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Quality of field collected and laboratory reared *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) for screening maize genotypes

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The quality of laboratory reared stem borer species for screening of maize varieties is usually questioned by end user cereal breeders. A quality check study was performed in a screen house at KARI-Katumani to evaluate the quality of eight-year old laboratory reared stem borer, *Chilo partellus* (Swinhoe). The evaluation was aimed at finding out the performance of the laboratory borers subjected to six-month interval of gene infusion in comparison with wild F1 generation of the same species collected from the field. One hundred (100) maize seedlings were grown on plastic pots of 5 by 5 cm and of 12 cm-height. The maize seedlings were infested with five first instar larvae on eight plants replicated four times for each borer ecotype. The wild ecotypes were collected from two different localities for comparison with eight-year old laboratory reared borers. Foliar damage, tunnel length on the maize stems and the recovered number of *C. partellus* larvae from the maize plants were used as the parameters for quality measure of the borer ecotypes. The laboratory-reared stem borer species had been subjected to frequent six-month gene-infusion interval from the wild. The results indicated feed-voracity drop of 3.8 and 21.5% for stem and foliar damage on the laboratory borer ecotype. The study established the need for continuous gene infusion to maintain high quality maize stem borer species as test organisms.

Key words: *Chilo partellus*, quality insects, insect rearing, maize genotypes.

INTRODUCTION

The spotted stem borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and the African stem borer *Busseola fusca* Fuller (Lepidoptera: Noctuidae) are the most important lepidopteran stem borer species causing substantial annual loss of maize (*Zea mays* L.) production estimated at 13.5%, and worth US $91 millions in Kenya (DeGroote, 2002). Hassan (1998) had given an estimate of 0.4 million tons of potential yield in the country. Grain maize is one of the most important food staples in sub-Saharan Africa, providing food and income to well over 300 million resource-poor smallholders (FAO, 2008). Its cultivation spans the entire continent and it is the dominant cereal food crop in many countries, accounting for 56% of total harvested area of annual food crops and 30 to 70% of total caloric consumption (FAO, 2008; World Bank, 2011). Among pests, stem borers play a considerable role in reducing maize yield in Africa through damaging the leaves, stem, ears and kernels. Various control mechanisms have been evaluated including chemical, cultural, host plant resistance, and classi-

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cal biological control in different parts of Africa (Akinsola, 1990; Reddy, 1998; Kfir, 2002; Tefera et al., 2011). With the introduction of the parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) into Africa, notable achievements were reported in suppressing *C. partellus* and *B. fusca* (Kfir, 2002). Various insecticides have been recommended in order to protect plants against the stem borers. However, in addition to posing health problems, insecticides are frequently unavailable or too expensive for subsistence farmers in Africa (Mugo et al., 2008). Therefore, an environmentally safe and economically feasible stem borer control practice needs to be available.

Host plant resistance is the most promising method to control stem borers, as it is environmentally benign, practically applicable and easy to adopt and to use by resource poor farmers in Africa (Mugo et al., 2008). Host plant resistance can be attained by using conventional breeding, marker assisted selection or by transformation (Reddy, 1998; Mugo et al., 2008; Tefera et al., 2010, 2011). In an effort to design effective and efficient methods to control the maize pests, the International Maize and Wheat Improvement Center (CIMMYT), developed and deployed resistant, high yielding, and adapted maize hybrids and open-pollinated varieties through conventional breeding. In collaboration with CIMMYT-Kenya, KARI has released 12 stem borer resistant open pollinated maize varieties and hybrids between 2006 and 2011 (CIMMYT, unpublished data). The resistant varieties show low stem borer damage, whereas, insect-cide use is justifiable to susceptible lines when borer density reaches the economic injury level of over 10% (Kfir, 2002). Unlike other pest infestations, stem borer damage on maize might look small on the foliage part of the plant but the major injury is usually on the stem as reported by Hassan (1998). The CIMMYT and KARI collaboration on maize breeding for resistance relies on mass supply of stem borers with acceptable quality from KARI- Katumani Insectary, for field testing of improved maize lines and hybrids. At the KARI-Katumani insectary, stem borer larvae and pupae are periodically collected from infested field maize stubbles and stalks (Tefera et al., 2010).

Observations at the KARI-Katumani laboratory indicated that the *C. partellus* species undergo about 11 to 12 generations in a year. The field collected insects are reared in isolation to F1 generation to avoid any contamination. Parasitized, diseased and deformed insects are discarded. The second generation is allowed to cross-breed with the laboratory colony to avoid genetic decay (Tefera et al., 2011). Despite periodic gene infusion done by mating the laboratory colony and the wild population, it has been observed that there is irregular infestation to maize plants when infested with laboratory reared neonates of *C. partellus* under field conditions. The objective of this study, therefore, was to assess the efficiency of *C. partellus* borers' subjected to continuous laboratory rearing in comparison to field collected populations.

MATERIALS AND METHODS

Test stem borers

An eight-year old laboratory-culture of *C. partellus* with six-month periodic gene infusion was compared with field collected populations of the same species from two different agro-ecological zones, Low/Medium 4 (LM4) at Kima and Upper Medium 3 (UM3) at Masii of the lower eastern Kenya. The two sites at Kima and Masii are different in altitude and thus the stem borer ecotypes were presumed to be different on voracity feeding on maize stems. *C. partellus* larvae were collected from 10 fields in each locality. Stem borer ecotypes at Kima were collected from farms of geographic areas of 01° 56.614 S, 037° 18.118 E and 01° 50.5 545 S, 037° 19.780 E sites in July 2011. Mean locality altitude was 1327 m above sea level. The farms within Masii locality were in 01° 28.992 S, 037° 23.394 E and 01° 32.183 S, 037° 17.368 E, with mean altitude of 1433 m above sea level. The mean altitude difference between the Masii and Kima sites was about 100 m.

Infestation of maize plants with larvae

The collected larvae were reared on artificial diet after removal from the plant stems for one month (August/November, 2011) before using them on potted maize plants to evaluate their quality. One hundred (100) seedlings of maize variety, Katumani Composite B (KCB) were grown on plastic pots of (5 cm-lenght × 5 cm-width × 12 cm-height) on well watered loam soil in September 2011. The plants were placed in a screen house (3 × 5 m), where day time temperatures ranging between 18 to 34°C and relative humidity between 36 to 78% (day and night). The plants were arranged in three groups, each group consisting of 24 plants. The plants were infested separately with three stem borer ecotypes (laboratory, Masii and Kima) with five first instar larvae per plant three weeks after emergence (late September and another lot November, 2011). The treatments were arranged in completely randomized design with four replicates. The plants were monitored daily and watering was done when required.

Data collection and analyses

Two months after infestation, foliar damage, tunnel length, number of surviving larvae and plant height were recorded after splitting the stems using a knife. Foliar damage was scored using a 1 to 9 scale (where 1 = least, 9 = highest) (Tefera et al., 2011). Analysis of variance (ANOVA) for plant height and tunneling lengths was performed using one-way (ANOVA) for each parameter across the three borer ecotypes. Non-parametric analysis of variance (Kruskal-Wallis) was used to determine foliar damage and number of borers. Plant percentage damage of leaf (out of total) and tunnel length (of total stem length) was calculated for borer ecotypes. T-test on significance difference of damage of pooled wild (Masii and Kima) and laboratory borers were explored to comparably measure the difference between the two ecotypes. GenStat Discovery 3 edition software was used for the significance difference test and Ms Excel for graphing of the percentage damage of leaf and stem.

RESULTS

Foliar damage and borer survival

The foliar damage was significantly different ($P<0.05$) among the *C. partellus* ecotypes as well as number of

Table 1. Mean foliar damage and *C. partellus* survival on maize stems.

C. partellus source	Foliar damage level (S.E)	Number of live larvae (S.E)
Laboratory	2.5 (0.14)	1.90 (0.32)
Kima	4.4 (0.22)	2.50 (0.30)
Masii	1.19 (0.70)	3.10 (0.35)

Table 2. Mean plant height and borer tunnel length.

C. parterllus source	Plant height (cm)	Tunnel length (cm)
Laboratory	51.1[a]	9.93[a]
Kima	50.8[a]	45.03[b]
Masii	47.2[a]	18.05[c]
P	*ns*	*<0.001*

Mean values within columns with the same letters are not significantly different (P>0.05).

Table 3. Mean borer numbers and tunnel length among borer ecotypes.

Borer source	Number of larvae per stem (± SE)	Mean tunnel length per stem (± SE)
Laboratory	1.9 ± 0.2[a]	9.9 ± 1.0[a]
Masii	3.1 ± 0.2[ab]	45.0 ± 1.2[b]
Kima	4.0 ± 0.1[b]	18.1 ± 0.7[c]

Mean values within columns with the same letters are not significantly different (P>0.05).

live borers recovered on maize samples was significantly different (P<0.001) (Table 1).

Plant height and tunnel length

Maize plants infested with the different ecotypes of *C. partellus* had fairly similar heights (Table 2). The tunnel length was significantly different (P<0.001) among the three different site sources. The Kima ecotype caused the highest stem damage followed by Masii and the laboratory borers 45.03, 18.05 and 9.93 cm, respectively as shown in Table 2.

Larvae numbers and tunnel length

There was higher number of Masii and Kima borer ecotypes (3.1 and 4.0) leading to subsequent higher tunnel lengths of 45.0 and 18.1 cm, respectively (Table 3). The laboratory culture borers (1.9/tem) had the least tunnel length of 9.9 cm/plant stem. This indicates that higher borer numbers led to increased tunnel length.

Quality of eight-year old laboratory borers as test organisms

The foliar damage index was a mean of 2.5 for the laboratory and 4.5, indicating 28.5 and 50% plant damage for the laboratory and wild borers, respectively (Figure 1). The stem damage percentage was 19.5% for the eight-year old Katumani laboratory borers compared to the 23.3% of the wild collected borers as shown in Figure 1. This was a mean short fall of 3.8% for the tunnel length and 21.5% level for the foliar damage, of laboratory borers as compared to voracity of wild borers. A t-test analysis of foliar and stem damage (tunneling) indicated a significant difference (<0.001) between the two *C. partellus* borer samples tested on KCB maize (Table 4). This was demonstrated by 8.62 of foliar damage and 12 value of t-statistic (df = 7) of stem damage between the laboratory-reared and field (wild) collected *C. partellus* borers.

DISCUSSION

Some workers on mass rearing of cereal stem borers have reported on the need to observe quality insect products by regular gene infusion of the laboratory cultured insects with the wild ecotypes which have diversified gene pool (Owens, 1984; Onyango and Ochieng-Odero, 1994). From the study, the Masii *C. partellus* ecotypes had twice stem damage (tunneling) in comparison to the laboratory reared borers. Both foliar and stem damage levels of wild ecotypes were higher indicating

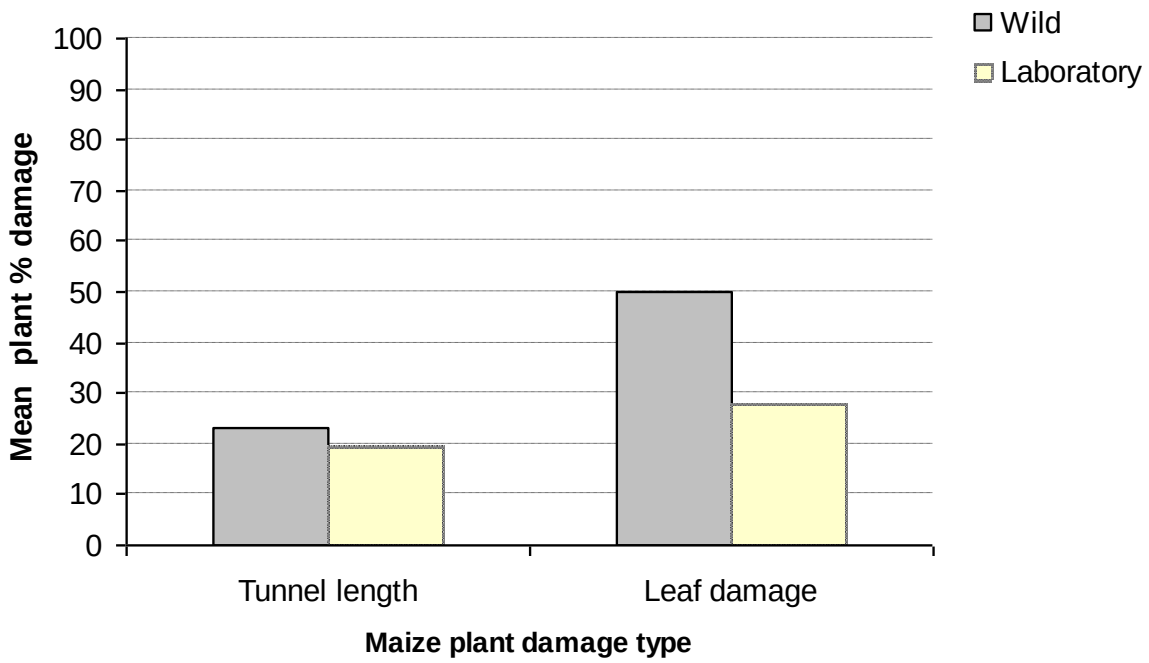

Figure 1. The damage (%) by laboratory reared and wild collected *C. partellus* on KCB maize variety.

Table 4. T-test comparison of eight-year reared and wild collected *C. partellus* borers.

***C. partellus* source**	**Mean foliar damage**	**Stem damage (%)**
Laboratory	2.6	19.5
Wild (fields)	4.5	23.3
t-test statistic	*8.62*	*12*
S.E.D	*0.411*	*1.781*
P	*<0.001*	*<0.001*

One-sample t-test at 95% confidence level (df = 7).

a slow deteriorating quality of the laboratory reared *C. partellus*. The wild borer numbers on test maize stems was higher than the eight-year laboratory reared ones. The study results show that the quality of the laboratory *C. partellus* borers had lower voracity of feeding on the maize plants. Though, minimal difference on stem damage from the wild borer ecotypes; it nevertheless demonstrated that in time laboratory, reared borers on artificial diet need frequent gene infusion to maintain their efficient quality as test organisms (Owens, 1984). The type of borer change which had occurred to *C. partellus*, whether physiological or morphological could not be ascertained from the study. If the artificial diet caused the low voracity of feeding of the borers on the maize plant whorls or the motility of the larvae on plants need to be answered in a similar study (Berger, 1993) and probably by including molecular studies.

Artificial diet formulated and prepared in the laboratory enable mass production of cereal stem borers like *C. partellus* (Tefera et al., 2011). Kega et al. (2011) reviewed the advantage of rearing stem borers on artificial diet leading to high numbers of eggs and larvae available for use by scientists and students involved in maize lines evaluation against specific stem borer pests. A year ago, the cost of producing one Chilo pupa was reported as KES 6.50 and *Busseola* sp as KES 13 (Kega et al., 2011). Nevertheless, the need to develop stem borer resistant maize varieties overrides the cost involved. Over decade ago, DeGroote (2002) estimated the yield loss in Kenya at US $ 76 million ha^{-1}. In the survey study, the marginal areas of Kenya led in maize deficit of the country's 0.39 million tones per year with stem borers contributing 15 to 21% loss (DeGroote, 2002). This emphasizes the likelihood of more laboratory produced stem borers for more research work towards developing stem borer resistant maize lines suitable to the varied agro-ecological zones of Kenya, and the larger south and eastern Africa region. Field experimental settings could reflect lower damage scores due to other biotic factors like increased predation on the borers by biological agents

agents in the environment (Bonhof et al., 1997). Early surveys in Ethiopia on cereal stems borers indicated that infestation levels depended on presence of natural enemies in the field (Gebre-Amlak, 1985). Reddy and Walker (1990) on a review of Chilo species damage of field cereals observed that this borer led other species in frequency in the South and Eastern African region. *C. partellus* has been reported to significantly reduce KCB variety yield under artificial infestation but escape such damage in natural settings where it matures early unlike late maturing varieties where higher damage occurs not only on the stem but also on the cobs (Kumar and Saxena, 1994).

It is important to continue with regular yearly gene infusion activities on laboratory reared *C. partellus* probably by reducing the interval from six to three months to continue providing quality insects to scientists carrying out maize screening tests both for the conventional and genetically modified varieties to enhance economic maize production (van de Berg et al., 1997; Tefera et al., 2011). The end user of the particular borer species needs to be assured of the quality of the test product, neonates, eggs or adult stages intended for maize genotype screening.

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