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Resistance of three-way cross experimental maize hybrids to post-harvest insect pests, the larger grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*)

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Abstract. The larger grain borer *Prostephanus truncatus* Horn and the maize weevil *Sitophilus zeamais* Motschulsky are important pests of stored maize in the tropics, particularly where maize is stored on-farm with little control of moisture content and without use of pesticides. This study was undertaken to determine level of resistance among new experimental maize hybrids against *P. truncatus* and *S. zeamais.* Out of the 54 experimental hybrids tested, eight hybrids were resistant, six were susceptible and the remaining 40 hybrids were moderately resistant. Five hybrids showed considerable reduction in losses for both *P. truncatus* and *S. zeamais* (CKPH08013, CKPH08021, CKPH08003, CKPH08004 and CKPH08009), suggesting that they contained genes that confer resistance to the two pests. Low grain weight loss, powder production and low insect multiplication on resistant grains reduce the negative impact of the two beetle pests. Therefore, host plant resistance can be used as a vital component of an integrated pest management strategy against *P. truncatus* and *S. zeamais*.

Key words: host resistance, maize, *Prostephanus truncatus*, *Sitophilus zeamais*, post-harvest loss

Introduction

The larger grain borer (LGB) *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) was accidentally introduced into Africa in the late 1970s from its area of origin in Mexico, where it had been recognized as an occasional pest of stored maize (Markham *et al.*, 1991). LGB is now widely recognized as the most destructive pest of farm-stored maize and dried cassava in Africa (Boxall, 2002). Losses to stored maize vary from 9 to 45% depending on periods of storage (Pantenius, 1988; Markham *et al.*, 1991; Schneider *et al.*, 2004; Gueye *et al.*, 2008).

The maize weevil (MW) *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an important pest of stored maize in the tropics, particularly when maize is stored on-farm, with no control of moisture content and without chemical protectants. Grain weight loss of 12–20% is common, and up to 80% loss may occur for untreated maize grain stored in traditional structures (Mutiro *et al.*, 1992; Boxall, 2002). There is also a health risk associated with consumption of weevil-infested maize grain, as damaged grain is

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prone to contamination by aflatoxins (Kankolongo *et al.*, 2009).

Application of synthetic insecticides has been recommended to protect grain against LGB and MW attack during storage. However, in addition to the health risk associated with insecticide use, these chemicals are frequently unavailable or too expensive for subsistence farmers in Africa (Golob, 2002; Dhliwayo and Pixley, 2003). Biological control is a suitable strategy, and two populations of *Teretrius nigrescens* Lewis, a histerid predator, were introduced into several African countries, among them Kenya, with varying degrees of success (Schneider *et al.*, 2004). Developing high-yielding maize varieties with resistance to LGB and MW has been regarded as a potential option to minimize the overall cost of production and storage of maize. Such varieties may also reduce the potential risk associated with consumption of treated maize with insecticides (Mugo *et al.*, 2008). Genetic variability exists among maize varieties for resistance to MW (Dobie, 1974; Serratos *et al.*, 1987; Tipping *et al.*, 1988; Arnason *et al.*, 1993; Derera *et al.*, 2001; Garcia-Lara *et al.*, 2004; Bamaiyi *et al.*, 2007) and LGB (Kumar, 2002; Mugo *et al.*, 2008; Mwololo *et al.*, 2010).

In an effort to design effective and efficient methods to control LGB and MW, the Insect Resistant Maize for Africa project was launched in 1999 by the International Maize and Wheat Improvement Center (CIMMYT) and the Kenya Agricultural Research Institute (KARI), with the aim of developing and deploying resistant, high yielding and adaptable maize hybrids through conventional breeding. Inbred lines, hybrids and open-pollinated varieties (OPVs) were developed and have been evaluated in preliminary trials and in the national performance trials, and seven hybrids and three OPVs were released for various maize growing ecologies of Kenya. The released hybrids and the current experimental hybrids originated from a cross between 'CubaGuard' and 'Kilima' as described by Derera *et al.* (1999). This paper reports on the level of resistance of new experimental maize hybrids to *P. truncatus* and *S. zeamais*.

Materials and methods

Development of parental lines and hybrid formation

Two susceptible elite inbred lines, CML 202 and CML 204, were crossed to 27 MW and LGB resistant advanced lines to obtain 54 single crosses (SC). The 54 SC served as parents in crosses with either CML 202 or CML 204 to obtain 54 three-way crosses hybrids (Table 1). The 27 selected lines were elite or advanced lines in the CIMMYT breeding programme, having above average resistance to MW in previous experiments (T. Tefera *et al.*, unpublished data). These resistant lines were developed from a cross between 'CubaGuard' and 'Kilima'. 'Kilima' is a Tanzanian OPV that has resistance to LGB (Derera *et al.*, 1999), whereas 'CubaGuard' was formed at CIMMYT in 1993 from the seed regeneration nursery of Caribbean landraces that had undergone more than four cycles of selection and inbreeding under infestation with LGB.

Field trial design and management

Two trials composed of 30 hybrids each were planted at Kiboko in Kenya, in 2009. Each trial involved 27 experimental hybrids, the parental lines carrying genes for resistance against *S. zeamais* and *P. truncatus*, along with a reference check (entry 28), a resistant check (entry 29) and a susceptible check (entry 30) (Table 1).

Kiboko is a semi-arid area located at latitude 2°1′S, longitude 37°7′E and altitude 975 masl. The trials were laid out in 6 × 5 alpha lattice designs with three replications. Two maize seeds were planted per hill in a row of 5 m length and thinned to one seedling per hill 2 weeks after emergence. There were two rows per plot spaced 75 cm apart with plant-to-plant distance of 25 cm to attain a population density of 53,000 plants/ha. Fertilizers were applied at the rate of 60 kg N and 60 kg P_2O_5/ha as recommended for the area. Nitrogen was applied in two splits. Supplemental irrigation was applied when needed. The fields were kept free of weeds by hand weeding.

Preparation of insects for infestation

Adults of *S. zeamais* and *P. truncatus* were obtained from KARI's Kiboko Postharvest Insect Pest Laboratory. Maize grains (400 g) were placed in 1-litre glass jars and covered with perforated lids. About 200 unsexed adult insects of the two species were separately introduced into the glass jars. After 10 days of oviposition, all adult insects were removed. Each glass jar was kept for progeny emergence. Progeny emergence was monitored daily and those emerged on the same day were transferred to fresh grain in glass jars with lids and kept at the experimental conditions until sufficient numbers of insects were obtained.

Preparation of grains and infestation with LGB and MW

The F_2 grain was used to evaluate resistance because this represents the generation that is stored by farmers and will be vulnerable to attack by MW and LGB. At harvest, a random bulk of about 500 g of grains was taken from each plot and fumigated with Phostoxin® for 7 days to disinfest from any

Table 1. List of entries evaluated for resistance to the maize weevil and to the larger grain borer

Entry	Name (set I)	Name (set II)
1	CKPH08002	CKPH09001
2	CKPH08003	CKPH08032
3	CKPH08004	CKPH09002
4	CKPH08005	CKPH08033
5	CKPH08006	CKPH09003
6	CKPH08008	CKPH08035
7	CKPH08009	CKPH08036
8	CKPH08010	CKPH08037
9	CKPH08011	CKPH08038
10	CKPH08012	CKPH08039
11	CKPH08013	CKPH08040
12	CKPH08015	CKPH08041
13	CKPH08017	CKPH08043
14	CKPH08018	CKPH08044
15	CKPH08019	CKPH08002
16	CKPH08020	CKPH08003
17	CKPH08021	CKPH08004
18	CKPH08023	CKPH09004
19	CKPH08025	CKPH08009
20	CKPH08026	CKPH08010
21	CKPH08027	CKPH08012
22	CKPH08028	CKPH08014
23	CKPH08029	CKPH08020
24	CKPH08030	CKPH08024
25	CKPH08031	CKPH08025
26	CKPH08036	CKPH08026
27	CKPH08043	CKPH08028
28	WH502 (RE)	WH502 (RE)
29	Local check (CKPH08020-resistant)	Local check (CKPH08020-resistant)
30	Local check (Duma-41 susceptible)	Local check (Duma-41 susceptible)

possible sources of infestation. The grains were sieved to remove any dirt, dust or broken grains. The moisture content of grains varied from 11 to 12%. About 100 g grains of each variety were kept in a glass jar at room temperature for 24 h before infestation with insects.

Single (no-choice) and multi-choice (free choice) tests were carried out. In the single choice test, thirty 7- to 10-day-old, unsexed MW or LGB adults were introduced into a glass jar containing 100 g grains of each hybrid. The insects were removed after 10 days of oviposition and feeding. The glass jars were covered with a lid made of wire mesh to allow adequate ventilation and prevent escape of the insects. The treatments were arranged in a completely randomized design with three replications kept on wooden shelves in a laboratory at 28 ± 2 °C and 65 ± 5% relative humidity (RH).

For the multiple choice test, de-husked but unshelled cobs, free from insect damage and cob rot, were fumigated in a plastic drum for 7 days with Phostoxin® tablets. There were three replications per entry, and three cobs per replication were labelled and put in a plastic net bag and hung about 2 m above the ground on a timber rail roof of a 2 × 3 m room. A total of 1000 adult insects, 500 individuals of each of the two species, were released on a plastic tray placed on the floor of the room, at three equidistant positions, with the supposition that the insects would crawl or fly to search the host of their choice. Small-scale farmers in Africa often store their maize cobs on the roof of their houses, where they are simultaneously exposed to an array of pests including LGB and MW. This method was, therefore, introduced to reflect a local practice. In the two tests, the mean temperature and RH were 28 ± 2 °C and 65 ± 5%, respectively. The treatments were incubated for 90 days.

Data collection and statistical analysis

In the single choice test, 90 days after incubation, the glass jars were opened, and the content was separated into grains, insects and dust using 4.7 and 1.0 mm sieves (Endecotts Ltd, UK). The number of live and dead insects and weight of the dust produced were recorded. The dust or flour produced due to insect feeding and the grains were weighed on a precision electronic scale. Dust weight was expressed as a percentage of the initial grain weight. Grain weight loss was determined using the bulk density method (Boxall, 2002). The percentage reduction in grain weight loss among the entries relative to the susceptible check was calculated as: 100 (% weight loss of entry − % weight loss of susceptible check) per % weight loss of susceptible check.

The number of insects was log transformed ($\log_{10}$), while dust production and weight loss were angular transformed (arcsine proportion). Significant differences among the means were detected using the *t*-test ($P < 0.05$). Data transformed back to original values are presented in the Tables. The susceptibility parameters (number of insects, % weight loss and % dust) were integrated to define the resistance reaction of the experimental hybrids. Therefore, selection index based on the three susceptibility parameters was done by summing the ratios between values and overall mean and dividing by 3 (number of parameters). The hybrids with selection index values less than 0.8 are regarded as resistant and those with a selection index greater than 0.8 as susceptible (Bergvinson *et al.*, 2004).

In the multiple choice test, the cobs were removed from the net bag and assessed for the insects' feeding damage based on a 0–10 visual scoring scale, where zero equals no visible damage and 10 equals 100% of the cobs totally damaged. Cobs with a scale of 0–2 were regarded highly resistant, 2.1–4 as resistant, 4.1–6 as moderately resistant, 6.1–8 as susceptible and 8.1–10 as highly susceptible (Kumar, 2002).

Table 2. Number of larger grain borers (LGB), % dust produced and grain weight loss 90 days after LGB artificial infestation of the seeds harvested for plants grown at Kiboko (Kenya)

	Set I hybrids			Set II hybrids		
Entry	No. of insects	% Dust	% Weight loss	No. of insects	% Dust	% Weight loss
1	105 ± 51	15.1 ± 6.8	23.45 ± 16.43	162 ± 13	26.19 ± 2.49	18.37 ± 1.7
2	95 ± 15	15.5 ± 1.1	23.27 ± 2.99	162 ± 44	31.8 ± 5.92	23.15 ± 4.43
3	90 ± 30	14.6 ± 3.0	22.02 ± 7.52	141 ± 31	26.94 ± 1.33	19.67 ± 1.04
4	139 ± 78	19.9 ± 5.4	28.93 ± 13.49	213 ± 65	36.83 ± 4.45	27.8 ± 3.24
5	118 ± 70	15.0 ± 4.3	22.71 ± 10.22	230 ± 64	38.87 ± 7.26	28.4 ± 5.93
6	111 ± 76	17.2 ± 4.1	25.90 ± 9.96	254 ± 40	40.41 ± 6.29	29.26 ± 4.67
7	70 ± 36	12.0 ± 4.2	18.85 ± 10.54	188 ± 0	32.7 ± 14.45	20.86 ± 9.58
8	178 ± 35	21.0 ± 3.6	30.12 ± 9.09	296 ± 36	44.05 ± 3.46	31.65 ± 2.12
9	136 ± 50	18.2 ± 3.6	27.05 ± 8.97	206 ± 70	32.59 ± 6.04	23.75 ± 4.57
10	135 ± 86	19.0 ± 5.4	28.35 ± 13.07	261 ± 18	46.44 ± 2.16	33.41 ± 1.67
11	41 ± 23	7.6 ± 2.5	12.05 ± 6.07	183 ± 50	33.7 ± 5.17	24.12 ± 3.98
12	111 ± 35	16.0 ± 4.0	23.90 ± 8.95	262 ± 25	38.69 ± 1.24	27.16 ± 7.86
13	98 ± 32	13.7 ± 3.3	20.70 ± 7.98	187 ± 34	33.04 ± 0.9	23.4 ± 0.81
14	69 ± 17	11.2 ± 2.6	16.69 ± 6.18	230 ± 19	35.07 ± 7.47	25.68 ± 5.37
15	160 ± 41	23.2 ± 3.0	33.33 ± 6.17	130 ± 45	25.32 ± 6.5	18.2 ± 5.02
16	86 ± 42	12.1 ± 3.7	18.21 ± 9.06	110 ± 30	14.93 ± 5.45	10.29 ± 3.39
17	64 ± 40	9.3 ± 3.9	14.65 ± 9.71	128 ± 45	19.67 ± 7.79	13.78 ± 5.43
18	130 ± 84	18.7 ± 6.6	27.38 ± 16.73	162 ± 57	30.58 ± 4.24	21.98 ± 3.19
19	132 ± 30	21.1 ± 2.1	31.24 ± 4.93	116 ± 40	21.95 ± 7.88	15.46 ± 5.56
20	108 ± 47	15.7 ± 3.8	23.46 ± 9.73	181 ± 73	34.96 ± 10.7	25.83 ± 8.02
21	94 ± 36	14.5 ± 4.7	21.99 ± 11.59	140 ± 72	25.7 ± 9.38	18.68 ± 6.73
22	77 ± 39	11.8 ± 4.7	17.68 ± 11.14	136 ± 10	26.41 ± 4.23	18.9 ± 3.18
23	88 ± 10	14.1 ± 1.0	25.22 ± 6.13	241 ± 27	40.25 ± 6.53	29.14 ± 4.67
24	114 ± 24	16.6 ± 1.9	26.25 ± 5.56	160 ± 80	29.72 ± 7.76	20.67 ± 5.93
25	104 ± 75	13.6 ± 6.4	21.33 ± 14.66	328 ± 53	48.56 ± 5.42	35.64 ± 4.08
26	161 ± 113	20.5 ± 8.3	29.98 ± 19.97	184 ± 41	36.66 ± 2.35	23.5 ± 7.4
27	125 ± 39	17.9 ± 3.6	26.62 ± 8.51	166 ± 18	25.79 ± 3.82	18.64 ± 2.77
28	153 ± 92	22.5 ± 6.2	33.55 ± 14.62	247 ± 31	44.36 ± 5.98	32.73 ± 4.26
29	110 ± 73	16.3 ± 5.7	25.29 ± 14.1	160 ± 25	30.95 ± 6.76	22.29 ± 5.09
30	141 ± 32	26.2 ± 1.8	37.69 ± 4.85	230 ± 19	33.02 ± 4.57	36.75 ± 3.53
Mean	111.46	16.38	24.59	189	32.5	23.3
t	−1.17	1.50	1.63	4.22	4.47	1.65
P	0.30	0.02	0.17	0.01	0.03	0.02

Results

No-choice test

There were significant differences among hybrids included in set I for LGB in % dust (Table 2). Entries 11 and 17 produced the lowest dust. There were also significant differences among hybrids in set II in number of insects, % dust produced and % grain weight loss due to LGB (Table 2). The lowest % grain weight loss of 1.8–2.9% was recorded for entries 2, 10, 11, 16, 21 and 22. On the other hand, entries 28 and 30 suffered relatively higher weight loss compared with the rest of the entries. Entries 16, 17 and 19 had lowest % dust production of 14.9–21% and weight loss of 10.2–15.4%; on the contrary, entries 8, 10, 25, 28 and 30 produced the highest % dust of 44.3–48.5% and weight loss of 31.6–35.6% due to LGB feeding. Fewer insects and lower percentages of dust and weight losses were observed in MW-infested entries (Table 3). The susceptible check (entry 30) harboured large numbers of insects and produced relatively high percentages of dust and weight losses.

Among the set I hybrids, reduction in grain weight loss over the susceptible check was 61.4–68.2% for entries 11 and 17 against LGB (Table 4). Entries 2, 10, 11, 21 and 22 had 60.4–74.5% reduction in weight loss against MW (Table 4). In the set II hybrids, entries 16, 17 and 19 had 72, 62.5 and 57.9% reduced weight loss over the susceptible check against the larger grain borer, respectively (Table 4). The set I hybrids appeared to be more tolerant to MW than LGB with over 90% reduction in grain weight losses for entries 15, 17 and 27. When the selection index was calculated, entries 2, 3, 10, 13, 14, 17, 21, 22 and 29 were resistant

Table 3. Number of maize weevils (MW), % dust produced and grain weight loss 90 days after MW artificial infestation of the seeds harvested for plants grown at Kiboko (Kenya)

	Set I hybrids			Set II hybrids		
Entry	No. of insects	% Dust	% Weight loss	No. of insects	% Dust	% Weight loss
1	40 ± 11	0.28 ± 0.06	5.47 ± 1.1	55 ± 19	4.44 ± 1.15	0.41 ± 0.14
2	15 ± 7	0.1 ± 0.03	2.91 ± 0.65	30 ± 9	2.6 ± 0.95	0.2 ± 0.05
3	15 ± 6	0.14 ± 0.05	3.36 ± 0.27	41 ± 1	3.48 ± 0.86	0.24 ± 0.04
4	30 ± 9	0.21 ± 0.07	3.91 ± 0.83	31 ± 17	2.91 ± 1.62	0.22 ± 0.12
5	28 ± 6	0.22 ± 0.04	3.64 ± 0.34	70 ± 27	5.7 ± 1.92	0.54 ± 0.31
6	38 ± 4	0.23 ± 0.02	3.82 ± 0.39	21 ± 5	1.52 ± 0.49	0.15 ± 0.01
7	35 ± 8	0.27 ± 0.08	4.09 ± 1.03	28 ± 11	2.69 ± 0.47	0.22 ± 0.09
8	66 ± 27	0.43 ± 0.19	6.95 ± 2.05	79 ± 30	6.72 ± 1.8	0.62 ± 0.29
9	35 ± 2	0.2 ± 0.01	3.73 ± 0.37	29 ± 12	2.49 ± 0.77	0.21 ± 0.08
10	6 ± 3	0.07 ± 0.03	1.87 ± 0.28	75 ± 37	5.53 ± 2.45	0.42 ± 0.17
11	12 ± 5	0.11 ± 0.04	2.40 ± 0.47	34 ± 8	3.49 ± 0.6	0.17 ± 0.03
12	43 ± 7	0.28 ± 0.04	5.04 ± 0.40	43 ± 22	4.31 ± 1.66	0.31 ± 0.14
13	22 ± 10	0.15 ± 0.05	3.22 ± 0.60	67 ± 31	5.56 ± 1.96	0.4 ± 0.15
14	26 ± 5	0.17 ± 0.04	3.26 ± 0.15	27 ± 11	3.09 ± 1.1	0.18 ± 0.07
15	50 ± 15	0.37 ± 0.08	5.60 ± 1.18	27 ± 20	2.64 ± 1.83	0.09 ± 0.01
16	19 ± 6	0.14 ± 0.04	2.98 ± 0.36	26 ± 9	2.25 ± 0.14	0.18 ± 0.07
17	29 ± 12	0.21 ± 0.10	3.49 ± 0.69	14 ± 4	2.17 ± 0.69	0.11 ± 0.03
18	44 ± 22	0.30 ± 0.10	4.96 ± 1.43	23 ± 18	2.06 ± 1.19	0.15 ± 0.09
19	39 ± 24	0.32 ± 0.22	4.64 ± 2.22	17 ± 7	2.41 ± 0.76	0.12 ± 0.02
20	32 ± 2	0.23 ± 0.02	4.10 ± 0.23	23 ± 16	2 ± 1.5	0.13 ± 0.08
21	15 ± 6	0.14 ± 0.05	2.45 ± 0.60	28 ± 12	2.63 ± 1.02	0.21 ± 0.04
22	18 ± 3	0.12 ± 0.02	2.40 ± 0.28	27 ± 21	1.96 ± 1.21	0.19 ± 0.11
23	64 ± 4	0.45 ± 0.05	6.10 ± 0.52	32 ± 11	3.45 ± 1.03	0.26 ± 0.1
24	34 ± 2	0.23 ± 0.01	4.19 ± 0.41	49 ± 27	5 ± 1.9	0.37 ± 0.17
25	25 ± 0	0.20 ± 0.02	3.61 ± 0.23	30 ± 21	3.28 ± 1.59	0.2 ± 0.11
26	50 ± 21	0.31 ± 0.15	5.20 ± 1.82	34 ± 22	3.38 ± 2.02	0.29 ± 0.14
27	32 ± 8	0.19 ± 0.02	3.66 ± 0.32	16 ± 8	1.54 ± 0.35	0.11 ± 0.04
28	79 ± 22	0.71 ± 0.22	8.56 ± 1.12	61 ± 29	4.82 ± 2.09	0.42 ± 0.21
29	32 ± 10	0.21 ± 0.06	3.85 ± 0.55	67 ± 39	5.42 ± 2.45	0.41 ± 0.22
30	61 ± 26	0.52 ± 0.18	7.35 ± 1.81	133 ± 30	9.18 ± 2	1.13 ± 0.4
Mean	34.45	0.25	4.22	41.2	3.6	0.28
t	1.72	1.87	1.33	−2.07	−1.91	2.13
P	0.01	0.01	0.01	0.10	0.12	0.03

Table 4. Percentage reduction in grain weight loss over the susceptible check

	Set I hybrids		Set II hybrids	
Entry	LGB	MW	LGB	MW
1	38.2	25.5	50.0	63.7
2	38.7	60.4	37.0	82.3
3	42.0	54.2	46.4	78.7
4	23.8	46.8	24.3	80.5
5	40.2	50.4	22.7	52.2
6	31.8	48.0	20.3	86.7
7	50.3	44.3	43.2	80.5
8	20.7	5.4	13.8	45.1
9	28.8	49.2	35.3	81.4
10	25.3	74.5	9.0	62.8
11	68.2	67.3	34.3	84.9
12	37.1	31.4	26.0	72.5
13	45.5	56.1	36.3	64.6
14	56.0	55.6	30.1	84.0
15	12.2	23.8	50.4	92.0
16	52.0	59.4	72.0	84.0
17	61.4	52.5	62.5	90.2
18	27.9	32.5	40.1	86.7
19	17.7	36.8	57.9	89.3
20	38.2	44.2	29.7	88.4
21	42.1	66.6	49.1	81.4
22	53.4	67.3	48.5	83.1
23	33.6	17.0	20.7	76.9
24	30.9	42.9	43.7	67.2
25	43.8	50.8	3.0	82.3
26	21.1	29.2	36.0	74.3
27	29.9	50.2	49.2	90.2
28	11.7	16.4	10.9	62.8
29	33.4	47.6	39.3	63.7
Mean	36.4	43.9	35.9	77.0

LGB, larger grain borer; MW, maize weevil.

for set I hybrids, while entries 4, 11, 15, 16, 17, 18, 19, 20, 21, 22, 26 and 27 were resistant for set II hybrids (Table 5). The number of insects, % dust production and grain weight loss were highly correlated (Table 6).

Multi-choice test

The majority of the hybrids were moderately resistant when subjected to feeding by mixed populations of the two beetles on unshelled cobs (Table 7). The resistant hybrids include entries 2, 22 and 29 from set I and entries 4, 16, 17, 18 and 26 from set II.

Discussion

This study demonstrated differences in resistance levels among maize hybrids with respect to number of insects, seed damage, seed weight loss and the susceptibility rating. These differences in the susceptibility of the hybrids indicate the inherent ability of a particular hybrid to resist *P. truncatus* and *S. zeamais* attack. Out of the 54 experimental hybrids tested against LGB and MW, eight hybrids were resistant, six were susceptible and the remaining 40 hybrids were moderately resistant using visual cob damage score. However, 37 hybrids were regarded susceptible and 23 were resistant using the selection index. The resistant hybrids showed considerably less % grain weight reduction for both beetles, suggesting that they contained genes that confer resistance to the two pests co-existing in farmers' stores. Resistance of stored maize to insect attack has been attributed to physical factors such as grain hardiness, pericarp surface texture and nutritional factors such as amylose, lipid and protein content (Dobie, 1974; Tipping *et al.*, 1988) or non-nutritional factors, especially phenolic compounds (Serratos *et al.*, 1987). The role phenolics play in resistance formation in these surface tissues may be both related to

Table 5. Selection index (SI) and reaction of set I and set II maize hybrids against larger grain borer and maize weevil

Entry	Set I hybrids SI	Reaction	Set II hybrids SI	Reaction
1	1.06	Susceptible	1.07	Susceptible
2	0.70	Resistant	0.83	Susceptible
3	0.72	Resistant	0.86	Susceptible
4	1.04	Susceptible	0.69	Resistant
5	1.09	Susceptible	1.47	Susceptible
6	1.00	Susceptible	0.88	Susceptible
7	0.86	Susceptible	0.84	Susceptible
8	1.55	Susceptible	1.70	Susceptible
9	1.01	Susceptible	0.87	Susceptible
10	0.73	Resistant	1.51	Susceptible
11	0.44	Resistant	0.70	Resistant
12	1.08	Susceptible	1.16	Susceptible
13	0.75	Resistant	1.26	Susceptible
14	0.69	Resistant	0.92	Susceptible
15	1.40	Susceptible	0.65	Resistant
16	1.07	Susceptible	0.56	Resistant
17	0.70	Resistant	0.53	Resistant
18	1.17	Susceptible	0.73	Resistant
19	1.20	Susceptible	0.57	Resistant
20	0.94	Susceptible	0.78	Resistant
21	0.69	Resistant	0.74	Resistant
22	0.61	Resistant	0.70	Resistant
23	1.29	Susceptible	1.06	Susceptible
24	0.99	Susceptible	1.08	Susceptible
25	0.83	Susceptible	1.18	Susceptible
26	1.30	Susceptible	0.71	Resistant
27	0.97	Susceptible	0.61	Resistant
28	1.87	Susceptible	1.39	Susceptible
29	0.75	Resistant	0.72	Resistant
30	1.66	Resistant	2.27	Susceptible

Table 6. Correlation between number of insects, % dust production and weight loss

	LGB (set I)		MW (set I)	
	% Powder	% Weight loss	% Powder	% Weight loss
No. of insects	0.901**	0.905**	0.924**	0.951**
% Powder	—	0.992**	—	0.919**
	LGB (set II)		MW (set II)	
No. of insects	0.915**	0.920**	0.852**	0.924**
% Powder	—	0.919**	—	0.886**

LGB, larger grain borer; MW, maize weevil. **Significant at $P < 0.01$.

structural components and antibiosis factors (Arnason *et al.*, 1993; Garcia-Lara *et al.*, 2004). For *Sitophilus oryzae* (L.), grain hardiness has been reported as the main resistance parameter (Bamaiyi *et al.*, 2007). Although resistance is governed primarily by genetics, physical and biotic factors in the environment also influence its expression (Altieri and Nicholls, 2003). The studied hybrids are currently being tested under different maize growing agroecologies in eastern and southern Africa to select best adaptive hybrids across environments.

Resistance to MW was reported for Mexican landraces, notably Si Naloa 35 and Yucatan-7

Table 7. Ear damage to maize hybrids by the larger grain borer and maize weevil 90 days after incubation of ears from two sets of trials

	Trial			
Entry	Set I	Category	Set II	Category
1	4.2	Moderately resistant	6.3	Susceptible
2	4.0	Resistant	4.7	Moderately resistant
3	5.4	Moderately resistant	4.3	Moderately resistant
4	5.2	Moderately resistant	3.7	Resistant
5	6.1	Susceptible	4.2	Moderately resistant
6	4.9	Moderately resistant	5.7	Moderately resistant
7	5.6	Moderately resistant	4.1	Moderately resistant
8	4.8	Moderately resistant	5.3	Moderately resistant
9	5.0	Moderately resistant	5.4	Moderately resistant
10	5.6	Moderately resistant	5.9	Moderately resistant
11	4.7	Moderately resistant	3.6	Resistant
12	6.0	Susceptible	6.4	Susceptible
13	4.7	Moderately resistant	5.7	Moderately resistant
14	5.1	Moderately resistant	4.5	Moderately resistant
15	6.6	Susceptible	4.5	Moderately resistant
16	6.5	Susceptible	3.5	Resistant
17	5.1	Moderately resistant	2.6	Resistant
18	4.9	Moderately resistant	4.0	Resistant
19	4.9	Moderately resistant	4.2	Moderately resistant
20	5.1	Moderately resistant	4.1	Moderately resistant
21	4.4	Moderately resistant	6.2	Moderately resistant
22	4.0	Resistant	4.2	Moderately resistant
23	4.5	Moderately resistant	5.3	Moderately resistant
24	5.3	Moderately resistant	6.5	Moderately resistant
25	4.9	Moderately resistant	5.2	Moderately resistant
26	5.1	Moderately resistant	3.2	Resistant
27	4.9	Moderately resistant	4.8	Moderately resistant
28	6.7	Susceptible	7.3	Susceptible
29	3.0	Resistant	5.5	Moderately resistant
30	7.5	Susceptible	7.5	Susceptible

Rating is on a 0–10 scale, with 0–2, highly resistant; 2.1–4, resistant; 4.1–6, moderately resistant; 6.1–8, susceptible; 8.1–10, highly susceptible.

(Arnason *et al.*, 1994), for the Tanzanian OPV 'Kilima' (Derera *et al.*, 1999), for tropical inbred lines Hi41, Hi34, ICA L29, KU1409, Hi39 and ICA L221 (Kim *et al.*, 1988) and for temperate inbred lines B37, B68, R805 and T220 (Tipping *et al.*, 1988). Grain factors reported to contribute to resistance include increased grain hardiness and sugar content (Sing and McCain, 1963).

The differences recorded among the hybrids in rating damage during the free-choice test indicated that LGB and MW preferred some hybrids relative to others. While our study did not investigate the chemical basis of the grain's non-preference resistance, Garcia-Lara *et al.* (2004) and Derera *et al.* (2001) reported that non-preference was based on the lack of feeding stimulants in the resistant grains. Our results suggest that it is possible to develop hybrids with improved resistance to *P. truncatus* and *S. zeamais*. There existed differences and similarities between the two ways of grouping (visual scoring and selection index) the genotypes into resistant and susceptible categories. For instance, from the set I hybrids, entries 2, 5, 12, 15, 16, 22, 28 and 29 and entries 1, 4, 11, 12, 16, 17, 18, 26, 27 and 30 from set II hybrids were grouped under the same category in the two tests. Since the two methods complement each other, we recommend advancing those hybrids that are consistently resistant in both tests. Although the free-choice test is relatively simple and cost-effective, the distribution of the insects might not be uniform and this could probably lead to escape by a few genotypes. The no-choice test set-up represents a rigorous exposure, in which the insects have no choice other than subsisting on a given genotype. In no-choice tests, confining insects with lower ranked hosts induces deprivation. Deprivation can induce some insects to accept a genotype it may reject if not deprived (Withers, 1997). Therefore, complete or partial acceptance of genotypes by an insect in no-choice tests does not necessarily reflect a lack of field specificity.

The number of insects and % dust produced for LGB exceeded that of MW, indicating that *P. truncatus* converts grain into powder within a short period of time. The extensive tunnelling in maize grain by LGB adults allows it to convert grain into flour within a short time. The flour produced during the insects' feeding consists of insect eggs, excreta and exuviae that are unfit for both livestock and human consumption. LGB belongs to the wood-boring family of Bostrichidae with strong mandibles (Hill *et al.*, 2004). *P. truncatus* has a remarkable ability to tunnel through hard materials including plastic thickness of 35 mm (Li, 1988).

The significant correlation among the number of insects, % dust and grain weight loss indicate that resistance in maize against LGB and MW can be expressed in terms of any of the three measured parameters. Resistant hybrids produced less powder, suffered less grain weight loss and had less multiplication of the two beetles. Grains from the resistant hybrids were not attractive to LGB and MW to feed on and reproduce as freely as the susceptible check, indicating that antibiosis was a mechanism of resistance operating within the resistant hybrids. In the present study, we used de-husked cobs although some farmers in Africa practice storing maize with husks. Demissie *et al.* (2008) stated that an exposed cob is more vulnerable to MW than one enclosed in the husk, and good husk cover is considered key to protecting the cob from insect and fungi attack.

Abraham (1991) indicated that the extent of damage during storage depends on the number of emerging adults during each generation and the duration of each life cycle, and grains permitting more rapid and higher levels of adult emergence will be more seriously damaged. Several maize varieties, including local land races, have been characterized as sources of resistance to MW (Giga and Mazarura, 1991) and some sources of resistance have been incorporated into elite maize lines (Bergvinson, 2001). Those hybrids with low rates of susceptibility can be stored for relatively longer periods of time. Resistant varieties, therefore, can be utilized as an environmentally friendly way to reduce damage by *P. truncatus* and *S. zeamais* under traditional storage conditions. The parental lines of the resistant hybrids identified in the present study can also be used as a source of resistance in breeding programmes to diversify the basis of resistance to the two pests. Low grain weight loss, powder production and low insect multiplication on resistant grains will reduce the negative impact of LGB and MW. Therefore, host plant resistance can be used as a vital component of an integrated pest management strategy against the two beetles. Large numbers of germplasm should be screened to identify more sources of resistance, particularly against *P. truncatus*.

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References

Abraham T. (1991) The biology, significance and control of the maize weevil, *Sitophilus zeamais* Motsch., (Coleoptera: Curculionidae) on stored maize. MSc Thesis, Alemaya University, Ethiopia.

Altieri M. A. and Nicholls C. I. (2003) Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. *Soil and Tillage Research* 72, 203–211.

Arnason J. T., Gale J., Conilh de Beyssac B., Sen A., Miller S. S., Philogene B. J. R., Lambert J. D. H., Fulcher R. G., Serratos A. and Mihm J. (1993) Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* Motsch. *Journal of Stored Products Research* 28, 119–126.

Arnason J. T., Baum B., Gale J., Lambert J. D. H., Bergvinson D., Philogene B. J. R., Serratos J. A., Mihm J. and Jewell D. C. (1994) Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxanomic and biochemical parameters. *Euphytica* 74, 227–236.

Bamaiyi L. J., Dike M. C. and Onu I. (2007) Relative susceptibility of some sorghum varieties to the rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Journal of Entomology* 4, 387–392.

Bergvinson D. J. (2001) Storage pest resistance in maize, pp. 32–39. Maize Research Highlights 1999–2000. *CIMMYT Technical Bulletin 6815* (edited by S. Pandey), CIMMYT, Mexico DF.

Bergvinson D. J., Vasal S. K., Singh N. N., Panwar V. P. S. and Sekhar J. C. (2004) Advances in conventional breeding for insect resistance in tropical maize, pp. 325–338. In *New Technologies for the New Millennium*. Proceedings of the 8th Asian Regional Maize Workshop, Bangkok, Thailand, 5–8 August 2002 (edited by G. Srinivasan, P. H. Zaidi, B. M. Prasanna, F. Gonzalez and K. Lesnick). International Maize and Wheat Improvement Center (CIMMYT), Mexico DF.

Boxall R. A. (2002) Damage and loss caused by the larger grain borer *Prostephanus truncatus*. *Integrated Pest Management Review* 7, 105–121.

Demissie G., Tefera T. and Tadesse A. (2008) Importance of husk covering on field infestation of maize by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) at Bako, Western Ethiopia. *African Journal of Biotechnology* 7, 3774–3779.

Derera J., Giga P. D. and Pixley K. (1999) Resistance of maize to the maize weevil: II. Non-preference. *African Crop Science Journal* 9, 441–450.

Derera J., Giga D. P. and Pixley K. V. (2001) Resistance of maize to the maize weevil: II. Non-preference. *African Crop Science Journal* 9, 441–450.

Dhliwayo T. and Pixley K. V. (2003) Divergent selection for resistance to maize weevil in six maize populations. *Crop Science* 43, 2043–2049.

Dobie P. (1974) The susceptibility of different types of maize to postharvest infestation by *Sitophilus zeamais* and *Sitotroga cerealella* and the importance of this factor at the small-scale farm level, pp. 98–113. In *Proceedings of the 1st International Working Conference on Stored-Product Entomology*. 7–11 October 1974, Savannah, Georgia, USA. (edited by E. U. Brady, J. H. Brower, P. E. Hunter, E. G. Jay, P. T. M. Lum, H. O. Lund, M. A. Mullen and R. Davis). IWCSPP, USA.

Garcia-Lara S. G., Bergvinson D. J., Burt A. J., Ramputh A. I., Pontones D. M. D. and Arnason J. T. (2004) The role of pericarp cell wall components in maize weevil resistance. *Crop Science* 44, 1546–1552.

Giga D. P. and Mazarura U. W. (1991) Levels of resistance to the maize weevil, *Sitophilus zeamais* in exotic, local open pollinated and hybrid maize germplasm. *Insect Science and Its Application* 12, 159–169.

Golob P. (2002) Chemical, physical and cultural control of *Prostephanus truncatus*. *Integrated Pest Management Reviews* 7, 245–277.

Gueye M. T., Goergen G., Badiane D., Hell K. and Lamboni L. (2008) First report on occurrence of the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Senegal. *African Entomology* 16, 309–311.

Hill M. G., Borgemeister C. and Nansen C. (2004) Ecological studies on the larger grain borer, *Prostephanus truncatus* (Horn) (Col.: Bostrichidae) and their implications for integrated pest management. *Integrated Pest Management Reviews* 7, 201–221.

Kankolongo M. A., Hell K. and Nawa I. N. (2009) Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. *Journal of Food Science and Agriculture* 89, 1366–1375.

Kim S. K., Brewbaker J. L. and Hallauer A. R. (1988) Insect and disease resistance from tropical maize for use in temperate zone hybrids, pp. 194–226. In *Proceedings of the 43rd Annual Corn and Sorghum Conferences*, Chicago, IL. 8–9 December 1988. American Seed Trade Association, Washington, DC.

Kumar H. (2002) Resistance in maize to the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Journal of Stored Products Research* 38, 267–280.

Li L. (1988) Behavioral ecology and life history evolution in the larger grain borer, *Prostephanus truncatus* (Horn). PhD thesis, University of Reading, UK. 229 pp.

Markham R. H., Wright V. F. and Rios Ibarra R. M. (1991) A selective review of research on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae) with an annotated and updated bibliography. *CEIBA* 32, 90 pp.

Mugo S., Mulaa M., Likhayo P. and Gichuki S. (2008) Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document no. 30. CIMMYT, Nairobi.

Mutiro C. F., Giga D. P. and Chetsanga P. (1992) Postharvest damage in small farmers' stores. *Zimbabwe Journal of Agricultural Research* 30, 49–59.

Mwololo J. K., Mugo S., Okori P., Tefera T. and Munyiri S. W. (2010) Genetic diversity for resistance to larger grain borer in maize hybrids and open pollinated varieties in Kenya, pp. 535–539. In *Second RUFORUM Biennial Meeting*. 20–24 September 2010. Entebbe, Uganda.

Pantenius C. U. (1988) Storage losses in traditional maize granaries in Togo. *Insect Science and Its Application* 9, 725–735.

Schneider H., Borgemeister C., Sétamou M., Affognon H., Bell A., Zweigert M. E., Poehling H.-M. and Schulthess F. (2004) Impact assessment of *Teretrius nigrescens* Lewis (Coleoptera: Histeridae), an introduced predator of the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Togo and Benin. *Biological Control* 30, 241–255.

Serratos A., Arnason J. T., Nozzolillo C., Lambert J. D. H., Philogene B. J. R., Fulcher G., Davidson K., Peacock L., Atkinson J. and Morand P. (1987) Factors contributing to resistance of exotic maize populations to the maize weevil, *Sitophilus zeamais*. *Journal of Chemical Ecology* 13, 751–761.

Sing D. N. and McCain F. S. (1963) Relationship of some nutritional properties of corn kernel to weevil infestation. *Crop Science* 3, 259–261.

Tipping P. W., Rodriguez J. G., Poneleit C. G. and Legg D. E. (1988) Resistance of dent corn inbreds to oviposition by the maize weevil. *Journal of the Kansas Entomological Society* 61, 131–134.

Withers T. M. (1997) Changes in plant attack over time in no-choice tests: an indicator of specificity. *Proceedings of the 50th New Zealand Plant Protection Society Conference* 50, 214–217.