REFEREED PAPER

THE EFFECT OF AN IMPROVED ARTIFICIAL DIET FORMULATION ON *ELDANA SACCHARINA* WALKER REARING, GROWTH AND DEVELOPMENT

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Abstract

Since 1970, *Eldana saccharina* Walker has been a key pest in South African sugarcane. Research projects on Integrated Pest Management (IPM), against this stalk borer are continually undertaken at the South African Sugarcane Research Institute (SASRI). A colony of *E. saccharina* is routinely reared on artificial diet for use in these IPM projects. The current diet was found to be expensive (R0.22 per *E. saccharina* larva), so investigations were undertaken to formulate an improved diet that produced more insects per volume of diet, without compromising insect quality. Two artificial diets were compared; namely, a diet developed for the European corn borer, *Ostrinia nubilalis* Hubner (referred to as rabbit pellet diet), and the current conventional diet (referred to as sugarcane diet). Quality assessment was conducted to determine dietary effects on *E. saccharina* growth and development. Texture, hardness, consistency and water content of the diets were important aspects in promoting good growth and development of the insects. Results showed that the rabbit pellet diet produced higher numbers of good quality pupae and moths (mean per 2 litres of diet = 89%) than the sugarcane diet (mean per 2 litres of diet = 16%). Time to pupation was also shorter (rabbit pellet diet = 27 days and sugarcane diet = 33 days). Given its lower cost (R0.05 per *E. saccharina* larva) and ease of preparation, the rabbit pellet diet can replace the current sugarcane diet as the medium on which to rear *E. saccharina* without compromising insect quality. This will facilitate the resulting IPM research that aims to provide sustainable control of *E. saccharina* in sugarcane.

Key words: crop protection, integrated pest management, biological control, sterile insect technique, diet formulation

Introduction

The stalk borer *Eldana saccharina*, first described by Walker in 1865, is an indigenous lepidopteran (Pyralidae) pest of graminaceous crops, several wetland sedges and grasses in various African countries (Chinheya *et al.*, 2009; Goebel *et al.*, 2005*). Eldana saccharina* has been the most destructive pest in sugarcane production, reaching key pest status in the South African sugarcane industry since 1970 (Conlong, 1990; Horton *et al.*, 2002; Potgieter *et al.*, 2013), and is known to cause direct losses estimated at R 344 000 000 per annum for South African sugarcane farmers (Rutherford, 2015). Losses result mainly from severe effects on sugarcane quality such as the reduction of soluble solids (Brix), sucrose content (Pol), and sucrose percentage in total solids (Purity) present in the plants juice (Potgieter *et al.*, 2013; Rutherford, 2015). Increased fibre caused by the combination of the borer and secondary

fungal infestations also has negative impacts on cane quality (Potgieter *et al.*, 2013; Rutherford, 2015).

At the South African Sugarcane Research Institute (SASRI) much research is focused on the control of *E. saccharina* (Mokhele, 2009). Research projects on Integrated Pest Management (IPM), which is a holistic agro-ecosystem interaction that incorporates practices for economic control of pests, are currently implemented at SASRI to investigate control options of this stalk borer (Conlong and Rutherford, 2009). Such control measures include host-plant resistance, push-pull technology, biological control and the sterile insect technique (SIT) (Chinheya *et al.*, 2009; Cockburn, 2013). At the SASRI Insect Rearing Unit (IRU), *E. saccharina* is routinely reared on artificial diet for use in these different IPM projects.

Large numbers of high quality insects have been reared on artificial diets for the benefit of humans for thousands of years (Wheeler and Zahniser, 2001). The use of artificial diets reduces time, space, labour and costs associated with growing host plants (Hervet *et al.*, 2016). Initially, artificial diets were developed as aids for bioassays of potential insecticides, entomopathogens and plant resistance trials (Morales-Ramos *et al.*, 2014; Hervet *et al.*, 2016). Today, insects reared on laboratory-produced diets are acknowledged as essential tools for insect control in SIT and biological control strategies (Morales-Ramos *et al.*, 2014; Hervet *et al.*, 2016). In order to successfully rear insects such as *E. saccharina* on such diets, it is important to have knowledge of the insect's biology, behaviour and nutrition (Davis, 2007; Divakara and Manjulakumari, 2015). It is vital to monitor the photoperiod, temperature, humidity and sanitation of their rearing facility to ensure optimal development and reduce susceptibility to microbial contamination (Davis, 2007; Divakara and Manjulakumari, 2015). Lastly, it is important to formulate a diet that provides all essential nutrients that allow complete insect development and reproduction (Davis, 2007; Divakara and Manjulakumari, 2015).

The aim of the project was to produce a more cost effective diet than the conventional diet currently used. The current diet was considered to be expensive, so investigations were initiated to formulate an improved diet that produced more insects per volume of diet, without compromising their quality. The objective of this study was to determine the effects of an improved artificial diet formulation that provides nutrients that are effective for supporting the growth of *E. saccharina* larvae from time of eclosion until pupation.

Materials and Methods

Experiment establishment

Eldana saccharina can develop on an *Ostrinia nubilalis* Hubner (Lepidoptera: Crambidae) diet (referred to as rabbit pellet diet), developed by Nagy (1970). This diet was much simpler to formulate and initial trials showed that it had no detrimental effects on *E. saccharina* fitness. To quantify the effects of this diet on *E. saccharina* growth and development, a modified form was used for comparison with the current conventional diet (referred to as sugarcane diet) (Walton and Conlong 2016) used to laboratory-rear *E. saccharina*. The study was conducted at the Insect Rearing Unit (IRU) of SASRI, in Mount Edgecombe, Durban, KwaZulu-Natal. This rearing facility provides reliable control of environmental conditions such as temperature, humidity and light (Conlong, 1992).

Insect-rearing laboratory conditions

At the SASRI-IRU, laboratory standard operational procedures (SOPs) are implemented to prevent contamination and contamination spread. These SOPs also create safe and pleasant working conditions for all staff members. Personal and facility hygiene standards are kept at a rigorous standard to avoid any risk of contamination. Clean laboratory coats, approved

protective garments, footwear and hair covers are worn at all times and appropriately. D-Germ hand disinfectant (0.5% Chlorhexidine gluconate) in 500 ml spray bottles are provided in all the rearing rooms. At the entrance of the rearing section and in all rearing passages, hand sanitiser (0.5% Chlorhexidine gluconate) is also provided in dispensers to prevent further contamination from external sources. Foodstuff, drinks, smoking and cell phones are not permitted inside the rearing section of the facility. Environmental conditions in the respective rooms are described in the relevant sections below.

Eldana saccharina *diet inoculation*

Various processes were involved during *E. saccharina* inoculation. These are described in more detail below. All processes and environmental conditions were kept similar for both the rabbit pellet diet and the sugarcane diet. Diet ingredients were kept at appropriate temperatures in the cold room (3°C) and host production area (IRU diet kitchen), which is maintained at human comfort conditions, i.e. 22 ± 2°C, ambient humidity and light provided through windows and supplemented by 65 W cool white fluorescent tubes.

Preparation of Eldana saccharina *eggs*

Eldana saccharina eggs were previously collected from the IRU adult emergence and oviposition room. The room is maintained at 26 ± 2°C, 75 ± 5% RH, and at an 8 hour light: 16 hour dark photo phase. Light was provided by 65 W colour 19 Triphosphate fluorescent tubes. The eggs used for inoculation were kept in an incubator at 21°C for three to four days in the IRU diet kitchen before they were surface sterilised. The eggs were counted under a light microscope, and cut into batches of 750 from the oviposition sheets. Once cut, the egg batches were placed into two 90 mm plastic petri-dishes. Working in the fume cupboard, 20 ml of undiluted D-Germ (0.5% Chlorhexidine Gluconate), was poured over the egg batches and left for 15 minutes (timed with a countdown timer). Using a 4 L container with a mesh lid, the eggs soaked in D Germ were poured on top of the mesh lid. The eggs were then rinsed for one minute using distilled water. Once rinsed the egg batches were placed in two clean petri-dishes lined with autoclaved blotting paper to absorb excess moisture. The eggs, still on their paper sections, were then kept in the fume cupboard for approximately two hours to dry.

Artificial diet components

The rabbit pellet diet was composed of rabbit pellets (the principal components of which are lucerne and maize) (Table 1), sourced from Mkondeni Animal Feeds CC. These pellets are used as food for rabbit pets. The rabbit pellets have a high protein content acting as the principal source of nitrogen when incorporated into the insect diet (Nagy, 1970). The rabbit pellet diet included wheat bran, which is a good source of minerals and vitamins (Cohen, 2015). Sucrose was added as an energy source in the rabbit pellet diet, and acetic acid served as an antifungal agent. In contrast, the sugarcane diet was composed of dried crushed sugarcane (the sugarcane stalks were disintegrated and the coarse sugarcane material was dried at 80°C for 24 hours and crushed into a powder using a Baby Hippo Hammer Mill). The sugarcane powder was tinderised/sterilised at 65°C for two days before adding to the diet. Instead of acetic acid, this diet used formalin (40% solution of formaldehyde) as an antiseptic, disinfectant and preservative. The remaining diet components of both the rabbit pellet diet and the sugarcane diet commonly included classes of carbohydrates, proteins, lipids, vitamins and minerals serving as energy bases, building blocks for synthesis, and co-factors for enzyme pathways (Cohen, 2015). Other ingredients included gelling agents, pH modifiers, preservatives and antimicrobial agents (Cohen, 2015).

Table 1. Ingredients and amounts required for preparing *Eldana saccharina* larval diet of different volumes according to the rabbit pellet and sugarcane diet recipes.

Ingredient	Unit	Rabbit pellet diet (2.8 L)	Sugarcane diet (45 L)	Sugarcane diet (2.8 L)
Lucerne pellets	g	600.0	0.0	0.0
Dried crushed cane	g	0.0	9 000.0	560.0
Wheat bran	g	100.0	0.0	0.0
Yeast extract	g	10.0	135.0	8.4
Ground chickpea	g	150.0	4 500.0	280.0
Full cream milk powder	g	66.0	900.0	56.0
Sodium propionate	g	25.6	411.3	25.6
Ascorbic acid	g	16.0	150.3	9.4
Calcium lactate	g	3.2	51.3	3.2
Tri-sodium citrate	g	6.4	103.1	6.4
Sodium chloride	g	1.6	25.7	1.6
Citric acid	g	6.4	103.1	6.4
Whole egg powder	g	80.0	1 125.0	70.0
Sucrose	g	160.0	0	0.0
Nipagin	g	16.0	90.0	5.6
Dithane M45	g	0.5	7.7	0.5
Terralon LA	ml	3.6	45.0	2.4
Denol (70%)	ml	98.0	1 575.0	98.0
Acetic acid	ml	20.0	0.0	0.0
Formalin (40%)	ml	0.0	202.5	12.6
Agar powder	g	11.2	180.0	11.2
Water for agar	ml	1 200.0	20 000.0	1 200.0
Water balance	ml	1 600.0	25 000.0	1 600.0

Rabbit pellet diet preparations

The rabbit pellets were first crushed into a powder form using a Baby Hippo Hammer Mill and sterilised/tinderised in an oven at 65°C for two days. Diet ingredients were weighed accordingly (Table 1). In a 2 L plastic jug, 1.2 L of boiling water was dispensed into which agar powder was poured slowly and stirred using a large spoon to avoid the formation of agar lumps. The agar solution was poured into a 5 L Schott Pyrex bottle and autoclaved at 121°C for one hour. Once autoclaved, the hot agar was poured into a food mixer (6.7 L Kenwood Titanium Major KMM060). The balance of the 1.6 L boiling water was added to the autoclaved agar solution. Sucrose and wheat bran was added next, followed by the crushed rabbit pellets. The remaining dry ingredients were added except for Nipagin and Dithane M45 (Mancozeb) which were first dissolved in Denol. The resulting mixture was poured into the food mixer, followed by acetic acid and Terralon LA (long acting tetracycline injectable antibiotic containing oxytetracycline 200 mg/ml). The diet was then mixed for approximately 10 minutes.

Diet dispensing

As the food mixer was running, 4 L plastic trays, which were previously soaked overnight in a 0.5% sodium hypochlorite solution, were set up on laminar flow benches (where they were further surface sterilised with ultraviolet germicidal lights behind ultra violet (UV) resistant welding curtains). On a daily basis, six trays were prepared. Using a plastic jug, 2 L of diet was dispensed per 4 L tray and left to cool for approximately one hour 15 minutes on the

running laminar flow bench. Once cool, the diet surface was scarified (using a plastic comb dipped in Denol) allowing the eclosed larvae to enter the diet. Two thousand g of sago was mixed with 0.4 g of Dithane M45, sealed in a 5 L Schott Pyrex bottle and placed on a tumbler for a minimum of five minutes to allow the Dithane to mix well with the sago. Two hundred and fifty ml of sago was then spread over the surface of the diet in each tray. The sago helped absorb excess moisture on the surface of the diet and also served as a refuge for the emerged *E. saccharina* larvae before they entered the diet. Sterilised egg batches on their pieces of paper were placed on top of the sago (750 eggs per tray). The trays were inserted into brown paper bags (550 mm in height x 205 mm in length x 124 mm in width; previously placed in the oven at 65°C for two days), the open end folded under the tray and labelled accordingly (i.e. mix number, larval room number, diet information and inoculation date). The trays were placed in the pass-through to the appropriate larval growth room (maintained at 28 ± 2°C, 75 ± 5% RH and zero light: 24 hour dark photo phase. In the larval growth room, the trays were stacked on clean 5-tier metal racks. A maxim iButton DS1923 (multi-trip temperature and humidity logger) was placed in the larval growth room to record temperature and humidity at 60 minute intervals. The iButton was configured using the Fairbridge Technologies ColdChain programme.

Diet pH testing

The 150 ml remaining diet was poured into a 250 ml glass beaker and used to test the diet's pH. The pH was tested using a flat probe pH meter (HANNA Instruments). The pH meter was first calibrated by inserting the probe into a neutral buffer solution (pH at 20°C ± 0.1°C of 7.00 ± 0.02). Once calibrated, the probe was rinsed again with distilled water and then inserted approximately 3 cm into the diet and the pH recorded.

Sugarcane diet preparations

The sugarcane diet (Walton and Conlong 2016) preparation was similar to that of the rabbit pellet diet, except that 45 L of diet was prepared each day in a 65 L Dayton ribbon blade mixer/blender. The diet was dispensed using a positive displacement pump set at dispensing 2 L of diet per tray.

Quality assessment

Quality assessment was conducted in the IRU harvest room (maintained at 22 ± 2° C, ambient humidity and light provided by 65 W cool white fluorescent tubes). This process involved checking for contamination by visual observation. The most common contaminant is a green fungus (*Aspergillus* sp.*)* known to compromise diet quality and lead to increased mortality (Graham, 1990). The second process involved development time to pupation (pupal quality control) which determined ***E. saccharina's*** growth rate. This took place at 27 days for the rabbit pellet diet and 33 days for the sugarcane diet. The insects were extracted from the diet and divided into size categories, namely small (1st and 2nd instar), medium (3rd and 4th instar), and large (5th and 6th instar), plus pre-pupal and pupal stages. One tray per diet was assessed from the six trays initially inoculated for this process. Details of the tray were recorded accordingly (i.e. mix number, diet inoculation date, QC date, larval room number, larval room temperature, relative humidity and diet treatments). Portions of the diet were scooped out into a large tray and the insects sorted from the diet were counted using a tally counter. Five plastic jars with lids, each labelled accordingly (small, medium, large, pre-pupa and pupa), were used to separate the life stages obtained. Dead insects found were counted as the diet was being assessed. Life stages attached to the paper bag were also removed and placed accordingly in the appropriate plastic jars. Once counted, the results were recorded.

Pupal harvesting

Pupal harvesting took place in the IRU harvest room. The process occurred 32 days after inoculation for the rabbit pellet diet and 43 days after inoculation for the sugarcane diet. The process took place early for the rabbit pellet diet due to early pupation of the insects. The pupae were harvested from the remaining diet trays using a custom-built horizontal vibrating sieving machine. The pupal harvester machine consists of two sieves of different sizes (smaller square mesh = 1 cm length x 1 cm breadth, and bigger diamond mesh = 1 cm length x 2.5 cm breadth) and a tray. Details of the five remaining harvested trays were recorded accordingly (i.e. mix number, diet type, number of trays harvested, trays contaminated, number of larvae, number of empty cocoons, and the number of pupae). The diet was scooped out of the trays using a spatula and placed in the upper sieve. When switched on, the diet was shaken sideways allowing the spent and unused diet and larvae/pupae to fall through the sieves into the bottom tray. The pupae were collected in the second sieve (smaller mesh) and the larger portions of the diet on the first sieve (larger mesh). The insects were collected and counted. Pupae and empty cocoons, which depict moth emergence, found on the brown paper bags were also removed and counted.

Pupal weighing

A further quality control measure involved weighing a sample of pupae. Naked pupae were obtained by removing the pupal cocoons and using a light dissecting microscope, 30 males and 30 females from each harvested batch were identified and selected based on the different structures on the ventral surface of their last abdominal segment (Atkinson, 1980). The pupae were weighed using a four decimal place balance. The results obtained were recorded. The harvested pupae were placed in 250 mm diameter plastic plant pot saucers (~500/saucer) and placed in 10 L ventilated plastic containers to check for moth emergence and female fecundity, and also to help maintain the *E. saccharina* culture. The containers were stored on 5-tier metal racks in the IRU adult emergence and oviposition room.

Statistical analysis

Using GenStat 18th edition (VSN International, 2015), statistical tests were conducted to compare data between the two artificial diets. To test whether the data sets for both the rabbit pellet diet and sugarcane diet were normally distributed, a W-test for normality was used. Once normality was confirmed, two-sample t-tests ($p<0.05$) were used to compare the means of *E. saccharina* life stages found in each diet, mortality at pupal quality control between the diets, *E. saccharina* life stage development at harvest of the diets, mean *E. saccharina* yield at harvest and *E. saccharina* pupal weights between the two diets.

Cost analysis

A cost analysis was undertaken to determine whether the rabbit pellet diet is more cost effective than the sugarcane diet. The cost analysis was based on the diet ingredient prices (Table 2), amounts of ingredients used in the diets, and the results obtained from the experiment.

The cost analysis for both the rabbit pellet diet and the sugarcane diet was calculated using the following formulae:

(1) Cost per ingredient package (ZAR) / Package quantity (g/ml) = Cost per unit (ZAR).
(2) Cost per unit (ZAR) x Diet ingredient quantity (g/ml) = Cost per diet ingredient (ZAR).
(3) Sum of cost per diet ingredient (ZAR) = Diet cost per litre (ZAR).
(4) Diet cost per litre (ZAR) / Average eldana yield per tray x 2 = Cost per eldana larva (ZAR).

Table 2. Diet ingredient costs per litre of the rabbit pellet and the sugarcane diet.

Rabbit Pellet Diet Ingredient	Quantity	Cost per Diet Ingredient (ZAR)	Sugarcane Diet Ingredients	Quantity	Cost per Diet Ingredient (ZAR)
Lucerne pellets	200.0 g	1.523	Ground chickpea	100.0 g	2.850
Wheat bran	50.0 g	0.683	Yeast extract	3.0 g	0.549
Yeast extract	3.0 g	0.549	Full cream milk powder	20.0 g	1.116
Ground chickpea	50.0 g	1.425	Sodium propionate	9.1 g	0.889
Full cream milk powder	20.0 g	1.116	Ascorbic acid	3.3 g	0.461
Sodium propionate	9.1 g	0.903	Calcium lactate	1.1 g	0.081
Ascorbic acid	5.7 g	0.789	Tri-sodium citrate	2.3 g	0.051
Calcium lactate	1.1 g	0.082	Sodium chloride	0.6 g	0.003
Tri-sodium citrate	2.3 g	0.051	Citric acid	2.3 g	0.045
Sodium chloride	0.6 g	0.003	Whole egg powder	25.0 g	3.058
Citric acid	2.3 g	0.045	Nipagin	2.0 g	0.373
Whole egg powder	25.0 g	3.058	Dithane M45	0.2 g	0.021
Sucrose	57.1 g	0.818	Terralon LA	1.0 ml	1.498
Nipagin	5.7 g	1.065	Denol	35.0 ml	0.969
Dithane M45	0.2 g	0.022	Formaldehyde 35%	4.5 ml	0.202
Terralon LA	1.3 ml	1.498	Agar powder	4.0 g	1.632
Denol	14.3 ml	0.395			
Acetic acid	7.1 ml	0.604			
Agar powder	4.0 g	1.632			

Results

Diet pH and contamination

The rabbit pellet diet had a lower pH (pH 4.9) with no contamination (0%) than the sugarcane diet (pH 5.2) (25% contamination). At a lower pH (i.e. acidic pH) microbial growth and contamination are known to be suppressed (Cohen, 2015).

Developmental time

Figure 1 shows the composition of *E. saccharina* life stages and mortality between the rabbit pellet diet and the sugarcane diet, at the time of pupal harvest. Development of *E. saccharina* was faster in the rabbit pellet diet, which is reflected by the significantly fewer 1st and 2nd instar ($t(22) = -2.45$, $p=0.022$) and the 3rd-4th instar ($t(16.50) = -2.41$, $p=0.028$), in the rabbit pellet diet compared to the sugarcane diet, and the significantly higher numbers of pupae ($t(11.08) = 5.59$, $p< 0.001$), in the rabbit pellet diet compared to the sugarcane diet. There were non-significant differences for the 5th-6th instar ($t(22) = 0.34$, $p=0.736$), the pre-pupal stage ($t(11.02) = 1.37$, $p=0.199$) and for mortality ($t(22) = -0.27$, $p=0.787$) between the two diets.

Pupal harvest analysis

Eldana saccharina pupae were harvested from the daily mixes and used to maintain the main laboratory culture and provide life stages for various entomological programmes. There were no significant differences between larvae of the rabbit pellet diet and the sugarcane diet ($t(11.15) = 2.17$, $p=0.053$), but there were significantly more pupae ($t(12.57) = 7.11$, $p<0.001$) and emerged moths ($t(11.02) = 2.46$, $p=0.032$) (Figure 2).

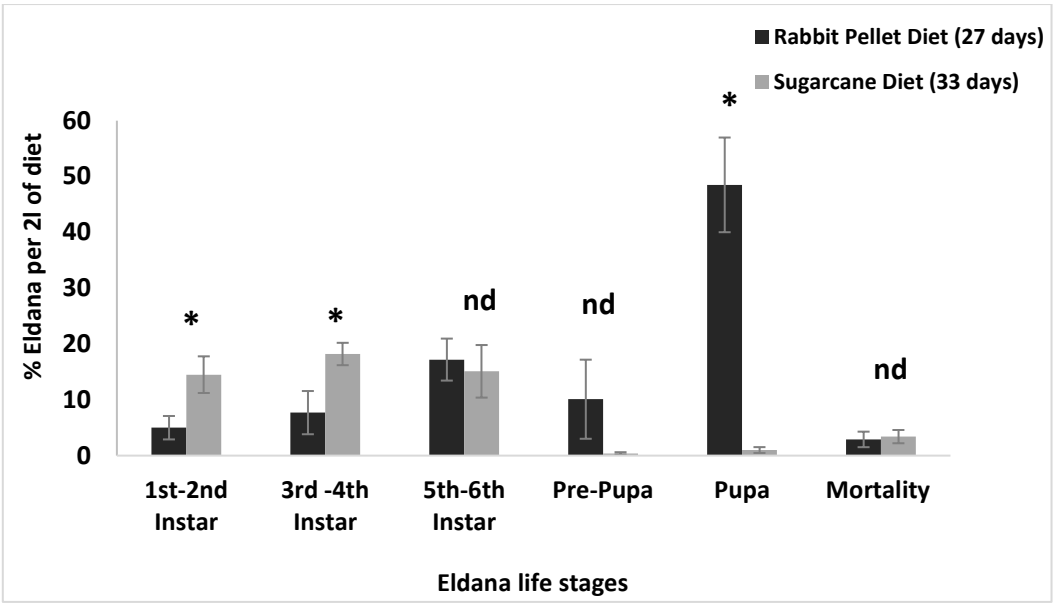

Figure 1. The distribution of life stages of *Eldana saccharina* recorded during pupal quality control (mean (± SE) numbers of 1st-2nd instar, 3rd-4th instar, 5th-6th instar, pre-pupa, pupa and dead larvae/pupa) on the rabbit pellet diet at day 27 and the sugarcane diet at day 33. The asterisk in the graph indicates significant differences (p<0.05) and the *nd* signifies non-significant differences (p>0.05) between the means (±SE) of the *E. saccharina* life stages and mortality for the rabbit pellet diet and sugarcane diet.

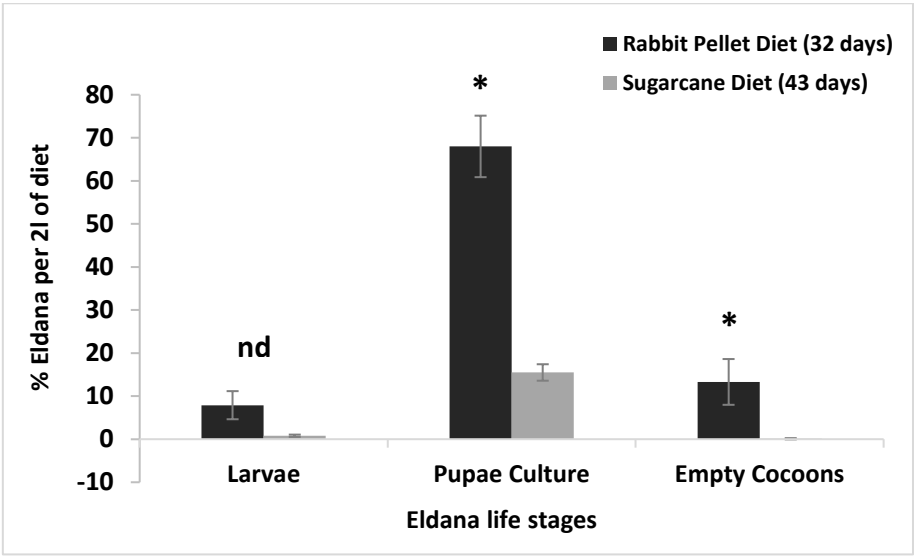

Figure 2. The distribution of growth stages (mean (± SE) number of larvae, pupae, emerged moths) of *Eldana saccharina* recorded at the time of pupal harvest on the rabbit pellet diet 32 days after inoculation, and the sugarcane diet 43 days after inoculation. The asterisk in the graph indicates significant differences (p<0.05), between the means (± SE) of the *E. saccharina* life stages for the rabbit pellet diet and sugarcane diet.

Mean pupal yield at harvest

The rabbit pellet diet yielded significantly more *E. saccharina* at different life stages than the sugarcane diet (t (39.17) = 4.26, p<0.001) (Figure 3).

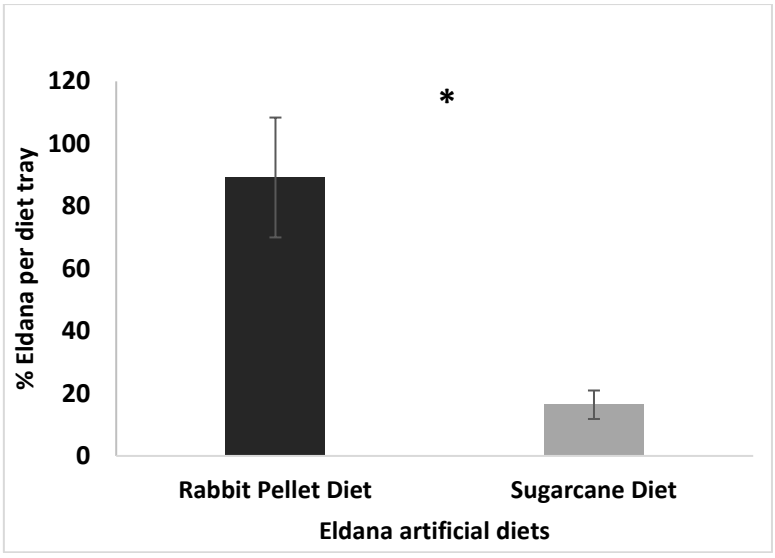

Figure 3. Mean (± SE) *Eldana saccharina* pupal yield at harvest for the rabbit pellet diet and the sugarcane diet. The asterisk in the graph indicates a significant difference (p<0.05) between *E. saccharina* yield means (± SE) for the rabbit pellet diet and sugarcane diet.

Analysis of Eldana saccharina *pupal weights at harvest*

There was a significant difference in male pupal weights (t (13.07) = -2.24, p=0.043), with those reared on the sugarcane diet being heavier than those reared on the rabbit pellet diet (Figure 4). There was no significant difference in female pupal weights (t (22) = 0.82, p=0.420) between the two diets.

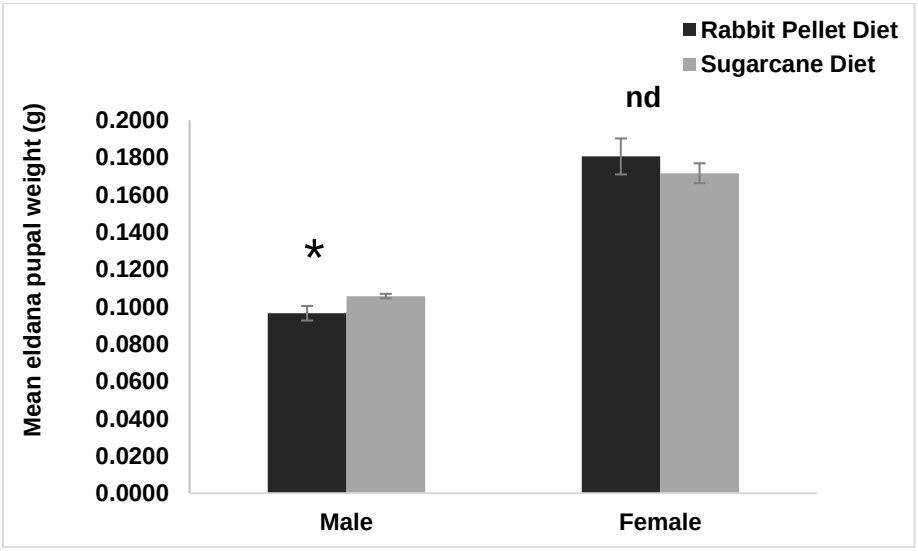

Figure 4. Mean (± SE) *Eldana saccharina* male and female pupal weights at harvest for the rabbit pellet diet and the sugarcane diet. The asterisk in the graph indicates a significant difference (p<0.05) and the *nd* signifies a non-significant difference (p>0.05) between the means (± SE) of the male and female pupal weights for the diet treatments.

It was calculated that the diet cost per litre of the rabbit pellet diet was higher than that of the sugarcane diet, with a difference of R2.46 (Table 3). However, the average *E. saccharina* yield per tray was much higher on the rabbit pellet diet than on the sugarcane diet. The diet cost

per *E. saccharina* life stage reared on the rabbit pellet diet is four times lower than that of life stages reared on the sugarcane diet.

Diet cost analysis per larva

Table 3. Diet cost comparisons of the Rabbit Pellet Diet and the Sugarcane Diet, in relation to the number of *Eldana saccharina* individuals produced per diet.

	Rabbit pellet diet	Sugarcane diet
Diet cost per litre (ZAR)	**16.26**	**13.80**
Average *E. saccharina* yield per tray (n=12) (%)	**89**	**16**
Cost per *E. saccharina* Larva (ZAR)	**0.05**	**0.22**

Discussion

For the past 15 years, a large body of literature has described artificial diets for more than 250 insect species (Taylor *et al.*, 1981). Hervert *et al.* (2016), for one, highlights the rearing of various Lepidopteran species on the McMorran diet. According to Davis (2007), the first known plant-feeding insect to be reared from egg to adult on artificial diet was the European corn borer (*Ostrinia nubilalis* Hubner) (Lepidoptera: Crambidae) in 1949, thus forming the basis for many phytophagous insect diets. Various Lepidopteran artificial diets have already been modified and improved as more information on the insects being reared, rearing techniques and dietary additives became known. This has certainly been true for the mass rearing of *E. saccharina*, with the present study being the most recent step in this process.

Following correct laboratory rearing SOPs made it possible to raise many *E. saccharina* individuals in a small space, with minimum risk of loss to disease or contamination (Conlong, 1992; Walton and Conlong, 2016). This was achieved as *E. saccharina's* mating habits, oviposition, fecundity, survival rate, sex ratio, environmental requirements and feeding preferences (Vanderzant, 1969) were well known. Nutritionally unbalanced diets are known to cause insect body defects, reductions in insect weight and increased mortality of all life stages (Hervert *et al.* 2016). This was also observed in the findings of Nagy (1970), who developed the rabbit pellet diet. The results presented in this paper are the latest in the continuing research on *E. saccharina* mass-rearing at SASRI-IRU, following the work of Atkinson (1978), Graham and Conlong (1988), Graham (1990), Gillespie (1993) and Walton and Conlong (2016).

Nagy (1970) developed a simplified form of an artificial diet for the rearing of the European corn borer, *O. nubilalis*, building a solid foundation for the current study. Using similar environmental conditions (28 ± 0.5 – 2ºC temperature and ambient humidity) and ingredients such as agar, lucerne meal, yeast, ascorbic acid, acetic acid and Nipagin (methyl paraben), allowed for the successful rearing of *E. saccharina*. The physical properties of the diets, such as texture, hardness, consistency, water content and the pH, played an important role in the growth and development of the insects (Vanderzant, 1969). The pH of the two diets (rabbit pellet diet = 4.9 and sugarcane diet = 5.2) in this study provides some insight into each diet's palatability, stability, activity of preservatives, solubility of nutrients and microbial activity (Cohen, 2015). According to Graham and Conlong (1988), the use of brown paper bags in diet containers causes condensation and contamination of the diet by yeasts and fungi. From their findings it was stated that yeast contamination may be traced to the presence of active yeast cells in the brewer's yeast powder in the diet, which contributed to the alternative use of yeast extract in the rabbit pellet diet, resulting in less contamination and less mortality. The use of ascorbic acid and Nipagin also played an important role in reducing contamination (Rutherford and Van Staden, 1991).

On average, the first *O. nubilalis* larvae feeding on Nagy's (1970) artificial diet, pupated after 26 days and the majority after 34 to 37 days. These results were similar to those of *E. saccharina* on the rabbit pellet diet, with high pupal production recorded from day 27 to day 32. Gillespie (1993) demonstrated *E. saccharina* survival in terms of larval density and suitability of the diet in terms of pupal weight. It was observed that there were no significant differences in larval density and pupal weights of *E. saccharina* developing in the diets containing approximately 0.3 -0.7 % of agar per litre. These results were similar to those of the rabbit pellet diet, implying that this diet is able to produce good quality insects using similar diet ingredient components of various tested artificial diets.

Conclusion

The major aim of the mass-rearing operation at the SASRI IRU is to rear large numbers of high quality insects on a cost effective diet (Gillespie, 1993). The rabbit pellet diet produced a higher number of good quality insects (mean per 2 litres of diet = 89 %) than the sugarcane diet (mean per 2 litres of diet = 16 %), and in a faster time at the same environmental conditions, without compromising their quality in terms of pupal weight, which is related to female fecundity, and was four times cheaper than the sugarcane diet. Therefore, based on these results, it is recommended that the rabbit pellet diet replace the sugarcane diet in future mass-rearing operations.

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