African Journal of Biotechnology Vol. 9(30), pp. 4725-4730, 26 July, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

A screening method for banana weevil (*Cosmopolites sordidus* Germar) resistance using reference genotypes

Kassim Sadik[1], Moses Nyine[1] and Michael Pillay[2]*

[1]International Institute of Tropical Agriculture (IITA), P.O. Box 7878, Kampala, Uganda.
[2]Vaal University of Technology, Private Bag X021, Gauteng, South Africa.

Accepted 17 May, 2010

The banana weevil (*Cosmopolites sordidus* Germar) is a serious pest in most banana-growing areas of the world. Host-plant resistance is considered to be the most feasible and sustainable method for its control. However, a quick and effective method for screening banana genotypes for resistance against the banana weevil to facilitate selection and/or development of resistant genotypes is lacking. The objective of the study was to develop an early screening method for weevil resistance by using a set of reference genotypes. Three susceptible genotypes ('Atwalira', 'Namwezi' and 'Kibuzi') and three resistant genotypes ('Calcutta 4', 'Yangambi Km5' and 'TMB3x1968-2') were used in screen-house experiments to assess weevil resistance/susceptibility. Healthy plantlets of the above genotypes were established in buckets in a screen house. Ten adult weevils (5 females and 5 males) were introduced at the base of each plant and the bucket was covered with a weevil-proof mesh. Weevil damage of the corms was estimated as a percentage at 35 and 60 days after the weevil introduction by estimating the peripheral and cross-section corm damage. The resistant genotypes had significantly lower ($p < 0.05$) peripheral and total cross-section corm damage, and less larvae than the susceptible genotypes. These results indicated that these genotypes can be used as reference genotypes in evaluating resistance or susceptibility against the banana weevil. These experiments were completed in five to seven months, depending on the source of planting material, as compared to field-screening experiments for the banana weevil that can take up to three or more years.

Key words: *Cosmopolites sordidus,* screening method, host-plant resistance, reference genotypes.

INTRODUCTION

The banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) is one of the most important pests of banana worldwide (Waterhouse and Norris, 1987). The larvae are the most destructive stage of the weevil when they develop from the eggs and bore their way into the corm and occasionally the pseudostem, making numerous tunnels. Corm damage of the plant interferes with root initiation and development, disruption of water and nutrient uptake, weakening of the plant and reduction in bunch weight (Gold et al., 1999). In extreme cases, weevil damage causes toppling and snapping of the pseudostem at the base, especially during windstorms (Rukazambuga et al., 1998) and consequently shortens the plantation life. The banana weevil can cause up to 100% yield loss in severe infestations (Sengooba, 1986). It can attain pest status in both stressed and well-managed banana plantations undermining most of the control strategies. Most edible bananas, including the East African highland banana (AAA) and plantains (AAB), have been reported to be highly susceptible to the banana weevil (Gold et al., 1994; Fogain and Price, 1994).

Attempts to control the weevil using cultural, chemical and biological methods have not been very successful (Gold et al., 1993). Although cultural control practices are readily available for small-scale farmers, they are ineffective because they are labour-intensive (Gold et al., 1993). Chemical control methods are effective but are expensive and dangerous to humans, domestic animals and the

*Corresponding author. E-mail: mpillay@vut.ac.za.

Abbreviations: AAA, East African highland banana; **AAB,** plantains; **PD,** peripheral damage; **XT,** total cross-section damage estimate.

Plate 1. Plants of susceptible and resistant banana genotypes wrapped in buckets after introducing adult weevils.

environment. Although there are prospects for biological control methods, no biological agent has yet been successfully and widely deployed against the weevil (Gold et al., 2001).

Host-plant resistance has been suggested as the most feasible and sustainable method for the control of the banana weevil, especially in developing countries where farmers lack the resources for other control measures (Frison, 1999). Several field-screening trials have been conducted to identify resistance to the weevil (Fogain and Price, 1994; Ortiz et al., 1995; Rajamony et al., 1995; Anitha et al., 1996; Kiggundu et al., 1999, 2003). Field screening for weevil resistance generally takes several years, and are labour-intensive and require large space as each banana plant occupies 4 to 9 m^2 depending on the planting density.

For breeding purposes, there is a need to develop a quick, reliable and effective screening method for resistance to the banana weevil. This will facilitate selection and/or development of resistant banana genotypes. The objective of the study was to develop an early screening for weevil resistance using a set of reference genotypes.

MATERIALS AND METHODS

This study was conducted at the IITA research station in Namulonge, 30 km North of Kampala in Uganda. Namulonge is located at 32° 27' E longitude and 0° 32' N latitude. Three susceptible AAA varieties, 'Atwalira', 'Namwezi' and 'Kibuzi' and three resistant accessions, '*Musa acuminata* 'Calcutta 4', 'Yangambi Km5' (Fogain and Price, 1994; Kiggundu et al., 2003) and 'TMB3x1968-2' (a hybrid derived from the cross 'Who-o-gu' x 'Calcutta 4'), were used in the screening experiments. The detached corm technique (Pillay and Tripathi, 2007) was used to produce the planting material from each cultivar.

Each plantlet was established in a 20-litre plastic bucket containing a mixture of sterilised topsoil and farm manure. Fifteen plants of each cultivar were arranged in a completely randomised design in an enclosed weevil-proof screen house. The plantlets were allowed to establish themselves for two months to attain a suitable corm size before the introduction of weevils. Weevils were obtained from old banana plantations by using the split pseudostem trapping method (Mitchell, 1978). The sex of the weevils was determined according to methods described by Longoria (1968). Ten adult weevils (5 females and 5 males) were placed at the base of each plant in the bucket. Each bucket was sealed off with a weevil-proof mesh to prevent the weevils from escaping or new weevils from entering the bucket (Plate 1). The experiment was replicated twice.

Weevil damage was estimated by using the peripheral damage and cross-section method (Gold et al., 1994) at 35 and 60 days after weevil introduction. The plants were uprooted and the corms pared to expose the weevil damage. Peripheral damage (PD) was determined by estimating the percentage of the pared corm area consumed by weevil larvae. The corms were then sectioned cross-wise at 3 and 6 cm {adjusted from 5 and 10 cm as described in Gold et al. (1994) to suit the corm sizes} below the collar (upper and lower positions, respectively). For each cross-section, weevil damage was assessed independently for the central cylinder and the cortex by estimating the percentage of corm tissue damaged by the weevil in each area. The mean of the four scores (upper cross-section inner, upper cross-section outer, lower cross-section inner and lower cross-section outer) was calculated to generate a total cross-section damage estimate (XT). The corm was then dissected and the number of eggs, larvae and pupae were recorded. The number of the adult weevils recovered from each bucket was also recorded. The data on PD and XT was transformed using arc sine transformation formula:

$$X^* = 100*\arcsin((\sqrt{(X+0.5)})/100*22/28$$

Where, X is the PD or XT and X* is the transformed data.
Similarly, the data on number of weevils, eggs, larvae and pupae recovered was transformed into logarithmic scale formula:

$$N^* = \text{Log}(N+1)$$

Where, N is the number of weevils, eggs, larvae or pupae and N* is

Table 1. Mean percent peripheral and cross-section corm damage observed after 35 days of weevil inoculation.

Genotype	Peripheral damage		Cross-section damage	
	% PD	(PD*)	% XT	(XT*)
Atwalira	26.67	(39.09ab)	19.10	(37.09a)
Namwezi	27.50	(39.49a)	13.04	(35.66ab)
Kibuzi	19.93	(37.47ab)	12.48	(35.43ab)
1968-2	12.50	(35.43bc)	4.30	(33.11bc)
Yangambi Km5	5.36	(33.39c)	3.09	(32.71c)
Calcutta 4	0.17	(31.89c)	0.04	(31.85c)

*Data in brackets was derived from arc sine transformation. Means with the same letter are not significantly different ($p > 0.05$).

Table 2. Mean number of adult weevils, eggs, larvae and pupae recovered from corms 35 days after inoculation.

Genotype	Weevil	(Weevil*)	Eggs	(Eggs*)	Larvae	(Larvae*)	Pupae	(Pupae*)
Atwalira	6.75	(0.81ab)	7.64	(0.74a)	6.08	(0.78a)	0.46	(0.11a)
Namwezi	6.58	(0.85a)	7.75	(0.77a)	4.58	(0.68a)	0.33	(0.06a)
Kibuzi	7.20	(0.87a)	8.67	(0.79a)	4.87	(0.68a)	0.20	(0.04a)
1968-2	5.43	(0.72ab)	3.36	(0.51ab)	4.07	(0.57ab)	0.14	(0.03a)
Yangambi Km5	5.87	(0.79ab)	3.93	(0.44ab)	1.47	(0.29bc)	0.00	(0.00a)
Calcutta 4	3.58	(0.49b)	0.05	(0.14b)	0.17	(0.05c)	0.00	(0.00a)

*Data in brackets was derived from arc sine transformation. Means with the same letter are not significantly different ($p > 0.05$).

the transformed data.

The transformed data was then subjected to analysis of variance using the PROC general linear model (GLM) procedure in statistical analysis system (SAS) software (SAS, 1991). Means were separated using the lines Tukey comparison test (SAS, 1991).

RESULTS

The results of the PD and XT damage by banana weevils 35 days after introduction are shown in Table 1. The mean PD ranged from 19.93 to 27.50% for the susceptible genotypes, and from 0.17 to 12.50% for the resistant genotypes. The mean XT damage ranged from 12.48 to 19.10% for the susceptible genotypes and from 0.04 to 4.30% for the resistant ones. With the exception of TMB3x1968-2, which showed an intermediate behaviour, significant differences ($p < 0.05$) were observed in weevil damage between susceptible and resistant genotypes. In the resistant group, TMB3x1968-2 had the highest PD and XT followed by 'Yangambi Km5' and 'Calcutta 4'.

Similarly, besides TMB3x1968-2 which did not differ from the susceptible group, the number of larvae recovered from the susceptible and resistant genotypes after 35 days (Table 2) were significantly different ($p < 0.05$). TMB3x1968-2 had the most larvae compared to 'Yangambi Km5' and 'Calcutta 4'. There were no significant ($p < 0.05$) differences in the number of adults, eggs and pupae recovered from both the susceptible and resistant genotypes.

Weevil damage 60 days after inoculation is presented in Table 3. PD ranged from 32.11 to 36.96% and from 3.87 to 13.43% for the susceptible and resistant genotypes, respectively. The XT ranged from 20.93 to 26.00% for the susceptible genotypes and from 1.46 to 6.44% for the resistant group. The PD and XT for 'Atwalira', 'Namwezi' and 'Kibuzi' were significantly ($p < 0.05$) larger than those for 'Yangambi Km5', 'Calcutta 4' and TMB3x1968-2 (Table 3). The level of damage was far larger among the susceptible genotypes when the assessment was done after 60 days rather than after 35 days (Plate 2). The number of adults, larvae and pupae recovered after 60 days of weevil introduction was similar to those recovered after 35 days. The number of larvae recovered from the corms was significantly ($p < 0.05$) higher among the susceptible genotypes than among the resistant ones, except in TMB3x1968-2 which had the highest number of larvae in the resistant group (Table 4). There were no significant ($p < 0.05$) differences in number of adults and pupae recovered between the susceptible and resistant genotypes. However, the number of adults and pupae recovered in both susceptible and resistant genotypes was generally lower when the assessment was done 35

Table 3. Mean percent peripheral and cross-section damage by weevils 60 days after inoculation.

Genotype	Peripheral damage		Cross-section damage	
	% PD	(PD*)	% XT	(XT*)
Atwalira	36.96	(41.52a)	26.00	(38.93a)
Namwezi	35.77	(41.41a)	20.93	(37.67a)
Kibuzi	32.11	(40.35a)	22.37	(37.87a)
1968-2	13.43	(35.67b)	6.44	(33.74b)
Yangambi Km5	3.87	(33.68b)	1.46	(32.27b)
Calcutta 4	6.36	(32.98b)	3.44	(32.84b)

*Data in brackets was derived from arc sine transformation. Means with the same letter are not significantly different ($p > 0.05$).

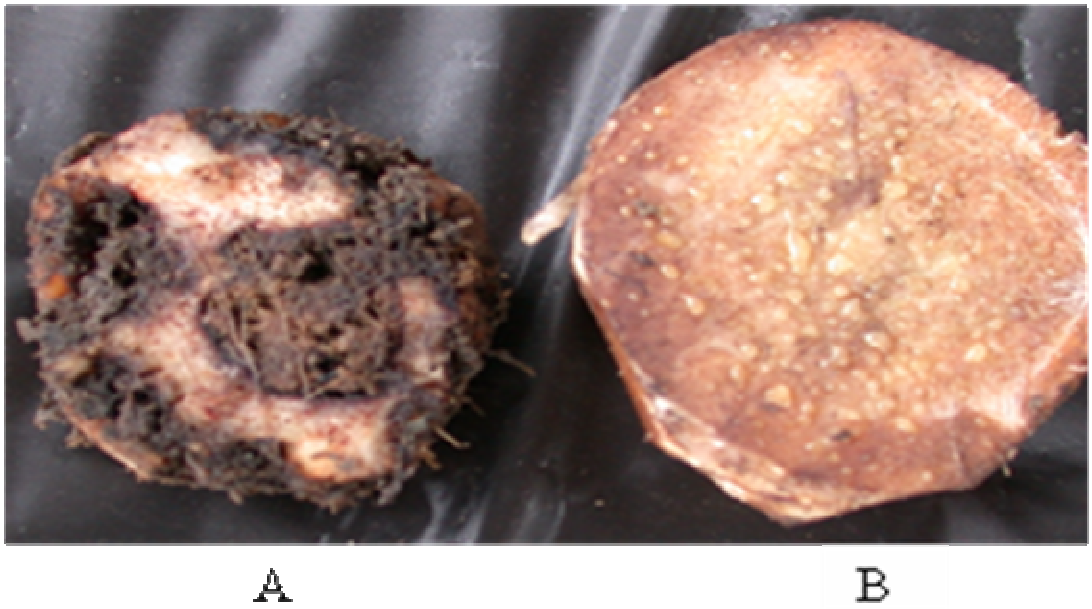

Plate 2. Cross-sections of corms of A, 'Atwalira' (susceptible) and B, *Musa acuminata* 'Calcutta 4' (resistant) genotypes showing presence and absence of weevil damage 60 days after inoculation.

Table 4. Mean number of adult weevils, larvae and pupae recovered from corms 60 days after inoculation.

Genotype	Weevil	(Weevil*)	Larvae	(Larvae*)	Pupae	(Pupae*)
Atwalira	5.67	(0.78a)	3.04	(0.47a)	0.56	(0.11ab)
Namwezi	4.61	(0.72ab)	2.18	(0.37ab)	0.18	(0.05ab)
Kibuzi	4.75	(0.71ab)	2.67	(0.44a)	0.67	(0.14a)
1968-2	3.30	(0.53bc)	1.00	(0.16bc)	0.07	(0.02b)
Yangambi Km5	2.93	(0.52bc)	0.47	(0.10c)	0.03	(0.01b)
Calcutta 4	2.50	(0.46c)	0.43	(0.10c)	0.07	(0.02b)

*Data in brackets was derived from arc sine transformation. Means with the same letter are not significantly different ($p > 0.05$).

days after weevil introduction. The number of eggs in both susceptible and resistant genotypes was also negligible and thus not included.

DISCUSSION

The results of the study revealed a wide range of genotypic

responses to the banana weevil between the susceptible and resistant genotypes. The resistant genotypes showed lower PD and XT and were clearly distinguishable from the susceptible group. The large difference in total cross-section damage between the two groups of genotypes is of particular interest for evaluating weevil resistance since it measures the extent to which the larvae could penetrate deep into the corm. Internal corm damage directly affects the yield and survival of the banana plant (Rukazambuga et al., 1998). Although external damage is not as important as internal damage, the difference in peripheral damage between the two sets of genotypes is useful for screening experiments. External damage of the corm is likely to affect root initiation, water and nutrient up take and stability of the plant (Kiggundu et al., 2003).

The trend in external and internal damage between the susceptible and resistant genotypes was similar both after 35 and 60 days of weevil introduction. However, as expected, the extent of PD and XT was greater after 60 days, especially among the susceptible genotypes. The increased corm damage with time was attributed to the increase in the number of larvae. On the basis of these results, it is recommended that screening for weevil resistance in bucket trials should be assessed 60 days after weevil infestation. At 35 days after weevil infestation, it was possible only to separate the susceptible genotypes from highly resistant genotypes such as 'Yangambi Km5' and 'Calcutta 4'.

There were differences in weevil survival rate and larval development among the genotypes. The number of adult weevils recovered from each bucket was generally less than ten, the number introduced per plant. Nevertheless, the numbers recovered from the susceptible genotypes were relatively higher compared to those recovered from the resistant ones. This may be attributed to differences in preference by weevils for the susceptible and resistant genotypes. The weevils possibly did not feed on the resistant genotypes and thus starved to death. This could also explain why the number of eggs recovered from the resistant genotypes was lower than those obtained from the susceptible ones. Mesquita et al. (1984) reported that the banana weevil preferred particular cultivars for feeding and oviposition. Classical resistance mechanisms have been investigated in *Musa* germplasm, and antibiosis (factors affecting larval performance), rather than anti-xenosis (attraction), appears to be the most important resistance mechanism in banana (Abera et al., 1999).

Differences were observed between the number of larvae recovered from the susceptible and those from resistant genotypes, in particular 'Yangambi Km5' and 'Calcutta 4', at both 35 and 60 days after weevil infestation. Besides lower egg density, the fewer larvae recovered among the resistant genotypes was attributed to low levels of penetration by the developing larvae due to the hard nature of their corms and other mechanisms of resistance such as antibiosis. Hardness of the corm has been suggested to be an important component of weevil resistance in some banana and plantain cultivars (Kiggundu et al., 1999). Furthermore, Lemaire (1996) showed that 'Yangambi Km5' has an antibiotic effect on developing larvae, causing substantial mortality and lengthening of the developmental stages. At 60 days after infestation, the actual numbers of larvae were relatively low in all the genotypes. This general reduction in the number of larvae with time may be due to their metamorphosis into pupae since the total developmental period of the larva is about 23 - 33 days (Gold et al., 1999).

There was no clear difference in the number of pupae recovered from the genotypes and the actual numbers were generally low at both 35 and 60 days after weevil introduction. The recovery of pupae from corms is normally difficult due to the short period they take to change into adults. Therefore the number of pupae recovered from corms cannot be used to separate susceptible and resistant genotypes. Similarly, differences in the number of eggs recovered may not be a good indicator of susceptibility or resistance since eggs also develop into larvae within a short time. It is worth noting that, although differences in the number of adults, eggs and pupae recovered from corms may reveal differences in host-plant response to the banana weevil, such differences may not be good indicators for screening banana genotypes because they do not directly cause damage to the plant.

In conclusion, the differences in cross-section and peripheral damage and larvae density clearly separated the susceptible ('Atwalira', 'Namwezi' and 'Kibuzi') from the resistant ('Calcutta 4', 'Yangambi Km5' and 'TMB3x1968-2), genotypes. This implies that 'Calcutta 4', 'Yangambi Km5' and 'TMB3x1968-2' can be used as reference genotypes in evaluating banana genotypes for weevil resistance and susceptibility. Internal and external damage and larvae density was almost uniform among 'Atwalira', 'Namwezi' and 'Kibuzi'. These genotypes were all reported to be highly susceptible to weevil damage (Kiggundu et al., 2003), and therefore any of them can be used as a reference genotype for susceptibility. Among the resistant group, 'TMB3x1968-2' had relatively higher external and internal damage and the number of larvae recovered compared to 'Yangambi Km5' and 'Calcutta 4'. 'TMB3x1968-2' is a triploid hybrid derived from the cross 'Who-o-gu' (susceptible) x 'Calcutta 4' (resistant). 'TMB3x1968-2' was selected for this study because of its long field resistance to weevils. However, its level of resistance in the screen house appeared to be lower than its male parent 'Calcutta 4'. This shows that this screen house technique of screening can reveal minor details and therefore it can be used for evaluating banana genotypes with different levels of resistance. The results further suggest that screening genotypes on the basis of external and internal damage can best be done 60 days after weevil infestation. If the assessment of resistance/ susceptibility is based on the number of larvae recovered

from the corms, then it can be done at 35 days after weevil introduction. Screening for weevil resistance in bucket experiments can be accomplished in 5 to 7 months, depending on the source of planting material as compared to the field-screening experiments that take more than three years.

This study provided an early screening method for weevil resistance using a set of reference genotypes.

ACKNOWLEDGEMENTS

We are grateful to P. Muchunguzi and L. F. Turyagyenda for assistance in carrying out the experiments. We thank Dr E. B. Karamura (Commodities for Livelihoods, Bioversity, previously known as INIBAP) and Dr. W. Tinzaara (formerly National Banana Research Programme, Kawanda, Uganda) for their useful comments on this manuscript during a Scientific Writing Workshop organised by Commodities for Livelihoods at Entebbe, Uganda.

REFERENCES

Abera AMK, Gold CS, Kyamanywa S (1999). Timing and distribution of attack by the banana weevil (Coleoptera: Curculionidae) in East Africa highland banana (*Musa* spp.). Fla. Entomol. 82: 631-641.

Anitha N, Rajamony L, Rahdakrishman TC (1996). Reaction of banana clones against major biotic stresses. The Planter, 72: 315-321.

Fogain R, Price NS (1994). Varietal screening of some *Musa* genotypes for susceptibility to the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). Fruits, 49: 247-251.

Frison EA (1999). Integrated pest management: An overview. In: Mobilization of IPM for sustainable banana production in Africa. Frison EA, Gold CS, Karamura EB, Sikora RA (eds) Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28 November 1998. INIBAP. Montpellier, France, pp. 9-22.

Gold CS, Pena JE, Karamura EB (2001). Biology and integrated pest management for the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). Integr. Pest Manage. Rev. 6: 79-155.

Gold CS, Rukazambuga NDTM, Karamura EB, Nemeye P, Night G (1999). Recent advances in banana weevil biology, population dynamics and pest status with emphasis on East Africa. In: Mobilization of IPM for sustainable banana production in Africa Frison EA, Gold CS, Karamura EB & Sikora RA (eds) Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28 November 1998. INIBAP. Montpellier, France, pp. 9-22.

Gold CS, Speijer PR, Karamura EB, Rukazambuga NDTM (1994). Assessment of banana weevils in East African highland banana systems and strategies for control. In: Banana Nematodes and Weevil Borers in Asia and the Pacific Valmayor RV, DavideRG, Stanton JM, Treverrow NL, Roa VN (eds) INIBAP, Los Banos, Philippines, pp. 170-190.

Gold CS, Ogenga Latigo MW, Tushemereirwe W, Kashaija I, Nankinga CM (1993). Farmer perceptions of banana pest constraints in Uganda: In: Results from a rapid rural appraisal. Biological and Integrated Control of Highland Banana and Plantain, Gold CS, Gemmil B (eds) Proceeding of research Co-ordination meeting, Ibadan, Nigeria, pp. 3-25.

Kiggundu A, Gold CS, Labuschagne MT, Vuylsteke D, Schalk L (2003). Levels of host plant resistance to banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), in Uganda *Musa* germplasm. Euphytica, 133: 267-277.

Kiggundu A, Vuylsteke D, Gold CS (1999). Recent advances in host plant resistance to banana weevil, *Cosmopolites sordidus* (Germar). In: Mobilization of IPM for sustainable banana production in Africa Frison EA, Gold CS, Karamura EB, Sikora RA (eds) Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28 November 1998. INIBAP. Montpellier, France, pp. 9-22.

Lemaire L (1996). Les relations sémiochimiques chez le charançon du bananier *Cosmopolites sordidus* Germar (Coleoptera : Curculionidae) et la resistance de sa plante-hôte, le bananier. Thèse de Doctorat, Université de Montpellier II, France.

Longoria A (1968). Diferencias sexuales en la morfologia externa de *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). Ciencias Biol. I, La Habana 1: 1-11.

Mesquita ALM, Alvers EJ, Caldas RC (1984). Resistance of banana cultivars to *Cosmopolites sordidus* (Germar). Fruits, 39(4): 254-257.

Mitchell G (1978). The estimation of banana borer population and resistance levels. Technical Bulletin 2, WINBAN, St. Lucia. p. 34.

Ortiz R, Vuylsteke D, Dumpe B, Ferris RSB (1995). Banana weevil resistance and corm hardness in *Musa* germplasm. Euphytica, 86: 95-102.

Pillay M, Tripathi L (2007). Banana breeding. In: Breeding Major Food Staples Kang MS & Priyadarshan PM, Blackwell Publishing, Iowa, USA,

Rajamony L, Anitha N, Rahdakrishman TC, George KC (1995). Variability of yield and yield components in the ABB group of bananas. The Planter, 71: 161-168.

Rukazambuga NDTM, Gold CS, Gowen SR (1998). Yield loss in east African highland bananas (*Musa* spp., AAA-EA group) caused by the banana weevil, *Cosmopolites sordidus* Germar. Crop Prot. 17: 581-589.

SAS Institute Inc., (1991). Cary, NC, U.S.A. Software Release 6.10 TS019.

Sengooba T (1986). Survey of banana pest problem complex in Rakai and Masaka districts, August 1986: Preliminary trip report. Ministry of Agriculture, Namulonge Research station, Uganda. Unpublished, p. 10.

Waterhouse DF, Norris KR (1987). Biological Control: Pacific Prospects: Australian Centre Int. Agr. Res, Melbourne, Inkata Press. p. 454.