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Measuring the nutritional cost of insect infestation of stored maize and cowpea

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Measuring the nutritional cost of insect infestation of stored maize and cowpea

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Abstract

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Keywords: nutrition, postharvest losses, grain storage losses, carbohydrate: protein ratio, *Sitophilus zeamais*, *Prostephanus truncatus*, *Callosobruchus maculatus*

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1 Introduction

Agricultural research predominantly focuses on increasing the productivity of food crops and livestock to meet the projected nutritional needs and changing dietary tastes of a rapidly growing and urbanising human population. Much less research attention has focused on ensuring that the quality and quantity of these harvests is maintained postharvest.

However, growing awareness of the socio-ecological costs of food production, food loss and the political ramifications of the food price hike associated food crises of the 1970s and 2007/08 have seen postharvest loss reduction reappearing as a development priority (World Bank et al., 2011; Gustavsson et al., 2011; Foresight Review, 2011; FAO, 2013; Hodges and Stathers, 2013; Mvumi and Stathers, 2014; Godfray and Garnett, 2014; Affognon et al., 2015; Sheahan and Barrett, 2017). In sub-Saharan Africa (SSA) where over 307 million people are already affected by severe food insecurity (FAO et al., 2017), the prevalence of undernourishment has recently started increasing again, reaching 22.8 % in 2018 (FAO et al., 2019). The region is considered highly vulnerable to the impact of climate change (Niang et al., 2014) since its population is projected to double to 2.4 billion people by 2050 (UNDESA, 2017), and is dependent on rain-fed agriculture. Given the compounding and increasing vulnerabilities and challenges facing SSA countries, reducing the losses in food crops, which occur both pre- and post-harvest, is imperative (Oerke, 2006; Gregory et al., 2009; World Bank et al., 2011; Gustavsson et al., 2011; Savary et al., 2012; Savary et al., 2017; Stathers and Mvumi, in press).

Following maturation of a crop in the field, harvests enter a series of stages prior to consumption, termed *postharvest activity stages*. These stages vary by crop and agricultural setting, but typically include: field drying, harvesting, transport, further drying, shelling or threshing, winnowing, storage, further processing (i.e. milling), transport to market, market storage and retailing. Postharvest losses occur when the quantity or quality (i.e. nutritional or financial value, grain viability or brewing ability etc.) of the crop decreases during these stages.

Good postharvest management to reduce postharvest losses that affect both the quantity and quality of food, can positively influence the main components of food and nutrition security: availability, stability, access and utility-safety-quality (see Stathers et al., 2013 and Sheahan and Barrett, 2017 for further discussion of the postharvest aspects of food security; and Schmiduber and Tubiello, 2007 and Savary et al., 2017 for the components of food security).

A large proportion of the grain postharvest research to date has focused on quantitative physical losses, typically expressed using dry weight loss, which is the standard international measure of grain loss (De Lima, 1979; Boxall, 1986). Additionally, most of the work which has measured (as opposed to estimated) quantitative postharvest losses in cereal or legume grain crops has focused on losses which occur while the crop is stored at the farmers' homestead or in warehouses or traders' stores (Hodges, 2013; Hodges et al., 2014; Affognon et al., 2015; Stathers et al., 2018). As significant quantities of grain crops are lost during storage due to attack by insects, rodents and/or fungi, or contaminated by toxins or chemical residues, considerable research and development efforts have concentrated on introducing technologies and skills to help farmers reduce crop storage losses. However, this focus on the physical quantitative loss underestimates the overall value and multi-dimensional nature of postharvest losses, as the quality as well as the quantity of the crop can diminish postharvest. Although postharvest quality loss due to fungal infestations and the associated mycotoxin problems (Wild and Gong, 2010; Ayalew et al., 2016) is a major global issue, there has been limited work on measuring the value of insect-mediated postharvest losses in quality (Hodges, 2013; Affognon et al., 2015). Such quality losses can affect the market price of the commodity and have an impact on household nutrition and income.

Science-based contextualised estimates of the quantitative postharvest losses occurring at each postharvest stage for the main cereal crops in each province of 38 SSA countries are provided by the African Postharvest Losses Information Systems (APHLIS www.aphlis.net) (Rembold et al., 2011; Hodges et al., 2014), to assist in better targeting of loss reduction investments. APHLIS is currently being expanded to include quantitative postharvest losses data on key legume and root and tuber crops, and the financial and nutritional values of postharvest losses (Stathers et al., 2018).

The ‘Missing Food’ study estimated that 13.5 % of the cereal grain produced across SSA is lost postharvest, equivalent to US$4 billion per year or the annual caloric requirement of 48 million people (World Bank *et al*., 2011). These financial and nutritional calculations assume that these losses vary linearly with weight loss, and convert the weight loss into kilo-for-kilo market value and/or the equivalence in number of people's annual nutritional requirements. However, the loss in quality of the remaining product causes additional nutritional and financial losses.

A set of trials were developed to improve our understanding of the nutritional consequences of postharvest losses, and specifically to quantify the effect of damage by storage insect pests on the nutrient value of stored maize and cowpea (two key SSA staple food grains). In these laboratory trials, we analysed the changes in the nutrient composition of smallholder farmer grown varieties of maize and cowpea grain (one white hybrid maize variety, one proVitamin A biofortified orange maize variety, and one cowpea variety) after different storage durations in the absence or presence of different combinations and initial infestation levels of the main storage insect pests. The associations we found between insect infestation and nutritional quality were used to create a prototype of a predictive tool to support more refined estimates of the nutritional losses associated with insect-infested stored maize and cowpea.

2 Overview of research on the impact of insect infestation on nutritional value of stored grain

Grain that is damaged or deteriorates postharvest will often be sold at a lower value at market, whether formal or informal grain quality standards are used. Quality losses can be more difficult to measure and express than quantity losses as the threshold for acceptance/rejection of the grain can depend strongly on the socioeconomic context in which the grain is being sold or consumed (Compton et al., 1998; Hodges and Stathers, 2013; Hoffman and Gatobu, 2014; Jones et al., 2018). This has led most economic loss studies to focus on the visible effect of postharvest insect damage (for further information see: Compton et al., 1998; Golob et al., 1999; Langyintuo et al., 2003, 2004; Jones et al., 2014, 2016, 2018; Kadjo et al., 2016; Mishili et al., 2007, 2011), and ignore invisible aspects such as mycotoxins or chemical residues (see Hoffman and Gatobu, 2014, Wu et al., 2011). Although it differs by location, crop and timing; when 5-10 % of grains are damaged by insects, moderate discounts typically occur, but when damage increases to 20-30 % of grains they may become unmarketable (Jones et al., 2018). Other factors such as discoloration, shrivelling, smell, evidence of chemical residues (Riwa et al., 2005), broken grains, and presence of foreign matter are also known to affect consumers' evaluation of grain quality and are key criteria in grain quality standards (Hodges and Stathers, 2012).

Substantial research on the chemical changes in insect-infested and uninfested stored cereal and legume grains took place between 1950 and the mid 1980's. These findings together with those from more recent work are summarised in Table 1.

Where whole grains are lost postharvest, such as when cobs/pods or scattered grains are left in the field after harvest, a 2 % loss (e.g. 2 kg of whole grains out of 100 kg) equates to the uniform loss of 2 % of all the nutrients in that volume of grain. Conversely, when insects attack grains, only the parts of the grain ingested or excavated by the insect are lost. Many insects however, feed on only part of the grain (e.g. the germ or endosperm). In many crops, nutrients are not evenly distributed throughout the grain (Rees and Hammond, 2002), therefore a 2 % loss due to insect feeding may result in a disproportionate loss of particular nutrients depending on which part of the grain is consumed by the insects.

The protein, carbohydrate, fibre and crude fat contents of the pericarp, endosperm and germ fractions of maize grains were analysed by Nuss et al., (2010; Fig. 1), with similar findings reported by Naves et al., 2011. Comparable analysis of the embryo, seed coat and cotyledons of cowpea grains was reported by Singh et al., (1968; Fig. 1). These studies highlight the differential distribution of nutrients within the grains.

Table 1 Overview of research findings on the effect of insect infestation on nutritional aspects of stored grains

Insect-infested stored grain	Un-infested stored grain
Protein, nitrogen and amino acids	
▲ total nitrogen content in wheat, finger millet, maize, grams, bean and cowpea (Pingale et al. 1954; Irabagon, 1959; Rajan et al., 1975; Murthy and Kokilvani, 1980; Francis and Adams, 1980) ▼ protein quality (Protein Efficiency Ratio (PER)) of maize and cowpea due to severe infestation (Rajan et al., 1975, 1975a) ▲ soluble protein, crude protein and total protein content (Francis and Adams, 1980; Tongjura et al., 2010)	▬ protein (Baldi et al., 1977 cited in Zhou et al., 2002; Dejene et al., 2006) ▼ protein solubility and *in vitro* digestibility ▲ amino-N
Fats and lipids	
▲ fat content in maize with heavy weevil infestation (Irabagon, 1959; Tongjura et al., 2010) ▲ free fatty acids in maize, sorghum and legumes (Pandey and Pandey, 1977; Venkat Rao et al., 1958, 1960).	▬ fats, protected from oxidation *At high temperatures or mc:* ▼ total lipids ▲ free fatty acids leading eventually to rancidity
Carbohydrate, fibre and calories	
● depending on insects' feeding habits and type of grain: ▼ caloric value by endosperm feeders (e.g. *Sitophilus* spp) ▼ vitamins by germ feeders (e.g. *Ephestia cautella*) ▼ outer bran and starchy endosperm when larvae are external grain feeders ▲ relative level of dietary fibre, when insects hollow out kernels and leave the pericarp ● between grain types due to differential nutrient distribution; in legumes, larval feeding affects carbohydrates, proteins and vitamins	▬ starch ▼ soluble carbohydrates due to respiration (Dejene et al., 2006) ▲ reducing sugars over time ▼ non-reducing sugars over time *At high temperatures or mc:* ▼reduced starch content, carbohydrate fermentation and sour odours (Zeleny, 1968)
Vitamins and minerals	
▼ vitamins by germ feeding insects (e.g. *Ephestia cautella*)	▼ carotenes, tocopherols, vitamin E, thiamine (vitamin B1), riboflavin (vitamin B2) *depending on storage conditions* (Kodicek et al., 1959; Weber, 1987; Burt et al., 2010; Mugode et al., 2014; De Moura et al., 2015; Bechoff and Dhuique-Mayer, 2017; Taleon et al., 2017) ▬ minerals
Other aspects	
▲ contamination due to insect fragments, excreta, dust and damaged grains ▼ baking qualities, taste, odour and flour appearance due to metabolic by-products e.g. quinones secreted by tenebrionid pests (Ladisch and Suter, 1968; Smith et al., 1971) ▲ entry by pathogens and toxin development, due to seed coat damage, insect carriage of fungal spores and mycotoxin link (Agrawal, 1957; Widstrom, 1979). Although, on wheat, it took several thousand insects per kg to cause an obvious increase in fungal populations (Fourar-Belaifa et al., 2011) ▼ grain palatability leading to reduced weight gain in rats (Rajan et al., 1975, 1975a; Irabagon, 1959; Jood and Kapoor, 1992) ▲ weight of chickens fed *S. zeamais* infested diet (Lopez-Verge et al., 2013)	

Key: ▲ = increase in; ▼ = decrease in; ● = varies; ▬ = no change in. (Data source: as specified and/or FAO, 1983)

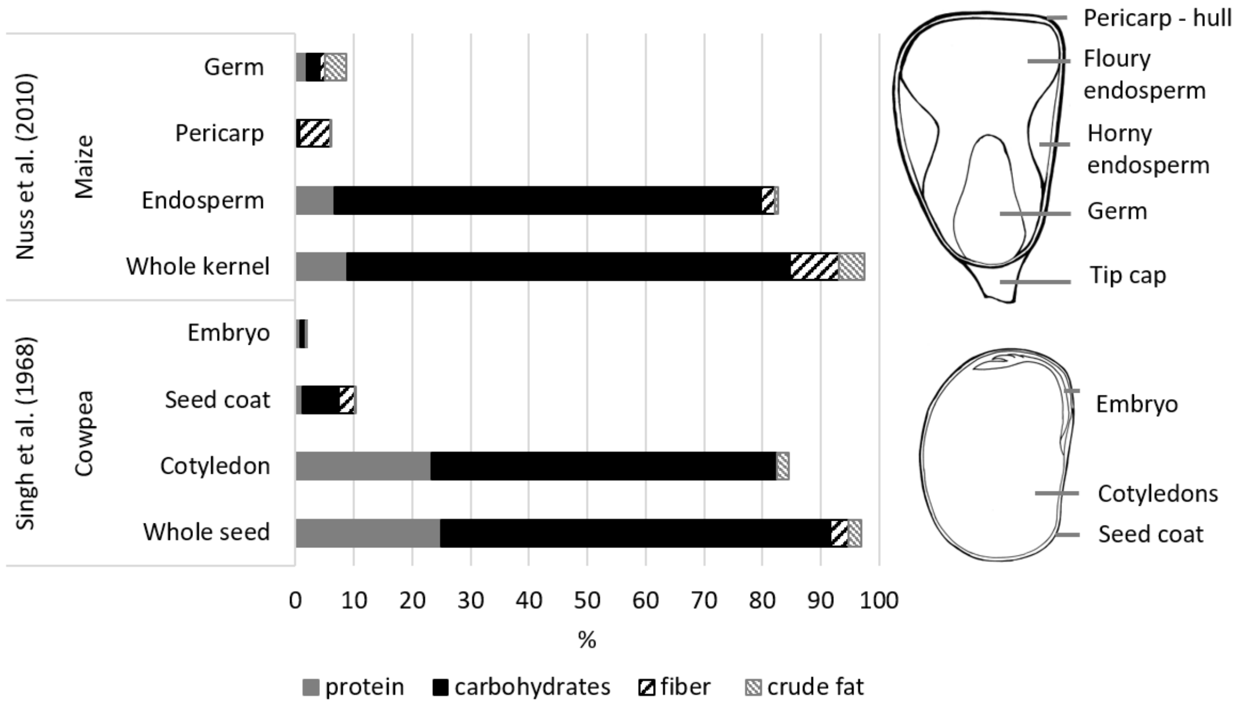

Fig. 1 Proportional distribution of macronutrients within maize and cowpea grains and diagrammatic structure of longitudinal section of a maize and a cowpea grain (Data source: Singh et al., 1968; Nuss et al., 2010)

Some storage pests (insects and rodents) are known to selectively feed on particular parts of the maize grain, for example the germ, which is relatively rich in protein, fat and some vitamins (Fig. 2). While the cowpea grain is somewhat more homogeneous, there are still spatial trends in where and how insect pests feed. Differential damage patterns by the storage insect pests, *Prostephanus truncatus* (the larger grain borer) and *Sitophilus* species (the weevils) have been reported, with *Sitophilus* tending to avoid the germ, and performing poorly if the larva has no access to the endosperm (Sharifi, 1972; Sharifi & Mills, 1971). Conversely, *P. truncatus,* is reported to feed and tunnel either randomly (Subramanyam et al., 1987) on the germ and the endosperm (Ramirez and Silver, 1983) or with an age-based preference changing from the endosperm in early instars to the germ in later instars in two studies (Demianyk and Sinha, 1988; Vowotor et al., 1998) where eggs were artificially introduced into endosperm of grains. However, some of these studies took place in wheat, rather than maize, the more important crop in SSA. Insect damage to different parts of the grain will result in different nutritional losses and therefore understanding how the insects use the grain has important implications for human health.

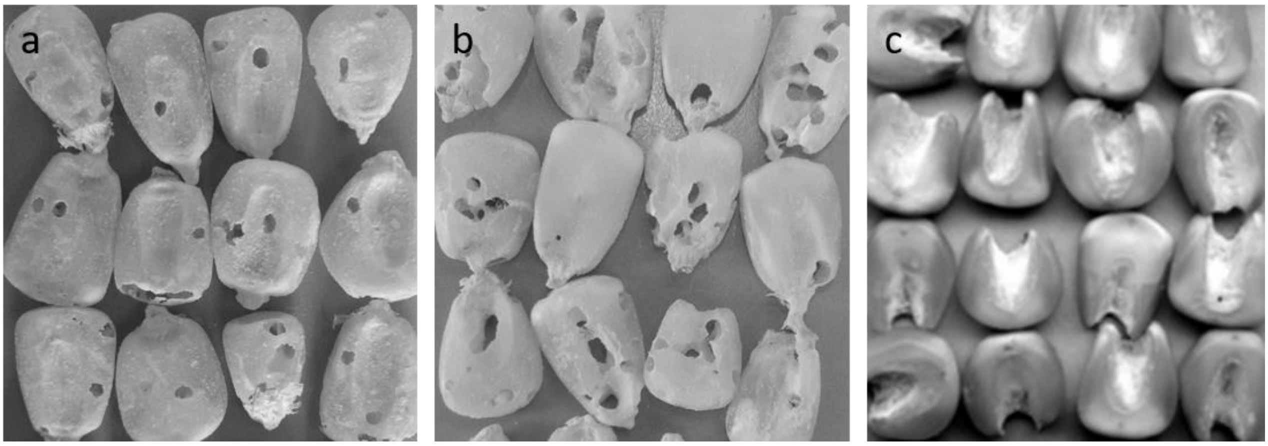

Fig. 2 Maize grains heavily damaged by a) *Sitophilus zeamais*, b) *Prostephanus truncatus*, c) rodents

3 Methods

3.1 Setting up grain storage bioassay jars

Glass jars (850 ml; Pattesons Glass Ltd., Grimsby, UK) were washed and then heat-sterilised, and once cool had fluon (Blades Biological Ltd, Edenbridge, UK) applied around the rim to prevent insect escapes. Sixty-six jars were assigned to the white hybrid maize trial, 15 to the pro-Vitamin A (pVA) biofortified orange maize trial, and 36 to the cowpea trial. Jars were numbered, provisioned with grain and then randomly assigned to one of the infestation treatments.

3.2 Source of grain and insects

Freshly harvested, sun-dried, shelled and hand-sorted maize and cowpea grains were sourced from smallholder farmers in Guruve and Mbire districts of Zimbabwe respectively, by the University of Zimbabwe postharvest team and then shipped in woven polypropylene sacks to the UK. The white hybrid maize variety used was SC719, the pVA biofortified orange maize variety used was ZS242, and the red cowpea variety used was CBC2. On arrival at the NRI laboratories in the UK, the maize and cowpeas were re-bagged into 5 kg lots and sealed inside two high density transparent polyethene bags and frozen to kill any live insects; the cowpeas were frozen for 72 hours and the maize for 1 week. Each grain type was sieved and hand-sorted to remove any foreign matter (e.g. chaff, small stones, insects, pieces of cob or seed pods, etc.) and damaged grains, to enable the trial to be set-up using grain without holes or signs of insect, fungal or rodent damage. The grain was then placed in clean metal trays (of ~10 kg capacity) in a controlled temperature and humidity room for one week (26 ± 1°C, 60 % r.h.) to allow it to equilibrate prior to setting up the experiments. The grains in the trays were turned and mixed twice per day during this equilibration period. The trays of each grain type were then mixed together to homogenise each of the grain types prior to measuring out 300 g of the grain into each jar for the experiment.

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) was sourced from Zimbabwe from infested maize grain and shipped to the UK under license. The species identity was checked by examination of the males' aedeagus (Dobie et al., 1991). *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) was also sourced from Zimbabwe from infested maize grain and species identity confirmed by observation under a microscope. Both species were maintained on white hybrid maize grain from Zimbabwe (the same as that used in experiments). *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) was sourced from an existing culture at the Natural Resources Institute, UK, originally obtained from infested cowpea in Ghana.

3.3 Insect infestation of grain

Five treatments were implemented on the white maize grain: (a) control (no insects); (b) *S. zeamais* (low level; two male plus two female 7-21 day old adults); (c) *S. zeamais* (high level; 20 unsexed 7-21 day old adults); (d) *P. truncatus* (two male plus two female 7-14 day old adults); (e) both insect pests (two male and two female *S. zeamais* and two male and two female *P. truncatus* 7-14 day old adults). For the pVA biofortified orange maize, two treatments were used: (a) control (no insects); (b) both insect pests (two male and two female *S. zeamais* and two male and two female *P. truncatus* 7-14 day old adults). For cowpea, three treatments were used: (a) control (no insects); (b) *C. maculatus* (low level; two male plus two female 0-3 day old adults); (c) *C. maculatus* (high level; 20 unsexed 0-3 day old adults).

Even the low infestation levels were anticipated to reach high population densities within 2-3 months, however, attempting experiments with a single female as the founder was considered to carry an unacceptable risk of early mortality or atypical fecundity so two females was considered the minimum required to ensure infestation occurred. Three jars were assigned to each combination of infestation type x grain type x duration of infestation (Table 2).

Table 2 Experimental design

Grain type	Infestation	Treatment code	No. of insects added to 300 g of grain	Storage duration (months)						
				0	1	2	3	4	6	8
White maize										
SC719	*S. zeamais* (high)	High Sz	20		X	X		X	X	
(66 samples)	*S. zeamais* (low)	Low Sz	4		X	X		X	X	
	P. truncatus	Low Pt	4		X	X		X	X	
	S. zeamais + P. truncatus	Low SzPt	4 Sz + 4 Pt		X	X		X	X	
	Non-infested control	Control	0	X	X	X		X	X	X
Orange maize										
ZS242	*S. zeamais + P. truncatus*	Low SzPt	4 Sz + 4 Pt		X			X		
(15 samples)	Non-infested control	Control	0	X	X			X		
Cowpea										
CBC2	*C. maculatus* (high)	High	20		X	X	X			
(36 samples)	*C. maculatus* (low)	Low	4		X	X	X	X		
	Non-infested control	Control	0	X	X	X	X	X		

Key: X indicates 3 replicates of that treatment

After addition of insects (if any), jars were sealed with a 70 mm filter paper (Schleicher & Schuell, Dassel, Germany, or Whatman No. 1 cut to fit the 70 mm jar opening) held in place by paraffin wax.

As the 0-month replicates for the infestation treatments were all identical, only three jars were used for the 0-month time point for each grain type.

Destructive sample analysis of the cowpea grains was done at 1, 2, 3 and 4 months' storage, while the white maize was destructively sampled at 1, 2, 4, 6 and 8 months' storage, and the pVA biofortified orange maize at 1 and 4 months' storage.

3.4 Storage conditions and sampling

All the jars of grain were stored in a controlled temperature and humidity chamber, set to 26°C and 60 % relative humidity with a 12:12 hour light: dark cycle. After the different assigned storage durations (Table 2), the relevant subset of jars (three replicates for each grain type x insect combination x storage duration) were removed from the chamber, opened and destructively sampled using the damage assessment procedure described below. The cowpea storage trial ran from September 2017 to January 2018, and the maize trials from September 2017 to May 2018.

3.5 Damage assessment of sample

The contents of each jar were weighed and sieved (nested metal sieves with apertures of 4.75 mm and 1 mm were used for white maize, and 2 mm and 1 mm for cowpea and pVA biofortified orange maize). The sieving process used involved one minute of manual shaking, one minute of rest and a further minute of shaking to separate the trash and insects from the grains. The weight of the trash and the insects were recorded along with the number and species of dead and live insects, except for the later storage duration cowpea jars as they contained several thousand of the flight-form insects.

The sieved grain was then poured through a riffle-divider to produce a sub-sample of ~90 g for damage assessment. Each grain in the sample was inspected and categorised as undamaged, insect damaged, broken (mechanical damage or damage not due to the storage insect pests), or insect damaged and broken. The total numbers and mass of the grains in each category were recorded. The percentage insect damaged grain was calculated according to the following equation:

$$\%\ insect\ damaged\ grain = \frac{Nd}{(Nd + Nu)} \times 100$$

with Nd representing the number of insect damaged grains, and Nu representing the number of undamaged or non-insect damaged grains (Boxall, 1986).

Percentage grain weight loss was calculated using the formula:

$$Attainable\ yield\ (Ya) = (Nu + Nd) \times Wu1$$

$$Actual\ yield\ (Y) = (Nu \times Wu1) + (Nd \times Wd1)$$

$$\%\, weight\ loss = \frac{Ya - Y}{Ya} \times 100 = \frac{(Nd \times Wu1)\ -\ (Nd\ \times Wd1)}{(Nd\ +\ Nu) \times\ Wu1} \times 100$$

with Wu1 = unit weight of unaffected grain, and Wd1 = unit weight of damaged grain.

Note: this formula gives the same result as the percentage weight loss (count and weigh) formula:

$$\%\, weight\ loss = \frac{(Wu \times Nd)\ -\ (Wd \times Nu)}{(Nd\ +\ Nu) \times\ Wu} \times 100$$

with Wu = total weight of undamaged or non-insect damaged grains in a sample, and Wd = total weight of insect damaged grains (Boxall, 1986; Adams and Schulten, 1978).

The whole sieved sample was then re-mixed, sealed inside two ziplock plastic bags and frozen at -20°C until nutrient content analysis.

3.6 Nutrient analysis of samples

Food proximate plus iron and zinc content analyses were used to determine the nutritional composition and energy value of each sample. A summary of the nutrient composition analysis methods is given in Supplemental Table S1.

A sub-sample of the 15 pVA biofortified orange maize samples were used for analysis of the carotenoid content. The maize carotenoids were extracted as previously reported (Ortiz et al., 2016; Nkhata et al., 2019). Liquid chromatography analysis was performed using authentic all-trans-carotenoid standards and comparison with spectral information from previous separations (Kean et al., 2008) to identify the carotenoid peaks. Quantification was completed using a seven-point response curve constructed with authentic carotenoid standards in the range of 0.01-8.0 µm.

3.7 Data analyses

The experiments enabled the insect damage-related attributes and nutritional content of each sample for the three focal grains and the different initial infestation levels to be compared during a storage period of up to eight months (see Table 1). Data were analysed using R version 3.5.1 (R Core Team, 2018).

Analyses were carried out using the grain in the state in which it had been stored, i.e. fresh-weight basis (FWB) from product previously sun-dried to <12 % moisture content, rather than analysing dry-weight basis (DWB) nutrient content. Using FWB values better reflects the nutritional situation for smallholder farmers, as food is prepared directly from stored grain rather than from the zero-moisture material that is used for DWB analysis.

For each of the three grain types, a two-way analysis of variance (ANOVA) was conducted to determine if the storage duration, the initial insect infestation level, or the interaction between them had a statistically significant effect on each of the variables considered (i.e. percentage damaged grains, percentage grain weight loss, protein (g/100 g), fat (g/100 g), iron (mg/100 g), zinc (mg/100 g), available carbohydrate (g/100 g), fibre (g/100 g), energy (kcal/100 g), % moisture content, % weight of trash, total insects/ kg). Variable values were plotted by treatment and storage duration. Multiple comparisons used the Least Significant Difference (LSD) LSD.test function in the R 'agricolae' package (de Mendiburu, 2019), applied to the output of a one-way ANOVA using a factor that combines the storage duration and infestation levels. This uses a Holm-corrected least significant difference method to generate groups of means which do not differ significantly at $p < 0.05$, identified by compact letter display codes (Steel et al., 1997).

For each grain type, the relationships between percentage insect damage and weight loss for each of the nutrients and storage duration (months) were analysed using plots of paired variables and Spearman's rank correlations, to detect correlations between insect damage variables and each of the different nutrients considered. In these analyses, the level of insect damage as opposed to the treatments (i.e. untreated control, low initial infestation, high initial infestation etc.) was used, as within each treatment there could be a wide range in the number of insects and the damage levels which had resulted.

An interactive storage insect damage-related nutrient loss prediction tool was developed. This tool requires the user to input i) the initial mass of grain and ii) the percentage of insect damaged grains at the sampling time, it then calculates the predicted nutritional content of the remaining stored grain using the nutrient: insect damage correlation data.

4 Results

4.1 Change in nutrient content of uninfested control commodities during storage

The proportional content of the different nutrients did not change significantly in the uninfested control white maize grain during the storage period (Fig. 3). The nutrient content of the white hybrid maize grain, the pVA biofortified orange maize grain and the cowpea grain at the time of the trial set-up are shown in Supplemental Tables S2 and S3. The moisture content of the white hybrid maize grain was 11.7 % at set-up and decreased slightly during the trial in the uninfested control grain, but not statistically significantly so.

In the uninfested control pVA biofortified orange maize grain no change occurred in the concentration of the different macronutrients, or the moisture content during the storage period. A decrease in the mean zinc content did occur in the uninfested grain, but not in the insect infested grain (Fig. 4). The concentration of all the carotenoids reduced during the four-month storage duration in both the uninfested control grain and the insect infested grain (Fig. 5).

Similarly, in the uninfested control cowpea grain, there was no significant change in the proportional content of any of the nutrients measured or the grain moisture content over the four-month storage period (Fig. 6).

4.2 Relationship between insect infestation, proportion of damaged grains and grain weight loss

4.2.1 White maize grain

Insect damage increased with storage duration in all the treatments to which insects were added at trial set-up (Fig. 3). The highest mean percentage of damaged grains (83 %) occurred following six months of storage of the white maize grain initially seeded with the high number of *S. zeamais* (i.e. High Sz = 20 *S. zeamais* adult insects added to 300 g of maize grain at set-up). Mean damage was slightly lower (58-70 %) at six months' storage in those treatments initially infested with lower numbers of insects (i.e. Low SzPt, Low Pt, Low Sz). A two-way analysis of variance (ANOVA) run on the 66 samples of white maize grain showed that storage duration ($F(5, 47) = 122.2$, $p < 0.0001$) and initial insect infestation level ($F(4, 47) = 34.3$, $p < 0.0001$) interacted and had a highly significant effect on percentage of damaged grains ($F(12, 47) = 11.4$, $p < 0.0001$).

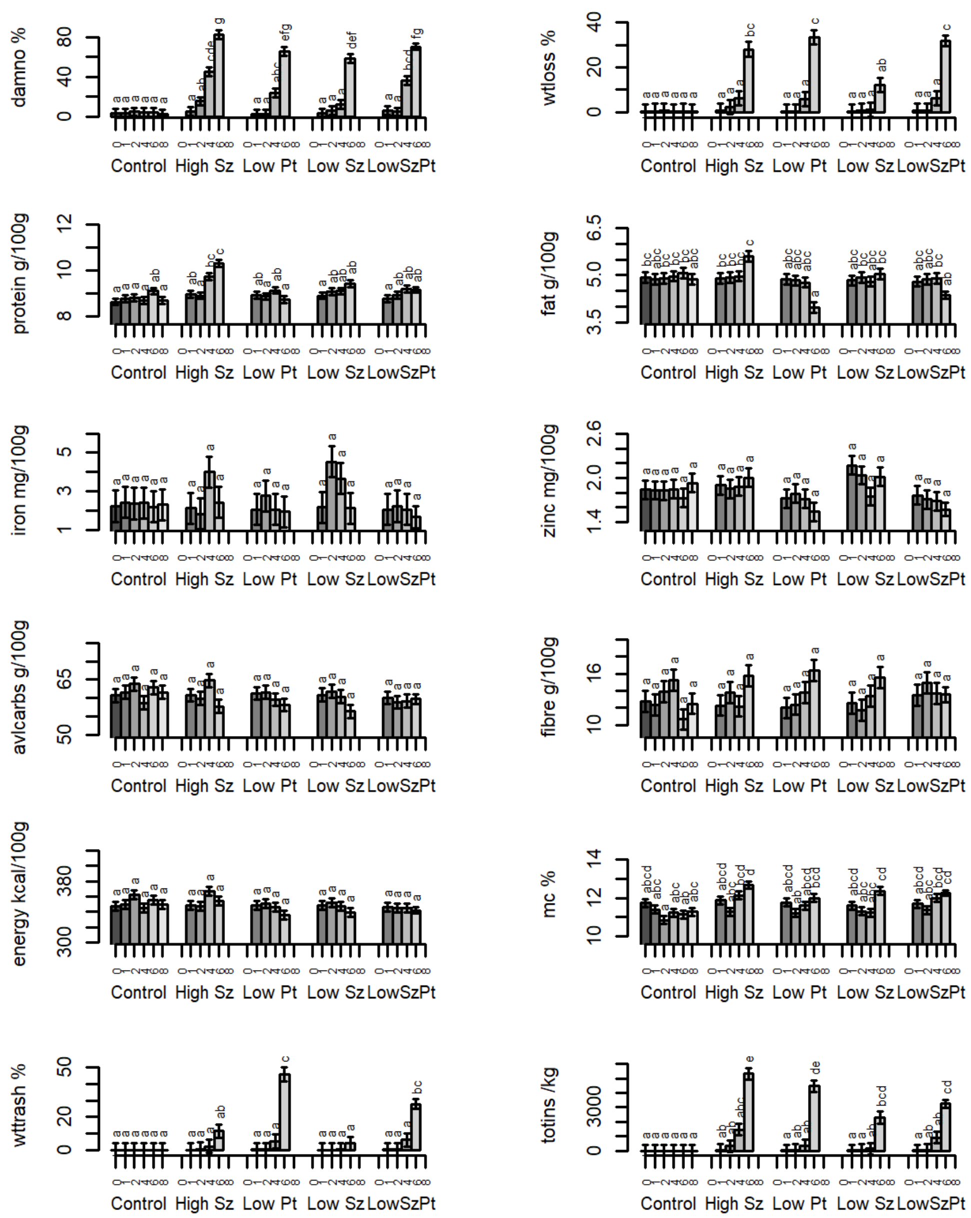

Fig. 3 Mean damage level and nutrient content of dried white maize grains infested with different numbers and species of the two main storage insect pests after different storage durations of up to 8 months (n=3) *(Note: x-axis shows treatments and storage duration - Control (0, 1, 2, 4, 6, 8 months), High Sz (*S. zeamais*), Low Pt (*P. truncatus*), Low Sz, LowSzPt (*S. zeamais *and* P. truncatus*) (at 1, 2, 4, 6 months); y-axis abbreviations: damno % = percentage damaged grain (by number); wtloss % = percentage grain weight loss; avlcarbs = available carbohydrates; mc % = percentage grain moisture content; wttrash % = percentage weight of trash; totins/kg = total number of insects (live and dead for both species)/ kg. Within each chart means which are statistically significantly different from each other are denoted by different lower-case letters (p < 0.05)*

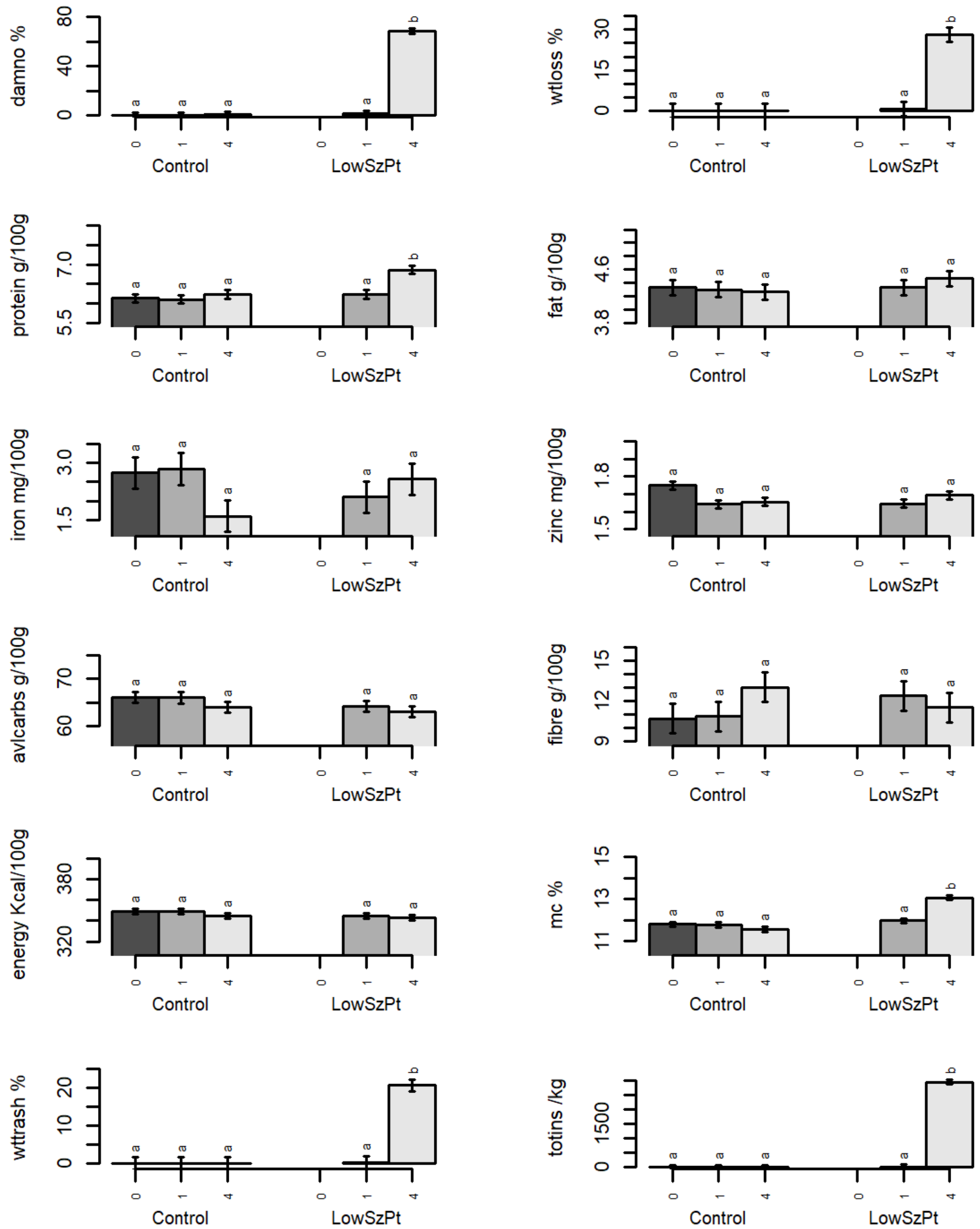

Fig. 4 Damage level and nutrient content of stored pro-Vitamin A biofortified orange maize grains with and without insect pest infestation after 0, 1 and 4 months storage *(Note: x-axis shows treatment and storage duration - Control (0, 1, 4 months), Low SzPt (1, 4 months); y-axis abbreviations as per figure 1 above. Within each chart means which are statistically significantly different from each other are denoted by different lower-case letters (p < 0.05)*

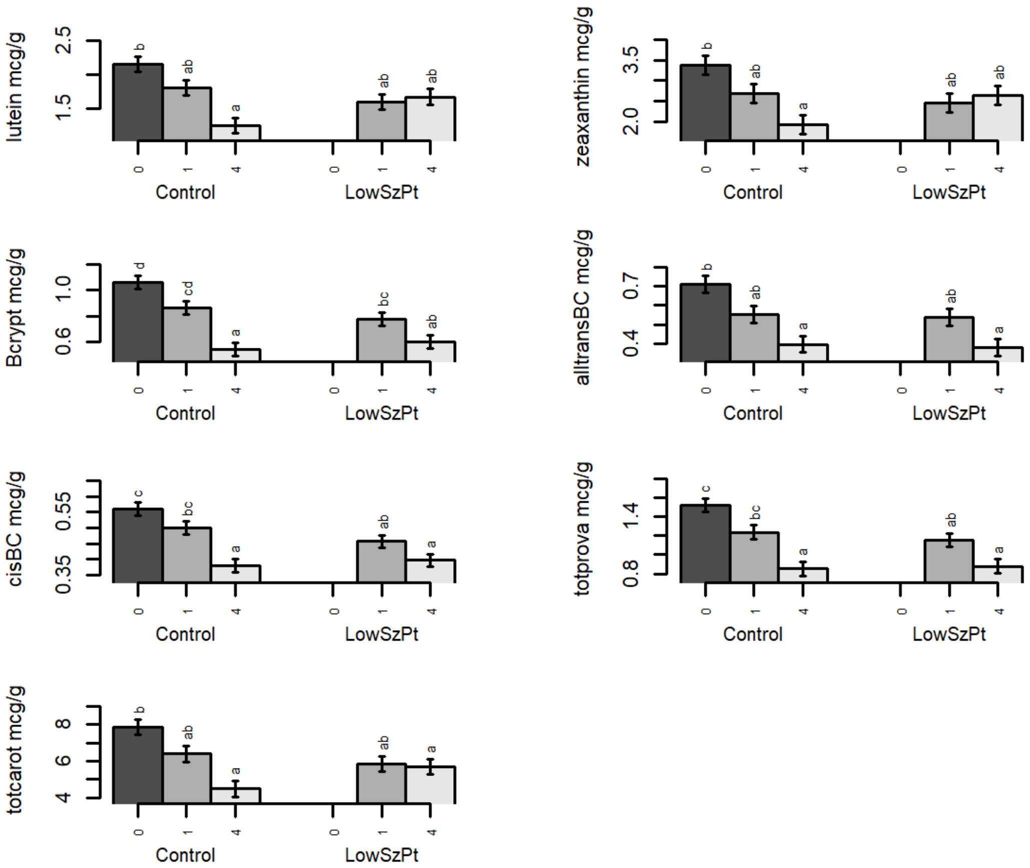

Fig. 5 Content of carotenoids in stored pro-Vitamin A biofortified orange maize grains with and without insect pest infestation after 0, 1 and 4 months storage *(Note: x-axis shows treatment and storage duration - Control (0, 1, 4 months), Low SzPt (1,4 months); y-axis abbreviations: Bcrypt = Beta-cryptoxanthin; alltransBC = all trans beta-carotene; cisBC = cis beta-carotene; totalprova = total pro-vitamin A; totalcarot = total carotenoids. Within each chart means which are statistically significantly different from each other are denoted by different lower-case letters ($p < 0.05$))*

At trial set-up, less than 5 % of grains were insect damaged; this damage was due to prior insect infestation in the field or during crop drying or transport. However, as the grain was frozen for 1 week prior to trial set-up, there were no surviving insects inside any of the damaged grains.

Grain weight loss due to insect damage also increased with storage duration, most rapidly between four and six months' storage. Grain weight loss differed significantly between treatments ($F (5, 47) = 40.8$, $p < 0.0001$), reaching extremely high levels of 28-34 % at six months' storage in the High Sz, Low SzPt and Low Pt treatments, but remaining lower (12 %) in the Low Sz treatment, and less than 0.7 % in the uninfested control grain (Fig. 3).

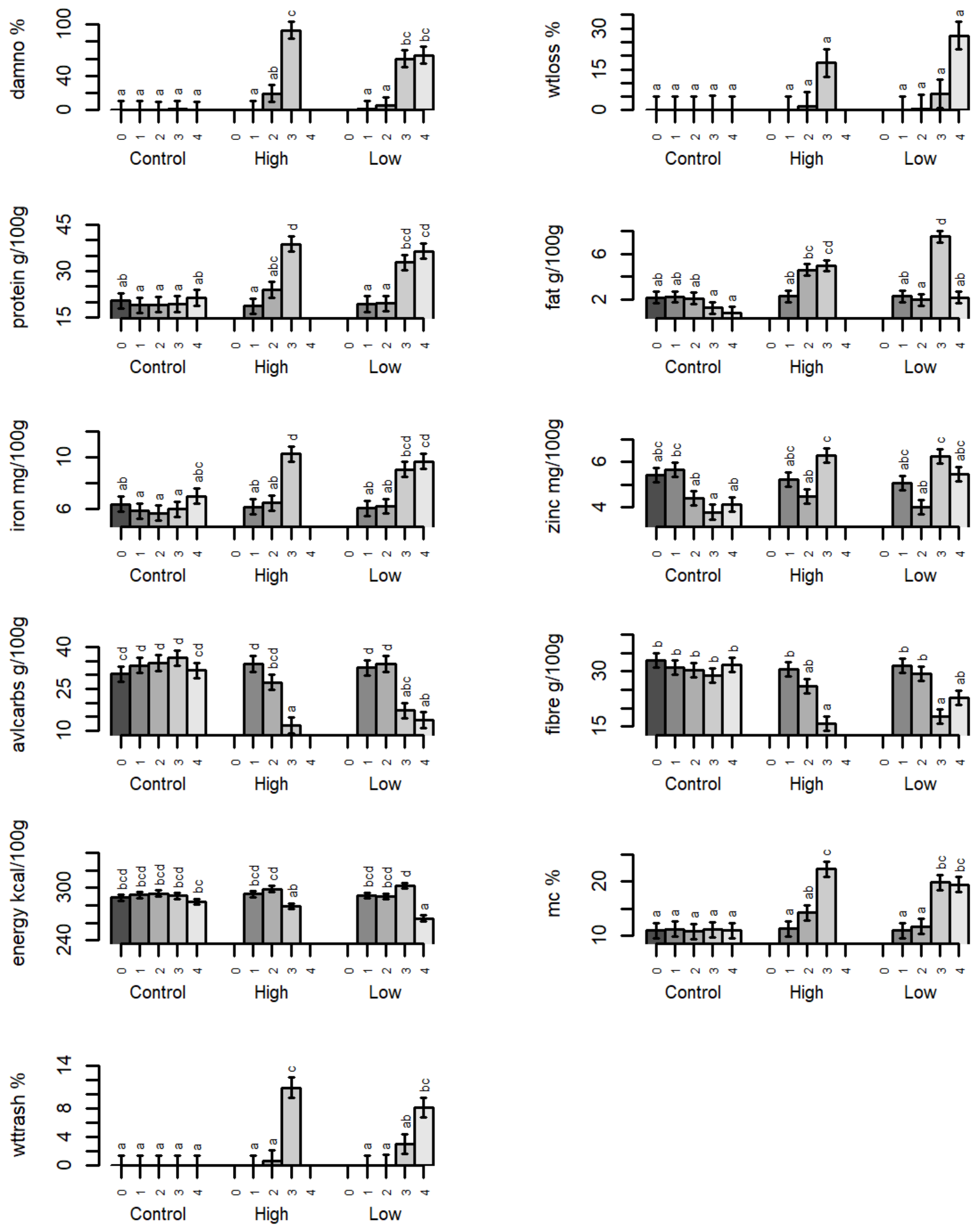

Fig. 6 Damage level and nutrient content of dried cowpea grains infested with different initial numbers of the insect pest *Callosobruchus maculatus* after 0, 1, 2, 3 and 4 months' storage *(Note: x-axis shows treatments and storage duration - Control (0, 1, 2, 3, 4 months), High* C. maculatus *(20 insects/ 300 g) (1, 2, 3 months), Low* C. maculatus *(4 insects /300 g) (1, 2, 3, 4 months); y-axis abbreviations as per figure 1 above. Within each chart means which are statistically significantly different from each other are denoted by different lower-case letters (p < 0.05).*

4.2.2 Pro-Vitamin A biofortified orange maize grain

Insect damage increased with storage duration in the infested pVA biofortified orange maize grain, resulting in a mean of 69 % of grains being damaged at four months' storage, equivalent to a mean grain weight loss of 28 % (Fig. 4). A two-way analysis of variance of the 15 pVA biofortified orange maize samples showed that storage duration ($F (2, 10) = 166.3$, $p < 0.0001$) and insect infestation ($F (1, 10) = 293.5$, $p < 0.0001$), had a significant effect on percentage insect damage, with a significant interaction ($F (1, 10) = 264.2$, $p < 0.0001$); and on grain weight loss (storage duration ($F (2, 10) =16.7$, $p = 0.0007$), insect infestation ($F (1, 10) =29.6$, $p = 0.0003$), with a significant interaction ($F (1, 10) =27.0$, $p = 0.0004$) (Fig. 4).

4.2.3 Cowpea grain

Insect damage increased with storage duration in both the high and the low levels of initial insect infestation level treatments, resulting in mean of 93 % and 60 % damaged grains at three months storage respectively (Fig. 6). A two-way ANOVA on the 36 samples of stored cowpea showed that storage duration ($F (4, 24) = 13.1$, $p = 0.00008$), initial insect infestation level ($F (2, 24) = 17.6$, $p = 0.0002$) and their interaction ($F (5, 24) = 6.3$, $p = 0.0007$) significantly affected the mean percentage of damaged grains. The mean percentage of damaged grains was significantly higher by 3 months storage in both the high and low treatments compared to the control. Although the percentage of damaged grain was higher in the high infestation treatment, than in the low infestation treatment, Least Significance Difference pairwise comparison tests did not detect significant differences (Fig. 6).

Grain weight loss of cowpea seeds also increased with storage duration in both the high and low insect infestation treatments. A two-way ANOVA showed that storage duration ($F (4, 24) = 3.1$, $p = 0.033$) and initial insect infestation level ($F (2, 24) = 3. 8$, $p = 0.037$) significantly affected the mean percentage grain weight loss. However, Least Significant Difference multiple comparison test did not detect significant differences between treatment means (Fig. 6).

4.3 Relationship between insect infestation and shifts in nutrient contents

The three insect damage variables considered (percentage damaged grains, percentage grain weight loss, and total insects /kg) were all significantly positively correlated with each other for all three grain types.

Moisture content of grain stored in non-airtight containers changes during the storage period in response to the relative humidity and temperature of the surrounding environment, and insect and/or fungal attack of the stored grain usually results in an increased grain moisture content. During the trial, grain moisture content ranged from 10.7 % to 13.1 % in the different white maize treatments (10.7 % to 11.9 % in the uninfested stored maize, and 10.9 % to 13.1 % in the insect infested stored maize), 11.4 % to 13.2 % in the pVA biofortified orange maize treatments (11.4 to 12.0 % in the uninfested pVA biofortified orange maize, and 11.4 % to 13.2 % in the insect infested pVA orange maize), and 10.5 % to 24.5 % in the cowpea treatments (10.7 % to 11.4 % in the uninfested stored cowpea, and 10.8 % to 24.5 % in the insect infested cowpea).

4.3.1 Shifts in nutrient contents in insect-infested white maize grain

The correlations between insect infestation and nutritional composition of white maize grain are shown in Fig. 7. Increasing storage duration (months) was positively correlated with increasing percentage damaged grain (damno; $p < 0.001$), percentage grain weight loss (wtloss; $p < 0.001$), and total number of insects /kg (tot_ins; $p < 0.001$) (Fig. 3). There was also a positive correlation between increasing storage duration and the protein content of samples (protein; $p < 0.01$).

The three insect damage variables (percentage damaged grains (damno), percentage grain weight loss (wtloss), and total insects /kg (tot_ins)) were all significantly positively correlated ($p < 0.001$) (Fig. 7). They were all also significantly positively correlated ($p < 0.001$) with increasing protein content (protein), increasing moisture content (mc) and with increasing storage duration (months). There was also a positive correlation between increasing relative fibre content (fibre) and percentage damaged grains (damno; $p < 0.05$) and total insects /kg (tot_ins; $p < 0.01$). There was a negative correlation between relative available carbohydrate content and these three insect damage variables ((damno; $p < 0.01$) (tot_ins; $p < 0.01$) (wtloss; $p < 0.05$)), and between all three insect damage variables (damno, wtloss, tot_ins) and the energy content (kcal; $p < 0.001$). There was a negative correlation between the insect damage variables and relative iron content (iron; $p < 0.05$). No correlation between the insect damage variables and the relative fat or zinc content of the white maize grain occurred.

The relationships between each of the insect species (*P. truncatus* and *S. zeamais*) and nutrient composition in the white maize were also studied using a Spearman's correlation (Fig. 8). While increasing numbers of each species were correlated with increasing percentage grain weight loss, percentage damaged grains and percentage moisture content (*P. truncatus* (Pt_pkg; $p < 0.05$), *S. zeamais* (Sz_pkg; $p < 0.001$)), only increasing numbers of *S. zeamais* were statistically significantly correlated with increased relative protein (Sz_pkg; $p < 0.001$) and fibre ($p < 0.05$) concentration. Increasing numbers of both species were significantly correlated with decreasing energy kcal (*P. truncatus* (Pt_pkg; $p < 0.001$), *S. zeamais* (Sz_pkg; $p < 0.01$)), and relative iron content (*P. truncatus* (Pt_pkg; $p < 0.01$), *S. zeamais* (Sz_pkg; $p < 0.05$)). Increasing numbers of *S. zeamais* (Sz_pkg; $p < 0.01$) were statistically significantly associated with decreasing relative available carbohydrate content. Increasing numbers of *P. truncatus* were also significantly (Pt_pkg; $p < 0.001$) associated with decreasing relative fat and zinc contents, but no significant relationship occurred between *S. zeamais* numbers and these two nutrients.

The significant correlations between insect damage, mass loss and nutrient composition could be used to create a maize storage nutrient loss predictive tool to assist in estimating the quantity and concentration of different nutrients in the remaining stored product at a range of different grain damage levels, with a prototype exemplar provided as Supplemental Tool 1.

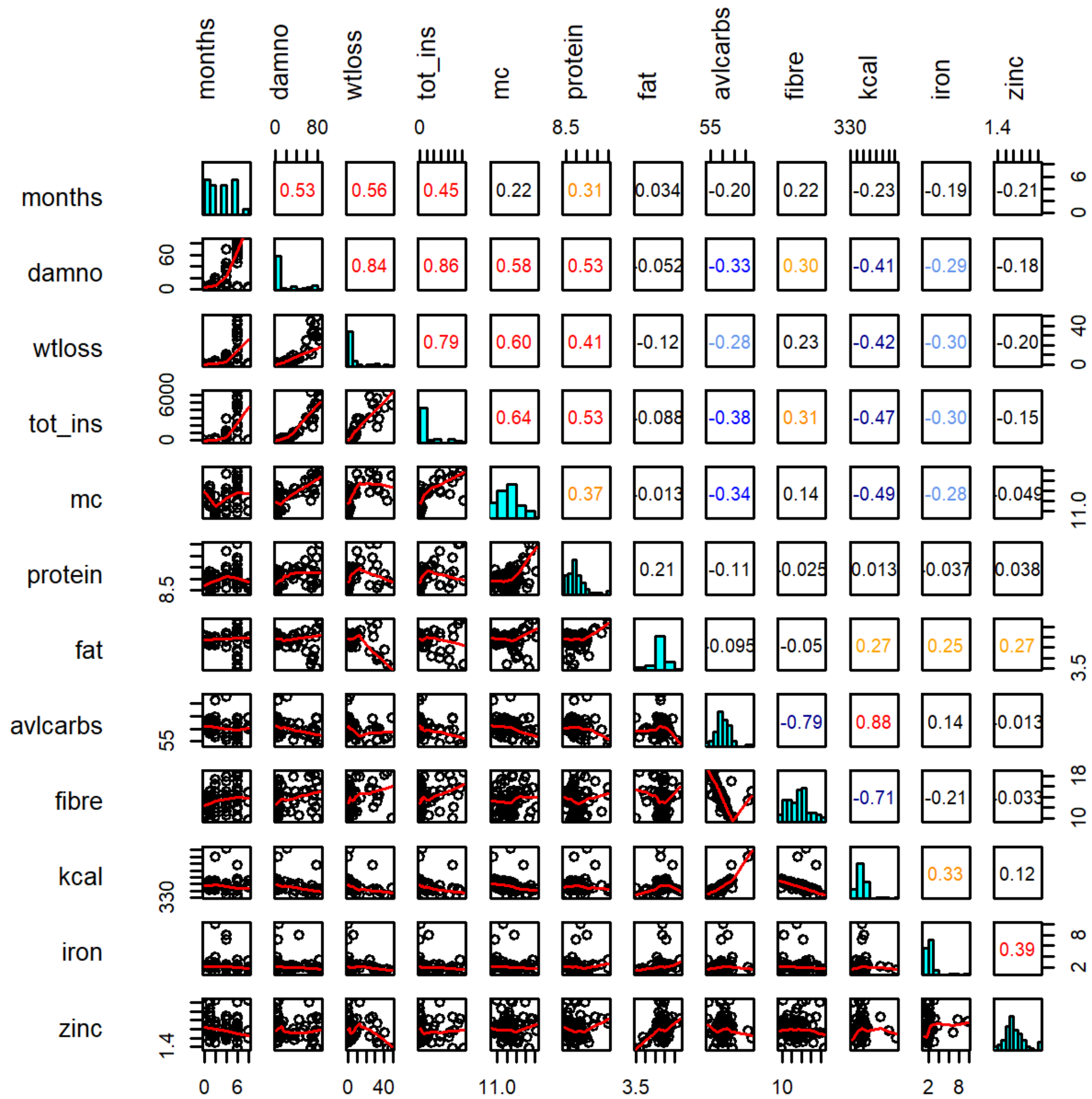

Fig. 7 Pairs plot and Spearman's rank correlations of relationships between storage insect damage factors and nutrients in white maize grain *[Spearman's rank correlation critical values for N = 69 are p = 0.05* 0.237; p = 0.01** 0.309*; *p = 0.001*** 0.390, the significantly positive correlations are shown in shades of yellow > red, and the significantly negative correlations in shades of blue; abbreviations= months = storage duration; damno = percentage damaged grain (by number); wtloss = percentage grain weight loss; totins = total number of insects/ kg; mc = percentage grain moisture content; avlcarb = available carbohydrates; kcal = energy (kcal/100g)]*

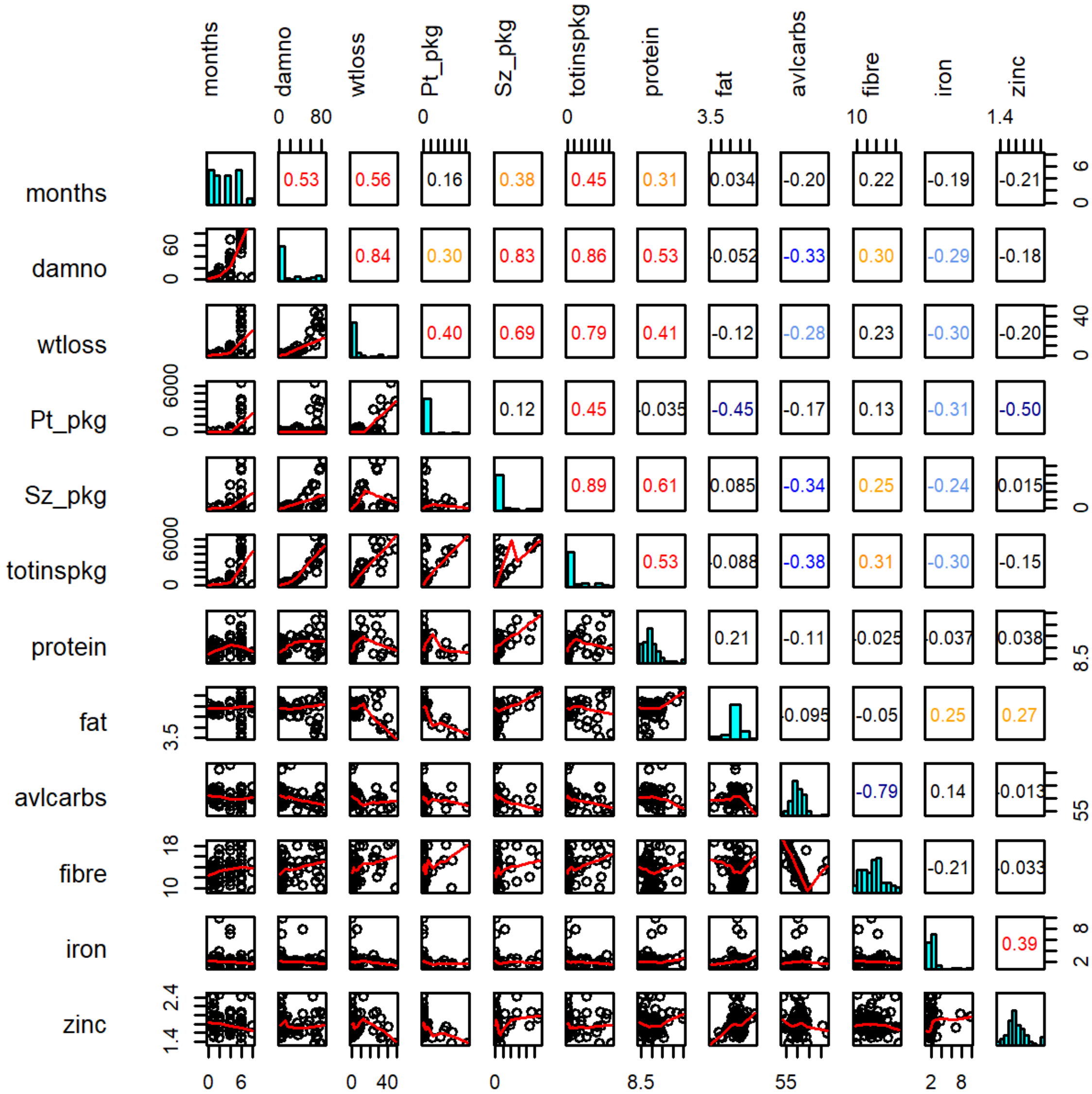

Fig. 8 Pairs plot and Spearman's rank correlations of relationships between the different storage insects and nutrients in white maize grain *[Spearman's rank correlation critical values for N = 69 are p = 0.05* 0.237; p = 0.01** 0.309*; *p = 0.001*** 0.390, the significantly positive correlations are shown in shades of yellow < red, and the significantly negative correlations in blue; abbreviations= months = storage duration; damno = percentage damaged grain (by number); wtloss = percentage grain weight loss; Pt_pkg = total* P. truncatus*/ kg; Sz_pkg = total* S. zeamais*/ kg; totins = total number of insects/ kg; avlcarbs = available carbohydrates]*

4.3.2 Shifts in nutrient contents in insect-infested Pro-Vitamin A biofortified orange maize grain

The relationships seen in the pVA biofortified orange maize samples were similar to those in the white maize, with storage duration positively correlated to percentage damaged grain (damno; $p < 0.01$), percentage grain weight loss (wtloss; $p < 0.01$), total insects /kg (tot_ins; $p < 0.05$), relative protein (protein; $p < 0.01$) and relative fibre content (fibre; $p < 0.05$).

The relationships between insect damage variables and nutritional composition and moisture content and storage duration in pVA biofortified orange maize grain are visualised in the pairs plot with the result of the Spearman's rank correlations tests (Fig. 9), with the data summarised by treatment shown in Fig. 4. As with the white maize grain, in the pVA biofortified orange maize the insect damage variables were positively correlated with increasing relative protein content (protein; $p < 0.05$) and increasing moisture content (mc; $p < 0.05$). While there was a negative correlation between percentage weight loss and available carbohydrates (avlcarbs; $p < 0.05$). No statistically significant relationship was found between the three insect-damage variables and the fat, fibre, energy, iron, or zinc contents of the pVA biofortified orange maize grain. Additional analyses (not shown) found no significant correlation between *P. truncatus* numbers and fat and zinc content nor with any of the other nutrients except an increase in relative protein content (protein; $p < 0.05$) in the pVA biofortified orange maize, and the same was found for *S. zeamais*.

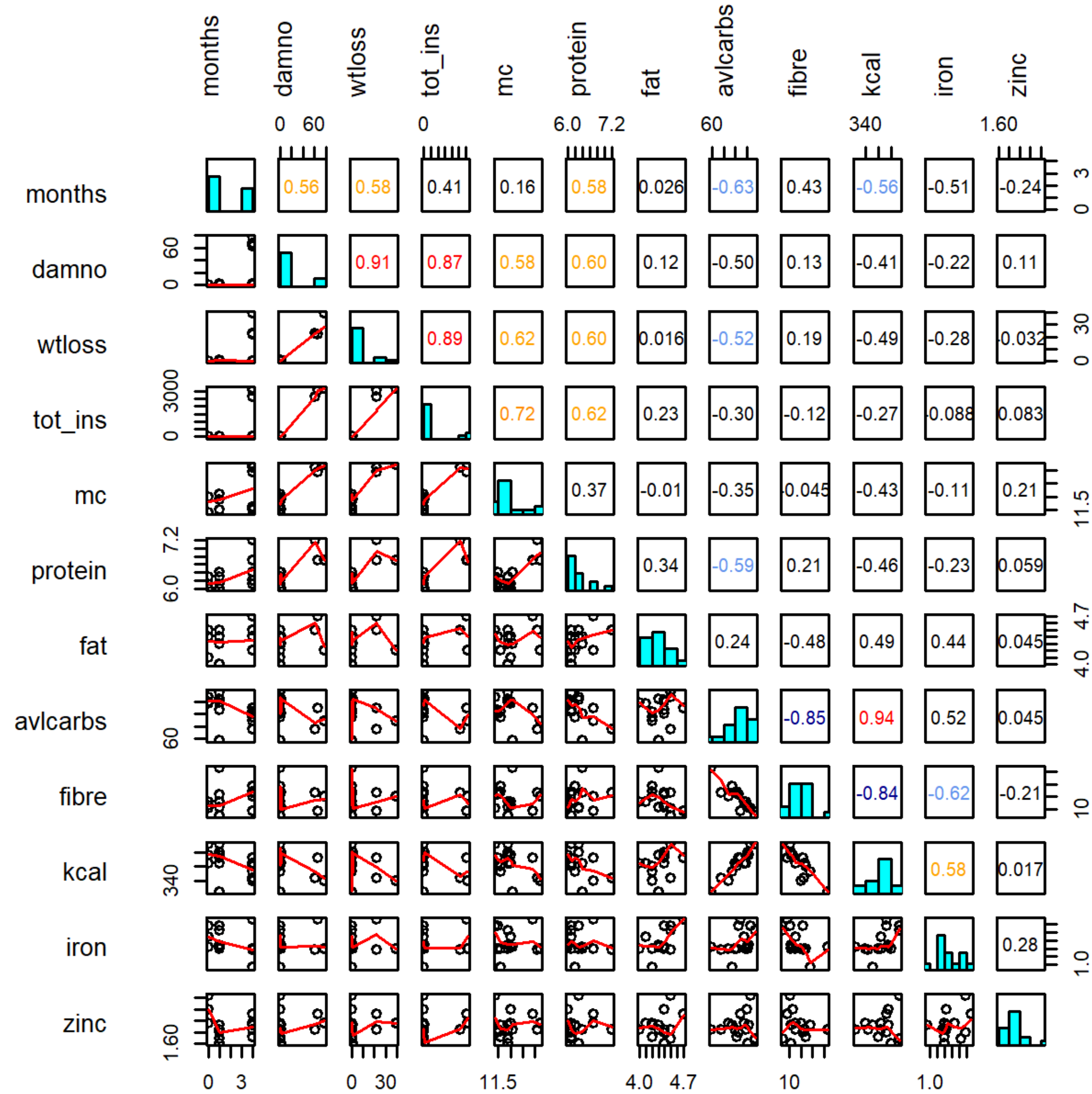

Fig. 9 Pairs plot and Spearman's rank correlations of relationships between storage insect damage factors and nutrients in pro-Vitamin A biofortified orange maize grain *[Spearman's rank correlation critical values for N = 15; p = 0.05* 0.521; p = 0.01** 0.654; p = 0.001*** 0.779, the significantly positive correlations are shown in shades of yellow < red, and the significantly negative correlations in shades of blue; abbreviations: months = storage duration; damno = percentage damaged grain (by number); wtloss = percentage grain weight loss; tot_ins = total number of insects /kg; mc = percentage grain moisture content; avlcarbs = available carbohydrates; kcal = energy (kcal/100g)]*

The relationship between the carotenoid composition of the pVA orange maize grain samples and the storage duration and insect damage variables was also analysed (Figs. 5 and 10).

All the carotenoid variables were negatively correlated with increasing storage duration (beta-cryptoxanthin (Bcrypt; $p < 0.001$); all-trans beta-carotene (alltransBC; $p < 0.001$); cis beta-carotene (cisBC; $p < 0.001$); total pVA (totprova; $p < 0.001$); lutein (lutein; $p < 0.01$); total carotenoids (totcarot; $p < 0.01$); zeaxanthin (zeaxanthin; $p < 0.05$)); highlighting their instability over time during grain storage (Fig. 10), whether the grain was infested with insects or not (Fig. 5). Increasing percentage grain weight loss was correlated with a decrease in: all trans beta-carotene (alltransBC; $p < 0.05$), cis beta-carotene (cisBC; $p < 0.05$), and total pro-Vitamin A (totprova; $p < 0.05$) (Fig. 10). All the carotenoids (lutein, zeaxanthin, beta-cryptoxanthin, cis beta-carotene, all trans beta-carotene, total carotenoids, total pro Vitamin A) were significantly ($p < 0.05$) positively correlated with each other (Fig. 10).

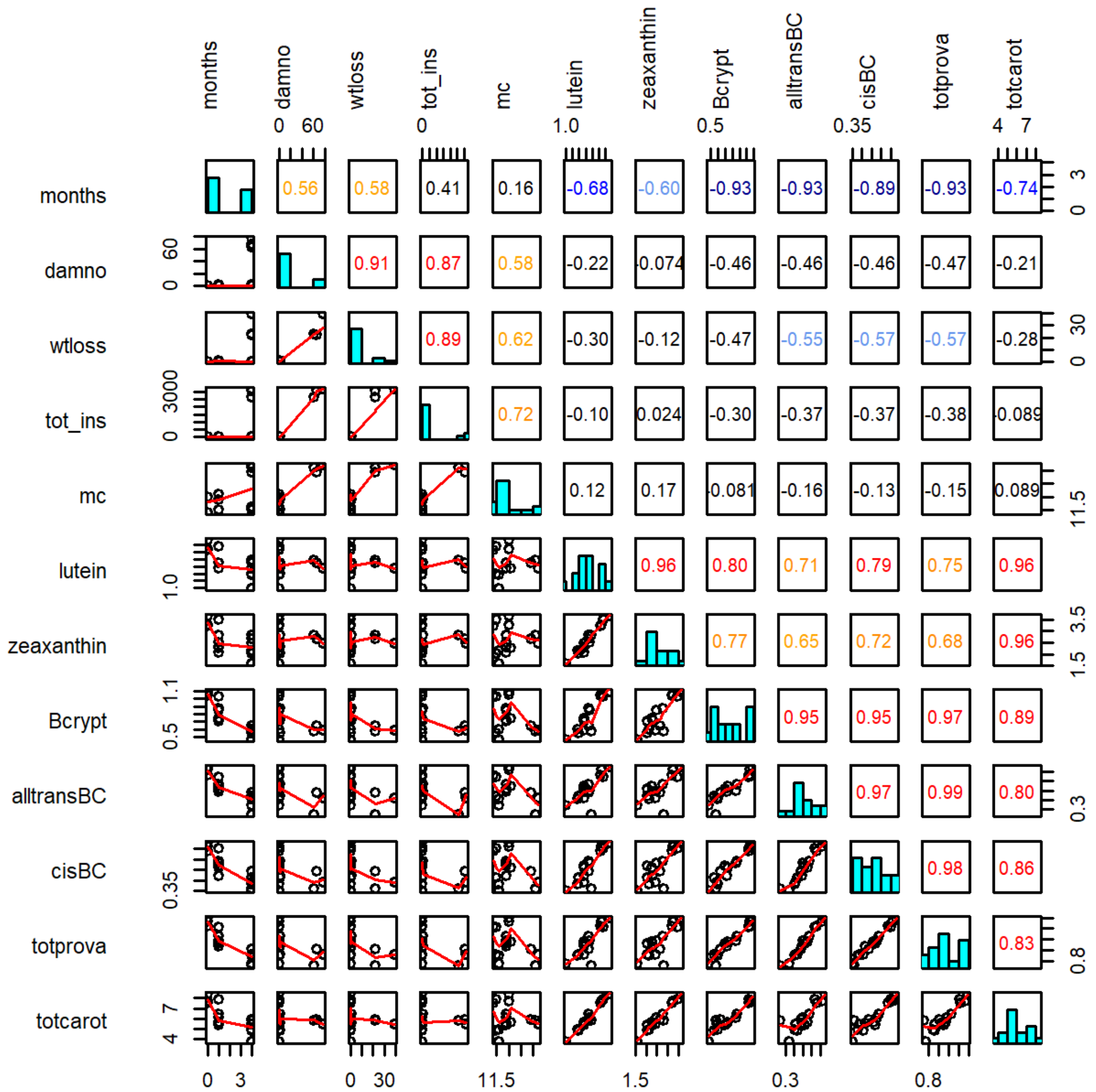

Fig. 10 Pairs plot and Spearman's rank correlations of relationships between storage insect damage factors and carotenoids in pro-Vitamin A biofortified orange maize grain *[Spearman's rank correlation critical values for N = 15; p = 0.05* 0.521; p = 0.01** 0.654; p = 0.001*** 0.779, the significantly positive correlations are shown in shades of yellow < red, and the significantly negative correlations in shades of blue; abbreviations: months = storage duration; damno = percentage damaged grain (by number); wtloss = percentage grain weight loss; tot_ins = total number of insects/ kg; mc = percentage grain moisture content; the carotenoids shown are lutein, zeaxanthin, beta-cryptoxanthin, trans beta-carotene, cis beta-carotene, total pro Vitamin A, total carotenoids]*

4.3.3 Shifts in nutrient contents in insect-infested cowpea grain

The relationships between insect damage variables and nutritional composition, moisture content and storage duration in cowpea grain are visualised in the pairs plot in Fig. 11. In the cowpea samples, storage duration (months) was not positively correlated with increasing percentage damaged grain, but was correlated with the percentage grain weight loss (wtloss; $p < 0.05$), and grain moisture content (mc; $p < 0.05$), as well as with protein (protein; $p < 0.001$) and iron (iron; $p < 0.01$) content, and negatively with fibre content (fibre; $p < 0.01$) (Fig. 6).

Percentage damaged grain and percentage weight loss were negatively correlated with the available carbohydrate (avlcarbs; $p < 0.05$) and fibre (fibre; $p < 0.05$) content of the cowpea grains. As with both types of maize grain studied, insect infestation in the stored cowpea grain was positively correlated with increasing protein content (protein; $p < 0.001$) and increasing moisture content (mc; $p < 0.001$) of the remaining material. Additionally, insect infestation variables were also significantly positively correlated with increasing fat (fat; $p < 0.001$), iron (iron; $p < 0.001$), and zinc (zinc; $p < 0.05$) content. No correlation was found between the insect damage variables and the energy (kcal) content of the cowpea grains.

Significant correlations between insect infestation and nutrients could be used to develop a cowpea storage loss prediction calculation tool, a prototype example is shown in Supplemental Tool 2.

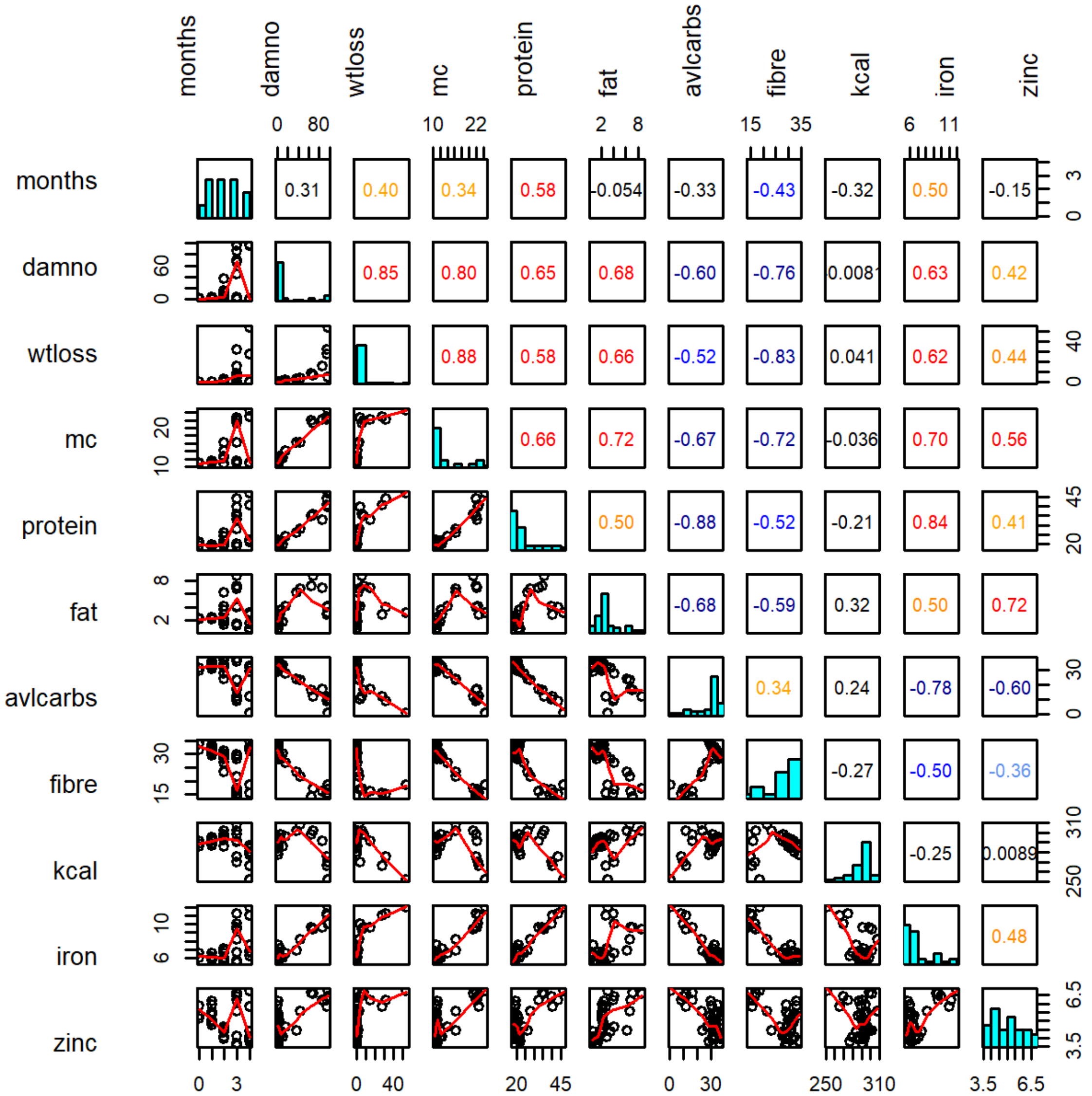

Fig. 11 Pairs plot and Spearman's rank correlations of relationships between storage insect damage factors and nutrients in stored cowpea grain *[Spearman's rank correlation critical values for N = 36* are *p = 0.05* 0.33*; *p = 0.01** 0.427*; *p = 0.001*** 0.533, the significantly positive correlations are shown in shades of yellow < red, and the significantly negative correlations in shades blue; abbreviations= months = storage duration; damno = percentage damaged grain (by number); wtloss = percentage grain weight loss; tot_ins = total number of insects /kg; mc = percentage grain moisture content; avlcarbs = available carbohydrates; kcal = energy (kcal/100g)]*

5 Discussion and conclusion

5.1 Impact of insect infestation on nutrient content of stored maize and cowpea grain

The results of our controlled laboratory bioassays can be summarised as follows:

1. Most nutrients in stored maize and cowpea grain remained stable over time in the absence of insect infestation with the exception of carotenoids, which degraded over the storage period
2. The three insect infestation variables measured (percentage damaged grains, percentage weight loss, and total number of insects per kg) were correlated
3. Correlations were found between insect infestation level (measured as percentage damaged grains, percentage weight loss, or total insects per kg) and some nutrients

a. Insect infestation level was negatively correlated with carbohydrate proportion as the storage insects consumed the carbohydrate over time
b. Insect infestation level was positively correlated with protein proportion as the carbohydrate proportion dropped
c. Insect infestation level was positively correlated with moisture content
d. Insect infestation level was largely independent of carotenoid quantities as they predictably degrade over time whether grain is infested or not
e. Changes in the proportion of fat, iron and zinc in insect-infested stored grain are insect species- and crop-specific.

The higher protein concentration associated with *S. zeamais* compared to *P. truncatus* might be due to different larval feeding patterns or other physiological and/or behavioural aspects. While *S. zeamais* larvae feed preferentially on the protein-poor endosperm (Sharifi, 1972; Sharifi & Mills, 1971; authors' laboratory investigations), *P. truncatus* larvae also feed heavily on the protein and fat-rich germ (Ramirez and Silver, 1983; authors' laboratory investigations) (Supplemental Fig. S1), and therefore metabolise a higher relative proportion of the protein and fat present in the original grain. As a result, the residual uneaten material tends to be enriched in proteins over time when the infestation is mainly caused by *S. zeamais*. Additionally, more of the developing larvae of *S. zeamais* than of *P. truncatus* remain inside feeding tunnels in the grain despite the sieving process, and if numerous, their presence may affect the nutrient composition of the complete remaining grain product. Extensive tunnelling damage is caused by the multiple *P. truncatus* larvae per infested grain (Ramirez and Silver, 1983), causing the grain to be more likely to break into small pieces. This may result in greater removal of *P. truncatus* than *S. zeamais* larvae during sieving. As a *S. zeamais* female only lays a single egg which it seals with an egg-plug in the cavity chewed into each cereal grain (Haines et al., 1991), the developing larva tends to leave the outer structure of the grain largely intact.

Similarly, increasing insect damage on stored cowpea led to decreased carbohydrate concentration and increasing concentration of protein, fat, iron, zinc and moisture. Cowpea has a more homogenous distribution of nutrients compared to maize grains. Thus, the increasing concentration of protein, fat and minerals in insect-infested cowpea is more likely to be linked to the presence of the protein, fat, iron and zinc-rich larval and pupal stages of the bruchid *C. maculatus*, which remain immobile and trapped inside the grains despite sieving (or winnowing), than to differential consumption of grain parts by the insects. In contrast, the iron content decreased in white and orange maize as insect damage increased. The unusually low carbohydrate values for cowpea probably result from the calculation used to derive carbohydrate by difference rather than direct measurement in the nutrient analysis, with some constituents being categorised as dietary fibre despite being carbohydrate (e.g. some resistant starches) (Haralampu, 2000).

While grain with a higher protein concentration would appear to be a positive nutritional outcome, insect infestation resulted in a higher concentration of protein but in a *smaller overall amount* of food, giving a net reduction in the overall amount of protein available (Fig. 12).

Our study confirmed the instability during grain storage of the nutritionally valuable carotenoids present in pVA biofortified orange maize, even without infestation (Mugode et al., 2014; De Moura et al., 2015; Bechoff and Dhuique-Mayer, 2017; Taleon et al., 2017). The pVA biofortified orange maize also sustained higher insect damage by 4 months' storage (69 % damaged grains) compared to the white hybrid maize grain (37 % damaged grains), despite identical numbers and species of insects being initially introduced. High susceptibility to storage insect damage combined with carotenoid instability during typical smallholder farmer postharvest drying and storage activities (Lividini and Fiedler, 2015), could limit the potential nutritional impact of pVA biofortified orange maize varieties. Improved grain storage practice and technologies such as hermetic grain storage bags can limit insect infestation. Recent research has shown the addition of oxygen scavengers to hermetic storage bags reduced carotenoid loss in pVA orange maize during storage durations of up to 6 months (Nkhata et al., 2019).

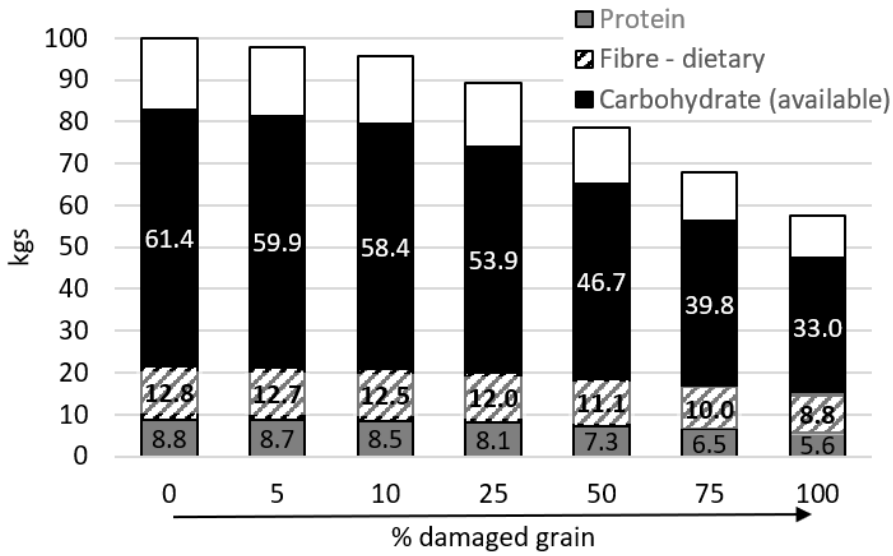

Fig. 12 Example of the quantity of protein, fibre and carbohydrate remaining in a stock of 100 kg of white maize grain at different insect infestation levels

5.2 Applicability of the work

In our study the crops were grown, harvested, threshed and dried by smallholder farming households. However, the study was laboratory-based and the grain stored under constant temperature and relative humidity conditions and in jars, therefore differing from farmer-stored grain which would experience varying temperature and relative humidity during the day and over seasons. In farmers' stores, insects disperse when high population densities are reached or food resources depleted, but could not disperse from our trial jars. Additionally, farmer-stored cereal and legume grains may be attacked by a wider range of insect species and/or by fungi and rodents. A similar experiment could evaluate the nutritional effect of rodent and fungal damage during crop storage. Rodents preferentially consume the grain's germ (Justice and Bass, 1979), cause contamination and by damaging packaging generate spillages (Mdangi et al., 2013). Given that mould developed in our cowpea and maize grain at the high insect damage levels, fungi which consume fat and carbohydrate for energy (Reed et al., 2007), will also have contributed to the nutrient changes. The fungi may also produce toxic secondary-metabolites and contaminate the grain with mycotoxins. In situations where food safety standards are implemented such contamination, if above the agreed maximum tolerable level, will result in a 100 % loss of all the grain and the nutrients it contained. In more typical SSA domestic food systems, where food safety standards are rarely monitored or enforced and the bulk of the population consume their own produce, grain infected by fungi may not be removed resulting in the consumption of grain with reduced nutritional content and the harmful effects of mycotoxin contamination (see Shephard, 2008, Ayalew et al., 2016, Omatayo et al., 2019 for further discussion).

Farm-level storage studies are needed to explore how our laboratory findings relating to the changes which occurred in the content and quantity of nutrients during storage of different grains, with different insect types and infestation levels, over different storage durations, compare to those experienced in farm-level stored grain. Further studies could also explore how the effect of storage insect-infestation on nutrient content differs between varieties and production locations, as variety and environmental factors such as soil quality and altitude are known to affect the nutritional composition of crops (Nuss et al., 2010; Charrondiere et al., 2013). These and other studies could further develop and validate our prototype grain storage nutrient loss calculation tools (Supplemental Tool 1 and 2), which require a user to input the percentage insect damage, to obtain a calculation of the remaining quantity and proportions of nutrients in their insect-infested stored grain.

In insect-infested stored grain, the percentage damaged grain and percentage grain weight loss are correlated. However, the relationship between them differs by grain and infesting insect species. For example, in maize a 5 % grain weight loss during storage equates to about 30 % of grains having insect holes in them (~30 % damaged grains), while for cowpeas a 5 % grain weight loss during storage equates to about 60 % damaged grains (Supplemental Fig. S2).

When preparing grain for consumption, some quality screening occurs. Grain is typically winnowed prior to being milled or cooked, this removes the trash portion (which contains insect waste, body parts, and grain dust generated during insect boring) which is not usually consumed. Additionally, for beans, cowpeas and other legumes in many SSA countries the damaged grains from a batch are removed by hand-picking (pers. obs.). In these cases where damaged legume grains are manually removed, the 'percentage damaged grains' will then represent the portion of the grain that is lost from human consumption. For example, cowpea can suffer a 5 % grain weight loss due to storage insect infestation but this may mean 60 % of the grains are damaged and thus removed from the batch and not consumed. Therefore, 60 % of all the nutrients would be lost from direct human consumption due to removal of damaged grains. Meanwhile, remaining early-stage infested grains, where damage is not easily externally visible, would be consumed rather than manually removed but contain less carbohydrate (and slightly increased concentrations of protein, fat, zinc and iron) compared to perfectly undamaged cowpea grain. Conversely, if a batch of a grain is winnowed, milled and processed into food without removal of damaged grains, as typically happens with many cereal grains in SSA countries, then the percentage grain weight loss figure will equate to the percentage weight of grain lost from human consumption, but the overall quality of the grain would also be reduced. However, a total loss can occur if stored grain is damaged by insects to such a degree that it becomes extremely mouldy, unappealing, and unfit for human consumption, although not all grains may exhibit visible insect damage.

A study in Tanzania found that although > 90 % of their sample of 120 households manually sorted out their insect-damaged and mouldy maize grains prior to storing the grain, 45 % of these households then consumed these insect-damaged and mouldy maize grains (Kimanya et al., 2008). Improving our understanding of consumers' behavioural responses and attitudes regards different types of postharvest quality deterioration is crucial for more accurately understanding and reducing the nutritional impacts of postharvest loss.

5.3 Further work and conclusions

Further work could validate and refine our findings and the prototype nutrient grain storage loss calculation tool and investigate the impact of different insect species and combinations on the nutrient composition of different stored staple crops and varieties of them. More detailed work could investigate the effect of insect infestation on essential amino acids (i.e. lysine and threonine which are limiting in cereals; methionine and tryptophan which are limiting in legumes (FAO, 1983)), and protein quality. Future studies could also quantify the extent to which the insect bodies themselves affect the grain product's nutrient composition when they are left in the grain after sieving or winnowing. Farm-level storage studies are needed to explore how similar our laboratory findings on the nutritional effect of insect-infestation are to those experienced in smallholder farmers' grain stores. Finally, it is important to understand more about consumer behaviour in response to different types of infestation – when, where, why and which damaged grains are or are not consumed. By quantifying the influence of insect infestation on the nutritional properties of stored grain we are better placed to understand how household nutrition and food security are affected by insect-mediated storage losses. In addition to the reduction in the quantity of grain stocks available for consumption due to insect damage, the protein-carbohydrate ratios and iron content can change significantly in insect-infested stored grain, with implications for the health of some of the most vulnerable groups of people.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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7 Supplemental information

Table S1 Nutrient composition analysis methods used

Determination	**Method description** (UKAS standard unless *)
Energy (kcal/100g) and (kJ/100g)	*Established by calculation 1g fat = 9 kcal/37kJ 1g protein = 4kcal/17kJ 1g carbohydrate = 4kcal/17kJ 1g fibre = 2kcal/8kJ
Protein (g/100g)	Established by calculation from nitrogen: protein = nitrogen x 6.25 *(as per Commission Regulation (EC) No 1169/2011, Annex I)*
Available Carbohydrate (by diffrn.) (g/100g)	Established by difference: 100 - (ash + moisture + fat + protein + fibre)
Fat (g/100g)	Fat determined by Soxhlet solvent extraction after pre-acid digestion. Fat determined by gravimetry *(based on VEMS (F/0177) procedure)*
Dietary Fibre (AOAC)	AOAC 985.29. Enzyme digestion followed by gravimetry
Iron (mg/100g)	Flame Atomic Absorption Spectrometry *(as per EC Commission Regulation No 152/2009)*
Zinc (mg/kg)	Flame Atomic Absorption Spectrometry *(as per EC Commission Regulation No 152/2009)*
Ash (g/100g)	Incineration followed by gravimetry *(based on VEMS (F/0003/5) procedure)*
Nitrogen (g/100g)	DUMAS technique- nitrogen determined by direct combustion and quantification by thermal conductivity
Moisture (%)	*Direct drying and moisture established by gravimetry *(as per EC Commission Regulation No 152/2009)*

*Key: * = not UKAS accredited; ^ = test method developed under UKAS flexible scope.*

Table S2 Nutrient content of test commodities at storage trial set-up (FWB, mean (±SEM), n=3)

Grain	**Energy** (kcal/ 100g)	**Protein** (g/ 100g)	**Fat** (g/ 100g)	**Dietary fibre** (g/100g)	**Available carbohydrate** (g/100g)	**Iron** (mg/100g)	**Zinc** (mg/100g)	**Moisture content** (%)
White hybrid maize	347.7 (±5.77)	8.63 (±0.15)	4.93 (±0.16)	12.80 (±1.22)	60.73 (±1.75)	2.23 (±0.80)	1.84 (±0.13)	11.7 (±0.21)
pro-Vitamin A biofortified orange maize	349.3 (±2.68)	6.13 (±0.11)	4.33 (±0.11)	10.67 (±1.11)	66.1 (±1.15)	2.73 (±0.42)	1.75 (±0.02)	11.8 (±0.12)
Cowpea	288.7 (±3.21)	20.43 (±2.50)	2.16 (±0.49)	32.93 (±1.94)	30.33 (±2.81)	6.37 (±0.59)	5.43 (±0.31)	10.9 (±1.38)

Table S3 Carotenoid content of pro-Vitamin A biofortified orange maize grain at storage trial set-up (µg/g, FWB, mean (±SEM), n=3)

Commodity	**Lutein**	**Zeaxanthin**	**Beta - cryptoxanthin**	**All-trans beta-carotene**	**cis-beta-carotene**	**Total pro-vitamin A**	**Total carotenoids**	**Moisture content (%)**
pro-Vitamin A orange maize grain	2.15 (±0.09)	3.38 (±0.14)	1.06 (±0.02)	0.71 (±0.03)	0.56 (±0.01)	1.52 (±0.04)	7.86 (±0.28)	11.8 (±0.12)

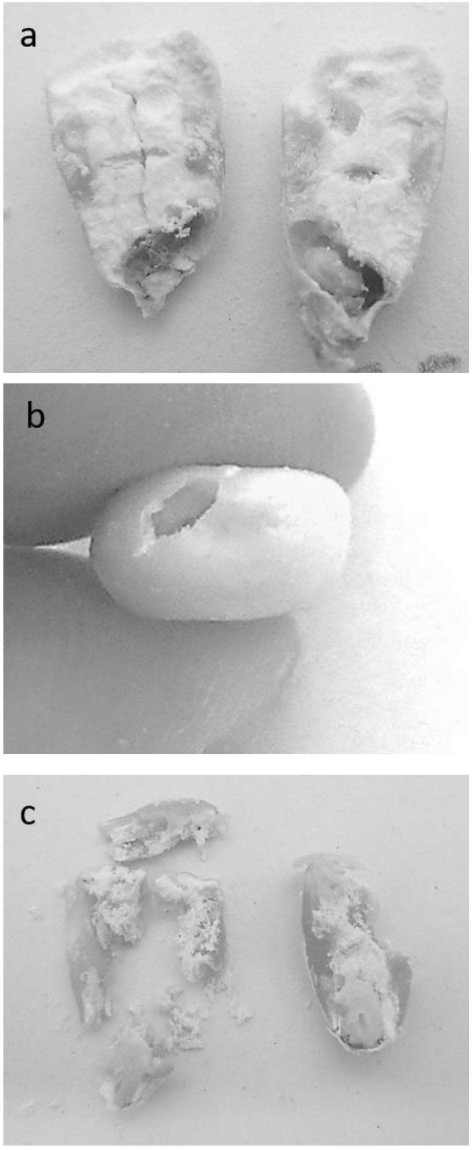


Fig. S1 Damage patterns to maize grain created by (a) *P. truncatus* and (b-c) *S. zeamais* larvae, showing (a) longitudinal section of grain with complete destruction of germ and presence of larva in the space previously occupied by the germ, (b) exit hole of *S. zeamais* from tip of grain and (c) longitudinal section of grain damaged by *S. zeamais* infestation showing superficial, if any, damage to germ but significant damage to floury endosperm

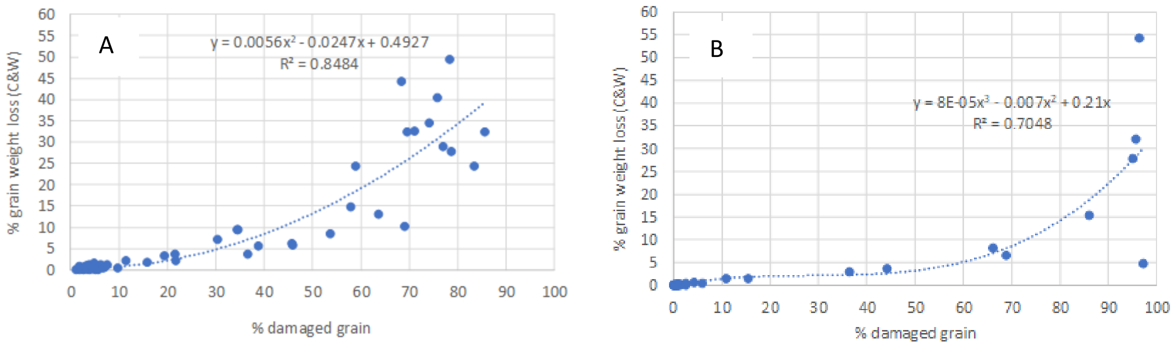


Fig. S2 Relationship between % grain weight loss and % damaged grains in a) insect-damaged stored maize and b) insect-damaged stored cowpea

Supplemental Tools: Prototype tools to estimate the remaining nutrient content of grain following storage insect pest attack

Using the relationships observed between insect damage, mass loss and nutrient composition in our laboratory studies, two simple interactive nutrient loss predictive tools were created to estimate the quantity and concentration of the different nutrients in the remaining stored product at a range of different grain damage levels. These prototype tools are shared to facilitate their testing and refining by other researchers.

White maize grain storage insect pest damage nutrient effect estimation tool

Two prototype interactive nutrient storage loss prediction tools (Supplemental Tool 1 and 2) were developed to help improve estimates of the change in the nutrient content of the stored crop due to insect damage. Using data generated during our laboratory trials, the tools aim to improve on the simplistic assumption that nutritional losses vary linearly with weight loss. The tools include those nutrients which during the trials correlated significantly with insect damage.

Example data for a range of white maize grain damage levels (i.e. 0, 5, 10, 25, 50, 75 and 100 %) are shown in Table S4. The decreasing mass of the stored white maize grain remaining as insect infestation increases is calculated using the final column of Table S4. The user enters: i) the percentage of damaged grain in their stored maize (based on a representative sample of their grain), and ii) the initial weight of the stored maize. The tool then calculates, A) the final quantity of maize remaining, B) the mass loss of each nutrient due to insect damage, C) the percentage change in the quantity of each nutrient between the initial and final amount, D) the absolute mass change of each nutrient in the stored maize due to the insect infestation. The tool uses data from samples where *S. zeamais* or *P. truncatus* or a mixture of the two insects were present; a similar tool could be constructed using data from samples where just one of the insect pests was present.

A similar tool was created for cowpea (Supplemental Tool 2) using the cowpea laboratory trial data. The relationships used to create this tool, and example data for a range of grain damage levels are shown in Table S5. Calculations in the ‘percentage damaged grain’ set of rows assume all the grain including the damaged grains is consumed. While in the ‘handpicking and removing damaged grain’ set of rows (shown in italic text), for grain damage levels between 5 to 75 % the assumption is that all *visibly* damaged grain is removed by handpicking and therefore not consumed by humans. The intention is for researchers to test these predictive tools and update them as new datasets become available.

Supplemental Tool 1 Storage nutrient loss due to insect infestation calculation tool for white maize

File name: Tool_1_WMz_Storage_N_Loss_V1_190511

MAIZE GRAIN STORAGE PEST INFESTATION NUTRIENT LOSS CALCULATION TOOL

This version is ONLY for stored white maize grain

Infested with non-specified insects

Version 1: Release 30 Mar 2019

UNIVERSITY of GREENWICH

NRI | Natural Resources Institute

Enter: initial weight of stored maize grain

Enter: % damage of stored maize grain

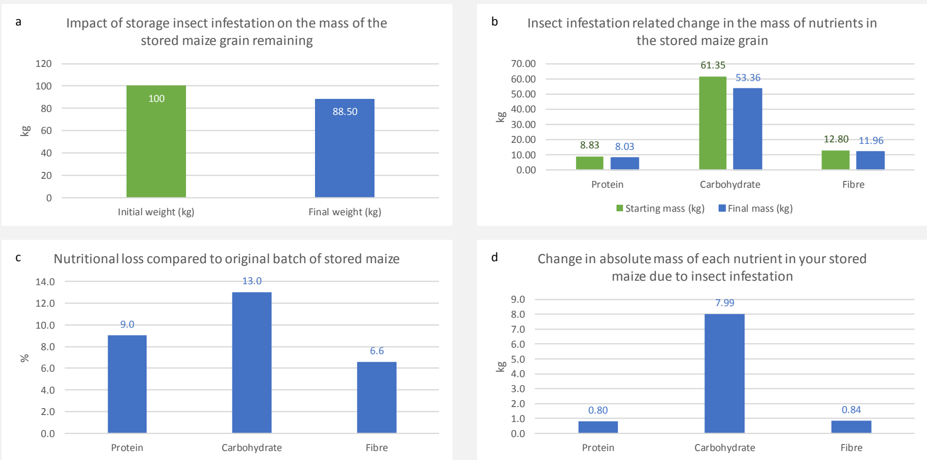
Commodity	Initial weight (kg)	% of damaged grains	Infesting insect	Final weight (kg)
White maize	100	27	Non-specific	88.50

Approximate change in the nutritional composition of your stored commodity

(Negative values indicate gains)

	Relative loss (% of original batch)	Absolute loss (kg)
Protein	9.0	0.80
Carbohydrate	13.0	7.99
Fibre	6.6	0.84
Energy	12.2	(kcals) 42,645

	Content model	Starting %	Starting mass (kg)	Final %	Final mass (kg)
Protein	y = 0.0091x + 8.8311	8.83	8.83	9.08	8.03
Carbohydrate	y = -0.0391x + 61.349	61.35	61.35	60.29	53.36
Fibre	y = 0.026x + 12.808	12.8	12.80	13.51	11.96
		Starting kcal/100g	Starting total kcals	Final kcal/100g	Final total kcals
Energy	y = -0.0971x + 350.59	351	350,590	348	307,945



Source: Stathers, T.E., Arnold, S.E.J., Rumney, C.J., Hopson, C., 2019. Measuring the nutritional cost of insect infestation of stored maize and cowpea.

Supplemental Tool 2 Storage nutrient loss due to insect infestation calculation tool for cowpea

File name: Tool_2_CP_Storage_N_Loss_V1_190511

COWPEA GRAIN STORAGE PEST INFESTATION NUTRIENT LOSS CALCULATION TOOL

This version is ONLY for stored cowpea grain

Version 1 Released: 04 May 2019

Enter: initial weight of stored cowpea grain

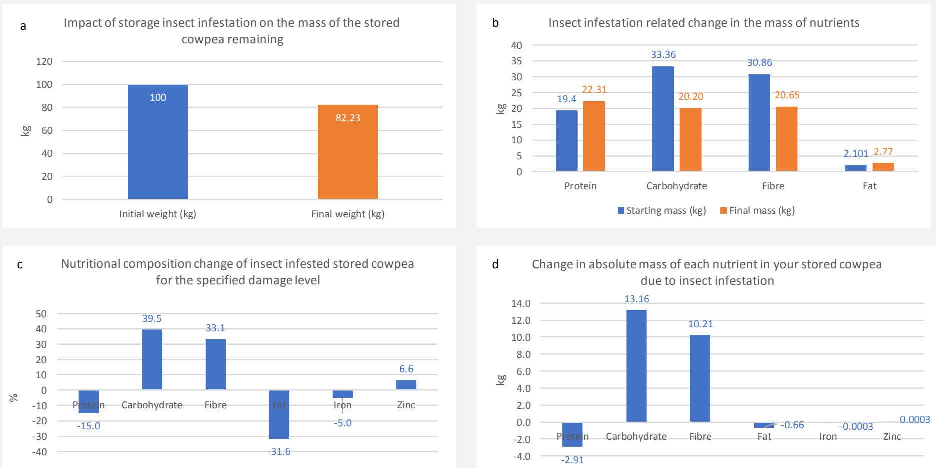
Enter: % damage of stored cowpea grain

Commodity	Initial weight (kg)	% of damaged grains	Infesting insect	Final weight (kg)
Cowpea	100	34	*Callosbruchus maculatus*	82.23

Approximate change in the nutritional composition of your stored commodity:
(Negative values indicate gains)

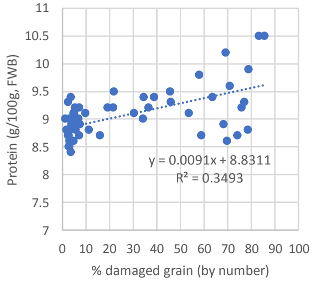
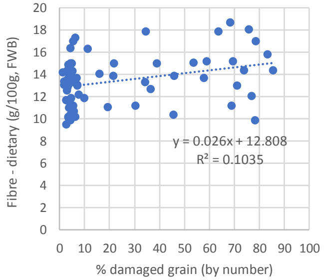
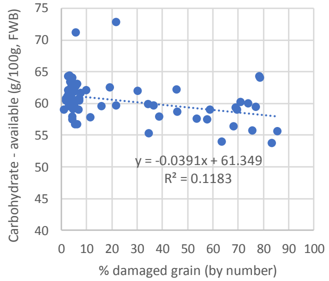
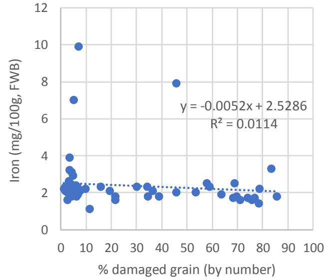
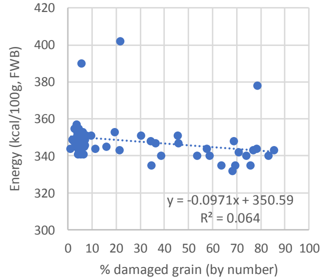
	Relative loss (% of original batch)	Absolute loss (kg)
Protein	-15.0	-2.91
Carbohydrate	39.5	13.16
Fibre	33.1	10.21
Fat	-31.6	-0.66
Iron	-5.0	-0.0003
Zinc	6.6	0.0003

	Content model	Starting %	Starting mass (kg)	Final %	Final mass (kg)
Protein	y = 0.2273x + 19.4	19.4	19.4	27.13	22.31
Carbohydrate	y = -0.2587x + 33.36	33.36	33.36	24.56	20.20
Fibre	y = -0.1693x + 30.864	30.86	30.86	25.11	20.65
Fat	y = 0.0371x + 2.1017	2.101	2.101	3.36	2.77
Iron	y = 0.0494x + 6.0449	0.00605	0.00605	0.0077	0.0064
Zinc	y = 0.0187x + 4.6366	0.00464	0.00464	0.0053	0.0043



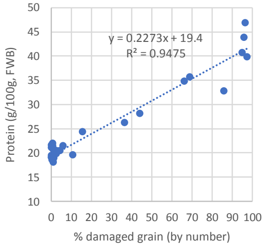
Source : Stathers, T.E., Arnold, S.E.J., Rumney, C.J., Hopson, C., 2019. Measuring the nutritional cost of insect infestation of stored maize and cowpea.

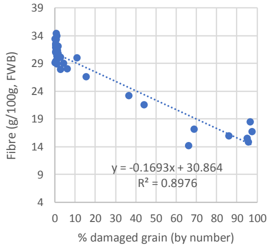
Table S4 Stored white maize grain insect pest damage nutrient effect estimation model

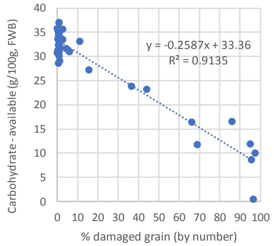
White maize stored grain *(FWB = 11.7%)*	Protein	Fibre	Carbohydrate (available)	Iron	Energy (kcal)	Grain mass remaining (%)
						
Relationship	y = 0.0091x + 8.8311	y = 0.026x + 12.808	y = -0.0391x + 61.349	y = -0.0052x + 2.5286	y = -0.0971x+ 350.59	
% damaged grain	*g per 100g*	*g per 100g*	*g per 100g*	*mg per 100g*	*kcal per 100g*	
0	8.83	12.81	61.35	2.53	350.59	100
5	8.88	12.94	61.15	2.50	350.10	98
10	8.92	13.07	60.96	2.48	349.62	96
25	9.06	13.46	60.37	2.40	348.16	89
50	9.29	14.11	59.39	2.27	345.74	79
75◆	9.51	14.76	58.42	2.14	343.31	68
100◆	9.74	15.41	57.44	2.01	340.88	57

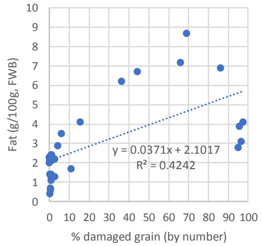
Key: ◆ = such heavily damaged grain is unappetising and unpalatable due to insects and mould and is unlikely to be consumed by humans unless there are extreme food shortages

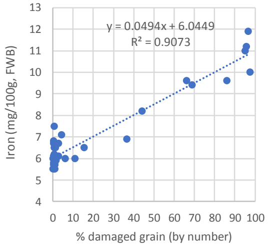
Table S5 Stored cowpea grain insect pest damage nutrient effect estimation tool model

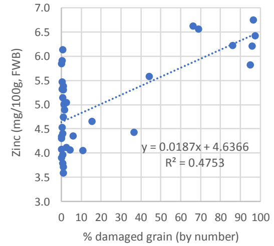












Cowpea stored grain	Protein	Fibre	Carbohydrate (available)	Fat	Iron	Zinc	Grain mass remaining (%)
Relationship	y = 0.2273x + 19.4	y = -0.1693x + 30.864	y = -0.2587x+ 33.36	y = 0.0371x+2.1017	y = 0.0494x + 6.0449	y = 0.0187x + 4.6366	
% damaged grain	g per 100g	g per 100g	g per 100g	g per 100g	mg per 100g	mg per 100g	
0	19.4	30.86	33.36	2.11	6.05	4.64	100
5	20.54	30.02	32.07	2.29	6.29	4.73	97
10	21.67	29.17	30.77	2.47	6.54	4.82	95
25	25.08	26.63	26.89	3.03	7.28	5.10	87
50	30.77	22.40	20.43	3.96	8.51	5.57	74
75◆	36.45	18.17	13.96	4.88	9.75	6.04	61
100◆	42.13	13.93	7.49	5.81	10.98	6.51	48

Handpicking and removing damaged grains will leave the following nutrient contents:

5	*18.43*	*29.32*	*31.69*	*2.00*	*5.75*	*4.41*	*95*
10	*17.46*	*27.77*	*30.02*	*1.90*	*5.45*	*4.18*	*90*
25	*14.55*	*23.15*	*25.02*	*1.58*	*4.54*	*3.48*	*75*
50	*9.70*	*15.43*	*16.68*	*1.06*	*3.03*	*2.32*	*50*
75◆	*4.85*	*7.72*	*8.34*	*0.53*	*1.51*	*1.16*	*25*

Key: ◆ = such heavily damaged grain is unlikely to be consumed due to its unpalatability and the high incidence of mould, etc.

Dr Tanya Stathers has worked as an agriculture for development researcher across sub-Saharan Africa for >25 years, with a particular focus on: postharvest systems and the reduction and understanding of postharvest losses (including their nutritional and financial value) in staple grains and root and tuber crops; multi-stakeholder learning processes; agricultural adaptation to climatic and other changes; creative training materials and approaches; rural-urban interdependencies; gender and diversity aspects of agri-food systems; and poverty impacts of market standards. Her earlier work focused on: participatory development of pre and postharvest integrated pest management options for a range of smallholder produced cash and food crops; including diatomaceous earth grain protectants, entomopathogenic fungi, pheromones, natural enemies, resistant varieties and the farmer field school approach. She also supervises postgraduate research students and has developed and teaches on several training courses. She is a principal scientist at the Natural Resources Institute (NRI) of the University of Greenwich.

Dr Sarah Arnold is a behavioural entomologist with a background in host- and food-seeking behaviours of economically important insects. After completing a BA at the University of Cambridge in Natural Sciences (Plant Sciences), followed by PhD at Queen Mary, University of London, she has carried out research at the Natural Resources Institute on the behavioural ecology of postharvest pests including their responses to odour and colour stimuli, and participates in research projects exploring the application of pesticidal plants in pre- and postharvest pest management in smallholder farming systems in sub-Saharan Africa.

Dr Corinne Rumney graduated in Applied Biology, specialising in Nutrition and Toxicology and subsequently completed a PhD in the microbial biochemistry of human gut microflora. For almost ten years she worked at the interface between diet, gut microflora and potential genotoxicity in the colon. After a career break of 7 years she spent five years working in the science department of a secondary school before taking on the role of research fellow / microbiologist / lab manager within the Food and Markets department of the Natural Resources Institute (NRI) of the University of Greenwich. As well as ensuring the smooth running of the labs, she gets involved in a wide variety of work in the fields of food safety and quality and postharvest storage, assists in the delivery of practical classes for MSc programmes in Food Safety and Quality Management and Food Innovation and supervises masters students in their lab-based projects.

Clare Hopson has worked in science for over thirteen years within a range of disciplines, from plant breeding, entomology, and physiology to consumer research, profile testing and commercial farm. She has extensive experience of working on a variety of top and soft fruit, vegetables, ornamental plants and flowers. Clare is particularly interested in supporting commerce through research, turning theories into practicalities that will improve industry. She is a research assistant, based at the Produce Quality Centre part of the Natural Resources Institute (NRI) of the University of Greenwich.