Behavioral characterization and alternative control methods of the Bamboo borer [Dinoderus minutus Fabricius (Coleoptera: Bostrichidae)]

Caracterización del comportamiento y métodos de control alternativos de la broca de bambú [Dinoderus minutus Fabricius (Coleoptera: Bostrichidae)]

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ABSTRACT

Bamboo wood is a widely used raw material for the manufacture of special furniture and handicraft products, and can also be used in construction and architecture. In Brazil, the usage of this material has gained great space in the last decades. However, it can suffer from the attack of the bamboo borer [Dinoderus minutus Fabricius (Coleoptera: Bostrichidae)], causing damages and making the use of this wood unviable. Hence, the purpose of this work was to verify the behavior of *D. minutus* in culms of three bamboo species (*Bambusa vulgaris, B. vulgaris var. vittata e B. multiplex*) in two seasons of the year, one dry and one rainy season, in addition to testing alternative methods of insect control. In relation to the behavior, we verified the damages caused by the larvae from the oviposition of *D. minutus* in nodes and internodes of the bamboo species. While the alternative control methods were based on the spraying of the bacterium *Proteus mirabilis*, as an entomopathogenic agent, on *B. vulgaris* nodes, and the immersion treatment of nodes of same bamboo species in aqueous extract of *Dracaena arborea* leaves, and in vinasse. In relation to the insect behavior, there was a higher activity (consumption and reproduction) during the rainy season and a higher attack preference for the species *B. vulgaris*. Regarding the alternative control methods, it was verified a better performance of the treatments with *P. mirabilis* and *D. arborea*, since they provided a mortality index superior to 90% of the insects.

Key words: starch, wood borer, bacteria, entomopathogen, plague.

RESUMEN

La madera de bambú es una materia prima ampliamente utilizada para la fabricación de muebles especiales y productos de artesanía. Además es empleada en la construcción civil y arquitectura. En Brasil, el uso de este material ha aumentado en las últimas décadas. Sin embargo, puede sufrir el ataque del barrenador de bambú Dinoderus minutus Fabricius (Coleoptera: Bostrichidae), causando daños e inutilizando la madera. El objetivo de este trabajo fue caracterizar el comportamiento de D. minutus en cañas de tres especies de bambú (Bambusa vulgaris, B. vulgaris var. vittata y B. multiplex) en dos estaciones del año, una seca y otra lluviosa, además de probar métodos alternativos de control de insectos. En relación con el comportamiento, verificamos los daños causados por las larvas a partir de la oviposición de D. minutus en nudos y entrenudos de las especies de bambú. Los métodos de control alternativos se basaron en la fumigación de la bacteria Proteus mirabilis, como agente entomopatógeno; en los nudos de B. vulgaris y el tratamiento de inmersión de nudos de la misma especie de bambú en extracto acuoso de hojas de Dracaena arborea y en vinaza. En relación con el comportamiento de los insectos, hubo una mayor actividad (consumo y reproducción) durante la temporada de lluvias y una mayor preferencia de ataque para la especie B. vulgaris. Respecto a los métodos de control alternativos, se verificó un mejor desempeño de los tratamientos con P. mirabilis y D. arborea, ya que proporcionaron un índice de mortalidad superior al 90% de los insectos.

Palabras clave: almidón, barrenador de bambú, bacterias entomopatógenas, plaga.

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Introduction

Bamboos are currently classified into three tribes: Arundinarieae, Bambuseae, and Olyreae. In Brazil, there are representatives of the Olyreae and Bambuseae tribes, with more than 1.3 thousand species, where 50 have already been domesticated and 38 are still under study. In the country, the most common exotic species are *Bambusa vulgaris* Schrad, *B. vulgaris* var. *vittata*, *B. tuldoides*, *Dendrocalamus giganteus* and some species of *Phyllostachys*. These species, all of Asian origin, were first brought by the Portuguese settlers, and later by the Orientals, spreading easily throughout the country (Sungkaew *et al.*, 2009).

As a monocot plant, it presents a high rate of lateral bud growth, high nutritional values and other promising characteristics that allow its wood to be used in the construction, biorefinery, renewable energy, bionanotechnology and environmental preservation sectors, making it suitable for scientific and social development studies (Watanabe *et al.*, 2016). Thus, bamboo is seen as an alternative to human needs due to its adaptation and versatility characteristics, as well as being a renewable resource, offering a high level of quality with low economic cost.

According to Beraldo and Rivero (2003), the bamboo production sector has been increasing and growing significantly worldwide since its production requires low capital investment and it is easy to learn. Developing countries in Asia, such as China, India, and Japan, and in South America, such as Colombia, Venezuela, and Ecuador, are the pioneers in the use of this raw material in several industrial sectors. In Brazil, the use of bamboo has gained prominence only in the last decades, and its wood is mainly used in the construction and architecture sectors (Souza, 2004).

Despite this potential, bamboo presents problems regarding its conservation. This is due to the structural anatomical constitution of the bamboo stems (culms), which presents parenchyma cells rich in starch reserves, which according to some authors is the main attraction to the bamboo borer [Dinoderus minutus Fabricius, 1775 (Coleoptera: Bostrichidae)] after harvesting (Abood and Norhisham, 2013). Due to its vulnerability to the attack of this insect, the bamboo wood may be reduced to dust in a short time, causing serious economic losses and making its use impracticable (Watanabe et al., 2018).

The infestation by *D. minutus* also occurs in several species of stored dry woods. This borer

opens galleries for oviposition and closes them with the wood powder. The larvae feed on the wood by excavating galleries parallel to the conducting vessels causing irreparable damages (Plank, 1951).

In the control of wood borers, chemical preservatives are usually introduced into the structure of the wood, making it toxic to fungi and xylophages insects. These products are classified into three types: oily, oil-soluble and water-soluble. Although effective, the environmental damage and the health of people handling traditional preservation products have worried the world and aroused interest in research that develops natural products for the treatment of wood (Koski, 2008). Thus, extracts and oils from plants with insecticidal potential represent an alternative to pest control.

However, it is important to highlight the need to characterize insect behavior and develop new research and technologies to preserve stored products, such as wood and grains, without the use of products harmful to health and the environment. The purpose of this study was to characterize the behavior of *D. minutus* in nodes and internodes of three bamboo species (*B. vulgaris, B. vulgaris* var. *vittata* and *B. multiplex*) in two seasons of the year, one dry and one rainy season, in addition to test alternative methods of insect control with the use of the bacterium *Proteus mirabilis*, the aqueous extract of *Dracaena arborea* leaves, and vinasse.

Material and Methods

The experiments were conducted at the Center of Agribusiness Technology and Analysis (CeTeAgro) of the Dom Bosco Catholic University (UCDB), located at the São Vicente Salesian Institute, in the city of Campo Grande - MS, Brazil, whose geo-coordinates are 20° 26' S, 54° 38' W, and 532 meters of altitude. According to Köppen classification, the Campo Grande climate can be classified as a transition between Subtropical Humid (Cfa), Tropical Humid (Aw) and Savana. The behavioral evaluation of the insect was split into two stages, I - Open field and II - Laboratory, while the bioassays of alternative methods of pest control were carried out only under controlled laboratory conditions.

Behavioral Assessment: Stage I - Open field

The experiment was carried out in a completely randomized design, with factorial 3x2x2, being three

bamboo species (*B. vulgaris*, *B. vulgaris* var. *vittata* e *B. multiplex*), two materials (node and internode), and two seasons (dry and rainy). The assay was repeated five times, with each replicate lasting for seven days (168 hours). Six samples of nodes and internodes with 20 cm length were used in each open field replicate.

The tests conducted in the rainy season occurred between October and November of 2011, using material (nodes and internodes) collected in September of the same year. In the dry season, the experiment was conducted between April and May of 2012 with the use of material collected in February of the same year. The culms collected were dehydrated in an air circulation oven at 50 °C for 48 horas, cut into nodes and internodes, and placed close to the bamboo clumps cultivated at the São Vicente Salesian Institute, and where previously the presence of the insect *D. minutus* had been verified. The samples were tied about 60 cm high and remained in place for 7 days.

At the end of each replicate, the number of perforations caused by the insect in each bamboo sample was estimated in order to characterize the behavior of the insect in relation to its feeding preference among the bamboo species and the types of material provided for infestation.

Behavioral Assessment: Stage II - Laboratory

The laboratory test was carried out in a completely randomized design, with factorial 3x2, being three bamboo species (*B. vulgaris*, *B. vulgaris* var. *vittata* e *B. multiplex*), and two seasons of the year (dry and rainy). The experiment was repeated three times, lasting 20 days each. As sample units were used bamboo nodes of the three species, and nine replicates in each laboratory test.

The assays of the dry season occurred from November to December of 2011, while those of the rainy season occurred from June to July of 2012. The bamboo culms were collected in the months of September of 2011 and February of 2012, for the tests of the dry and rainy seasons, respectively.

The nodes of the three bamboo species were dehydrated in an air circulation oven at 50 °C for 48 hours and sectioned in stem cuts of 9 cm. Subsequently, the material was randomly arranged in a circle in a glass arena with the dimensions of 1.48 x 1.48 x 0.15 m, and which was coated with white cardboard paper and installed in a ninemeter-square room. Room conditions were adjusted

throughout the experiment at 27±2 °C and 70±5% humidity in the dry season, and at 27±2 °C and 85±5% in the rainy season, with the aid of an air humidifier and a portable thermal conditioner. One hundred non-sexed adult insects were deposited in the center of the circle formed by the bamboo stem cuts in order to cause infestation and give the same chance of choice towards the nodes.

At the end of every 20 days, the behavior of the insect in relation to the three bamboo species was verified taking into consideration the number of perforations, node dry mass consumption, the population of adult insects, and the number of larvae of *D. minutus* found inside the nodes.

Alternative control methods

Stem cuts of *B. vulgaris* nodes were used for the tests of alternative control of the bamboo borer. The experiment was conducted in a completely randomized design with four treatments (I - *Proteus mirabilis*, II - aqueous extract of *Dracaena arborea* leaves, III - vinasse, and IV - control), and six replications. The bioassays were repeated three times with a duration of 50 days each, to verify if there was an influence on the insect reproduction.

Test tubes containing pure liquid culture of the bacterium *P. mirabilis* were sprayed, in aseptic media, directly onto bamboo stem cuts and left to rest for 24 hours. In the treatment with aqueous extract of *Dracaena arborea* (100 grams of dried leaves grounded in 1 L distilled water) and with vinasse, the stem cuts were immersed in each product for 24 hours and then dehydrated for 24 hours in an air circulation oven at 50 °C. The control treatment consisted of stem cuts of *B. vulgaris* nodes only dehydrated, thus providing the material in the form that it is naturally infested by the insect.

Then, the treated bamboo stem cuts were placed in glass jars. One hundred non-sexed adults of *D. minutus* were inserted in each flask. After 50 days, the birth rate and mortality of insects were recorded, and the dry node mass consumed during the experiment was measured.

Statistical analysis

The data obtained were analyzed by ANOVA, and when significant, the means were compared by Tukey test at 5% of probability using the software SISVAR 5.3.

Results and Discussion

Behavioral Assessment: Stage I - Open field

The analysis of variance of the open field experiments shows that there was no significant interaction among the factors: species x materials x seasons (Table 1). However, there was an interaction between species and seasons, demonstrating that the behavior of *D. minutus* in open field conditions is not influenced by the type of material (node or internode) provided by the three species of bamboo.

From the significant interaction between species x season of the year, it is verified that the species B. vulgaris and B. vulgaris var. vittata influence the behavior of the bamboo borer, in relation to perforations caused in both dry and rainy seasons (Figure 1). It is important to point out that in the rainy season the insect caused a greater number of perforations in the species B. vulgaris followed by B. vulgaris var. vittata, which presented on average 196 and 55 perforations per culm, respectively, differing statistically from the dry season results.

Although the lowest number of perforations per bamboo culm was found in the dry season, the behavior of the insect followed the same pattern in relation to the bamboo species, with *B. vulgaris* and *B. vulgaris* var. *vittata* presenting the average of 90 and 24 perforations, in the same sequence. No perforations were found at the nodes and internodes of *B. multiplex* in both dry and rainy seasons.

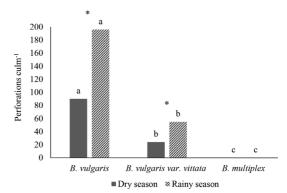


Figure 1. Number of perforations caused by the bamboo borer [Dinoderus minutus Fabricius (Coleoptera: Bostrichidae)] in culms of the bamboo species B. vulgaris, B. vulgaris var. vittata and B. multiplex, in the rainy and dry seasons of 2011 and 2012. (*) there is a statistical difference between the seasons by Tukey test ($\alpha \le 0.05$). Lowercase letters do not differ from each other comparing the bamboo species by Tukey test ($\alpha \le 0.05$).

These results indicate that the bamboo borer activity is higher during the rainy season. Results that are in agreement with Hidalgo-Lopez (2003), which states that the attack of this borer, in regions of the tropical climate, occurs mainly in warm and humid seasons.

Brito *et al.* (2017) evaluated the seasonal variation in carbohydrate contents and phenolic compounds in bamboo species over two years. The authors verified that in the month of September (when the material was collected for the tests of rainy season), the average starch contents in nodes of

Table 1. Analysis of variance for the main effects (bamboo species, material used and seasons) and Interactions for the number of perforations caused by the bamboo borer *Dinoderus minutus* Fabricius (Coleoptera: Bostrichidae).

Souce of Variation	DF	SS	MS	F	P
Species (S)	2	218259.30	109129.65	7311.87**	< 0.0001
Material (M)	1	14.02	14.02	0.94^{NS}	0.3374
Seasons (SE)	1	31970.42	31970.42	2142.07**	< 0.0001
Int. S x M	2	7.03	3.52	0.24^{NS}	0.7910
Int. S x SE	2	29935.43	14967.72	1002.86**	< 0.0001
Int. M x SE	1	33.75	33.75	2.26^{NS}	0.1392
Int. S x M x SE	2	32.50	16.25	1.09^{NS}	0.3448
Treatments	11	280252.45	_	_	
Residual	48	716.40	14.92	-	
Total	59	280968.85	-	_	
General Mean		60.95			
Standard Deviation		3.86			
CV (%)		6.34			

^{**} Significant at 5% of probability (p < 0.05). Not significant.

B. vulgaris, *B.* vulgaris var. vittata and *B.* multiplex were 23.0, 30.0, and 20.1 μg g⁻¹ of dry matter (DM), while the internodes of the same species presented lower starch levels, with 10.5, 10.9, and 7.6 μg g⁻¹ DM, respectively. As for the month of February (harvest season of the bamboo samples used in the dry season), the starch contents in *B.* vulgaris, *B.* vulgaris var. vittata e *B.* multiplex were 54.3, 89.6, and 15.5 μg g⁻¹ DM for the nodes, and 40.0, 23.7, and 15.6 μg g⁻¹ DM for the internodes, respectively.

Several authors argue that the presence of starch in the parenchyma cells of the bamboo stem is the main attraction to the attack of the bamboo borer (Plank, 1951). However, the highest activity of the insect, observed in this study through the number of perforations found in open field conditions, was in the species *B. vulgaris* during the rainy season, which presented a lower content of starch in relation to *B. vulgaris* var. *vittata*.

It is also worth noting that the starch content in this species was lower in September (rainy season) than in February (dry season), which indicates that the starch present in the bamboo wood may not be what characterizes the behavior of the insect in relation to its choice of attack and consumption. It is also observed that the starch content in nodes of the three bamboo species was always higher than the starch content in their internodes, and the insect had no preference for any of the material (node or internode) in specific (Table 1), corroborating the idea that the starch really is not primarily responsible for the borer feeding behavior.

Another important fact observed by Brito *et al.* (2017) relates to the content of phenolic compounds in bamboo species. The authors observed that *B. multiplex* presents higher levels of phenolic compounds than *B. vulgaris* and *B. vulgaris* var. *vittata* in both dry and rainy seasons, which may explain why this species is not infested. according

to Koul *et al.* (1990), some secondary metabolites present in bamboos, such as phenolic compounds, act as repellents to coleopteran attack, reducing the need to apply chemical products for the conservation of their wood.

Silveira *et al.* (2017a) verified the influence of diets based on different bamboos in the feeding preference of the bamboo borer. The authors reported similar results, with a preference for *B. vulgaris* and lower consumption of *B. multiplex*. These results indicate that *B. multiplex* is the most resistant species to pest attack, presenting greater durability, which can be commercially more interesting to the producer or industry that wants to use this wood in the most diverse industrial sectors, such as construction, architecture or even for the production of handicraft products.

Behavioral Assessment: Stage II - Laboratory

Taking into account the behavior of the bamboo borer in relation to the perforations of the different types of materials, and noting that there was no infestation preference for the node or internode of the three species in open field conditions (Table 1), it was decided for the use of only bamboo nodes in the laboratory tests.

Under the controlled conditions of the laboratory, the three species of bamboo (*B. vulgaris*, *B. vulgaris* var. *vittata* e *B. multiplex*) were also evaluated in two seasons of the year, one dry and one rainy season. However, unlike the results observed under open field conditions, only *B. vulgaris* was infested and consumed by the borer *D. minutus* in the laboratory, which justifies the results of dry mass consumption, number of perforations, population of adult insects and number of larvae of *D. minutus* be expressed only for this species in particular (Table 2).

Table 2. Dry mass consumption, number of perforations, number of adult insects, and number of larvae of *Dinoderus minutus* Fabricius (Coleoptera: Bostrichidae) found inside *B. vulgaris* nodes in the rainy and dry seasons of 2011 and 2012.

Season	Dry Mass Consumption (g)	Perforations	Population	Larvae
Dry season	10,8 a	20 b	14 b	15 b
Rainy season	14,6 a	29 a	38 a	51 a
CV (%)	12,4	15,2	8,6	14,3

Lowercase letters do not differ from each other comparing the different seasons by Tukey test ($\alpha \le 0.05$).

Thus, the highest value of dry mass consumption by the insects was observed in the rainy season, with 14.6 grams consumed per node; this value is not statistically different from the dry season, which had an average consumption of 10,8 g. However, it was in the rainy season that the highest number of perforations and the largest population of larvae and adult insects were observed inside the nodes of *B. vulgaris*, statistically differing from the dry season.

The results indicate that it is in the rainy season the greatest activity of the bamboo borer. According to Silveira *et al.* (2017b), the greatest presence of the borer corresponds to the days with higher temperatures and greater rainfall rates, where there is a natural occurrence of the insect *D. minutus*. The authors also observed a smaller number of insects captured in seasons with a lower relative air humidity, which is similar to the data obtained in the present study.

Norhisham et al. (2013) analyzed the effect of moisture on egg hatching and reproductive biology of the bamboo borer. The authors report that the reproductive capacity of D. Minutus is higher in in periods of high relative air humidity, where the adult female presents better fertile characteristics, occurring a higher oviposition and incidence of the insect than when compared to periods of low humidity, which cause the dehydration of the eggs, leading to its death or failure in hatching, reducing the natural occurrence of the insect in the dry seasons. This information corroborates the results obtained in the present study, where the highest consumption occurred in periods of higher relative air humidity, which is related to the greater activity and presence of the insect.

The relation between the number of naturally occurring borers and the favorable environmental

conditions was also reported by Bousquet (1990), who verified the highest occurrence of *D. minutus* in samples collected in regions with higher temperatures and rainfall.

The results obtained in this stage show that it is in the season of the rainfalls that the highest incidence of the bamboo borer occurs. This information is important for the correct management of the bamboo harvest, and on which seasons the species *B. vulgaris* would require treatments with products that are preventive to the attack of *D. minutus*. Nevertheless, it can be noticed that the species *B. multiplex* presents greater resistance to the insect, being able to be characterized then as the bamboo wood of greater economic interest among the three tested in the present study.

Alternative pest control methods

The treatments for *D. minutus* control using the bacterium *P. mirabilis* and the extract of *D. arborea* did not differ between them, presenting the lowest values of node dry mass consumption with 2.68 and 0.27 grams consumed, respectively (Table 3). Moreover, these treatments were also not statistically different for the mortality and birth rate factors.

These results demonstrate that, when bamboo nodes were treated with *P. mirabilis* and *D. arborea*, most of the insects died before the reproduction period, indicating that in addition to causing death, the treatment with both products directly influenced the reproductive capacity of these insects, being characterized as efficient methods in the control of the bamboo borer.

The mortality of the insects due to the use of *P. mirabilis* probably occurs because of the action of protease inhibitors released by the bacterium,

Table 3. Dry mass consumption, mortality, and birth rate of *Dinoderus minutus* Fabricius (Coleoptera: Bostrichidae) exposed to diets containing nodes of *B. vulgaris* treated with the bacterium *P. mirabilis*, aqueous extract of *D. arborea* leaves, and vinasse.

Treatment	Dry mass consumption (g)	Mortality (%)	Birth rate (%)
Proteus mirabilis (bacterium)	2.68 b	94 a	1 b
Dracaena arborea (extract)	0.27 b	98 a	0 b
Vinasse	32.4 a	32 b	92 a
Control (B. vulgaris)	29.2 a	25 b	88 a
CV (%)	19,34	8,59	9,11

Lowercase letters do not differ from each other comparing the alternative pest control methods by Tukey test ($\alpha \le 0.05$).

since several insects use digestive proteases in the degradation of ingested proteins (Pilon et al., 2009), leading the insects to death. Burgess et al. (2002), used protease inhibitors in tests with Nebria brevicollis and achieved similar results, such as the increase of the insect mortality. Another example to be cited is Annadana et al. (2012), who managed to decrease the oviposition of the insect Frankliniella occidentalis. Similar results were also observed by Oliveira et al. (2016), that using P. mirabilis obtained a mortality rate of bamboo borer superior to 80%.

In relation to the aqueous extract of *D. arborea*, the results suggest that the effect of this plant on repelling the insects is related to the presence of polyphenols in this species. The polyphenols inhibit several digestive enzymes, and because they are involved in lignin bindings with carbohydrates of the cell wall reduce the digestibility of the food (Lopes, 1990), the reason why the consumption of *B. vulgaris* nodes treated with *D. arborea* by the insect was low, directly influencing the insect mortality rate.

According to Okunji et al. (1996), the ornamental species D. arborea is rarely attacked by insects, which is due to its possible antifungal and antiparasitic properties. Similar results regarding the mortality of insects with the use of this plant were reported by Epidi et al. (2008), who used dehydrated leaf extracts in the control of Sitophilus zeamais and Callosobruchus maculatus. Udo et al. (2011) suggest that D. arborea also exhibits ovicidal and larvicidal activity, inhibiting the development of insect eggs and larvae, a factor that may be related to the lack of birth of insects inserted in this treatment. More recently, Udo et al. (2011) verified the mortality efficiency of 83% of C. maculatus exposed to the leaf extract of *D. arborea*, which is characterized as an efficient alternative method of pest control of stored products.

The treatment with less efficiency in the control of *D. minutus* was observed with the use of vinasse, since the dry mass consumption, mortality and birth rate of the insects were not statistically different from the control treatment, in which no alternative control methods were used. According to Camargo *et al.* (1983), vinasse may increase nutrient availability and water retention on substrates, which may have occurred with the bamboo nodes immersed in vinasse, increasing nutrients and moisture, providing a propitious

environment for the development of the insects in this type of treatment.

According to Precetti and Arrigoni (1990), the excessive application of vinasse can promote the reproduction and occurrence of the sugarcane weevil (*Sphenophorus levis*). The promotion of the reproduction was also observed in the present study, where the treatment with vinasse promoted a birth rate of approximately 92% of insects, in comparison to a birth rate of 88% in the control treatment, not being characterized then as an efficient alternative method of control of the bamboo borer.

Conclusion

In laboratory and open field conditions, the presence of starch in the parenchyma cells is not characterized by influencing the choice, consumption and reproduction behavior of *D. minutus* in the bamboo culms, since the insect opted for some of the species with lower starch content (*B. vulgaris*) in both dry and rainy seasons. Despite this, the greater presence and activity of the bamboo borer occurs in the rainy season, when the temperature and the relative air humidity are higher.

It is believed that the presence of phenolic compounds reduces the activity and infestation of the bamboo borer in *B. multiplex*, which makes it an excellent bamboo species to be used in industrial branches such as construction, architecture, and handicraft products. When taking into account the species *B. vulgaris* and *vulgaris* var. *vittata*, the different seasons demonstrated that the harvesting and storage of this material should be carried out in the dry season to avoid minor infestations by *D. minutus*.

The bacterium *P. mirabilis* and the aqueous extract of leaves of the ornamental plant *D. arborea* are efficient alternative methods to control the bamboo borer when compared to the vinasse, which in turn ends up promoting the development of the insect. However, new research in the area of alternative control of stored product pests still needs to be carried out in order to reduce the use of chemical pesticides in the agribusiness.

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