Journal of Stored Products Research 71 (2017) 72–80

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Maize storage insects (*Sitophilus zeamais* and *Prostephanus truncatus*) prefer to feed on smaller maize grains and grains with color, especially green

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A R T I C L E I N F O

Article history:
Received 18 July 2016
Received in revised form
26 January 2017
Accepted 27 January 2017
Available online 11 February 2017

A B S T R A C T

Maize is the major food staple in Eastern and Southern Africa, and since production is seasonal, on-farm storage is practiced by farmers. Because stored maize is susceptible to pests, improved storage methods are being developed which need to be evaluated for their effectiveness in reducing losses. The standard count and weigh method (CWM) assumes storage insects attack grains of different sizes in equal measure, although this has not been tested. This study therefore analyzed storage insects' feeding preferences for maize grains of different sizes. Maize grain was sieved and split into four size categories, colored with food dyes, remixed, and distributed into 48 jars, each with about 300 g of grain, artificially infested with maize weevils (*Sitophilus zeamais)* or larger grain borers (*Prostephanus truncatus)*, 40 insects per jar. After two months, the grains were separated into their size categories (visually, by color), and grain damage and weight loss was estimated with the CWM. Results show that storage insects have a preference for feeding on smaller, rather than larger grains; and on colored rather than uncolored grains. Small-sized and colored grains were more likely to be perforated by insects and suffered more weight loss. This preference, observed for both species, is more pronounced in *S. zeamais,* who also distinguish between different colors, and prefer green. The size effect is, however, comparatively small and the average weight loss calculated by a size-adjusted formula is not significantly different from that calculated by the standard CWM. However, the resulting differences in weight losses between grains of different sizes are small. Weight loss calculated by weighted CWM, while in principle more accurate, does not in practice lead to significantly different estimates. Therefore, the results do not warrant a change in the conventional CWM for weight loss in stored grain.

1. Introduction

Maize is the major food staple in East and Southern Africa (Nuss and Tanumihardjo, 2010), and most of it is produced seasonally under rain-fed conditions. Farmers therefore need to store their maize to maximize consumption until the next harvest and to provide a buffer against fluctuating prices (Gitonga et al., 2013). However, on-farm storage entails substantial risks; losses from storage pests can be large and threaten food security (Hodges and Maritime, 2012; Tefera, 2012), especially from the maize weevil (*Sitophilus zeamais*) and the larger grain borer (*Prostephanus truncatus*), the most serious pests of stored maize in Africa (Holst et al., 2000). Therefore, many research and development projects have engaged in improved storage technologies, in particular hermetic bags (Baoua et al., 2014; De Groote et al., 2013) and metal silos (Tefera et al., 2011a). To justify research on, and dissemination of, new storage technologies, the cost of these technologies needs to be compared to their benefits, which are largely composed of the abated storage losses.

Measuring grain loss in on-farm storage is difficult; there is a high variation over space as well as time, including both between and within seasons (Affognon et al., 2015; Hodges and Maritime, 2012; Tefera, 2012). The most reliable method of weight loss

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http://dx.doi.org/10.1016/j.jspr.2017.01.005

determination is probably the standard volume weight (SVW) method (Boxall, 1986), but it is cumbersome, and requires baseline data and moisture measurement. The count-and-weigh method (CWM)(Adams and Schulten, 1978) is more convenient; it does not require a baseline or moisture measurement, and is therefore more commonly used for on-farm storage losses in cereals. With this method, grain samples are taken from the storage under study, and the grains of each sample are divided into two subsamples: one with damaged grains and one with undamaged grains. For each of the subsamples; the grains are counted and weighed, and the average grain weight for each subsample calculated. Weight loss (%) is then calculated as the average weight loss per damaged grain, multiplied by the percentage of damaged grain. The CWM has a major drawback: because each grain sample contains grains of different sizes, 1% weight loss in large grains weighs more than 1% weight loss in small grains, so extrapolating an average weight loss assumes implicitly that the damage is equally spread over grains of different sizes. This also implies that there is no insect preference during maize storage to perforate grains of different sizes.

For the sake of argument, assume maize comes in two grain sizes: small (with a weight of 0.6 g) and large (1 g) with half of the grains in each size category, so the average grain weight is 0.8 g. Assume the storage insects only perforate the larger grains; they perforate half of them, and cause a weight loss of 50% in damaged grains. In a sample of 100 grains, total loss would be 0.5 (% perforated) × 0.5 (% loss) × 50 (number of large grains) × 1 (grain weight)/80 (initial grain weight) = 15.6%. To estimate the weight loss using CWM, a researcher would take a sample of 100 grains, separate the damaged from the undamaged and find that 25% of grains are damaged, that damaged grains weigh on average 0.5 g, compared to undamaged grains which weigh on average 0.73 g (25 large undamaged grains of 1 g and 50 small undamaged grains of 0.6 g), or an average weight loss for damaged grains of 31% $\left(\frac{0.73-0.5}{0.73)}\right)$. The researcher calculates the overall percentage weight loss as 0.25 (proportion perforated) × 0.5 (% loss) × 31% = 4%. This example shows how ignoring insect preferences for, in this case, large grains leads to a large underestimate of real loss. It illustrates how the average weight of damaged grain can easily be larger than the average weight of undamaged grain. If the damaged grains suffer a loss of only 25%, with the same assumptions as above, the damaged grains would weigh on average 0.75 g, while the average weight of the undamaged grain would remain the same at 0.73 g. In this case, the CWM would result in a negative weight loss.

The preference of insects during maize storage to perforate grains of different sizes has never been analyzed empirically. However, the effect of grain size on oviposition has been studied; experiments have shown that the wheat weevil (*Sitophilus granarius)* is able to differentiate between larger and smaller wheat kernels, preferring the larger ones for oviposition (Ewer, 1945; Stejskal and Kučerová, 1996). But if insects prefer to oviposit on larger kernels, they might also perforate larger grains more often and cause more damage among larger, rather than smaller, grains. In that case, as shown in the example above, the extrapolation of the average percentage weight loss in damaged grains over the average undamaged grain, as in the standard weight loss calculation in the CWM, will lead to an underestimation of weight loss. Inversely, if insects prefer smaller grains, extrapolation will lead to an overestimate.

Similarly, as illustrated with the second example, the CWM method can lead to negative results. Because, in principle, weight loss cannot be negative, it is common practice to ignore negative losses in the CWM or set them equal to zero (De Groote et al., 2013). However, negative numbers could just be a result of the probability distribution of grain sizes; if grain sizes vary substantially, and the grain numbers in one of the sub-samples is small (either from a damaged or undamaged category), and the total number of samples is larger, the probability of the average weight of damaged grains to be larger than that of the undamaged grain is not zero. In this case, setting these samples to zero would overestimate the weight loss, and should be avoided. An alternative cause for negative results could be insects' preference for larger grains. If, in a sample, the average size of the damaged grains was larger than that of the undamaged grains, and the weight loss less than the initial weight difference, weight loss calculated by the CWM would be negative. In this case, setting the negative results to zero would be an improvement of the estimate, but would still result in an underestimate of the weight loss.

To address this problem, this study was undertaken with two objectives. The first was to assess if maize storage insects have a preference for maize grain of different sizes. The second was to assess, given such a preference, if it affects the storage loss measurement by the count and weigh method. To distinguish grains of different sizes while keeping them in the same jar during the experiment, food dyes were used and, because these dyes might themselves affect insects' preferences, two more research objectives were added: to assess whether insects have a preference for maize with food coloring and, if so, which colors they prefer.

2. Methodology

2.1. Measuring storage weight loss and the effect of grain size

The count and weigh method (CWM) or gravimetric method (Boxall, 1986) is one of the most popular methods for crop loss assessment in storage. Because of its simplicity, ease of use and limited requirement for equipment, the CWM is popular in Africa, especially for on-farm research (GSARS, 2015). To test the method's susceptibility to bias from insects' grain size preference, we compared its results to those of the adjusted, weighted CWM in which the losses are analyzed separately for different size categories first, and then combined into an aggregate weight loss estimate.

To analyze the damage and weight loss by size, we therefore first used sieves to divide the maize grains into different size categories, then marked the different size categories with different food dyes, and mixed them back together. The maize was then artificially infested, and after a two-month storage period, the grains were separated by size, followed by a determination of damage and weight loss.

2.2. Study site

The experiment was conducted in the maize postharvest lab of the International Maize Improvement Centre (CIMMYT), based at the Kiboko research station of the Kenya Agricultural Research Organization (KALRO) (2.15° S, 37.90° E).

2.3. Preparation of materials

One standard 90 kg bag of maize grain, variety Duma 43, was purchased from a local farmer. All grain used in the experiment was first stored in a metal silo and fumigated with one tablet (1 g) of Phostoxin (a.i. ¼ aluminum phosphide, 57% w/w; produced by Detia Freyberg Gmbh, in Laudenbach, Germany) and left for seven days. The grain was subsequently divided into four size categories with three sieve sizes (CISA CEDACERIA Industrial SL SC/Alaba Barcelona Spain) that were available at the station (8 mm, 9.5 mm, and 11 mm), resulting in four grain categories: small (diameter <8 mm), medium (8.0–9.5 mm), large (9.5 mm - 11 mm), and very large (>11 mm). The proportions in weight were 5%, 40%, 52%, and 3%, respectively.

2.4. Experimental design

A complete randomized block design was used, with grains of different sizes as the treatments for each block or jar. In each block, one portion was left undyed and three portions were colored, each with a different dye, and all four categories were randomly assigned.

In practice, each size category was split into four equal parts. One part was left undyed or ‘clear’, and the others were dyed green (Brilliant Blue FCF and Tartrazine), blue (Indigo Carmine) or red (Ponceau 4R and Carmoisine), which are standard food colors obtained from a local producer (Galaiya Food, Nairobi). These colors were maintained after testing all available food colors and selecting those that provided the clearest distinctions. The dyes were dissolved in water (5 mg/l) and the grains were submersed for a few minutes and then air dried.

For a balanced experimental design, we combined each of the four size categories with each of the four color categories. Mathematically, this results in 24 permutations (technically the number of possibilities is determined by n! (n − r)! where n = k = 4 or n! = 4 × 3 × 2 = 24), all of which were assembled in the original proportions of the size categories. As a result, each color category was used six times for each size category, but with different combinations of colors for the other size categories.

Grains of each of the 24 permutations (or combinations in which the order matters) were used to fill two glass jars of 1l each with about 100 g of grains, or 48 in total; one jar bearing a particular combination was artificially infested with 40 adult *S. zeamais*, the other with 30 adult *P. truncatus*. These insects were obtained from the Kenya Agricultural Research Institute's (KARI) post-harvest insect laboratory in Kiboko. The two insect species were raised separately on maize variety H-513, a popular hybrid susceptible to storage insects, using standardized procedures (Tefera et al., 2011b).

The top of the container was covered with a fine mesh to retain the insects but allow air movement, and the jars were stored on shelves for two months in an air conditioned room with humidifier. Average temperature was 27 ± 1 °C and average relative humidity 65 ± 5%, as measured by a combined thermometer/hygrometer (produced by Brannan, in Cleator Moor, UK).

2.5. Data collection and calculation of damage and weight loss

After two months, all 48 jars were opened, and the grains removed and divided into the four categories s (with 1 = small, 2 = medium, 3 = large and 4 = very large), through visual inspection by color, resulting in 96 batches for the two insect species. Each color category k (clear, blue, red, or green) was therefore assigned six times to each size category s for the two species. For each of the resulting 192 sub-samples, the grains were divided into damaged and undamaged grains by visual inspection. The weight was measured for all undamaged grains (W_u) and damaged grains (W_d) separately, and for each batch the number of grains was counted (N_u and N_d), and the average grain weight for undamaged grains $\overline{w}_u (= W_u/N_u)$ and for damaged grains $\overline{w}_d$ $(= W_d/N_d)$ calculated.

Insect damage levels D were calculated as the percentage of damaged grains, for each sub-sample. Relative weight loss L was defined as the percentage of absolute weight loss over the original weight. In the CWM, absolute weight loss is estimated by multiplying the number of damaged grains by the average weight loss per grain, while the original weight is estimated by multiplying the total number of grains by the average weight for undamaged grains:

$$L = \frac{N_d\,(\overline{w}_u - \overline{w}_d)}{(N_u + N_d)(\overline{w}_u)} \quad (1)$$

If we define the relative loss in the damaged grains as:

$$L_d = \frac{\overline{w}_u - \overline{w}_d}{\overline{w}_u} \quad (2)$$

it is easy to see that relative weight loss over all grains (as calculated by the CWM) equals:

$$L = DL_d \quad (3)$$

In other words, the relative loss in the damaged grain is extrapolated over all damaged grains. This also explains the possible bias of the method: it assumes that if the grain were not damaged, if would have, on average, the weight of the undamaged grains $\overline{w}_u$, which implicitly assumes grain weight and probability of being perforated are not related, or that damage is equally distributed over size categories. If this is not the case, the weight loss would need to be calculated by size category s. To calculate an overall relative weight loss, taking into account size differences, we calculate a weighted relative weight loss L. The average absolute weight loss per grain (in g) for each category s was calculated and multiplied by the number of damaged grains in that size category. Losses for all categories were added up and divided by the total estimated undamaged weight, for each category calculated as the average undamaged grain weight multiplied by the total number of grains:

$$L_w = \left(\sum_{s=1}^{S} (\overline{w}_{us} - \overline{w}_{ds}) N_{ds} \right) \Big/ \sum_{s=1}^{S} \overline{w}_{us} (N_{ds} + N_{us}) 100 \quad (4)$$

2.6. Analysis

First, the relationship between damage and weight loss on the one hand, and grain size and color on the other hand, was analyzed by simple tabulation of the means, comparison of means by pairwise t-tests, and a graphical presentation to explore possible trends.

Second, a mixed random effects model was estimated for appropriate statistical analysis of the data. Assuming both dependent variables are an approximately linear function of size category s, this variable can be entered directly into the model with coefficient β. The four color categories k can be entered as binary variables x_k with coefficients γ_k. Further, four observations were taken from each jar, one for each size category, and these could be correlated. Therefore, a random effect u_i was added for each jar i, resulting in:

$$D_{ski}\ (\text{or} L_{ski}) = \alpha + \beta' s + \sum_{k=1}^{4} \gamma_k x_k + u_i + e_{ski} \quad (5)$$

The same model was used to analyze both the damage D and the weight loss L caused by the storage insects, and the analysis was conducted separately for each species.

Third, the possible relationship between negative weight loss results and size preference was explored by calculating the proportions of negative weight loss in the different categories. Finally, the practical implications of the effect of grain size on weight loss were analyzed by comparing the results of Equation (1) (standard CWM) and Equation (4) (CWM adjusted for size).

For all analysis, the software IBM - SPSS Statistics version 24 (IBM Corp. Released, 2016. IBM SPSS Statistics for Windows, Version 24. Armonk, NY: IBM Corp.) was used. For statistical analysis, a significance level of 95% was generally used, and results at that level (with $p < 0.05$) were referred to as significant. Although that level is the standard norm, it is rather arbitrary, so we also looked at 90% significance levels ($p < 0.10$), in particular where the results seem to indicate a trend. Results at that level (with $0.05 < p < 0.10$) were considered marginally significant.

3. Results

3.1. Damage caused by *S. zeamais* and *P. truncatus*, by size and color categories

Two months after artificial infestation, the grains were separated into the four size categories, and split into undamaged and damaged grains (Table 1). Most of the grains were in the two middle categories (44% in the medium and 46% in the large category), with relatively few grains in the small (8%) and very large category (2%) (as can be calculated by adding up the grains in the categories from both insect species and dividing by the total number of grains). The jars infested with *S. zeamais* contained on average 195 grains with an average weight of 70 g (the sum of the weight of the undamaged gains, 39.0 g, and those of the damaged grains, 30.8 g), while those infested with *P. truncatus* contained on average 189 grains, with an average weight of 61 g. The lower weight of the jars infested with *P. truncatus* was likely caused by a higher damage rate (63%) than those infested with *S. zeamais* (47%).

The results clearly show that damage levels by both species decrease with grain size (Fig. 1). The effect is more pronounced in grains infested with *S. zeamais*, as can be observed by the steeper slope of the decrease in damage from small (52%) to very large grains (41%). A pairwise t-test shows a statistical difference in grain damage by *S. zeamais* between small and very large grains ($p = 0.015$), but the difference is only marginally significant between small and large grains ($p = 0.091$) and between medium and large grains ($p = 0.092$). For *P. truncatus*, damage is also highest for small grains and smallest for very large grains, with a 6% difference but only marginally significant ($p = 0.088$). Otherwise, there were no significant differences between any of the other pairs of categories.

To distinguish between the size categories, one category was left clear (without any dye), and the other three were dyed with food colors: green, blue and red. Both species showed a preference for the colored grains, with higher damage in colored grains than in clear grains, but the difference is more pronounced for *S. zeamais* than for *P. truncatus* (Fig. 2). Weevils have a strong preference for colored grain, and distinguish between the different colors. Grains dyed with any of the three colors were more damaged by *S. zeamais* than clear grains, and these differences were significant for all

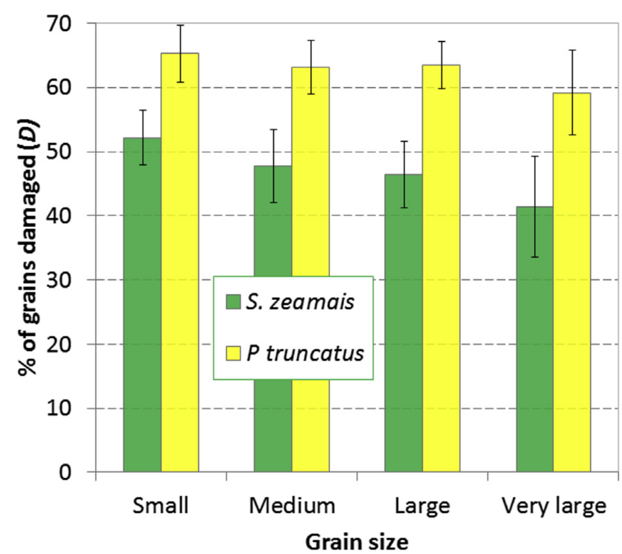

Fig. 1. Damage *D* (% of grains damaged) by grain size (error bars are 5% confidence intervals for the mean).

Table 1
Number of grains, batch weight and average grain weights of damaged and undamaged grains two months following infestation (N = 24 jars for each species, each size once per jar).

	Size		*N*	Number of grains		Total	% damaged	Batch weight (g)		Grain weight (g)	
				Undamaged (N_u)	Damaged (N_d)			Undamaged (W_u)	Damaged (W_d)	Undamaged (w_u)	Damaged (w_d)
S. zeamais	Small	Mean	24	30.7	33.5	64.1	52.2	7.0	6.5	0.23	0.20
		Std.		7.1	7.2			1.7	1.3	0.01	0.02
	Medium	Mean	24	179.8	163.2	343.0	47.6	61.4	49.8	0.34	0.31
		Std.		53.2	49.1			16.7	13.6	0.03	0.03
	Large	Mean	24	189.9	164.5	354.4	46.4	82.1	63.4	0.43	0.39
		Std.		49.7	49.1			21.4	18.9	0.04	0.05
	Very large	Mean	24	10.8	7.7	18.5	41.8	5.4	3.5	0.50	0.45
		Std.		3.5	3.8			1.8	1.6	0.03	0.06
	All	Total	96	411.1	368.9	780.0	47.3	155.9	123.2		
		Mean	96	102.8	92.2	195.0	47.3	39.0	30.8	0.38	0.34
		Std.		90.4	80.3			36.3	28.8	0.11	0.10
P. truncatus	Small	Mean	24	21.3	39.8	61.1	65.2	5.0	6.9	0.24	0.17
		Std.		7.0	7.1			1.7	1.3	0.01	0.01
	Medium	Mean	24	121.9	208.9	330.8	63.1	41.7	55.3	0.34	0.27
		Std.		35.7	35.4			12.5	6.6	0.01	0.02
	Large	Mean	24	127.5	220.9	348.4	63.4	56.0	73.2	0.44	0.33
		Std.		32.9	32.4			14.9	12.1	0.06	0.04
	Very large	Mean	24	7.1	10.5	17.6	59.5	3.6	4.1	0.51	0.39
		Std.		2.7	3.0			1.4	1.3	0.02	0.06
	All	Total	96	277.8	480.0	757.8	63.3	106.3	139.5		
		Mean	96	69.4	120.0	189.5	63.3	26.6	34.9	0.38	0.29
		Std.		60.8	99.0			24.9	31.0	0.11	0.09

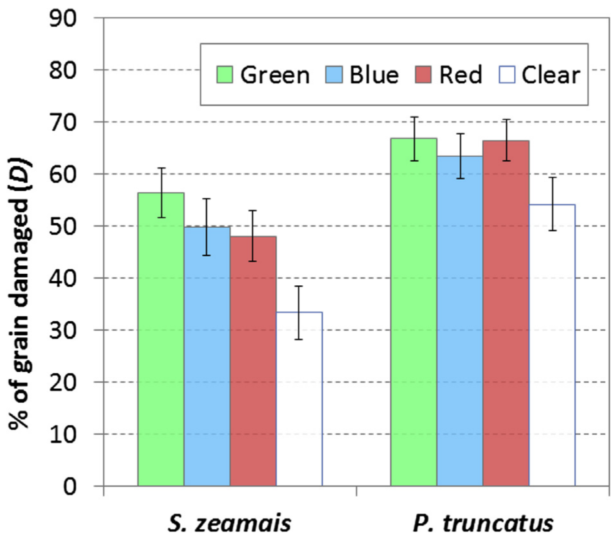

Fig. 2. Damage *D* (% of grain damaged) by color and species (bars represent 95% confidence intervals). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

colors ($p < 0.001$). Green-colored grains were the most damaged by *S. zeamais* (56.5%), followed by those colored blue (50%) and red (48%), while clear grains were the least damaged (33%). Green maize suffered significantly more damage than blue maize ($p = 0.018$) or red maize ($p = 0.004$), but there was no significant difference in damage between blue and red maize. Damage by *P. truncatus* was also significantly higher in colored grain (on average 66% over the three colors) than in clear grain (54%), ($p < 0.05$), but no significant differences in damage between the three colors were observed.

3.2. Statistical analysis of damage caused by storage insects with a mixed effects model

Because of the significant effects of food colors on damage, they need to be included in the regression model as binary variables, with size as a continuous variable (from 1 = very small to 4 = large), as in Equation (5). The regression results show that size has a negative and significant effect on damage, for both insect species (Table 2). For *S. zeamais*, damage decreases by 3.38% for each increase in size category (for example going from very small to small, or from small to medium). For *P. truncatus*, the reduction is 1.79% per category. The regression analysis confirms the analysis of the color effect with t-tests for both species; all colors show a significantly higher damage than clear maize (the base category). Further, the differences between colors are much larger for *S. zeamais* than for *P truncatus*; and these differences are significant for green (*23 vs. 13)* and for blue (16 vs. 9). The results also indicate that the observations from the same jar are strongly correlated ($\rho = 0.6$), supporting the use of the random effects model.

3.3. Weight loss caused by S. zeamais and P. truncatus, by size and color category

The effect of grain size on weight loss due to insect pests followed roughly the same pattern as its effect on damage (Fig. 3). For ease of presentation, we distinguish between weight loss as a percentage of damaged grain only (L_d), and losses as a percentage of all grain (L). Losses were substantial for both species, but more for *P truncatus*, which on average caused 23% weight loss in damaged grains only and 15% losses over all grain; whereas *S. zeamais* caused on average 11.1% weight loss in damaged grain and 5.2% losses over all grain.

As with damage, weight loss by both species generally decreased with increasing grain size (Fig. 3). For *S. zeamais*, there is a clear difference in weight loss in damaged grains between the small grains (14%) and the other categories (10%), although the difference is only marginally significant between small and medium grain sizes ($p = 0.074$). When the weight loss from *S. zeamais* is calculated over all grains, there is a gradual reduction of losses from small grains (7%) to very large grains (4%). These differences are significant between small and very large grains ($p = 0.029$), but only marginally significant between small and large grains ($p = 0.057$).

For *P. truncatus*, weight loss of damaged grains (L_d) is also highest in the small grains, but no further trend with relation to grain size can be seen in the other categories, and only the difference between small and medium grains is significant ($p = 0.011$). Weight loss from *P. truncatus* calculated over all grains (L) is highest in the small grains (17%) and lowest in the very large grains (13%), and only this difference is significant ($p = 0.028$); these losses are also lower in medium grains than in small grains, although the difference is only marginally significant ($p = 0.050$), but otherwise there are no significant differences between categories.

The effect of color on weight loss is less pronounced than on damage (Fig. 4). Weight loss caused by *S. zeamais* on damaged grains (L_d) is higher on colored than on clear grains; blue grains suffer most (15% loss) followed by red (12%), green (10%) and clear (7%) grains. The differences are statistically significant between

Table 2
Regression analysis of damage *D* (% of grain) caused by *S. zeamais*, by size and color, using a mixed-effects model.

	S. zeamais			*P truncatus*		
	Coefficient	Std. Error	P>|z|	Coefficient	Std. Error	P>|z|
Size	−3.38	0.70	0.000	−1.79	0.69	0.009
Green	23.06	2.22	0.000	12.57	2.18	0.000
Blue	16.48	2.22	0.000	9.31	2.18	0.000
Red	14.70	2.22	0.000	12.29	2.18	0.000
Constant	41.82	3.05	0.000	58.71	2.83	0.000
σ_u	9.46			8.04		
σ_e	7.69			7.54		
ρ	0.60			0.53		
Number of observations	96			96		
Number of groups	24			24		
R^2	0.38			0.21		

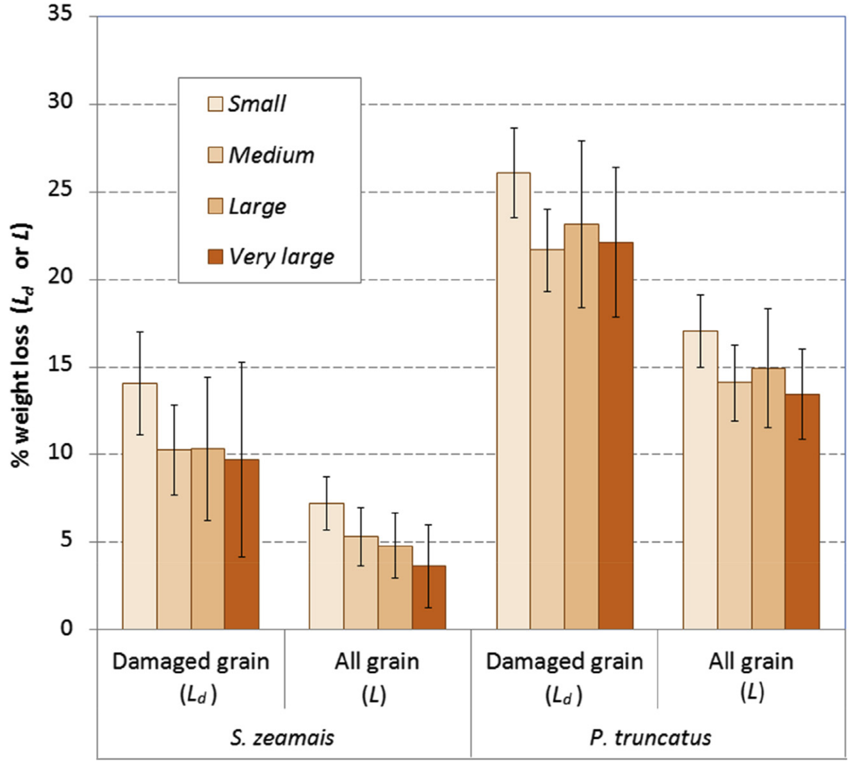

Fig. 3. Weight loss (%) in damaged grains (l_c) and in all grains (L_c), in % of weight, by grain size (bars represent 95% confidence intervals).

clear and blue grains (p = 0.022), and between clear and red grains (p = 0.011), but not between clear and green (0.151). The differences between weight loss of damaged grain from different colors are, however, not significant. For weight losses over all the grain (L), *S. zeamais* also caused more weight loss to colored grains (6%) than to clear grains (2%), and the differences are significant for all colors (p = 0.013 for green, p = 0.003 for blue and p = 0.001 for red). For *P. truncatus*, on the other hand, weight losses calculated over all grains (L) in colored grains (green, blue and red, combined) (15.5%) is are on average significantly larger than those for clear grains (13.0%). However, the difference is not significant between clear grains and grains of a particular color (green, blue or red, individually).

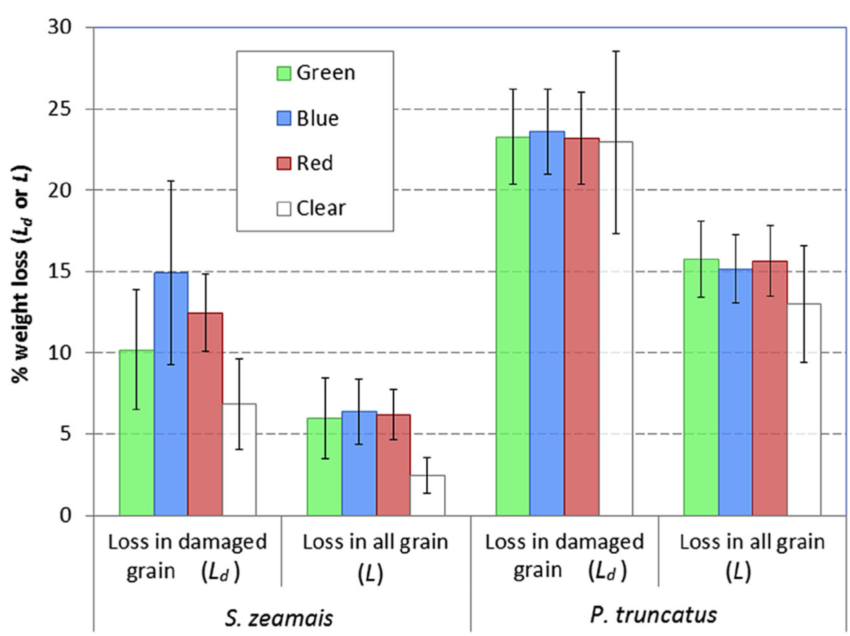

Fig. 4. Weight loss by color, in damaged grains (L_u) and in all grains (L) (bars represent 95% confidence intervals errors). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Regression analysis of weight loss (%) in damaged grains only (L_d) and all grains (L), from *S. zeamais* and *P truncatus* (96 observations, four ordered size categories from 24 jars), using a mixed effects model.

Group	Variable	Weight loss (%) in damaged grains(L_d)						Weight loss (%) calculated over all grain(L),								
		Model 1: *S. zeamais*			Model 2: *P truncatus*			Model 3: *S. zeamais*			Model 4: *P truncatus* - separate colors			Model 5: *P truncatus* - colors combined		
		Coef.	SE	P>|t|	Coef.	SE	P>|t|	Coef.	SE	P>|t|	Coef.	SE	P>|t|	Coef.	SE	P>|t|
Grain size	Categories (1–4)[a]	−1.30	0.87	0.135	−1.04	0.76	0.170	−1.13	0.39	0.003	−0.99	0.50	0.046	−0.99	0.49	0.043
Color	Green	5.62	2.74	0.040	0.24	2.40	0.921	3.75	1.22	0.002	2.76	1.57	0.079			
	Blue	3.33	2.74	0.224	0.32	2.40	0.893	3.53	1.22	0.004	2.14	1.57	0.174			
	Red	8.07	2.74	0.003	0.67	2.40	0.782	3.94	1.22	0.001	2.63	1.57	0.094			
	Colored													2.51	1.26	0.047
	Constant	10.09	2.92	0.001	25.55	2.68	0.000	5.26	1.33	0.000	15.48	1.81	0.000	17.98	1.56	0.000
Model	σ_u	0.905			3.963			0.025			3.53			3.56		
	σ_e	9.502			8.329			0.042			5.44			5.37		
	ρ	0.009			0.185			0.262			0.30			0.31		
	Number of obs.	96			96			96			96			96		
	Number of groups	24			24			24			24			24		
	R^2	0.183			0.057			0.184			0.058					

[a] Size category by grain diameter: 1 = grains with diameter ø < 8 mm, 2 = grains with 8 mm <ø ≤ 9.5 mm, 3 = grains with 9.5 mm ≤ 11 mm, 4 = grains with ø>11.

3.4. Statistical analysis of weight loss caused by storage insects with the mixed effects model

To analyze the effect of grain size and color on weight loss in storage, we use the same mixed effects model as for damage (Equation (5), Table 3). The results show that the effect of grain size on weight loss in damaged grain (L_d) is not significant, neither for *S. zeamais* (Model 1) or *P. truncatus* (Model 2). The effect of color, on the other hand, is significant on the grain loss caused by *S. zeamais* in damaged grain for green (5.6% more loss than clear maize, p = 0.040) and for red (8.1% more loss, p = 0.003) (Model 1, Table 3), but not for *P truncatus* (Model 2), confirming the results of Fig. 4.

The analysis of weight loss calculated over all grain (L), however, shows a clear and significant effect of size, for both *S. zeamais*, as indicated by the negative and significant coefficients in the random effects model (Model 3, Table 3) and *P. truncatus* (Models 4 and 5). For both species, weight loss decreased by about 1% for each increase in size category (going from one category to the next, for example very small to small, or small to medium, and so forth) (p = 0.003 for *S. zeamais* and p = 0.046 for *P. truncatus*). The effect of color on loss over all grain caused by *S. zeamais* is also significant for all colors; the effect ranges from 3.5% for blue (p = 0.004) to 3.9% for red (p = 0.001) (Model 3). For losses over all grains caused by *P. truncatus*, the individual colors are not statistically significant (Model 4). However, when we combine them into a single binary variable, the effect is significant; colored grains suffer, on average, 2.5% more weight loss on overall grains than clear grains (p = 0.047) (Model 5).

3.5. Analysis of negative losses and comparing calculation of weight loss

As the CWM regularly produces negative losses, we analyzed the occurrence of these observations and explored the relationship between negative weight loss and grain size. Out of 96 observations, only six cases of negative weight loss were found, and all in the grain infested with *S. zeamais*. Two of the negative losses were found among large grains and four among very large grains, and all were small; one observation reached −0.11%; the others fell between −0.01% and 0%. The low number of observations did not warrant further analysis.

Finally, weight loss calculated by the standard CWM formula of Equation (1) were compared to that calculated by the size-adjusted formula of Equation (4). Weight loss calculated by standard CWM is apparently higher than that calculated with the size-adjusted formula (Table 4), as can be expected with a negative effect of the grain size on weight loss. However, the difference between the two calculations was not significantly different.

4. Discussion and conclusions

We conclude that storage insects, in particular *S. zeamais* and *P truncatus*, have a preference for feeding on smaller, rather than larger grains, which are more likely to be perforated and suffer more damage and relative weight loss. This preference is observed for both *S. zeamais* and *P. truncatus*, but is more pronounced in the former. This main result is in contrast to storage insects' documented preference for ovipositing on larger grain sizes (Ewer, 1945; Stejskal and Kučerová, 1996), and against our initial expectations, in part motivated by the occurrence of negative weight loss in storage studies. However, the oviposition studies were conducted with another species, *Calandragranaria (*syn. *Sitophylus granarius*), in which population density was also found to be important. The limitations of our resources for this study did not allow for analysis of oviposition by size, so the link between the preference of *S. zeamais* and *P truncatus* for smaller grain exploitation and oviposition cannot be answered at this point, and will need to be explored by further research. Further, this study was conducted under artificial infestation with high population density, which might also have affected the results. Another limitation was that the sieves used (with mesh sizes of 8, 9.5 and 11 mm), resulted in most of the grain falling in the two middle categories. It would be preferable to use more size categories or use sieves that spread the

Table 4
Comparing weight loss estimates from the standard CWM formula (L, Equation (1)), and from the size-adjusted formula (L_w, Equation (4)) (both in % of weight, calculated for all grain).

		Standard CWM	Size-adjusted CWM
Weevil	Mean	5.46	5.12
	Std	3.24	3.13
	SE	0.66	0.64
	N	24	24
P truncatus	Mean	14.73	14.71
	Std	7.90	6.99
	SE	1.61	1.43
	N	24	24

distribution of the grains better over the categories. For this maize variety, for example, mesh size of 8.5, 9.5 and 10.5 would likely result in a better distribution of the grains over the four categories.

The results of this experiment show that food dyes are a convenient way to distinguish different grain categories, and in particular different grain size categories as used for this study. However, insects are not indifferent to these dyes; both insect species used in this study prefer to feed on colored grain rather than on clear grain, and again the effect is more pronounced with *S. zeamais*. Moreover, *S. zeamais* distinguishes between different colors, and has a preference for maize dyed green.

While attraction of humans to colorants made from insects is well studied (in particular carmine) (Burrows, 2009) the attraction (or repellence) of insects to colorants made by humans has so far not received much attention in the literature. Some species of insects are attracted to particular colors, which has been shown with colored traps, for example for thrips (Beckham, 1969) fruit flies (Chang, 1990; Epsky et al., 1995), and tsetse flies (Green, 1993). Tsetse flies were more attracted to black and blue colored objects than white objects (Green, 1993), Caribbean fruit flies were more attracted to orange sticky traps, followed by yellow and yellow-green (Greany et al., 1977), while Psyllid insects preferred yellow sticky traps over other colored ones (Brennan and Weinbaum, 2001). Visual cues can be more important than olfactory cues in some attraction flights (Magnarelli, 1979).

There is also an established link between color, host location and oviposition. For example, mosquitoes responded to blue at the time of egg deposition (Williams, 1962), and colored substrates elicited more ovipositions and more eggs from wasps than uncolored substrates (Eller et al., 1990). In storage pests, differential preference for a colored surface has been shown for red flour beetles (Reza and Parween, 2006), and several visual stimuli have been shown to elicit positive orientation behavior from *S. zeamais* (Arnold et al., 2015). None of these studies used food dyes. Still, food dyes are commonly used in entomology, but to mark individual insects, usually by mixing the dye in their food, and recapture them later to study their behavior, movements and dispersal. Examples include feeding behavior in ants (Heller et al., 2008), food intake in flies (Wong et al., 2009) and fruit flies (Min and Tatar, 2006), and mating in fruit flies (Avent et al., 2008). Food dyes (in particular red) have been shown to affect mating behavior in fruit flies (Verspoor et al., 2015). However, none of these studies analyzed if insects were attracted or repelled by food dyes.

As far as we were able to establish, therefore, the results presented here are the first to document the effect of food dyes on damage and weight loss caused by storage insects. This study, nor the literature reviewed, allows us to distinguish whether the preference is related to the color itself, or to other chemical or physical characteristic of the dyes used, and therefore this preference needs to be interpreted with caution. In any case, the significant effect of food dyes on insect loss in storage requires that different colors should be properly randomized over the treatments to balance their effects in experiments using food dyes with storage insects.

If insects preferred larger grains, this would help to explain the occurrence of negative weight losses as measured by CWM. Therefore, another implication of storage insects' preference for smaller grain sizes is that these preferences cannot be responsible for the negative weight loss often found when using the CWM. Until the cause of negative weight losses has been properly assessed, it would be most prudent to treat them as the outcome of the random distribution of size among damaged and undamaged grains. Under such hypothesis, however, it would not be correct to ignore them or set them to zero, because it would result in a bias and lead to an overestimate of storage losses. Alternatively, if the negative losses were caused by human error, it would be most prudent to consider them as missing values, but not to convert them to zero.

Finally, the demonstrated preference of insects for smaller grains, in principle, would lead to an underestimate of storage weight loss when using the CWM; in practice, however, the effect seems to be small, as shown by our results. In our study, adjusting the calculation to compensate for differences in size categories did not lead to a statistically different result. For practical purposes, therefore, the standard count and weigh method should be maintained, but the practice of setting negative values to zero should be discouraged.

Acknowledgements

This study was supported by a travel grant from the Vlaamse Interuniversitaire Raad (VLIR), while the operational expenses were covered by the Efficient Grain Storage Project, supported by the Swiss Development Corporation (SDC) (contract no. 81015000). We thank Prof. Wilfred Mwangi, CIMMYT country liaison officer at the time of the study, his successor Dr. Stephen Mugo, and Prof. Joos Latré from the Gent University College for facilitating this study. We thank Kathleen Sinclair for editing the manuscript, and the staff at Kiboko, particularly Sotero Bumagat, for their help and support.

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