3 and BM 709 PRO2, germination is preserved at minimum commercialization standards for up to 360 days of storage, regardless of the seed industrial treatment with the chlorantraniliprole, cyantraniliprole, clothianidin insecticides or of the storage temperatures of 10, 20, and 30°C.

2. The storage temperature of 10°C preserves seed vigor and minimizes the negative effect caused by insecticides, regardless of the active ingredient used; however, at 20 and 30°C, vigor is cumulatively impaired as storage is extended, with a significant effect at 30°C.

3. Among the tested insecticides, chlorantraniliprole leads to a greater preservation of the vigor of the stored corn seeds, regardless of genotype and storage temperature, whereas clothianidin causes a greater loss of vigor in industrially treated corn seeds, mainly when stored at 30°C.

Conclusions

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variedades, girassol cultivares híbridas, juta, linho, mamona variedades, mamona cultivares híbridas, milho variedades, milho cultivares híbridas, painço, soja, sorgo variedades, sorgo cultivares híbridas, tabaco, trigo, trigo duro, triticale e de espécies de grandes culturas inscritas no Registro Nacional de Cultivares - RNC e não contempladas com padrão específico, a partir do início da safra 2013/2014, na forma dos Anexos I a XXX desta Instrução Normativa]. **Diário Oficial da União**, 18 set. 2013, Seção1, p.6-27.

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