

Effect of vacuum storage on shelf life of a grain protector based on *Peumus boldus* Molina foliage powder and lime against *Sitophilus zeamais* Motschulsky

Paulina Rivera¹, Gonzalo Silva^{1*}, Inés Figueroa¹, Maritza Tapia¹, and J. Concepción Rodríguez²

The maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae) is a key pest of stored grain maize. As an ecological pest control alternative, the use of botanical insecticides, such as powder from boldus (*Peumus boldus* Molina) foliage singly or mixed with lime, has been evaluated. Unfortunately, its shelf life is very short and does not exceed 15 d. The effectiveness of vacuum storage on insecticidal properties of a natural grain protector produced with boldus powder:lime at proportions of 50:50 and 60:40 against adults of *S. zeamais* was assessed under laboratory conditions. Treatments were evaluated at 1% and 2% (w/w) for 150 d of storage. All treatments based on boldus powder kept the level of mortality by contact activity over 80% at 150 d of storage. The highest toxicity, as a fumigant, was observed in treatments 50:50 at 2% and 60:40 at 1% and 2% with mortality over 60%. The grain weight loss was less than 1% and seed germination was not affected. With the exception of 0:100 at 2% without vacuum storage, all treatments were repellent to *S. zeamais*. Vacuum storage extended shelf life of the grain protector for 150 d.

Key words: Botanical insecticides, contact toxicity, fumigant, maize weevil, repellent.

INTRODUCTION

Cereals are a very relevant part of the human and animal diet (FAO, 1993). Annually, insect pests of stored products cause losses of approximately 30% although in developing countries this impact reaches up to 50% (García-Lara et al., 2004). Insect pests of stored grains are mainly controlled by synthetic contact insecticides, such as organophosphates or pyrethroids, and fumigants, such as methyl bromide and aluminum phosphide. However, the use of synthetic insecticides has disadvantages such as toxic residues, human intoxication, environmental pollution, and the development of insecticide resistance (White and Leesch, 1966). Additionally, the use of methyl bromide is banned because it depletes the ozone layer (USDA, 2000).

The maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae) is considered to be a severe worldwide pest of stored products. The attack of this insect species begins in the field and continues once the grain is stored. It may cause complete grain destruction in only 6 mo. The larvae and adult feed on the endosperm, which leads to the attack of secondary insect pests

and fungi (Larraín, 1994). It is usually controlled by synthetic insecticides with the consequent development of resistance as it has occurred with phosphine (Pimentel et al., 2009), organophosphates (Pereira et al., 2009), and pyrethroids (Ribeiro et al., 2003). According to the arthropod pesticide resistance database (www.pesticideresistance.org), *S. zeamais* has 32 cases of field resistance reported for the carbaryl, chlorpyrifos-methyl, cypermethrin, DDT, deltamethrin, HCH-gamma, malathion, phosphine, and permethrin insecticides. Hence, the use of botanical insecticides should be considered as a viable alternative. Malik and Mujtaba (1984) and Makanjuola (1989) indicated that these pesticides have shown contact, fumigant, antifeedant, and repellent insect activity. According to Weaver and Subramanyam (2000), botanical insecticides against stored grain pests are used as a dry vegetable powder mixed with the grain.

The perennial boldus tree, *Peumus boldus* Molina (Monimiaceae), is native to Chile and its powder has insecticidal activity against *S. zeamais* (Páez et al., 1990; Silva et al., 2003a; 2005; Pérez et al., 2007), and third instar larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Zapata et al., 2006), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Silva-Aguayo et al., 2010). The efficacy of botanical insecticides is usually accomplished by mixing them with mineral compounds such as lime or calcium carbonate. Silva et al. (2003a; 2005) and Nuñez et al. (2010) indicated that ratios of 50:50 and 60:40 (boldo powder:lime) at concentrations of 1.0% and 2.0% (w/w) caused 95% mortality of *S. zeamais*. However, Bustos-Figueroa et al. (2009) observed that the

¹Universidad de Concepción, Facultad de Agronomía, Av. Vicente Méndez 595, Chillán, Chile.

*Corresponding author (gosilva@udec.cl).

²Colegio de Postgraduados, Campus Montecillo, km 36,5 Carretera México-Texcoco, Montecillo, Estado de México, México.

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toxicity of *P. boldus* powder mixed with lime decreases after 30 d of storage. Pizarro et al. (2013) indicated that boldus powder stored under refrigerated conditions does not exceed 65% mortality after 15 d. This impact of storage on biological efficacy limits the commercial use of this type of pesticide. The aim of this research was to evaluate the effect of storage under vacuum conditions on the contact, fumigant, and repellent activity against *S. zeamais* of a natural grain protector elaborated with a mixture of *P. boldus* foliage powder and lime.

MATERIALS AND METHODS

The study was carried out from December 2012 to April 2013 in the Laboratory of Entomology, Faculty of Agronomy, Universidad de Concepción in Chillan, Biobío Region, Chile. Bioassays using boldus powder alone or mixed with lime stored under and without vacuum conditions at eight storage times against *S. zeamais* were assessed.

Plant material and grains. *Peumus boldus* foliage was collected in Pinto (36°42' S, 71°54' W, 286 m a.s.l.), province of Ñuble, Biobío Region, Chile, with criteria used by Vogel et al. (1997). Once collected, leaves were dried for 48 h in a stove (UNB 500, Memmert GmbH, Schwabach, Germany) at 40 °C. After that, foliage was ground in an electric coffee grinder (Moulinex, A5052HF, Alençon, France) to obtain a fine powder with a 20 mesh (0.841 mm) sieve (Dual Manufacturing, Chicago, Illinois, USA).

Maize grains with 14% moisture were used as food substrate. The maize was obtained in the local market. To avoid any prior infestation, grains were washed and frozen at -4 ± 1 °C for 48 h.

Insects. The insects used for the bioassays are susceptible to insecticides and were obtained from the Laboratory of Entomology, Faculty of Agronomy, Universidad de Concepción in Chillan. They were reproduced in 1 L glass flasks containing maize as a food source. Insects were maintained in total darkness at 30 ± 1 °C and 60% RH in a bioclimatic chamber (IPS 749, Memmert GmbH, Schwabach, Germany).

Treatments. We used boldus foliage powder:lime mixture at two proportions of 50:50 and 60:40. According to Silva et al. (2006), these ratios are the most effective against *S. zeamais*. The boldus powder and lime were also assessed separately. Treatments were set up in transparent plastic bags (Oster Rol20, Sunbeam Products, Miami, Florida, USA). One set was stored under vacuum conditions using a food vacuum sealer machine (Oster FoodSaver V2240, Sunbeam Products); the other was kept without vacuum conditions. Both types of treatments (under and without vacuum conditions) were stored in a paperboard box to

simulate warehouse conditions at room temperature (20 ± 5 °C). Eight storage times were assessed: 0, 7, 15, 30, 60, 90, 120, and 150 d. All treatments were evaluated at concentrations of 1.0% and 2.0% (w/w) (Nuñez et al., 2010). After the respective storage time, the insecticidal effect of each treatment was evaluated.

Contact toxicity. These bioassays were carried out using the methodology by Silva et al. (2006) where 100 g maize grains were placed into 250 mL jars, the respective treatment was added, and the container was hand shaken for 1 min. After that, 20 insect couples, not older than 10 d, were added. The untreated control was a jar with 100 g maize grains infested with 20 insects. The sex was determined using the criteria proposed by Halstead (1963). After insect infestation, containers were transferred to a bioclimatic chamber (25 ± 1 °C, 60% RH).

Adult mortality. The percentage of mortality was assessed 7 d after infestation (DAI). To consider the bioassay as valid, the maximum level of mortality accepted for the untreated control was 10%; in cases when insects dying of natural causes in the control were lower than the threshold, mortality was corrected by Abbott's formula (Abbott, 1925), but if the control exceeded 10% mortality, the complete bioassay was discarded and repeated. An insect was considered dead when it failed to move after being prodded gently with a needle for 30 s. After collecting the data, all the insects were removed and the flasks returned to the bioclimatic chamber.

F₁ adult emergence. After 55 DAI, the number of F₁ adults was recorded and percentage emergence was calculated by considering the values of the untreated control as 100%.

Grain weight loss and germination. Both variables were assessed 55 DAI. Grain weight loss was determined based on the difference between initial (100 g) and final grain weight. So as not to take into account grain weight reduced by moisture loss, the bioassay included four jars with 100 g maize without insects and at 55 DAI the weight difference was recorded and discounted from the cereal weight loss recorded in jars infested with *S. zeamais*. The impact of the treatments on maize germination was evaluated by selecting 10 apparently healthy seeds per treatment, which were germinated for 7 d on wet filter paper and kept under conditions of 24 ± 2 °C and $60 \pm 5\%$ RH in a bioclimatic chamber. The grains used in the bioassays were not certified seeds; hence, relative germination was estimated by considering the untreated control as 100%.

Repellent effect. The methodology proposed by Mazzonetto and Vendramim (2003) was used with slight modifications. The experimental unit was a plastic

Petri dish (5 cm diameter) containing 20 g maize grains mixed with the respective treatment. Treatments were intercalated on a Petri dish without powder (untreated control) in a circle around a central Petri dish containing 20 individuals of *S. zeamais* 48 h of age without sexing. The central Petri dish was connected to the treatments through tubes 10 cm long and 0.5 cm in diameter (Procópio et al., 2003). The experimental batch was maintained in a bioclimatic chamber for 24 h at 30 ± 1 °C. Subsequently, the number of insects present in each treatment was counted. Each treatment had 10 replicates and in each replicate the treatment locations were randomly rotated to avoid external interference. The repellency index was calculated according to Mazzonetto and Vendramim (2003), who classified powder as neutral if the index = 1, attracting if > 1, and repellent if < 1.

Fumigant effect. The methodology to evaluate the fumigant effect was adapted from Tavares and Vendramim (2005). At the bottom of 200 mL plastic containers, a PVC tube, 5 cm long and 2.5 cm in diameter and containing the respective treatment, was inserted vertically. Then, PVC tubes were covered with a piece of fine organza fabric to prevent the direct contact of insects with the powder, but allowing release of volatile compounds into the environment. Outside, the tube and the inner edge of the plastic containers were filled with 20 g of maize, which were infested with 20 unsexed insects. The controls were plastic containers with 20 g maize grains infested with 20 insects without powder in the tube. Mortality by the fumigant effect was evaluated 5 DAI and each treatment included five replicates.

Experimental design and statistical analysis. We used a completely randomized experimental design with a factorial arrangement of 2×8 with two types of storage (under and without vacuum conditions) and eight treatments (four formulations 50:50, 60:40, 100:0, and 0:100 at concentrations of 1% and 2%) during a storage period of 150 d. Each treatment had four replicates and the percentage of mortality by contact toxicity and fumigant effect, emergence (F_1), and germination were transformed with the equation $\arcsin(x)^{1/2}$, while grain weight loss was transformed to the function $(x+0.5)^{1/2}$ prior to ANOVA ($\alpha = 0.05$) with the statistical software InfoStat® (Balzarini et al., 2008). Statistical differences were determined by Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The assessed treatments produced with *P. boldus* powder and stored under vacuum conditions were effective after 150 d storage (Figures 1 and 2). The interaction between treatment factors and type of storage was significant ($p \leq 0.05$) for the variables of contact toxicity, emergence of adult insects (F_1), and fumigant effect (Table 1).

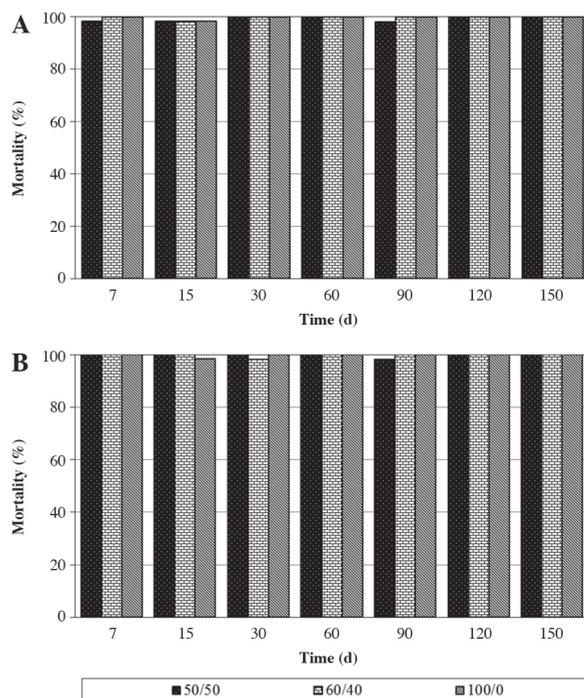


Figure 1. Mortality of *Sitophilus zeamais* exposed to *Peumus boldus* powder mixed with lime at different proportions (boldus:lime): 50:50, 60:40, and 100:0 at concentrations of 1.0% (A) and 2.0% (B) stored under vacuum conditions for 150 d.

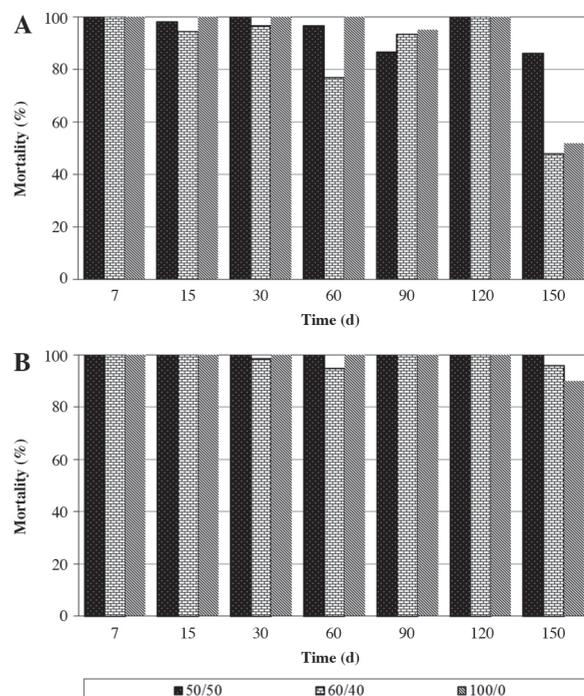


Figure 2. Mortality of *Sitophilus zeamais* exposed to *Peumus boldus* powder mixed with lime at different proportions (boldus:lime): 50:50, 60:40, and 100:0 at concentrations of 1.0% (A) and 2.0% (B) stored without vacuum conditions for 150 d.

Table 1. Mean minimum squares and coefficient of variation for assessed variables in the evaluation of biological activity of *Peumus boldus* powder singly and mixed with lime at proportions of 0:100, 50:50, 60:40, and 100:0 at concentrations of 1% and 2% (w/w) stored under environmental and vacuum conditions for 150 d.

Sources of variation	DF	MC	F ₁	GG	FE	GWL
Treatment	7	0.41*	0.90*	0.01	0.46*	0.15
Package	1	1.60*	1.61*	0.05	4.18*	0.01
Treatment × Package	7	0.12*	0.42*	0.02	0.29*	0.03
Error	32	0.02	0.11	0.01	0.02	0.06
CV, %		10.21	75.25	7.97	43.60	23.07

*Significant according to Tukey's test ($P \leq 0.05$).

CV: Coefficient of variation; DF: degree of freedom; MC: mortality by contact; F₁: emergence; GG: grain germination; FE: fumigant effect; GWL: grain weight loss.

Contact toxicity

Adult mortality. *Peumus boldus* mixed with lime and stored under vacuum conditions induced 100% mortality (Table 2); therefore, vacuum storage increases the time that the formulation remains toxic. Treatments without vacuum conditions exhibited better results than those observed by Silva et al. (2005) and Bustos-Figueroa et al. (2009), who indicated that *P. boldus* powder does not have a residual effect beyond 30 d of storage. This difference may be due to the type of container used. We used special bags for vacuum storage, while other authors used paper bags or opaque plastic containers allowing greater gas exchange. However, lime used alone under vacuum conditions was the only treatment that did not reach 100% mortality. Similar results were documented by Bustos-Figueroa et al. (2009) and Nuñez et al. (2010), but our results are better than those found by Silva et al. (2004), who did not exceed 60% mortality of *S. zeamais* treated with lime.

Adult insect emergence (F₁). The only treatment that showed significant differences ($p \leq 0.05$) with its counterpart was lime alone at 2.0%, which showed F₁ emergence of 11.1% and 98.8% under and without vacuum conditions, respectively (Table 2). Treatments under vacuum conditions had lower emergence of F₁

Table 2. Mortality and adult emergence (F₁) of *Sitophilus zeamais* fed with maize treated with *Peumus boldus* powder alone and mixed with lime at proportions of 0:100, 50:50, 60:40, and 100:0 at concentrations of 1% and 2% (w/w) stored under and without vacuum conditions for 150 d.

Formulation (%:%) ¹	Concentration	Mortality		Emergence (F ₁)	
		Vacuum	Without vacuum	Vacuum	Without vacuum
0:100	1.0	69.5Ab	44.5Ab	44.4Aa	94.0Aa
0:100	2.0	72.9Ab	49.5Ab	11.1Ba	98.8Aa
50:50	1.0	100.0Aa	85.9Aa	22.2Aa	3.6Ab
50:50	2.0	100.0Aa	100.0Aa	0.0Aa	0.0Ab
60:40	1.0	100.0Aa	47.8Bb	33.3Aa	60.7Aab
60:40	2.0	100.0Aa	95.8Aa	0.0Aa	1.2Ab
100:0	1.0	100.0Aa	51.7Bb	0.0Aa	44.0Aab
100:0	2.0	100.0Aa	89.9Aa	11.1Aa	3.6Ab

¹Ratio boldo:lime (%:%).

Different uppercase letters indicate significant differences between columns for each treatment and lower-case letters indicate significant differences between rows for each type of package (under vacuum/without vacuum) according to Tukey's test ($P \leq 0.05$).

adult insects than treatments without vacuum conditions; a higher mortality-lower emergence trend was observed. These results agree with Silva et al. (2004), Pérez et al. (2007), and Cruzat et al. (2009), who concluded that higher concentrations of powder led to a higher mortality level and lower F₁ adult emergence. Treatments stored under vacuum conditions did not have any significant differences in F₁ emergence. In the case of treatments without vacuum conditions, those formulated with a higher concentration of *P. boldus* powder showed a lower F₁, which coincided with data reported by Silva et al. (2005; 2006) and Bustos-Figueroa et al. (2009), this is perhaps achieved by reducing the oviposition rate or impeding male and female encounter and then avoiding copula.

Grain weight loss and germination. Grain weight loss did not show any significant differences between types of storage. Losses under and without vacuum conditions were 0.72% and 0.67%, respectively (Table 3). Treatments 50:50 and 100:0 at 1.0% were the only ones significantly different from the control (Table 4). Our results are similar to those found by Silva et al. (2003a) and Cruzat et al. (2009), who observed weight losses above 1.0% at concentrations of 1.0% and 2.0% (w/w) with boldus powder and lime used singly. The lower weight loss may be due to high mortality produced by treatments, which then reduced grain damage.

Treatments with *P. boldus* powder used alone or mixed with lime at both storage conditions did not affect significantly maize germination (Tables 3 and 4). Percentage germination was 96.95% and 95.13% under and without vacuum conditions, respectively.

Table 3. Grain weight loss and germination of maize infested with adults of *Sitophilus zeamais* stored under and without vacuum conditions for 150 d.

	Germination	Weight loss
	%	g
Under vacuum	96.95a	0.72a
Without vacuum	95.13a	0.67a

Different lower-case letters indicate significant differences between columns for each type of package (under vacuum/without vacuum) according to Tukey's test ($P \leq 0.05$).

Table 4. Grain weight loss and germination of maize treated with *Peumus boldus* powder alone and mixed with lime at proportions of 0:100, 50:50, 60:40, and 100:0 at concentrations of 1% and 2% (w/w).

Formulation (%:%) ¹	Concentration	Weight loss	Germination
		%	%
Control (0:0)	--	1.50a	--
0:100	1.0	0.39ab	95.56a
0:100	2.0	0.67ab	97.23a
50:50	1.0	0.41b	93.34a
50:50	2.0	0.93ab	96.67a
60:40	1.0	0.68ab	96.11a
60:40	2.0	0.66ab	94.45a
100:0	1.0	0.34b	97.78a
100:0	2.0	0.75ab	97.23a

¹Ratio boldo:lime (%:%).

Different lower-case letters indicate significant differences between formulations according to Tukey's test ($P \leq 0.05$).

All treatments show germination percentage > 90%, which agrees with data reported by Pizarro et al. (2013). However, our results disagree with Silva et al. (2003b) and Pérez et al. (2007), whose values did not exceed 75% germination at concentrations of 1.0% and 2.0% (w/w) and concluded that germination values decrease if powder concentration increases. This may be due to the time of year when plant material was field-collected since bioactivity of *P. boldus* foliage varies between seasons (Pérez et al., 2007); it is possible that the chemical compound that affects germination is absent or has a lower concentration in January, which was our collection date. The higher germination values obtained (> 90%) comply with the requirement for export seeds (González, 1995); thus, this increases the possibility of using more effective treatments to protect grain and seed.

Repellent effect. The only treatment that did not show repellent effect was lime used alone (0:100) at 2.0% (w/w) stored without vacuum conditions (Table 5). The rest of the treatments showed a repellency index < 1, which, according to Mazzoneto and Vendramim (2003), classifies as repellent activity. Results match those found by Betancur et al. (2010) and Nuñez et al. (2010), who evaluated essential oil and boldus powder at concentrations of 1.0% and 2.0% and reported a repellency index of 0.45 and 0.18 for oil and powder, respectively; both values are similar to our research results.

Fumigant effect. Treatments 50:50 (1.0% and 2.0%), 60:40 (2.0%), and 100:0 (1.0% and 2.0%) stored under vacuum conditions showed significantly higher mortality than fumigants as compared with treatments without vacuum conditions (Table 6). However, the highest mortality was obtained with boldus used alone (100:0) with 86.6% and 93.3% dead insects at concentrations of 1.0% and 2.0%, respectively. These results agree with Nuñez et al. (2010), who reported the highest mortality in the same treatments but with values just over 47%. Treatments stored without vacuum conditions did not surpass 10% of mortality, which may be due to degradation of plant compounds by oxygen present in the bags (Morales and García, 2000).

Table 5. Repellent effect of *Peumus boldus* powder alone or mixed with lime against adults of *Sitophilus zeamais* at proportions of 0:100, 50:50, 60:40, and 100:0 and concentrations of 1.0% and 2.0% (w/w) stored under and without vacuum conditions for 150 d.

Formulation (%:%) ¹	Concentration %	RI	RI
		Under vacuum	Without vacuum
0:100	1.0	0.25	0.41
0:100	2.0	0.43	1.03
50:50	1.0	0.32	0.49
50:50	2.0	0.42	0.47
60:40	1.0	0.68	0.39
60:40	2.0	0.34	0.32
100:0	1.0	0.25	0.37
100:0	2.0	0.27	0.24

¹Ratio boldus:lime (%:%).

RI: Repellency index (> 1 attracting; = 1 neutral; < 1 repellent).

Table 6. Toxicity against adults of *Sitophilus zeamais* by the fumigant effect of *Peumus boldus* powder alone or mixed with lime at proportions of 0:100, 50:50, 60:40, and 100:0 and concentrations of 1.0% and 2.0% (w/w) stored under and without vacuum conditions for 150 d.

Formulation (%:%) ¹	Concentration	Mortality	Mortality
		Under vacuum	Without vacuum
0:100	1.0	3.33Ad	0.0Aa
0:100	2.0	3.33Ad	0.0Aa
50:50	1.0	23.30Ac	0.0Ba
50:50	2.0	63.30Abc	3.33Ba
60:40	1.0	10.00Ad	0.0Aa
60:40	2.0	40.00Ac	3.33Ba
100:0	1.0	86.60Aab	6.66Ba
100:0	2.0	93.30Aa	3.33Ba

¹Ratio boldus:lime (%:%).

Different uppercase letters indicate significant differences between columns for each treatment and lower-case letters indicate significant differences between rows for each type of package (under vacuum/without vacuum), according to Tukey's test ($P \leq 0.05$).

CONCLUSIONS

Vacuum storage extends insecticidal activity by 150 d as a contact insecticide against adult and immature *Sitophilus zeamais* as well as a repellent effect against adults. There was no impact on maize germination by the natural grain protector produced with *Peumus boldus* foliage powder mixed with lime.

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