Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Corn physicochemical properties affect *Sitophilus zeamais* losses

Propriedades físico-químicas do milho afetam as perdas causadas por *Sitophilus zeamais*

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Abstract

The maize weevil, *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae), is one of the primary insect pests affecting maize in Brazil. Due to the limited availability of active compounds for insect control, there is a pressing need to explore alternative sources of resistance, potentially harboring genetic reservoirs for material improvement. This study aimed to identify resistant maize varieties by assessing *S. zeamais* emergence, amylase enzyme inhibitors, susceptibility indices, and nutritional analysis of the grains. Eight maize varieties, namely Azteca, Caiano, Colorado, Maisena, Oaxaca, Oito Carreira, Palha Roxa, and Rajado, were evaluated. Unsexed *S. zeamais* adults were confined in 250 mL plastic bottles containing grains of each maize variety for ten days, with ten replications for copulation and oviposition. After the incubation period, the insects were removed, and the infested grains stored for an additional thirty days. The emergence of *S. zeamais* was monitored every two days, and the pots were discarded after three consecutive evaluations without emergence. The amylase enzyme inhibitors, as a potential factor influencing resistance, was also analyzed. The susceptibility index was the primarily parameter used to evaluate resistance of maize varieties to S. *zeamais* adults. The Colorado, Oaxaca, and Oito Carreira maize varieties were resistant through antibiosis and the Azteca, Caiano and Rajado were moderately resistant. In contrast, the Maisena and Palha Roxa varieties were susceptible to *S. zeamais*. The Azteca variety was moderately resistant, despite its high levels of amylase enzyme inhibitors.

Keywords: insect pest management, storage, weevil, infestation.

Resumo

O gorgulho do milho, *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae), é um dos principais insetos pragas do milho no Brasil. A disponibilidade limitada de compostos ativos para o controle de insetos aumenta a necessidade de se pesquisar fontes alternativas de resistência, potencialmente abrigando reservatórios genéticos para melhoria de materiais. O objetivo foi avaliar emergência de *S. zeamais*, inibidores da enzima amilase, índices de suscetibilidade e qualidade nutricional dos grãos para identificar variedades de milho resistentes a esse inseto. As oito variedades de milho, Azteca, Caiano, Colorado, Maisena, Oaxaca, Oito Carreira, Palha Roxa e Rajado, foram avaliadas. Adultos não sexados de *S. zeamais* foram confinados em garrafas plásticas de 250 mL comgrãos de cada variedade de milho durante dez dias, com dez repetições para cópula e oviposição. Após o período de incubação dos ovos, esses adultos foram retirados e os grãos infestados armazenados por mais trinta dias. A emergência de *S. zeamais* foi monitorada a cada dois dias, sendo os vasos descartados após três avaliações consecutivas não emergência desse inseto. Inibidores da enzima amilase, como um fator potencial influencia a resistência, foram analisados. O índice de suscetibilidade foi o principal parâmetro para se definir a resistência das variedades de milho a *S. zeamais*. As variedades de milho Colorado, Oaxaca e Oito Carreira foram resistentes por antibiose e as Azteca, Caiano e Rajado moderadamente, resistentes e as Maisena e Palha Roxa suscetíveis a *S. zeamais*. A variedade Azteca, embora com níveis elevados de inibidores da enzima amilase foi moderadamente resistente considerando outros fatores.

Palavras-chave: controle de pragas de insetos, armazenamento, gorgulho, infestação.

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Received: November 12, 2024 – Accepted: June 5, 2025
Editor: Takako Matsumura Tundisi

1. Introduction

The maize weevil, *S. zeamais*, is a major pest of maize in Brazil. It damages kernels in the field before harvest and facilitates infestation in storage facilities (Sileem et al., 2020; Martins et al., 2024). Its ability to penetrate deep into grain masses and its high reproductive potential increase its significance as a pest (Napoleão et al., 2011; Suleiman et al., 2015).

Grains damaged by insect pests facilitate the development of secondary and opportunistic organisms such as fungi and bacteria (Marsaro Júnior et al., 2005; Norambuena et al., 2016). Chemicals are mostly used to manage pests, but they can select populations resistant insects damaging maize grains by *S. zeamais* and leaving harmful residues to humans and animals (Kasozi et al., 2015; Pinto et al., 2016; Haddi et al., 2018) what increases the need of safer methods to manage pests in storage facilities (Greco et al., 2024) .

The use of resistant maize varieties is important in pest management strategies (Kim et al., 2022). Insect digestive enzymes play a crucial role, as the insect gut is the primary interface with the environment. Digestive enzyme inhibitors, particularly those targeting α-amylases, show promise in pest management (Darvishzadeh et al., 2012).

α-Amylases (α-1,4-glucan-4-glucanhydrolases) are hydrolytic enzymes found in microorganisms, plants, and animals, catalyzing the breakdown of α-D-(1,4)-glucan bonds in starch and other carbohydrates. α-Amylase activity has been observed in various insect orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Darvishzadeh et al., 2012, 2013).

The enzyme trypsin, found in insect intestines, breaks down proteins into peptides, making its inhibitors in plants potential insecticides. Proteins like lectins may also play a role in plant defense against insects, and inhibitors of these digestive enzymes can inhibit insect growth and development (Frazão et al., 2018).

The genetic variability of maize enables the development of breeding programs for resistance to pests and diseases (Nascimento et al., 2014). Resistant cereal varieties offer a promising and environmentally friendly alternative in pest management (Corrêa and Salgado 2011; Frazão et al., 2018; Astuti et al., 2019).

This study aimed to identify maize varieties resistant to S. zeamais based on their emergence rates, the presence of amylase enzyme inhibitors, susceptibility indices, and nutritional analysis of their grains.

2. Materials and Methods

The experiment was conducted at the Laboratório de Entomologia, Campus Pato Branco, Federal University of Technology-Paraná (UTFPR), in Pato Branco, Paraná state, Brazil.

Maize used in the study included Azteca, Caiano, Colorado, Maisena, Oaxacan, Oito Carreira, Palha Roxa, and Rajado. Caiano and Colorado varieties were obtained from Epagri, Chapecó, Santa Catarina state, Brazil, while the others were sourced from the Municipality of Dois Vizinhos, Paraná, Brazil.

Adults of *S. zeamais* were collected from a grain storage facility in Pato Branco, Brazil, and reared for two months in the laboratory at UTFPR. They were kept in plastic pots with corn, covered with voile fabric, and maintained at 36°C ± 2°C and humidity from 45% to 50%.

To eliminate field insect infestations, maize grains were stored in plastic bags in a freezer at -10°C for 10 days. Then, they were kept at room temperature in the laboratory.

For infestation, sixty grams of grains of each maize variety and fifteen un-mated adult weevils, aged between seven and fourteen days, were placed in plastic bottles (250 mL) for ten days for copulation and oviposition. After this period, the adults were removed, and the grains were incubated in B.O.D type chambers at 27 ± 1°C and 55% ± 10% RH without light for thirty days.

The number of emerged *S. zeamais* adults per maize variety was recorded every two days, starting after the incubation period, until no adults emerged for three consecutive evaluations. Samples of maize and emerged insects were weighed together on a precision scale to estimate mass losses due to insect damage.

For the amylase assay, the Bioclin kit (Quibasa-Química Básica Ltda., Belo Horizonte, Minas Gerais, Brazil) was used. Fifteen un-mated adult weevils from each treatment were first washed in a 1.5% KCl solution and then placed in a container with 5.0 mL of pH 3.0 water acidified with HCl. The weevils were then macerated and filtered through cotton gauze. The filtrate was centrifuged at 10,000 g for 15 minutes. The pH of the solution was adjusted using a pH regulator and meter. Aliquots of the supernatant were collected to determine protein concentration, while the pellet was discarded.

The susceptibility index (SI) was used to evaluate the resistance of maize varieties. The biological cycle of *S. zeamais* was also determined.

Nutritional analysis of maize samples was conducted at the Agroindustrial Quality Laboratory (LAQUA) of UTFPR (Lanara, 1981; Zenebon et al., 2008). This straightforward nutritional analysis was conducted to demonstrate the quantitative differences among the corn materials, with the aim of better characterizing them in the presentation of the results.

Data from eight maize varieties were analyzed using a completely randomized design with ten replications, each with fifteen adult weevils. Analysis of variance (ANOVA) was performed, and means were compared using the Scott-Knott test at a 5% probability of error with the statistical program GENES.

Enzyme activity data were analyzed using a completely randomized design with three replications. ANOVA was conducted, and means were compared using the Scott Knott test at a 5% probability of error with the statistical program SASM-Agri. 8.2 (demo).

3. Results

The number of *S. zeamais* adults, emerged, was higher in the Palha Roxa maize (64.6), followed by the Maisena

(30.6) than in the other six varieties, with similar values between these last ones (Table 1).

The number of emerged *S. zeamais* adults was higher in the Palha Roxa maize (64.6), followed by Maisena (30.6), than in the other six varieties, which had similar values (Table 1).

The total weight of *S. zeamais* adults was higher in Palha Roxa (205.5) than in Azteca (19.6), Caiano (21.2), Maisena (94.2), and Rajado (51.0), with lower values in Colorado (5.2), Oaxacan (15.4), and Oito Carreira (19.2) (Table 1).

The biological cycle of *S. zeamais* did not differ between the eight maize varieties (Table 1).

The susceptibility indices of Maisena and Palha Roxa varieties were similar and classified as susceptible to *S. zeamais*. Colorado and Rajado were moderately resistant, while Oaxacan and Oito Carreira, with the lowest averages, were resistant to this weevil (Table 1).

The weight loss of the Palha Roxa variety was greater, reinforcing its susceptibility to *S. zeamais*, compared to Azteca, Caiano, and Rajado, which had similar values, and to Colorado and Oito Carreira, which had lower values (Table 1).

The percentages of ash, carbohydrates, crude fiber, lipids, moisture, and proteins in the eight maize varieties ranged from 0.52 to 1.75, 50.07 to 67.08, 4.43 to 23.84, 1.50 to 4.76, 8.71 to 15.85, and 5.07 to 11.47, respectively (Table 2).

4. Discussion

The differences in the number of emerged adults among the maize materials indicate a clear variation in susceptibility among them. Palha Roxa, for example, shows a higher preference and potential for infestation than the other materials, indicating greater sensitivity to the action of *S. zeamais*, as confirmed by the higher total mass of the insects. On the other hand, materials such as Colorado, Rajado, Oaxacan, and Oito Carreira demonstrated greater resistance, with a lower biotic potential of these insects resulting in a lower mass of adults. This parameter is essential considering that storage conditions favor population growth of insects, making materials that restrict this growth important additions to other management tactics and strategies.

Palha Roxa (purple corn) was initially selected for the study due to its significant amounts of anthocyanins, which were believed to confer resistance to *S. zeamais*. However, the results did not support this hypothesis. On the other hand, anthocyanins have been associated with higher mortality and reduced damage caused by *Sitophilus granarius* (L.) in purple wheat compared to yellow durum wheat (D'Isita et al., 2023). Studies on the susceptibility of corn genotypes to *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) also suggest that resistance

Table 1. Number of emerged individuals (EI), weight (PI), amylase enzyme inhibition (AI), biological cycle in days (CB) of *Sitophilus zeamais* (Coleoptera: Curculionidae) and susceptibility index (SI) and weight losses of the grains (PP) (g) of eight varieties (Varieties) of maize in laboratory at 27 ± 1 °C, RH: 75 ± 5%, photophase: 12 h.

Varieties	EI	PI (mg)	AI	CB (ns)	SI	PP (g)
Asteca	6.4$^{1.56}$c	19.6$^{4.74}$c	147.40$^{21.06}$a	36.51$^{0.91}$	4.37$^{0.55}$c	2.11$^{0.21}$b
Caiano	7.9$^{2.20}$c	21.2$^{5.78}$c	19.34$^{10.78}$c	29.97$^{5.04}$	4.37$^{0.92}$c	1.99$^{0.10}$b
Colorado	1.6$^{0.89}$c	5.2$^{2.87}$d	66.22 $^{10.58}$b	24.12$^{8.28}$	0.88$^{0.59}$d	1.0$^{0.09}$d
Maisena	30.6$^{7.31}$b	94.2$^{21.83}$b	0.00$^{0.00}$c	37.24$^{1.03}$	7.73$^{1.09}$a	1.91$^{0.24}$b
Oaxacan	4.5$^{1.58}$c	15.4$^{5.18}$d	32.14$^{16.73}$c	30.80$^{6.91}$	2.65$^{0.76}$d	1.42$^{0.11}$c
Oito Carreira	5.7$^{2.62}$c	19.2$^{8.90}$d	67.83$^{21.02}$b	31.74$^{5.35}$	2.86$^{0.94}$d	0.97$^{0.12}$d
Palha Roxa	64.6$^{10.26}$a	205.5$^{31.61}$a	40.10 $^{05.43}$c	43.11$^{1.55}$	9.4$^{0.52}$a	2.64$^{0.24}$a
Rajado	14.6$^{4.10}$c	51.0$^{14.09}$c	63.18 $^{11.46}$b	34.58$^{4.08}$	5.71$^{0.92}$b	1.74$^{0.18}$b

Means followed by the same letter, per column, do not differ by the Scott-Knott test (p≤ 0.05). ns = Non-significant by the Kruskal Wallis mean test.

Table 2. Percentage of ash, carbohydrates (Carb.), crude fiber (CB), lipids, moisture (%) (Moist.), and proteins, of eight maize varieties analyzed at the Federal University of Technology - Paraná in Pato Branco, Paraná, Brazil, 2019.

Varieties	Ash	Carb.	CB	Lipids	Moist.	Proteins
Asteca	1.08	54.77	18.50	3.74	13.53	8.38
Caiano	0.84	56.67	18.54	4.73	8.92	10.30
Colorado	1.30	53.10	23.58	4.76	8.71	8.55
Maisena	1.30	57.71	14.51	4.67	13.00	8.81
Oaxacan	1.56	55.81	18.51	3.89	12.40	7.83
Oito Carreira	1.30	67.08	4.43	4.47	12.79	9.93
Palha Roxa	0.52	50.07	20.59	1.50	15.85	11.47
Rajado	1.75	53.24	23.84	3.60	12.50	5.07

Methodologies used: Lanara (1981) and Zenebon et al. (2008).

may be influenced by complex genetic factors, not just physical aspects like material hardness (Locatelli et al., 2019). The influence of texture and nutrient composition interfere with insect populations in interaction with corn genotypes. Thus, while Palha Roza and Maisena are sensitive to *S. zeamais*, Colorado, Rajado, Oaxacan, and Oito Carreira show resistance.

Therefore, Palha Roxa maize exhibited greater susceptibility, with higher adult emergence and more significant grain weight loss than varieties such as Colorado, Rajado, Oaxacan, and Oito Carreira, demonstrating relative resistance to insect infestation. Careful selection of maize varieties for storage systems should consider not only physical attributes like grain hardness but also nutritional composition, as it can play a determining role in modulating insect infestation.

Although anthocyanins may initially suggest resistance to pest attack, the results of this research highlight the complexity of the phenomenon, showing that other factors exert significant influence on the susceptibility of maize varieties to insects.

When analyzing the presence of amylase enzyme inhibitors in different corn materials, it was observed that the Asteca variety exhibited the highest activity, followed by Colorado, Oito Carreiras, and Rajado. Conversely, materials such as Caiano, Maisena, Oaxacan, and Palha Roxa showed lower activities. The presence of these α-amylase inhibitors in grains and seeds can compromise enzymatic activity in insects, resulting in reduced nutrient assimilation by them (Zhang et al., 2023). However, in this study, the presence of α-amylase inhibitors in the different corn materials was not the predominant factor for their resistance. This is because insects have important physiological mechanisms of adaptation in hosts, such as detoxification, the presence of antioxidants, and other digestive enzymes (Zhang et al., 2023).

The greater susceptibility of the Maisena and Palha Roxa varieties to S. *zeamais* may be due to their grains being softer than those of the Colorado, Oaxacan, and Oito Carreira. On the other hand, the high ash content in the grains of the Colorado, Oaxacan, and Oito Carreira varieties may have contributed to their resistance to *S. zeamais.*

The greater weight losses of the Palha Roxa variety grains may be related to the greater emergence and weight of *S. zeamais* adults, contrary to that of the Colorado and Oito Carreira grains, which had reduced emergence of this insect. The lower weight loss in the Colorado and Oito Carreira varieties classifies them as resistant to *S. zeamais*.

The Colorado, Oaxaca and Oito Carreira corn showed resistance to *S. zeamais*. This study reinforces the importance of considering a wide range of factors, including nutritional and genetic composition, when selecting maize varieties for post-harvest conservation. Furthermore, the materials identified as more resistant to *Sitophilus zeamais* represent sources for the improvement of maize varieties to mitigate losses resulting from infestations of this species.

Acknowledgements

To the “Universidade Tecnológica Federal do Paraná (UTFPR)” for the finantial support and the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for the scholarship.

Data Availability Statement

The entire data set that supports the results of this study was published in the article itself.

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