

Effect of treatment with thiamethoxam and cyantraniliprole on the germination and storage of forest tree seeds

Thayane Ferreira Carvalho¹, Patricia Doerl Barroso^{1*}, Everson Reis Carvalho¹, Jose Marcio Rocha Faria¹, Anderson Cleiton José¹

ABSTRACT: Seed predation is a problem that limits the use of direct sowing of tree species, and it is necessary to develop studies involving products used in seed treatment. The objectives of this study were to evaluate the effects of treatment with insecticides on the storage and germination of seeds of six forest species. Seeds were treated with thiamethoxam and cyantraniliprole and germinated on paper and sand substrates at 25 °C under constant light. For storage, the seeds were treated and analyzed at 0, 3 and 6 months. For both tests, the following variables were evaluated: germination, mortality, hard seeds, abnormal seedlings, average length of shoot and primary root, and germination speed index (GSI). For *Senna multijuga*, treatment with insecticides had no effect. *Peltophorum dubium* and *Enterolobium contortisiliquum* showed positive effects of the treatments on seedling vigor and development, while *Guazuma ulmifolia* displayed a negative effect on primary root length. *Bowdichia virgilioides* and *Hymenaea courbaril* seeds were sensitive to the treatments. It is possible to treat and store *P. dubium* and *E. contortisiliquum* seeds with thiamethoxam for up to six months.

Index terms: chemical control, phytotoxicity, seed treatment.

RESUMO: A predação de sementes é um problema que limita o uso da semeadura direta de espécies arbóreas, sendo necessário o desenvolvimento de estudos envolvendo produtos utilizados no tratamento dessas sementes. O objetivo do presente trabalho foi avaliar o efeito do tratamento com inseticidas sobre o armazenamento e germinação de sementes de seis espécies florestais. As sementes foram tratadas com tiametoxam e ciantraniliprole e colocadas para germinar nos substratos papel e areia, a 25 °C, sob luz constante. Para o armazenamento as sementes foram tratadas e com o tempo de 0, 3 e 6 meses foram realizadas análises, para ambos os testes foram avaliadas as variáveis: germinação, mortalidade, sementes duras, plântulas anormais, comprimento médio de raiz primária, parte aérea e índice de velocidade de germinação (IVG). Para a *Senna multijuga*, o tratamento com inseticidas foi indiferente. No caso de *Peltophorum dubium* e *Enterolobium contortisiliquum*, os tratamentos proporcionaram resultados positivos para vigor e desenvolvimento de plântulas. Em *Guazuma ulmifolia* foi observado um efeito negativo dos tratamentos sobre a variável comprimento de raiz primária. Sementes de *Bowdichia virgilioides* e *Hymenaea courbaril* apresentaram sensibilidade aos tratamentos. Foi possível armazenar sementes de *P. dubium* e *E. contortisiliquum* tratadas com tiametoxam por até seis meses.

Termos para indexação: controle químico, fitotoxicidade, tratamento de sementes.

Journal of Seed Science, v.46,
e202446027, 2024



<http://dx.doi.org/10.1590/2317-1545v46279811>

*Corresponding author
patricia.barroso@estudante.ufla.br

Received: 10/23/2023.
Accepted: 08/16/2024.

¹Universidade Federal de Lavras,
Campus Universitário, Lavras,
Minas Gerais, Brasil.

INTRODUCTION

Direct seeding is a technique that has rarely been explored in forest restoration. The method is still very limited, given that some species can have low germination rates, uneven germination, slow seedling development, seed and seedling predation and low seed vigor, leading to failure in most cases (Silva and Vieira, 2017).

To minimize these problems, the search for preventive measures is of fundamental importance. A viable alternative to the problem of predation by insects is seed treatment with insecticides (Martins et al., 2009). Its use can maintain physiological quality, making a significant contribution to proper initial sowing development (Barros et al., 2005).

Among the most widely reported insecticides in the literature are nicotine-derived thiamethoxam, which has a systemic action, high water solubility, is biodegradable, has a broad spectrum of action, can be used at low concentrations, has a long residual effect and has moderate toxicity to humans and the environment. Another insecticide is cyantraniliprole, which belongs to the diamide group, has a systemic action through contact and ingestion, a widespread spectrum of action, low toxicity to mammals and is effective at low doses (Murillo et al., 2015; Zhang et al., 2015; Costa et al., 2018).

Although research on the effects of insecticides on seeds has shown favorable results, and even bioactivation has been reported, before opting for treatment, some points must be considered, such as the effectiveness of the insecticide selected, the presence of phytotoxicity, and the viable storage time of the treated seeds, so that there are no negative effects on seed vigor and viability, causing damage to sowing and seed storage (Carvalho et al., 2020).

The aim of this study was to evaluate the effects of seed treatment with insecticides on the germination and initial development of seedlings of six forest species and to study the influence of thiamethoxam on the physiological performance of forest seeds during storage.

MATERIAL AND METHODS

The experiments were carried out at the Forest Seed Laboratory (LSF) of the Department of Forest Sciences and Central Seed Laboratory (LCSEM) of the Department of Agriculture, both at the *Universidade Federal de Lavras (UFLA)*.

Seeds of *Bowdichia virgilioides*, *Guazuma ulmifolia*, *E. contortisiliquum*, *Hymenaea courbaril*, *P. dubium* and *Senna multijuga* were used. The dormancy of *B. virgilioides*, *S. multijuga* and *P. dubium* seeds was overcome in sulfuric acid for 10, 15 and 20 minutes, respectively. Seeds of *E. contortisiliquum* and *H. courbaril* were mechanically scarified. *G. ulmifolia* seeds were immersed in hot water (100 °C).

For the treatment of the seeds with insecticides, the volume of spray used was 300 mL of the commercial product (thiamethoxam), with 700 mL of water for every 100 kg of seeds for the species *P. dubium*, *S. multijuga*, *B. virgilioides* and *G. ulmifolia* and 400 mL of the commercial product topped with 700 mL of water for every 100 kg of seeds for the species *E. contortisiliquum* and *H. courbaril*. For the insecticide cyantraniliprole, the dosages were 200 mL of the commercial product and 700 mL of water for every 100 kg of seeds for the species *P. dubium*, *S. multijuga*, *B. virgilioides* and *G. ulmifolia* and 350 mL of the commercial product and 700 mL of water for every 100 kg of seeds for the species *E. contortisiliquum* and *H. courbaril*.

To treat the seeds, the mixture (product + distilled water) was applied to the bottom of a transparent plastic bag via a graduated pipette. The bags containing the seeds plus the syrup were flushed with air and shaken vigorously for three minutes to evenly distribute the insecticides across the surface of the seeds. The seeds were then removed and spread on plastic trays so that the mixture could dry for approximately 12 hours in an air-conditioned room with an average temperature of 20 °C and a relative humidity of 50%. Afterward, the seeds were allowed to germinate on two substrates: germitest paper and sand.

The experiment consisted of a completely randomized design (DIC) in a 2x3 factorial arrangement, with two substrates (sand and paper) and three groups of seeds (seeds treated with thiamethoxam, seeds treated with cyantraniliprole and

the control), with four replications of 25 seeds each. Each species was subjected to a different experiment. Germitest paper was moistened with distilled water at a rate of 2.5 times the weight of the dry paper and placed in Gerboxes. Sand was sterilized, moistened and then placed in gerboxes with lids. The experiment was carried out in a germinator at 25 °C and constant light.

For storage, only the seeds of *P. dubium* and *E. contortisiliquum* were used. The seeds were packed in plastic bags and then stored in a cold and dry room, with temperatures ranging from 5 to 8 °C and a relative humidity of 40%. The physiological quality of the stored seeds was assessed at 0, 3 and 6 months via germination tests. The experiments were set up in a 2x3 factorial arrangement (seeds treated and not treated with thiamethoxam × three storage times: 0, 3 and 6 months, with four replications of 25 seeds, at 25 °C, with constant light (Brasil, 2013).

Germination was measured for up to 14 days for *P. dubium* and *E. contortisiliquum*, 7 days for *S. mutijuga*, 21 days for *G. ulmifolia*, 28 days for *H. courbaril* and 60 days for *B. virgilioides* (Brasil, 2013). The percentage of hard seeds, dead seeds, germination speed index, length of shoot and primary root, and percentage of abnormal seedlings were determined.

The data from both experiments were subjected to the Shapiro–Wilk test for normality and Bartlett’s test for homogeneity. When there was no normality or homoscedasticity of the data, nonparametric data analysis was used, the Kruskal–Wallis test was used, and when a significant difference was found ($p < 0.05$), the means were compared via the Student–Newman–Keuls (SNK) test at 5% significance. When the ANOVA assumptions were respected, Tukey’s test was used at 5% significance when significant ($p < 0.05$). The data were processed via R for Windows version 3.6.2 (R Core Team, 2023).

RESULTS AND DISCUSSION

The effect of treatment with cyantraniliprole on germination was observed in *B. virgilioides* seeds. When the final averages were compared, regardless of the substrate used, the seeds treated with cyantraniliprole presented a lower germination percentage and hard seeds than did the control (SNK at 5% significance). In *H. courbaril*, the control had a greater percentage of germination than did the other treatments ($p > 0.05$). For the mortality variable, a significant difference was observed when the final averages were evaluated, regardless of the substrate used, in *B. virgilioides*, where mortality was higher in seeds treated with thiamethoxam and cyantraniliprole, and in *H. courbaril*, where mortality was higher in the treatment where thiamethoxam was applied. The action of the insecticides on the formation of abnormal seedlings was observed only in *H. courbaril* when germitest paper was used as a substrate, where the highest percentage of abnormal seedlings was observed in the control ($p > 0.05$) (Table 1).

Similar response patterns in terms of germination, mortality, abnormal seedlings and hard seeds between the control and thiamethoxam and cyatraniliprole treatments in both substrates used revealed that there was no negative interference from the insecticides on the performance of *P. dubium* seeds, *S. multijuga*, *E. contortisiliquum* or *G. ulmifolia* when subjected to the respective treatments. These results are similar to those reported in agricultural species such as cotton (Abdel-Hamid et al., 2016) and rice (Tang et al., 2017) treated with thiamethoxam. Rice seed treatment also promoted an increase in crop yield, demonstrating the beneficial effects of the insecticide. On the other hand, soybean seeds treated with thiamethoxam and cyantraniliprole presented no physiological changes compared with those of the control, indicating that thiamethoxam had no positive or negative effects, corroborating the results presented here (Soares et al., 2019). Notably, in this first stage, the forest seeds were treated with the insecticides and evaluated immediately afterwards, i.e., they were not stored. The most common type of phytotoxicity involves prolonged storage of seeds after treatment with insecticides (Carvalho et al., 2020).

The physiological attributes of seeds, whether harmful or beneficial, may or may not be altered by chemical treatment (Almeida et al., 2011). These characteristics depend not only on the active ingredient but also on the intrinsic characteristics of the species to be treated, as well as the quality of the seeds (Tonin et al., 2014).

Table 1. Average germination percentage, mortality, hard seeds and abnormal seedlings of *P. dubium*, *S. multijuga*, *E. contortisiliquum*, *G. ulmifolia*, *B. virgilioides* and *H. courbaril*. Control and seeds treated with thiamethoxam (Ttx) and cyantraniliprole (Ctp) were germinated on paper (SP) and sand (S) substrates.

Species	Treat.	Germination (%)			Mort. (%)			Hard (%)			A.S.		
		P	S	χ	P	S	χ	P	S	χ	P	S	χ
<i>P. dubium</i>	Cont.	81 Aa	92 Aa	87 a	14 Aa	4 Aa	9 a	5 Aa	4 Aa	5 a	7 Aa	6 Aa	7a
	Ctp	90 Aa	87 Aa	89 a	7 Aa	7 Aa	7 a	3 Aa	6 Aa	5 a	14 Aa	7 Aa	11 a
	Ttx	89 Aa	88 Aa	89 a	9 Aa	8 Aa	9 a	2 Aa	4 Aa	3 a	6 Aa	12 Aa	9 a
	Avg.	87 A	89 A		10 A	6.3 A		3 A	5 A		9 A	8 A	
	CV (%)		10.5			84.2			114			84.6	
<i>S. multijuga</i>	Cont.	82 Aa	90 Aa	86 a	7 Aa	4 Aa	6 a	11 Aa	6 Aa	9 a	11 Aa	6 Aa	9 a
	Ctp	86 Aa	94 Aa	90 a	5 Aa	1 Aa	3 a	9 Aa	5 Aa	7 a	9 Aa	5 Aa	7 a
	Ttx	88 Aa	94 Aa	91 a	5 Aa	2 Aa	4 a	7 Aa	4 Aa	6 a	7 Aa	4 Aa	6 a
	Avg	85 A	93 A		6 A	2 A		9 A	5 A		8 A	6 A	
	CV (%)		9.9			129.1			87.3			85	
<i>E. contortisiliquum</i>	Cont.	74 Aa	79 Aa	77 a	14 Aa	13 Aa	14 a	12 Aa	8 Aa	10 a	0 Aa	2 Aa	1 a
	Ctp	75 Aa	72 Aa	74 a	11 Aa	16 Aa	14 a	9 Aa	12 Aa	13 a	2 Aa	3 Aa	3 a
	Ttx	73 Aa	81 Aa	77 a	18 Aa	8 Aa	13 a	9 Aa	11 Aa	10 a	0 Aa	4 Aa	2 a
	Avg	76 A	77 A		14 A	12 A		10 A	10 A		1 A	3 A	
	CV (%)		12.5			64.1			82			183.6	
<i>G. ulmifolia</i>	Cont.	74 Aa	79 Aa	77 a	4 Aa	3 Aa	3 a	23 Aa	18 Aa	20 a	0 Aa	1 Aa	0 a
	Ctp	75 Aa	72 Aa	74 a	3 Aa	4 Aa	3 a	22 Aa	24 Aa	23 a	1 Aa	1 Aa	1 a
	Ttx	73 Aa	81 Aa	77 a	5 Aa	2 Aa	3 a	23 Aa	17 Aa	20 a	0 Aa	1 Aa	1 a
	Avg	74 A	73 A		4 A	3 A		22 A	20 A		1 A	1 A	
	CV (%)		12.5			66.1			39.0			183.6	
<i>B. virgilioides</i>	Cont.	89 Aa	86 Aa	88 a	3 Aa	4 Aa	3 b	9 Aa	11 Aa	10 b	1 Aa	0 Aa	1 a
	Ctp	74 Aa	80 Aa	77 b	6 Aa	5 Aa	6 a	20 Aa	15 Aa	17 a	12 Aa	1 Aa	1 a
	Ttx	80 Aa	85 Aa	83 ab	4 Aa	4 Aa	4 ab	16 Aa	11 Aa	14 ab	1 Aa	1 Aa	18 a
	Avg	88 A	84 A		4 A	4 A		15 A	12 A		2 A	1 A	
	CV (%)		9.9			41.3			39.1			91.7	
<i>H. courbaril</i>	Cont.	46 Aa	80 Aa	63 a	54 Aa	20 Aa	37 c	0 Aa	0 Aa	0 Aa	32 Aa	5 Ba	19 a
	Ctp	11 Aa	59 Aa	35 b	89 Aa	41 Aa	65 b	0 Aa	0 Aa	0 Aa	8 Ab	8 Aa	8 a
	Ttx	0 Aa	27 Aa	14 c	100 Aa	73 Aa	87 a	0 Aa	0 Aa	0 Aa	0 Bb	20 Aa	10 a
	Avg	19 B	55 A		81 A	45 B		0 Aa	0 Aa		13 A	11 A	
	CV (%)		27.7			16.4			0			86.5	

Averages followed by the same lowercase letter vertically and uppercase letter horizontally do not differ according to the SNK test at 5% significance. Avg: average. Cont.: Control (untreated seeds). Mort. (%): mortality (%). Hard: Hard seeds (%). A.S.: abnormal seedlings (%)

The effects of the various treatments on the growth of the shoot were observed in *E. contortisiliquum*, where the highest average growth was found in the seeds treated with thiamethoxam and cyantraniliprole ($p>0.05$) in the sand substrate (Table 2). In *G. ulmifolia* and *S. multijuga*. The highest average was observed in the sand substrate, regardless of the treatment ($p>0.05$). In terms of the primary root growth variable, *P. dubium* seeds treated with thiamethoxam presented greater average growth than did the control seeds ($p>0.05$).

Table 2. Average length of primary roots and shoots from seeds of *P. dubium*, *S. multijuga*, *E. contortisiliquum*, *G. ulmifolia*, *B. virgilioides* and *H. courbaril* treated with thiamethoxam and cyantraniliprole.

Species	Treatments	Root (mm)			Shoot (mm)		
		Paper	Sand	Avg	Paper	Sand	Avg
<i>P. dubium</i>	Control	35.2 Bb	52.6 Aa	43.8 a	30.6 Aa	38.6 Aa	34.5 a
	Ciantraniliprole	38.9 Bb	46.9 Aa	42.9 a	35.6 Aa	32.3 Aa	33.0 a
	Tiametoxam	51.0 Aa	50 Aa	50.6 a	39.0 Aa	38.4 Aa	38.7 a
	Avg	41.4 A	50.2 A		35.8 A	36.4 A	
	CV (%)	11.3			16.1		
<i>S. multijuga</i>		Paper	Sand	Avg	Paper	Sand	Avg
	Control	16.3 Aa	23.5 Aa	19.9 a	29.7 Aa	37.3 Aa	33.5 a
	Ciantraniliprole	19.1 Aa	27.5 Aa	23.3 a	29.2 Aa	38.1 Aa	33.6 a
	Tiametoxam	19.5 Aa	23.7 Aa	21.6 a	28.2 Aa	37.2 Aa	32.6 a
	Avg	18.3 B	24.9 A		28.9 B	37.5 A	
	CV (%)	30.1			12.5		
<i>E. contortisiliquum</i>		Paper	Sand	Avg	Paper	Sand	Avg
	Control	54.4 Aa	76.0 Aa	65.2 a	87.9 Aa	70.2 Bb	79.1 a
	Ciantraniliprole	58.7 Aa	99.2 Aa	78.9 a	90.6 Ba	108.2 Aa	99.4 a
	Tiametoxam	42.9 Aa	75.5 Aa	59.2 a	82.5 Aa	95.0 Aa	88.8 a
	Avg	51.9 B	83.5 A		87 A	91.2 A	
	CV (%)	28.9			10.7		
<i>G. ulmifolia</i>		Paper	Sand	Avg	Paper	Sand	Avg
	Control	29.5 Aa	29.1 Aa	29.7 a	24.2 Aa	31.1 Aa	29.8 a
	Ciantraniliprole	19.0 Bb	29.8 Aa	24.0 a	27.4 Aa	31.0 Aa	27.6 a
	Tiametoxam	20.5 Bb	29.9 Aa	25.2 a	26.6 Aa	32.9 Aa	29.2 a
	Avg	23.0 A	29.6 A		26.1 B	31.6 A	
	CV (%)	13.6			6.2		
<i>B. virgilioides</i>		Paper	Sand	Avg	Paper	Sand	Avg
	Control	16.3 Aa	23.5 Aa	19.9 a	29.7 Aa	37.3 Aa	33.5 a
	Ciantraniliprole	19.1 Aa	27.5 Aa	23.3 a	29.2 Aa	38.1 Aa	33.6 a
	Tiametoxam	19.5 Aa	23.7 Aa	21.6 a	28.2 Aa	37.2 Aa	32.7 a
	Avg	18.3 A	24.9 A		38.9 A	37.5 A	
	CV (%)	25.2			14.0		
<i>H. courbaril</i>		Paper	Sand	Avg	Paper	Sand	Avg
	Control	---	89.9 a	---	---	120.8 a	---
	Ciantraniliprole	---	167.2 a	---	---	190.4 a	---
	Tiametoxam	---	63.9 a	---	---	65.3 a	---
	Avg	---	107.0 A	---	---	125.5 A	---

Averages (Avg) followed by the same lowercase letter vertically and uppercase letter horizontally do not differ according to the Tukey test at 5% significance.

For the *G. ulmifolia* species, the highest average was in the control ($p>0.05$). For both *P. dubium* and *G. ulmifolia*, however, the differences between the means were only in the paper substrate ($p>0.05$) (Table 2). In *Hymenaea courbaril*, it was not possible to assess the primary root and shoot length variables due to low germination and not enough seedlings to be sampled.

The positive effects on the growth of the primary root and GSI of *P. dubium* and the primary root of *E. contortisiliquum*, when subjected to treatments with insecticides, may be associated with the bioactivating effects of some insecticides (Lauxen et al., 2016). According to Annamalai et al. (2018), some molecules associated with insecticides have beneficial physiological effects on the development of seedlings and are able to modify their metabolites and morphology, thus influencing their growth.

A reduction in the physiological potential of seeds treated with insecticides, as observed for some variables in the species *G. ulmifolia*, *B. virgilioides*, *H. courbaril* and *E. contortisiliquum*, may be associated with the application of dosages unsuitable for the species (Gafar et al., 2011), since for forest species, there is a lack of recommendations for the use of insecticides, and in this work, we used dosages based on agronomic species with similar characteristics to their seeds.

Costa et al. (2018), studying the effect of thiamethoxam on lettuce seeds, reported that the physiological effect is directly associated with the dosage used. Similarly, in rice seeds, as the concentration of the product increased, there was an associated beneficial physiological response until they began to have phytotoxic effects, highlighting the need to define appropriate dosages for each crop (Almeida et al., 2014).

The germination speed index (GSI) significantly increased in *P. dubium*, where the seeds treated with thiamethoxam and cyantraniliprole presented a greater GSI than did the control seeds ($p>0.05$). The overall averages were evaluated, regardless of the substrate used. For *E. contortisiliquum*, the treatment with thiamethoxam was inferior to the control (Tukey test at 5% significance) when the overall averages were evaluated, regardless of the substrate used. For *P. dubium* and *S. multijuga*, there was a greater GSI when the sand substrate was used ($p>0.05$), whereas for *E. contortisiliquum*, the highest average was found in the paper substrate ($p>0.05$). (Table 3).

The divergence between the GSI and shoot development in *E. contortisiliquum* shows that although the germination speed was reduced, shoot growth increased when the seeds were treated.

Table 3. Germination speed index of seeds of *P. dubium*, *S. multijuga*, *E. contortisiliquum*, *G. ulmifolia*, *B. virgilioides* and *H. courbaril*. Control (untreated) and seeds treated with thiamethoxam and cyantraniliprole.

Treatment	<i>P. dubium</i>			<i>S. multijuga</i>		
	Paper	Sand	Avg	Paper	Sand	Avg
Control	13.8 Aa	16.5 Aa	15.1 b	18.2 Aa	24.8 Aa	21.5 a
Cyantraniliprole	17.7 Aa	22.4 Aa	20.1 a	19.7 Aa	30.8 Aa	25.2 a
Tiametoxam	17.9 Aa	24.2 Aa	21.1 a	20.9 Aa	26.1 Aa	23.5 a
Avg	18.8 B	21.0 A		19.6 B	27.2 A	
CV (%)	11.3			34.4		
	<i>E. contortisiliquum</i>			<i>G. ulmifolia</i>		
	Paper	Sand	Avg	Paper	Sand	Avg
Control	14.3 Aa	15.2 Aa	14.8 a	19.1 Aa	20.1 Aa	19.6 a
Cyantraniliprole	14.7 Aa	11.7 Aa	13.2 ab	19.7 Aa	17.7 Aa	18.7 a
Tiametoxam	13.1 Aa	10 Aa	11.5 b	17.5 Aa	16.4 Aa	16.9 a
Avg	14.1 A	12.3 B		18.8 A	18.1 A	
CV (%)	15.17			21.02		

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Table 3. Continuation.

Treatment	<i>B. virgilioidis</i>			<i>H. courbaril</i>		
	Paper	Sand	Avg	Paper	Sand	Avg
Control	7.9 Aa	8.5 Aa	8.2 a	14.7 Aa	29.4 Aa	22 a
Cyantraniliprole	7.2 Aa	8.0 Aa	7.6 a	1.2 Aa	16.3 Aa	8.7 b
Tiametoxam	9.6 Aa	10 Aa	9.8 a	0 Aa	14.4 Aa	7.2 b
Avg	8.2 A	8.8 A		5.3 B	20.0 A	
CV (%)	46.9			6.2		

Averages (Avg) followed by the same lowercase letter vertically and uppercase letter horizontally do not differ according to the Tukey test at 5% significance.

According to Dan et al. (2010), a higher GSI is a preponderant factor for rapid seedling establishment under field conditions; however, the reduction in germination speed is offset by the ability of insecticides to protect seeds from predation. The fact that some variables performed better in the sand substrate may be related to the characteristics of the substrate itself, such as the type of structure provided, aeration, water retention capacity, degree of pathogen infestation and porosity, which favor germination and seedling development.

After storing the *P. dubium* seeds (Table 4), there was no significant interaction effect between germination and mortality ($p < 0.05$). When the storage time factor was analyzed separately, there was a significant difference ($p < 0.05$) between the times evaluated (Tukey's test at 5% significance; SNK at 5% significance), where the germination percentage of seeds stored for six months, regardless of the treatment received, was lower than that of seeds stored for three months and those not stored.

Mortality was greater at six months of storage, regardless of treatment. Seeds treated with thiamethoxam had significantly greater germination than the control (Tukey test at 5% significance) and a lower percentage of dead seeds. The variable primary root length, when compared at six months of storage, was higher in seeds treated with thiamethoxam than in the control (Tukey test at 5% significance).

The analysis of the variable percentage of hard seeds, whose interaction was significant between the factors ($p < 0.05$), revealed a greater average percentage of hard seeds at three months of storage where there was treatment with thiamethoxam (SNK at 5% significance). When germination is greater than 80%, germination levels are considered adequate, as this is the minimum value established as the threshold for attributing a good germination level when assessing the quality of a batch of seeds (Brasil, 2009). Therefore, for *P. dubium*, after up to three months of storage, there were no negative effects on the stored seeds, both treated and untreated. However, at six months, there was a decrease in the overall average germination, suggesting that storage should not be extended for more than three months, regardless of the treatment.

According to Dan et al. (2010), a long storage period can lead to a reduction in the physiological quality of the seeds, since the increased contact time of the insecticide with the seeds can generate a phytotoxic effect, leading to a reduction in the quality parameters assessed. The germination speed index (GSI) was significantly affected ($p < 0.05$). Over the course of the storage period, the GSI decreased in both the treated seeds and the control (Tukey test at 5% significance). Before storage, the seeds treated with the insecticide had a significantly higher GSI when compared to the untreated seeds (Tukey test at 5% significance) (Table 5). For the other variables, there was no significant difference between the factors (SNK at 5% significance; Tukey at 5% significance).

The fact that the GSI was higher in seeds treated with thiamethoxam but not stored corroborates the beneficial action of insecticides, such as thiamethoxam, which have a biotactivating function and act in the pentose phosphate metabolic pathway, leading to an increase in the hydrolysis of reserves and making energy available for germination processes (Horii et al., 2007). However, the positive effect only occurs when the seeds are not subjected to long-term

storage, which can lead to the opposite effect on the seeds, ranging from beneficial to harmful (Lorenzetti et al., 2014). These molecules have electrons, which interact with cellular compartments such as DNA, proteins and lipids.

However, the reduction in germination, increase in mortality and decrease in the GSI over time generally occurred regardless of the presence or absence of thiamethoxam. Thus, the presence of the insecticide was not responsible for the negative effects presented but rather for storage and deterioration over time, indicating that these implications may be linked to the negative effect of storing the seeds after breaking dormancy. When mechanical or chemical scarification is carried out to break dormancy, the tegument is damaged, which can interfere with the normal flow of water in and out of the seeds (Marcos-Filho, 2015).

It is also suggested that thiamethoxam plays a bioactivating role by providing a higher overall germination average than the control, lower mortality (with a greater number of hard but living seeds), higher GSI and greater primary root length at six months after storage.

Table 4. Germination, dead seeds, hard seeds, abnormal seedlings and shoot and root length of normal seedlings of *P. dubium*. Both untreated seeds (control) and thiamethoxam-treated seeds were used after different storage times (0, 3 and 6 months).

Storage time (months)	Germination (%)			Mortality (%)		
	Control	Ttx	Avg	Control	Ttx	Avg
0	85 Aa	90 Aa	88 a	12 Aa*	8 Aa*	10 b*
3	88 Aa	83 Aa	86 a	11 Aa*	5 Aa*	8 b*
6	58 Aa	78 Aa	68 b	31 Aa*	22 Aa*	27 a*
Avg	77 B	83.7 A		18 A*	12 B*	
CV (%)		11.9			36.7	
Storage time (months)	Hard seeds (%)			Abnormal seedlings (%)		
	Control	Ttx	Avg	Control	Ttx	Avg
0	3 Aa*	2 Ab*	3 a*	6 Aa*	7 Aa*	7 a*
3	1 Ba*	12 Aa*	7 a*	8 Aa*	6 Aa*	7a*
6	2 Aa*	0 Ab*	1 a*	16 Aa*	1 Aa*	9 a*
Média	2 A*	4.7 A*		10 A*	5 A*	
CV (%)		95.9			134.7	
Storage time (months)	Root length (mm)			Shoot length (mm)		
	Control	Ttx	Avg	Control	Ttx	Avg
0	29.8 Aa	30.2 Aa	30.0 a	34.6 Aa	29.7 Aa	32.1 a
3	30.4 Aa	27.9 Aa	29.2 a	31.7 Aa	19.1 Aa	25.4 a
6	19.1 Ba	34.3 Aa	26.7 a	20.7 Aa	28.7 Aa	24.7 a
Avg	26.4 A	30.8 A		29 A	25.8 A	
CV (%)		23.4			31.8	

Averages (Avg) followed by the same uppercase letter horizontally and the same lowercase letter vertically for each variable do not differ according to the Tukey test at the 5% significance level.

Averages followed by the same capital letter with an asterisk (*) horizontally and the same lowercase letter with an asterisk vertically for each variable do not differ according to the SNK test at 5%. Ttx: thiamethoxam.

Table 5. Germination speed index of untreated (control) and thiamethoxam-treated seeds of *P. dubium* at different storage times (0, 3 and 6 months).

Storage time (months)	Control	Tiametoxam	Average
0	23.9 Ba	32 Aa	27.9 a
3	12.6 Ab	6.6 Ab	9.6 b
6	4.3 Ab	9.1 Ab	6.7 b
Average	13.6 A	15.9 A	
CV (%)	35.2		

Averages followed by the same uppercase letter horizontally and the same lowercase letter vertically for each variable do not differ according to the Tukey test at the 5% significance level.

Table 6. Germination, dead seeds, hard seeds, abnormal seedlings and shoot and root length of control (untreated seeds) and thiamethoxam-treated (Ttx) seeds of *E. contortisiliquum* at different storage times.

Storage (Months)	Germination (%)			Mortality (%)		
	Control	Tiametoxam	Avg	Control	Tiametoxam	Avg
0	95 Aa	87 Aa	91 a	5 Aa	12 a	9 a
3	91 Aa	91 Aa	91 a	9 Aa	9 Aa	9 a
6	90 Aa	97 Aa	94 a	10 Aa	3 Aa	6.5 a
Average	92 A	92 A		8 A	8 A	
CV (%)		8.6			94.3	

Storage (Months)	Hard seeds (%)			Abnormal seedlings (%)		
	Control	Tiametoxam	Avg	Control	Tiametoxam	Avg
0	0 Aa*	4 Aa*	2 a*	8 Aa*	14 Aa*	11 ab*
3	0 Aa*	0 Aa*	0 a*	14 Aa*	21 Aa*	17 a*
6	0 Aa*	0 Aa*	0 a*	0 Aa*	0 Aa*	0 b*
Average	0 A*	1 A*		7 A	12 A	
CV (%)		489.9			159.6	

Storage (Months)	Root length (mm)			Shoot length (mm)		
	Control	Tiametoxam	Avg	Control	Tiametoxam	Avg
0	23.3 Aa	24.1 Aa	23.7 b	41.6 Aa	45.5 Aa	43,5 a
3	31.2 Aa	24.8 Aa	28.8 b	40.0 Aa	24.8 Aa	32,4 a
6	62.0 Aa	35.9 Aa	48.9 a	58.7 Aa	40.2 Aa	49,5 a
Average	38.3 A	28.3 A		46.8 A	36.8 A	
CV (%)		43.7			53.5	

Averages followed by the same uppercase letter in the row and the same lowercase letter in the column for each variable do not differ according to the Tukey test at the 5% significance level. Averages followed by the same capital letter with an asterisk horizontally and the same lowercase letter with an asterisk vertically for each variable do not differ according to the SNK test at 5%.

Table 7. Germination speed index of untreated (control) and thiamethoxam (Ttx) treated seeds of *E. contortisiliquum* at different storage times.

Storage (months)	Control	Tiametoxam	Average
0	9.9 Aa	5.2 Aa	7.6 a
3	12.7 Aa	12.9 Aa	12.8 a
6	5.9 Aa	14.1 Aa	10 a
Average	10.7 A	9,5 A	
CV (%)	59.4		

Averages followed by the same uppercase letter horizontally and the same lowercase letter vertically for each variable do not differ according to the Tukey test at the 5% significance level.

For *E. contortisiliquum* seeds (Table 6), when the variance of the data was analyzed, for the variables germination percentage, mortality, percentage of hard seeds, percentage of abnormal seedlings, shoot length and GSI, no significant differences were observed (Tukey test at 5% significance; SNK at 5% significance). Analysis of primary root length revealed no significant interaction between the factors tested. However, when each factor was examined separately, the average primary root length of the seeds stored for six months was greater than that of those subjected to the other storage times (Tukey test at 5% significance) (Table 7). The presence of abnormal seedlings at time zero and after three months of storage in *E. contortisiliquum*, regardless of the treatment, may be related to factors intrinsic to the seed, as there may have been some damage to the embryo or to its cotyledons (Cicero et al., 2003).

The absence of negative effects on the other variables evaluated under thiamethoxam treatment and the higher average primary root length found at 6 months indicate that, for this species, treatment with thiamethoxam and storage of the seeds for six months does not seem to negatively affect their quality. When evaluating the performance of hybrid maize seeds treated with thiamethoxam and stored for 60, 120 and 180 days, Rosa et al. (2012) reported that physiological quality was maintained during the storage period, both in seeds subjected to insecticide application and in the control (untreated seeds).

In general, the treated seeds presented values equal to or higher than those of the control, even after storage, which makes it possible to use the treated seeds after storage.

CONCLUSIONS

The use of the insecticides thiamethoxam and cyantraniliprole on seeds of *G. ulmifolia*, *B. virgiloides* and *H. courbaril* caused phytotoxic effects during germination.

No positive or negative effects of thiamethoxam or cyantraniliprole were detected in *S. multijuga* seeds.

The treatment of *E. contortisiliquum* seeds resulted in both negative and positive effects, whereas only positive physiological effects were observed in *P. dubium* seeds for the two insecticides evaluated.

It is possible to treat and store *P. dubium* and *E. contortisiliquum* seeds for six months.

ACKNOWLEDGMENTS

The National Council for Scientific and Technological Development (CNPq) and Minas Gerais State Research Foundation (FAPEMIG) supported this work under Grant number: CAG-APQ-01337/17 (ACJ); Grant number: 317242/2021-0 (ACJ) and Grant number: 317013/2021-1 (JMRF).

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