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IS FLINT CORN NATURALLY RESISTANT TO MAIZE WEEVIL INFESTATION?

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Abstract.

Sitophilus zeamais (maize weevil) is one of the most destructive pests of maize stored in tropical and subtropical regions. This study determined the resistance of flint corn and dent corn to infestation by S. zeamais (Motschulsky), *the maize weevil. Improved King Philip hybrid flint corn and Fontanelle 6T-510 hybrid dent corn were used in this experiment. Two temperature conditions (10 °C and 27 °C) and two storage times (15 days and 30 days) were used. Results showed that flint corn was more resistant to insect damage than dent corn at 27 °C and 30 day storage time. After 30 days storage time and 27 ºC death rate was significantly higher in flint corn (R^2= 0.945) compared to (R^2 = 0.634) in dent corn. Damaged seed was 10% higher in dent corn then in flint corn at 27 ºC and 30 days. However, no significant difference was observed for seed weight loss between flint corn and dent corn at the same storage conditions. Both dent and flint corn are extensively cultivated in developing countries. It appears that storage of flint corn may be one promising solution to reducing corn damage and infestation problems in the tropics and in developing countries, but more research is needed.*

Keywords. *Sitophilus zeamais; flint corn; dent corn; corn damage; corn storage.*

Introduction

Corn (*Zea mays L.*) is a unique crop in its versatility; it is the only food grain that is eaten from flower to flour (Boutard, 2012). It is the principal staple food and major source of calories in many developing countries (FAO, 2009), and the biggest source of feed, biofuel, and raw material for many industries in developed countries. It is the third most important cereal crop globally after wheat and rice (Adarkwah et al., 2012). According to the United Nations Food and Agriculture Organization (FAO), in 2010-2011 over 800 million metric tons of corn was produced (FAO, 2011); this is predicted to double by 2025, and corn is predicted to become the greatest crop in terms of production by 2050 (Rosegrant et al., 2008). Nearly half is produced in North America, with over 35% of total world production occurring in the United States Corn Belt, followed by China, European Union (EU-27), Brazil, and Argentina (USDA, 2012).

Despite increases in production, post-harvest losses due to biotic factors such as insects and molds remain a huge challenge worldwide (FAO, 2009). It is estimated that 14% to 50% of the total corn produced each season in developing countries is lost due to insect infestation, compared to only 1% to 2% in developed countries (Ojo and Omoloye 2012).

Corn is classified into groups based on endosperm characteristics, kernel color, maturity, and final uses (Paliwal et al., 2000). There are six main varieties of corn grown worldwide for commercial and human consumption: dent corn, flint corn, flour or soft corn, sweet corn, waxy corn and popcorn (Singh et al., 2009). Dent corn is the most widely grown corn in the United States (US) Corn Belt, and most parts of the world (Boutard, 2012). The kernel contains both corneous and soft starches, characterized by very hard, vitreous, horny endosperm at the sides and back (Singh et al., 2009). The central core extends to the top, or crown of the kernel, which collapses on drying, resulting in the distinctive indentation (dent) (Paliwal et al., 2000). Dent corn has a fairly wide range of colors, from yellow to white, but yellow is the most common and is extensively grown for seed, silage, biofuel, and other commercial uses in the US. Dent corn is susceptible to grain insect infestation by insects such as *S. zeamais* (Paliwal et al., 2000).

Flint corn is less popular than dent corn. The kernels of flint corn range from small (<5 mm long) to large (>11 mm long) in size, are rounded on the top, smooth, hard and thick with no indentation of the crown at maturity (Boutard, 2012). Flint corn exhibits an extended range of colors from white through yellow, orange, red, mahogany, blue, purple, and black (Boutard, 2012), and it is widely grown in Latin America, Northern Europe, and some parts of Asia for commercial purposes (Gujral et al., 2001).The endosperm of flint corn is primarily vitreous, with little soft starch, and is enclosed by a corneous outer layer. Starch is more concentrated at the periphery than in the center, which gives the endosperm hard external layers (Haros et al., 2001). The hard outer layer of flint corn may make it less prone to insect damage (Paliwal et al., 2000) and less water absorbent than dent corn (Haros et al., 2001). In terms of nutrients, flint corn typically contains more protein than dent corn, (9.2% versus 7.0% dry basis respectively), while flint corn contains less starch (63%) than dent corn (76%), but its quality is good and the ratio of amylose - amylopectin is about the same as that of dent corn (Haros et al., 2003; White and Johnson, 2003). Compared to dent corn, flint has lower yield, is less cultivated, and farmers normally receive a higher price from millers and brokers (Cirilo et al., 2011).

Corn and other cereal grains account for over 70% of the total crops produced in developing countries. Smallholder, subsistence farmers produce most of these grains; unfortunately, significant amounts are often lost after harvest, resulting in increased hunger and human labor (FAO, 2011). Africa Postharvest Losses Information System (APHLIS) statistics showed that nearly 17% of the total corn produced in Africa was lost in 2011-2012 (APHLIS, 2013). FAO estimates about $4 billion lost each year in sub-Saharan Africa due to post harvest grain losses (FAO, 2011). The biggest cause of grain loss is infestation by insects such as *S. zeamais* during storage (Ukeh et al., 2012).

S. zeamais Motschulsky, the maize weevil, is among the most destructive pests in stored grain, especially corn in tropical regions (Paes et al., 2012). *S. zeamais* are regarded as internal feeders of grains. Adult female *S. zeamais* cause damage by boring into the kernel and laying eggs (ovipositing). Then, larvae and pupae eat the inner parts of the kernel, resulting in a damaged kernel and reduced grain weight (Ojo and Omoloye 2012). Apart from weight losses, the feeding damage caused by weevils leads to severe reductions in nutritive and economic values, reduced seed viability, as well as contamination by chemical excretions (silk) and insect fragments (Ukeh et al., 2012). The infestation also elevates temperature and moisture content in the stored grain mass, which can lead to mold growth, including toxigenic species such as *Aspergillus flavus* (Chu et al., 2013). *S. zeamais* cause extensive losses in quality and quantity of the grain in the field as well as in storage (Sabbour, 2012). Several studies have examined storage infestation in dent corn; little work, however, has been reported on infestation of flint corn by *Sitophilus zeamais*. Therefore, the objective of this research was to determine the resistance of flint and dent corn to *S. zeamais* infestation.

Materials and Methods

Experimental design

In this experiment, three replications of two corn varieties (dent and flint), and twenty-four glass jars with screened lids were used, with two temperature conditions (10 °C and 27 °C) and two storage/opening times (15 days and 30 days) (Table 1). The moisture content of each corn variety was determined with samples of 30 g in three replications at 103 ºC for 72 h, following ASAE Standard S352.2 (ASAE, 2001).

Treatment and storage trials

The dent corn was a commercial hybrid (Fontanelle 6T-510) harvested during 2012, and flint corn was Improved King Philip hybrid from crop year 2009-2010. The moisture contents of all corn samples were adjusted to 13.5± 0.5% (wet basis) prior to initiating the storage trials. Two identical environmental chambers with different temperature settings (10 °C and 27 °C) were used (Model 23-988 126 GW, Fisher Scientific Inc., Waltham, MA 02454). *S. zeamais* used in these experiments were obtained from the stock of *S. zeamais* already feeding on dent corn in the Department of Agricultural Biosystems Engineering at Iowa State University (Yakubu et al., 2011). Twenty-four 246-mL glass jars, with screened lids to allow air flow (i.e., 12 each of dent and flint) were each loaded with 230 g of corn; then 20 unsexed adult *S. zeamais* were introduced into each jar, based on Yakubu et al., (2011). The 12 jars for each hybrid were then stored in each experimental chamber.

Data collection and analysis

Mortality was assessed after 15 days and 30 days of storing the weevil-infested maize. All weevils were separated and removed (by hand) from the corn at the end of these two periods. Numbers of live and dead weevils were recorded at this time. By visual inspection, the number of damaged and undamaged kernels (seeds) in each treatment was recorded, as were the weights of damaged and undamaged kernels. Damaged kernels meant that visible physical damage caused by *S. zeamais* was present (Fig 1 and 2). Percent (%) kernel weight loss was determined by using the count and weigh method developed by Adams and Schulten (1978).

The factorial design consisted of three main effects, two corn types, two temperatures, and two storage times. Analysis of variance (ANOVA) was performed using the Statistical Analysis System (SAS) version SAS 9.3, with a general linear model (GLM), using PROG GLM (2011) at α of 5%, to determine the main and interaction effects and least significant differences (LSD) between treatment means. Additionally, treatment effects were examined at α of 0.05%.

Results and Discussion

The results for the main effects (Table 2), show that all independent variables had significant effects (P <0.05) on *S. zeamais* infestation parameters, except for live *S. zeamais* (LSZ), dead *S. zeamais* (DSZ) at 10 °C and 27 °C, and seed weight loss (SWL) for dent and flint corn. For the interaction effects (Table 3), the results show significant effects due to corn type and time, but mixed results for the other independent variable interactions., No significant effects were observed for the three-way interaction (corn by time by temperature). Furthermore, all independent variables showed significant effects for treatment combinations except for the LSZ (Table 4).

S. zeamais mortality

There were significant (P <0.05) differences seen with corn type and time for mortality (i.e. LSZ and DSZ (Table 2). However, there were no significant effects on mortality between 10 °C and 27 °C. The numbers of LSZ were significantly higher in flint corn at 15 days storage time. This concurred with a study by Paliwal et al., (2000), who examined dent corn susceptibility to grain insect infestation. Likewise, as expected, there was a higher number of DSZ observed in flint corn with the 30 days storage time; this was attributed to end of life cycle of *S. zeamais,* hardness of kernel and compounds such as phenolic acids that caused damage to midgut cells of the insects (Kevin, 2002). Kernel hardness was found to be the biggest factor contributing resistance to *S. zeamais* infestation on flint corn. Several studies reported results that concurred with our study. Golob, P., 1984; Kossou et al., 1993; Dombrink-Kurtzman and Knutson, 1997 reported that maize kernel hardness has strong correlation with insect damage during harvesting, handling, and storage and concluded that establishing maize varieties with higher kernel hardness is necessary for reducing insect infestation and improving protein quality of maize. Similar results were reported by Kossou et al., (1993) who reported that grain kernel hardness has a significant effect upon *S. zeamais* infestation, and Serratos et al., (1987) who described that out of four varieties they studied, two varieties were less susceptible to weevils. These were found to be those with harder kernel structure. High correlation between kernel hardness and pericarp cell wall of maize on *S. zeamais* resistance was observed by García-Lara et al., (2004).

In addition, significant effects (P <0.05) were observed for time and the interaction of corn type and time (Table 3) for LSZ and DSZ; however, no significant effects were detected for temperature, temperature-time interaction, corn type temperature interaction, or the three ways interaction (i.e. corn type by time by temperature). Moreover, no significant differences were found for the treatment combination effects for LSZ (Table 4), while there were some higher significant differences for DSZ, amongst treatments. Results also show that the growth of *S. zeamais* in dent corn (Fig. 3) follows a linear growth curve (R^2= 0.574), while different results were observed for flint corn (Fig. 3) whereby *S. zeamais* growth decreased exponentially with time (R^2= 0.945); this was believed due to shortage of food due to hard structure of flint corn.

Furthermore, the first derivative of the death curves in dent and flint (equation 1 and 2) respectively, show that after 30 days storage time death rates for *S. zeamais* in flint corn are almost three times higher than those of dent corn (Fig. 4 and Fig. 5), due to the same reasons mentioned previously.

$$\frac{\text{d Dead(dent)}}{\text{dt}} = (-0.002\text{t} + 0.233) \ldots\ldots\ldots (1)$$

$$\frac{\text{d Dead(flint)}}{\text{dt}} = (0.052\text{t} - 0.189) \ldots\ldots\ldots (2)$$

Results also revealed that growth rates decreased over time as shown on Fig. 3. The rate seems higher on flint corn (R^2 = 0.945) than in dent corn, the main reasons believed to be structural differences between flint and dent corn as flint corn exhibits hard endosperm (Maiorano et al., 2010) which makes them harder for *S. zeamais* to bore into the kernel and oviposit and also due to decreased food as the weevil population increased in dent corn.

Damaged and undamaged seed

For the case of damaged seed (DS) and undamaged seed (UDS), there were significant differences among all three main effects (Table 2). The highest DS was observed in dent corn, while the lowest DS was observed in flint corn. As time and temperature increased, DS increased, and UDS decreased. Examining treatment effects, dent had greater DS for all times and temperatures. Higher temperature led to greater insect activity. As described by Monstros et al., (1999) the main factors influencing propagation and development of insects are temperature and moisture content. Hayma, (2003), found that favorable temperature for most grain storage insects to develop is between 25 °C to 30 °C. Likewise, stated by Gudrups et al., (2001) factors like kernel hardness, husk protection, kernel size and texture, play significant roles on maize protection from insect attack, and these agreed with our finding. As shown on Table 4, damaged seed (DS) on dent corn were higher compared with flint corn both at 15 and 30 days storage times as well on 10 and 27 ºC temperature conditions.

The numbers of DS were directly related to LSZ. With an increasing number of LSZ, there was an increase in DS. Similar results were observed by Singh and McCain (1963), who found positive correlations between kernel nutrient contents, reproduction, and weights of weevils (i.e., as nutrients of kernels increased, weevil reproduction rate and weevil weights increased, and thus seed damage increased). Clearly, significant differences (P <0.05) were observed for all three main effects (Table 3) for DS, while only two main effects (corn and time) exhibited significant differences for UDS, while opposite results were observed for their interaction.

Weight of damaged and undamaged seed

There were significant differences (P <0.05) in the weight of damaged (WD) and undamaged (WUD) seed (Table 2). Higher WD was observed in dent corn than in flint corn for both 27 °C and 30 days storage time. As expected, more DS and LSZ were found in dent than in flint. Corn type and time were the only significant effects (Table 3) on *S. zeamais* infestation. Similarly, temperature and all other interactions were not actors influencing WD. For the case of WUD, significant effects were observed for corn type, time temperature, and the interaction of time and temperature. However, no significant effects were detected for corn time, corn by temperature, or the three way interaction of corn-time temperature (Table 3).

Seed weight loss (SWL)

Results showed few significant differences between dent and flint corn; the only significant differences (P <0.05) detected were due to temperature and storage time. The highest percentages of SWL were recorded at 27 °C and 30 days storage time, for both dent and flint corn. It is suspected a higher number of LSZ corresponds with high SWL in dent corn, according to a study conducted by Abebe et al., (2009), that found direct relationships between seed damage and weight loss with the number of weevils emerged, for different maize varieties. For this study, mixed results were observed for the interaction results, ranging from highly significant to no significance for some factors (such as type of corn, corn by time, and corn by time by temperature) (Table 3). Treatment combinations showed that dent corn at 10 °C and 15 day storage time had similar results to flint corn under the same conditions (Table 4). The results also showed that dent corn at 10 °C and 15 days storage time were similar (P >0.05) to flint corn at 27 °C and 30 days storage time.

Resistance of stored grain insects such as *S. zeamais* to protectants has recognized as an increasingly important problem in tropical countries. Studies conducted by Samson et al., 1988 and Arnason et al., 1992, show that most of the chemicals used to protect corn against stored product insects in tropic climates have low effectiveness and insects build resistance to them. To avoid creating stronger pests and yet reducing postharvest losses of corn in developing countries, the use of resistant varieties like flint corn remain the best option, and many scientists considered it as a sustainable way of integrated pest management strategy (García-Lara et al., 2010; Arnason et al., 1992; Adebe et al., 2009).

Conclusions

This experiment was conducted to determine the resistance of flint and dent corn to *S. zeamais* infestation. The results suggest that dent corn is more susceptible to *S. zeamais* than flint corn. Other factors, such as time and temperature, play large roles in corn infestation, as this study revealed that most of the damage occurred at 27 °C and 30 days storage time. Therefore, flint corn, or a hybrid of flint and dent, could be a viable approach to reduce the problem of infestation and damage in developing countries. Further study is needed to look at different varieties of flint, especially for longer storage times. These studies are ongoing.

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Figure 1. Flint corn shows damage caused by *S. zeamais* during 30 days of storage.

Figure 2. Dent corn shows damage caused by *S. zeamais* during 30 days of storage.

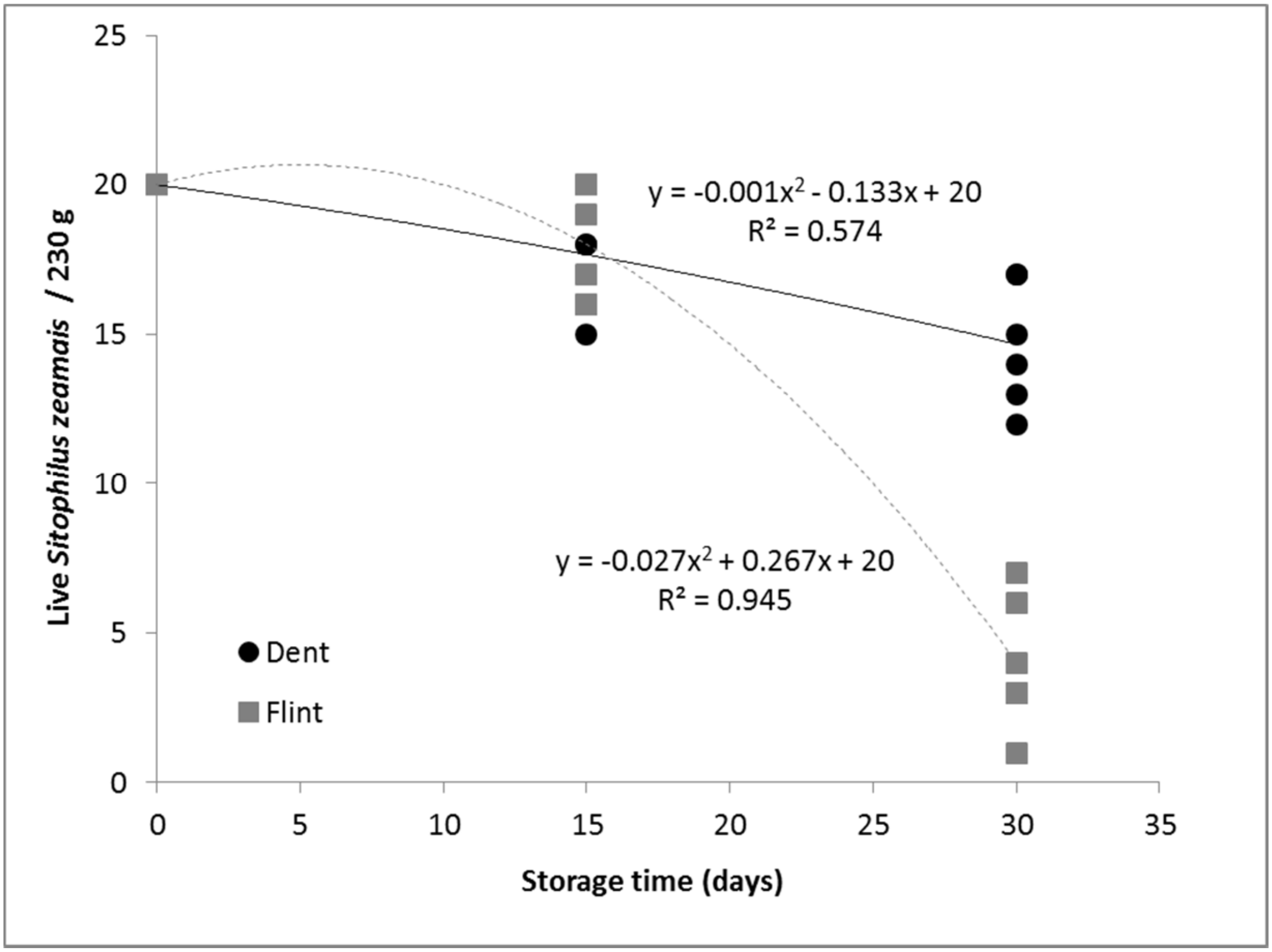

Figure 3. Number of live *S. zeamais* over time.

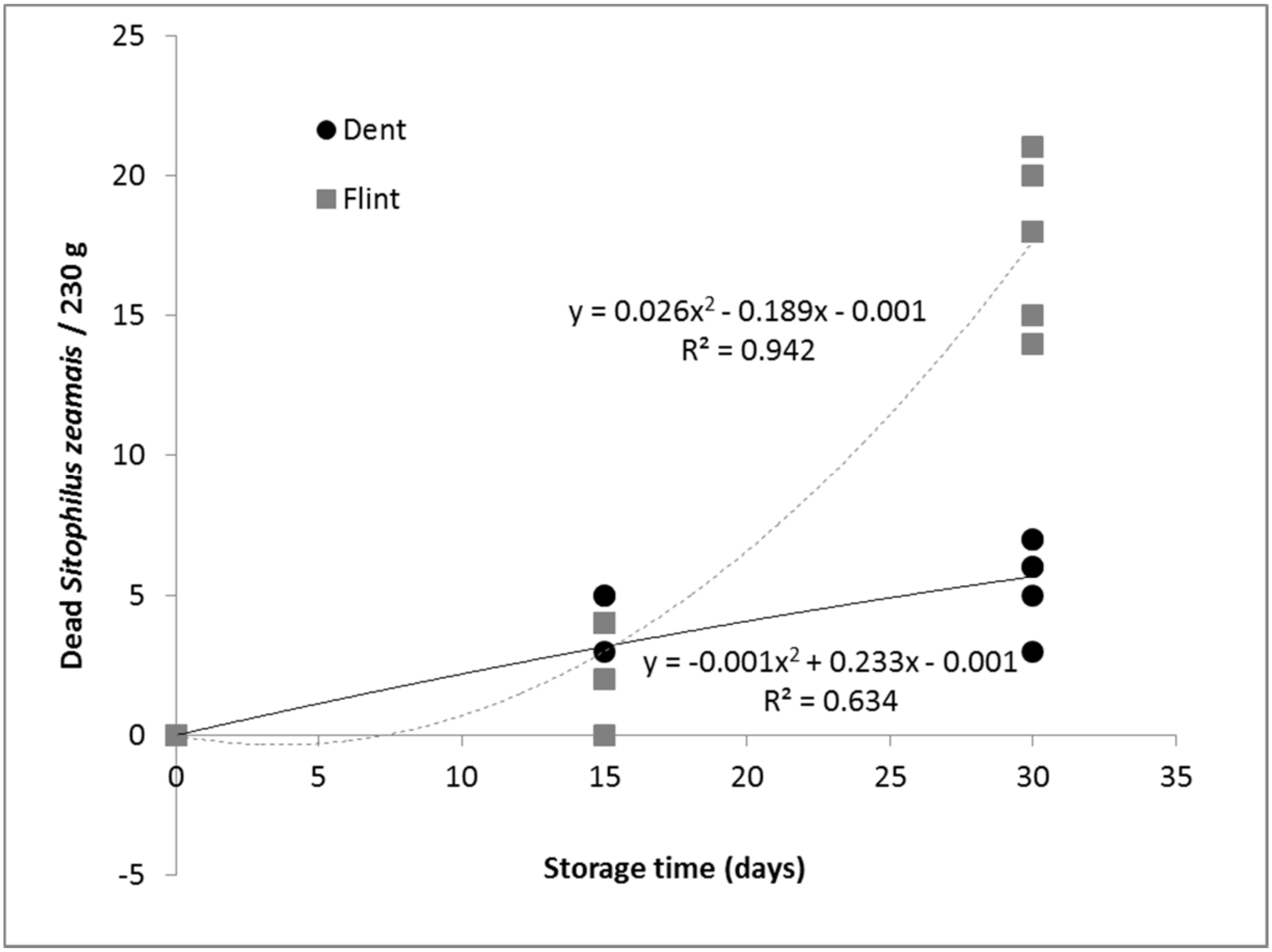

Figure 4. *S. zeamais* mortality (number dead) over time.

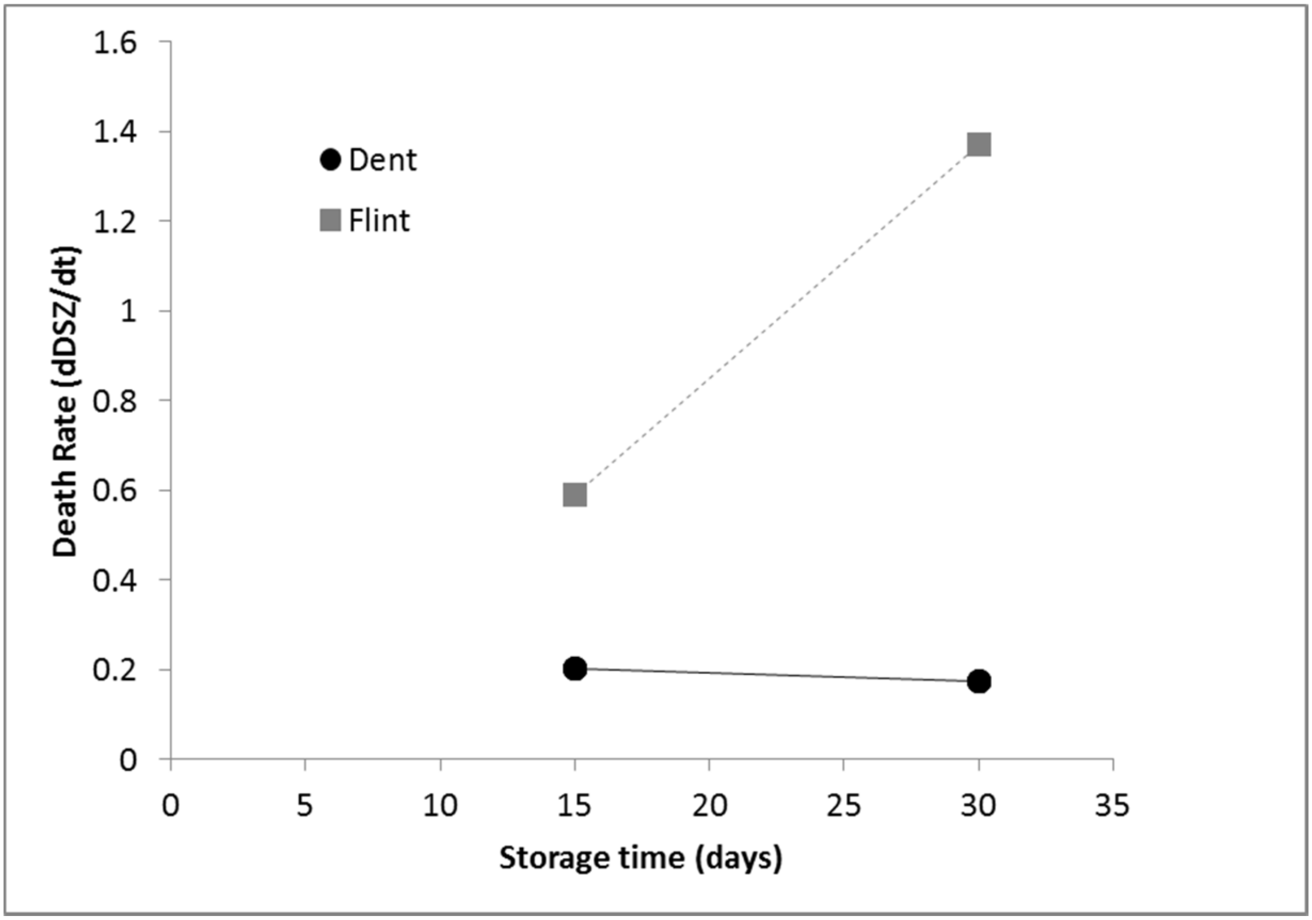

Figure 5. First derivatives (increase in death rate), denoted as dDSZ/dt, for *S. zeamais* mortality over time.

Table 1. Experimental design.

Treatment	Corn type	Time (days)	Temp (°C)
1	dent	15	10
2	dent	15	27
3	flint	15	10
4	flint	15	27
5	dent	30	10
6	dent	30	27
7	flint	30	10
8	flint	30	27

Table 2. Main effects of Corn types, temperature and time on *S. zeamais* infestation. The values in the table are means ± standard deviation. [+]

	LSZ	DSZ	DS	UDS	WD(g)	WUD(g)	SWL (%)
Corn							
Dent	16.2 ± 2.4^{a}	4.1 ± 2.1^{b}	56.7 ± 15.1^{a}	639.1 ± 19.2^{a}	14.3 ± 3.5^{a}	207.8 ± 6.2^{b}	1.8 ± 1.0^{a}
Flint	11.0 ± 7.6^{b}	10.3 ± 7.9^{a}	36.3 ± 13.7^{b}	708.9 ± 17.2^{b}	7.7 ± 3.1^{b}	215.4 ± 6.2^{a}	1.5 ± 0.8^{a}
Temp (ºC)							
10	13.8 ± 6.1^{a}	6.8 ± 5.8^{a}	39.9 ± 14.8^{b}	675.6 ± 38.8^{a}	10.3 ± 4.1^{b}	216.1 ± 4.5^{a}	1.1 ± 0.9^{b}
27	13.4 ± 6.4^{a}	7.9 ± 7.2^{a}	53.0 ± 18.2^{a}	672.4 ± 42.6^{b}	11.7 ± 5.3^{a}	207.1 ± 6.6^{b}	2.1 ± 0.7^{a}
Time (d)							
15	17.8 ± 1.8^{a}	3.1 ± 1.7^{b}	35.7 ± 14.8^{b}	688.3 ± 35.6^{a}	8.3 ± 3.4^{b}	214.8 ± 4.9^{a}	1.3 ± 1.1^{b}
30	9.3 ± 5.9^{b}	11.7 ± 6.6^{a}	57.3 ± 13.0^{a}	659.7 ± 40.2^{b}	13.6 ± 4.4^{a}	208.4 ± 7.7^{b}	2.0 ± 0.5^{a}

[+] Values with the same letter for a given property, within each independent variable, are not significantly different (P <0.05) for the dependent variable. LSZ= live *S. zeamais*, DSZ= dead *S. zeamais*, DS = damaged seed, UDS = undamaged seed, WD = weight of damaged seed (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.

Table 3. Interaction results (*P* values) for corn types, temperature and time on *S. zeamais* infestation.[+]

Variable	LSZ	DSZ	DS	UDS	WD	WUD	SWL (%)
Corn	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0547
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0011
Temp	0.7187	0.1398	0.0001	0.3874	0.0295	0.0001	0.0001
Corn x Time	0.0001	0.0001	0.4388	0.2981	0.3683	0.8677	0.1103
Corn x Temp	0.7187	0.0919	0.0586	0.7132	0.5253	0.8077	0.0146
Time x Temp	1.0000	0.9063	0.9188	0.0024	0.0060	0.0001	0.0002
Corn x Time x Temp	1.0000	0.0232	0.4008	0.9266	0.1034	0.3690	0.2552

[+] A significance level of $P < 0.05$ was used. . LSZ= live *S. zeamais*, DSZ= dead *S. zeamais*, DS = damaged seed, UDS = undamaged seed, WD = weight of damaged seed (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.

Table 4. Treatment combination effects due to corn types, temperature and time on *S. zeamais* infestation

The values in the table are measn ± standard deviation.

Trmt	Corn	Time	Temp	LSZ	DSZ	DS	UDS	WD	WUD	SWL (%)
1	dent	15	10	17.8±2.5[a]	2.3±2.5[d]	36.7±4.5[e-d]	649.7±1.5[d]	10.8±1.6[c]	214.3±0.7[c]	0.5±0.2[d]
2	dent	15	27	17.8±1.5[a]	4.0±1.0[d-c]	57.0±9.5[b]	661.0±11[c]	11.8±1.5[c]	208.1±1.4[e-d]	2.7±0.8[a]
3	flint	15	10	18.3±2.1[a]	2.7±2.3[d]	21.3±1.5[e]	717.3±4.7[b-a]	6.3±0.4[e-d]	220.9±1.9[a]	0.1±0.1[d]
4	flint	15	27	17.7±2.1[a]	3.3±1.2[d]	27.7±2.1[e-f]	725.3±12[a]	4.4±0.7[e]	216.2±0.7[b-c]	1.7±0.0[b-c]
5	dent	30	10	14.7±2.1[a]	6.7±0.6[c]	58.6±7.2[b]	630.3±6.0[e]	15.8±2.6[b]	210.6±2.6[d]	1.6±0.3[c]
6	dent	30	27	14.6±2.5[a]	4.6±1.5[d-c]	74.3±4.9[a]	615.3±4.2[e]	18.5±0.6[a]	198.6±1.1[f]	2.4±0.8[b-a]
7	flint	30	10	4.3±1.5[b]	15.6±2.1[b]	43.0±7.5[c-d]	705.0±15[b]	8.0±1.9[d]	218.7±2.1[b-a]	2.3±0.1[b-a-c]
8	flint	30	27	3.6±3.1[b]	19.7±1.5[a]	53.0±5.3[c-b]	688.0±7.5[c]	12.0±1.1[c]	205.7±0.9[e]	1.7±0.3[c]

[+] Values with the same letter for a given property are not significantly different (P <0.05) for the dependent among the treatment combinations. LSZ= live *S. zeamais*, DSZ= dead *S. zeamais*, DS = damaged seed, UDS = undamaged seed, WD = weight of damaged seeds (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.