Uganda Journal of **Agricultural Sciences**
ISSN: 1026-0919 (Print); 2410-6909 (Online)
Volume 18 Number 2 (2018) pp. 103 – 109
DOI: http://dx.doi.org/10.4314/ujas.v18i2.4

https://www.ajol.info/index.php/ujas

Use of Artificial Diets with Plant Material to evaluate Banana Cultivars for Resistance to *Cosmopolites sordidus*

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Abstract. Artificial diets rapidly establish the effectiveness of chemical-based control strategies. Diets permit preliminary evaluation of active compounds, study *in-vitro* larval growth cycles that are usually inaccessible and produce uniform large consistent numbers of insects as needed. With no known artificial diet, banana weevils, have always been reared on field-collected banana rhizome (corm). This study, therefore, developed and examined the effect of commercial diet recipes fortified with susceptible banana corm powder on weevil growth and development. Subsequently corm powders from different banana cultivars were also evaluated for weevil performance. Successful laboratory rearing of the weevils to adult stage on diet was achieved in 48 days compared to 36 days in the natural banana stem. The difference in weevil larvae performance reared different corm powder, presented a novel screening method for banana genotypes. For example genotypes, Culcatta-4 (AA), Cavendish (AAA) and Kayinja (ABB) showed 0-35% of adult emergences compared to 65% in susceptible genotypes. The diet developed can be used to perform rapid bioassay experimentation to screen potential candidate proteins or molecules for a transgenic approach. It has also shown potential for rapid screening of genotypes for resistance.

Keywords: Resistant banana, Laboratory weevil rearing, Resistance screening.

INTRODUCTION

Artificial diets play important roles in research and development of technologies to control pests and evaluate chemical compounds. For example it has been used in bioassay experiments to test the effectiveness of *Bacillus thuringiensis* proteins against sweet potato weevils *C. puncticollis* (Boheman) and *C. brunneus* F. (Coleoptera: Brentidae) Ekobu *et al.,* (2010). Insect diet allows the study of developmental stages (growth cycle) of inaccessible instar stages of some insects once they bore into the tissue. The diet further provides insects of the same size, age and in large numbers throughout the year for experiments. Banana weevil previously lacked the diet to evaluate the different control strategies for instance evaluation of effective chemical compounds as it has been reported that weevils have developed resistance to commonly used pesticides. With no known artificial diet for banana weevils, insect rearing required considerable space and use of field-collected banana stems to maintain insects in the laboratory which further pose difficulties in uniform incorporation of active proteins. This not only hampered research on preliminary evaluation of active compounds and testing candidate genes but also evaluating germplasm and other breeding lines for resistance (Forgain and Price, 1994; Kiggundu *et al*., 2003).

Banana weevil (*Cosmopolites sordidus* Germar) is one of the disastrous pests of bananas and plantain (Rubaihayo, 1992). It is black measuring 10-15 mm and lives between leaf sheaths and at the base of the mat or crop residues. Through its damage on the corm/rhizome, prevent crop establishment, causes reduction in ratoon cycle and contribute to disappearance susceptible bananas and plantains (Gold *et al.* 1999; Rukazambuga and Mbwana, 1999; Gold *et al.*, 2000). Any research that is directly or indirectly targeting the reduction of weevil damage effect, contributes to food security. The objective of this study, therefore, was to develop a diet that supports large numbers of weevil of normative growth and development for experimentation into its control strategies.

Knowledge about the biology, nutrition and environmental composition of feeds for banana weevil, allows approximation of their growth requirement (Moran *et al.* 1998). Working with diet recipes other than fresh host plant materials, allow precise control of nutritional factors needed by insect development. Besides that, quantity, rate, and quality of the diet fed on by the insects affect not only their growth rate but also the developmental time, bodyweight, movement and survival (Kogan. 1986). Louis et al. (2005) established the usefulness of recipes such as Agar, cellulose and other nutritionally inert substance in adding the texture to liquid food to give a firm surface similar to host plant parts and roughages needed for food passages in the insect gut. According to Louis et al. (2005), diets are indispensable tools when studying insect behavioural response, especially where standardizing host plant materials for laboratory studies is difficult.

MATERIALS AND METHODS

Insects and the diet

Cosmopolites sordidus used in this study carried out at National Agricultural Research Laboratories (NARL), Kawanda, were collected from banana plantations within 1 km of NARL by setting traps as previously described by Ogenga and Bakyalire, (1993). They were maintained in the laboratory as previously described by Kiggundu *et al.* (2000).

Banana weevil artificial diets Table 1; were formulated based on the sweet potato weevil diet (Shimoji and Yamagishi, 2004; Ekobu et al. 2010) with modification. The modification involved; additional banana corm powder of susceptible or resistant cultivar and reducing the amount of preservatives and antimicrobial agents in the diet. Corm powder from either susceptible; Mbwazirume (AAA- EA), Atwalira (AAA-EA) and Bogoya (AAA), or resistant cultivars; Kayinja (ABB), Culcutta4 (AA) and Cavendish (AAA) were used.

Preparation of an artificial diet for banana weevil neonates

The banana corm powder was made from a maiden sucker of about 2-3m tall. The fresh corm was chopped into small slices of about 5x5x1cm and dried using a cabinet solar dryer for four sunny days. The sliced pieces were turned regularly to attain uniform drying without the loss of nutrients. Prior to pounding, corms were put in an oven at 70^{o}c for 1 hour to make them thoroughly dry. It was pounded using pulverisette 15 grounding machine. The powder was stored at 22-24^{o}c in an airtight container until required. The complete diet was prepared as follows: Agar was added to 510ml of distilled water and methyl *p*-hydroxybenzoate (Nipagin) then agitated until mixed. The 490ml of distilled water was reserved for dissolving other components. The water-agar-Nipagin mixture was autoclaved for 15 min until the agar was completely dissolved. The medium was poured into a 2-liter beaker and placed into a water bath at 70^{0}C. The inositol, stigmasterol, potassium sorbate and

tetracycline were later dissolved in 100% ethanol and added to agar mixture, and blended for 2min at 40-75%.

Table 1: Composition of *C. sordidus* artificial diet

Ingredients	Quantity (g)
Agar	40.0
Banana rhizome powder	80.0
Dextrose	40.0
Yeast extract	9.0
Cellulose	14.4
Casein	21.6
Vitamin B	0.045
Salt mixture	2.7
Ascorbic acid	1.8
Chlorine chloride	0.45
Methyl 4-hydroxybenzoate (Nipagine)	0.67
Inositol	0.36
Stigmasterol	0.72
Potassium sorbate	0.67
Tetracycline	0.2
Ethanol	10.0[1]
Distilled water	1000.0[1]

[1] Millilitres

Oviposition and hatching of eggs to larvae

Eggs were obtained by allowing female *C. sordidus* to oviposit overnight into fresh young pseudostem sheath placed in a 10 L plastic container whose cover was perforated. Freshly laid eggs were carefully exercised from thin pseudostem sheath using a knife, rinsed in distilled water to remove any plant material, then sterilized in 70% ethanol and finally rinsed with distilled water and placed on moistened filter paper in sterile Petri dishes and incubated at room temperature for 5 - 8 days to hatch into larvae.

Growth determination of banana weevil neonates on diet

Head capsule measurements and developmental rates were determined using a binocular dissecting microscope fitted with a calibrated ocular micrometre as described (Gold *et al.,* 1999).

Larvae retrieval from the diet for head capsule measurements and weight determination using micro scale was done after observed tunnelling in diet, at 3-day intervals until pre-pupa stage. All experiments were replicated three times.

Screening for resistant banana cultivars with banana weevil

Banana powder was obtained from maiden suckers of susceptible cultivars, like Gonja (AAB), Atwalira (AAA-EA), Mbwazirume (AAA-EA), Bogoya (Gros Micheal-AAA) and resistant cultivars, Cavendish (AAA), Culcata-4 (AA) and Kayinja (Pisang Awark ABB) and processed following procedures described in 3.2.

RESULTS

Effect of artificial diet on the life cycle of Banana weevil

The eggs hatched within 7 days and the resultant larvae bore into the diet via the scratched grooves. Larvae pupated into the cocoon chamber at the base of the plate about 30 days after hatching and adults emerged after about 12 days Fig. 1. The hatchability and adult emergences were 85% and 70% respectively. The percentage survival of banana weevils was 69% at pupa and through adult thus stable. Big variations were observed at instar stage 1 as 85% and at instar stage 6 as 70%. (Table 2). The cycle took 48 days for the growth and development of banana weevil neonates to adult on diet and 36 days on natural plant tissue as control. The development from larvae through to adult as determined based on head capsule width (HCW) and body weight, 1-6 instar stages are as presented in Table 3.

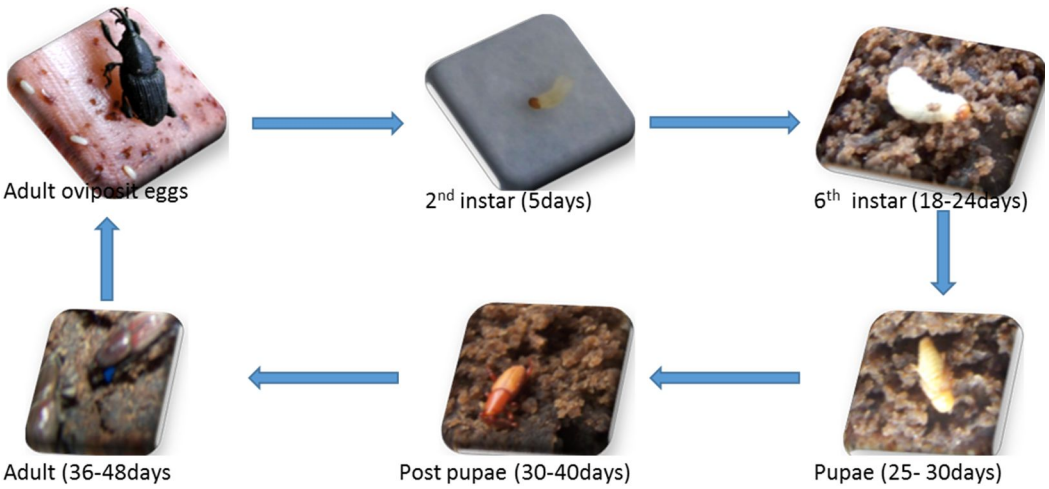


Figure 1. Life cycle of banana weevil on the diet which takes 48 days to emergence of the adult with a growth interval of 3-4 days between each instar stage.

Table 2. Survival rates of *Cosmopolite sordidus* neonates on artificial diet

Developmental stage	Number alive	Number dead	Survival rate
Egg	200	30	85
Larva	170	51	70
Pupa	119	2	69
Adult	119	0	69

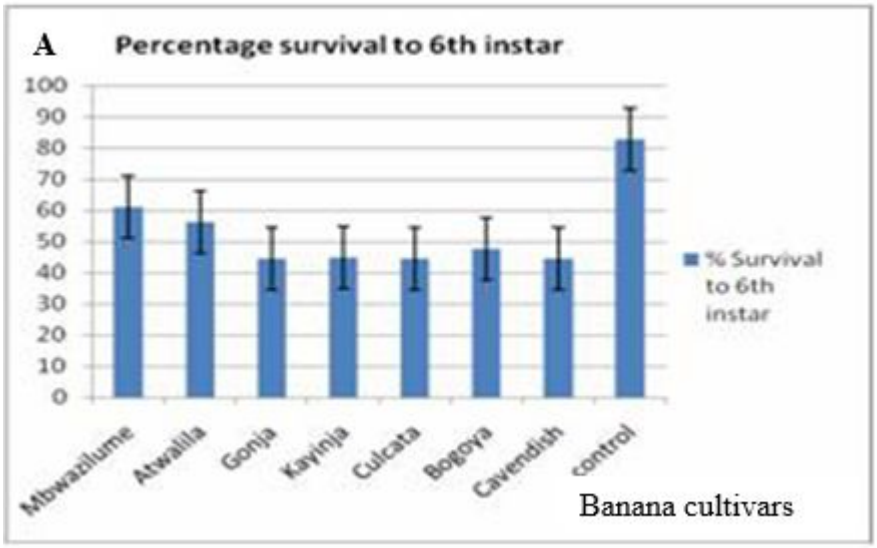
Table 3. Development of banana weevil on artificial diet

Days	**HCW**	**Growth stages**	**Weight (g)**
4-	25.2±8	1	0.01
10	33.0±6	2	0.01-0.015g
15	43.1±4	3	0.015-0.02g
16-20	49.1±2	4	0.02-0.03g
21-23	60.3±9.7	5	0.04g
24-27	>69.8	6	0.07g
28-30	N/A	Pre-Pupation	N/A
31-33	N/A	Pupation	N/A
34-48	N/A	Post-pupation	N/A
49..	N/A	Adult	N/A

HCW = head capsule width

Effects of corm powder from different banana cultivars

Fortifying the diet with corm powder from different banana cultivars varieties significantly ($P < 0.01$) affected the rate of adult emergence. Diets with corm powder of susceptible cultivars "Mbwazirume" (AAA- EA), "Atwalira" (AAA-EA), "Gonja" (AAB) and "Bogoya" (AAA) had higher survival rates than resistant cultivars "Kayinja" (ABB), "Culcutta-4" (AA) and "Cavendish" (AAA), throughout their developmental stages as shown for 6th larvae Instar in panels 2A and B for adult emergence Fig. 2. Corm powder from banana cultivars that are resistant showed 0-35% adult emergence. Corm powder from susceptible cultivars showed 65% adult emergence.



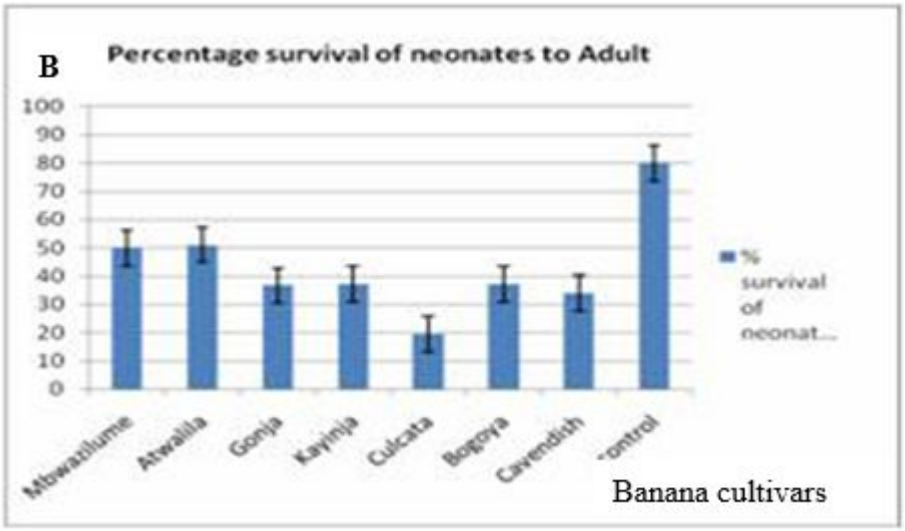


Figure 2: Percentage survival of banana weevils on diets fortified with corm powder of different banana cultivar; survival from 1st to 6th instar (2A), from neonates to adult emergence (2B). "Mbwazirume" (AAA- EA), "Atwalira" (AAA-EA), "Gonja" (AAB) and "Bogoya" (AAA) resistant cultivars "Kayinja" (ABB), "Culcutta-4" (AA) and "Cavendish" (AAA).

DISCUSSION

Most of the developed insect diets are modified from a mixture of recipes that successfully support related insect species. Similarly, *C. sordidus* diet in this current study was a modification of *Cylas puncticollis* diet. This study demonstrated a successful rearing of banana weevil on a modified diet through its life cycle in 36-48 days at room temperature (28°C), which is comparable to 46-56 days (Nankinga, 1999). The demonstrated result in the study indicated larvae development to 6th instar stage in 18-24 days with no significant weight gain difference between diets. It took fewer days

than 30-40 days taken by the larvae on natural host or 55-65 days (Bakyalire & Ogenga-Latigo, 1994). Still on this current diet, pupae took an average of 5 days to develop into adults which are comparable to 5-8 days (Traoré et al., 1993) or 8-10 days (Bakyalire & Ogenga-Latigo, 1994). On the other hand, the soft brown adult weevil that emerged from the pupal case took averagely 6 days to become adult black weevil. This suggests its good representation of the natural host. The duration taken, however, is dependent on temperature, relative humidity, the susceptibility of the plant and diet quality (Gold et al. 1999; Nankinga, 1999), for extreme conditions prolong the cycle to even 89 days (Traoré et al 1993).

Research to improve this diet should be done to reduce the duration of 48 days taken by adult weevil emergence to less than 36 days observed on the natural host (Gold *et al.,* 1999). Nevertheless, the described diet could still be used to evaluate the efficacy of new pesticides, other active compounds and contribute to genetic engineering against banana weevils. It may also permit early screening of conventionally bred line against weevil as it was demonstrated; resistant cultivars supported 36% adult weevil while susceptible cultivars 65%. In conclusion the diet created will not only perform rapid bioassay experimentation to screen potential candidate proteins or molecules for a transgenic approach but also shows potential for rapid screening of genotypes for resistance.

ACKNOWLEDGMENTS

Financial support for this study was provided by Bioversity International through the National Banana Research program. We are grateful to the coordinator Diversity International, Head of National Banana Research program, Head of National biotechnology Laboratory Centre, Kawanda and our fellow scientists (Mrs Alice Bukenya, Mr Moses Matovu, Mr Stephen Bua and Mr Abu Muwonge) for their technical support and cooperation towards the success of this study.

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