A Simplified Low-Cost Diet for Rearing *Spodoptera exigua* (Lepidoptera: Noctuidae) and Its Effect on *S. exigua* Nucleopolyhedrovirus Production

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ABSTRACT A low-cost simplified diet has been successfully developed for rearing *Spodoptera exigua* larvae under laboratory conditions. The cost of ingredients was lower than that of the standard diet based on a modified tobacco hornworm, *Manduca sexta* (L.), diet. The simplified diet fulfilled larval nutritional requirements without apparent adverse effects on the reproductive capacity of the insect. Survival, pupal sex ratio, and fecundity registered in insects that were reared on the simplified diet did not differ from those observed on the standard diet. The mean larval development period of insects that consumed the simplified diet was also similar to that of insects that consumed the standard diet, whereas weight of pupae and adult longevity were significantly higher in insects reared on simplified diet. Larvae consumed ≈11% more of the standard diet compared with the simplified diet and a corresponding increase was observed in the number of larvae that could be reared through to pupation on each liter of simplified diet. The production of *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) occlusion bodies (OBs) in insects grown on each type of diet was also evaluated. Weights of larvae at inoculation and at death, OB yields and biological activity of OBs did not differ significantly for each type of diet. The simplified low-cost diet appears suitable for large-scale in vivo production of SeMNPV OBs.

KEY WORDS *Spodoptera exigua*, artificial diet, growth and reproduction, SeMNPV, OB yield

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera; Noctuidae), is an important insect pest of many vegetable and ornamental crops in temperate and subtropical regions worldwide. The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV, Baculoviridae) is a highly effective biological control agent against *S. exigua* larvae, especially on greenhouse crops (Smits et al. 1987, Kolodny-Hirst et al. 1997, Lasa et al. 2007). However, as highly insecticidal batches of SeMNPV can only be produced in living insects, commercial scale production can be costly compared with synthesized chemicals or natural compounds that are produced during fermentation (Black et al. 1997, Szewczyk et al. 2006). This, coupled with problems of consistent insect quality, has been an important factor limiting the development of these viruses as the basis for biological insecticides (Shapiro 1982, 1986).

Efficient laboratory rearing of *S. exigua* is the key to ensuring a continuous supply of large numbers of insects for commercial virus production. Numerous techniques for rearing Lepidoptera have already been developed (Baumhover et al. 1966, Dutky et al. 1962; Griffith and Smith 1977, Poitout and Bues 1974; Shieh 1989, Singh and Moore 1985, Shelton et al. 1991, Waldbauer et al. 1984), but most of these are feasible only for small-scale laboratory rearing. For many species, adult requirements for optimal survival and oviposition, pupation, and immature development still need considerable study (Shieh 1989, Szewczyk et al. 2006). In this respect, research aimed at developing cost-effective artificial diets for larval rearing under controlled environmental conditions holds much promise (Szewczyk et al. 2006).

The development of artificial diets, pioneered by Vanderzant et al. (1962), facilitated the continuous production of insects. Since then, numerous species of dipterans, lepidopterans and coleopterans have been successfully reared under controlled laboratory conditions (Bell et al. 1981, Burton and Perkins 1972, Smith 1976, Singh and Moore 1985, Chu and Wu 1992, Blossey et al. 2000, Gupta et al. 2005).

Insect meridic diets usually contain numerous ingredients that are expensive and time consuming to prepare. Costs and preparation times can be reduced by careful selection of diet ingredients for rearing both

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Table 1. Composition of the standard and simplified diets used for rearing *Spodoptera exigua* larvae and labor costs to prepare 10 liters of each type of diet

Ingredients	Standard diet			Simplified diet		
	Cost (€/kg)	Quantity (g/liter)	Total cost (€/10 liters)	Cost (€/kg)	Quantity (g/liter)	Total cost (€/10 liters)
Water (ml)	—	760.00	—	—	800.00	—
Wheatgerm	1.48	72.00	1.07	1.48	120.00	1.78
Brewer's yeast	5.83	14.25	0.83	—	—	—
Casein	31.67	33.00	10.45	—	—	—
Soy protein	—	—	—	1.68	25.00	0.42
Sucrose	0.95	29.25	0.28	—	—	
Wesson's salt mixture[a]	28.89	9.40	2.72	28.89	8.00	2.31
Sorbic acid	12.89	1.50	0.19	12.89	4.00	0.52
Cholesterol	135.60	0.94	1.27	—	—	—
Linoleic oil (ml)	23.60	1.87	0.44	—	—	—
Agar	31.20	18.75	5.85	—	—	—
Carrageenan	—	—	—	12.53	15.00	1.88
Nipagin	18.32	0.94	0.17	18.32	1.00	0.18
Streptomycin	85.60	0.18	0.15	—	—	—
Chlortetracycline	605.52	1.65	9.99	—	—	—
Ascorbic acid	26.10	3.69	0.96	26.10	3.69	0.96
Choline	16.65	0.94	0.16	16.65	0.94	0.16
Vitamin mixture[b]	900.66	0.09	0.81	900.66	0.09	0.81
Total cost of ingredients			35.34€			9.02€
Labor cost[c]			15.81€			14.79€
Total cost[d] per 10 liters			51.15€			23.81€

[a] Wesson's salt mixture contains: $CaCO_3$ 1.55 g, $CuSO_4 \cdot 5H_2O$ 0.0029 g, $FePO_4$ 0.1103 g, $MnCl_2$ 0.0015 g, $MgSO_4$ 0.675 g, $KAI(SO_4)$ 0.0007 g, KCl 0.9 g, KH_2PO_4 2.325 g, KCl 0.0038 g, NaCl 0.785 g, NaF 0.0043 g, $Ca_3(PO_4)_2$ 1.12 g.

[b] Vitamin mixture contains: 30.77 g niacin, 30.77 g calcium pantothenate, 15.38 g riboflavin, 7.69 g thiamin hydrochloride, 7.69 g pyridoxine hydrochloride, 7.69 g folic acid.

[c] Determined for a technician's salary in Spain of 10.20 €/h as calculated for a 37.5 h working week.

[d] Exchange rate at time of writing: 1 € = US$ 1.35.

healthy and virus infected insects (Shapiro et al. 1981, Shapiro 1986). Balanced nutrient intake plays a very important role in the normal growth and reproductive cycle of insects and allows continuous rearing over a many generations. Most of the diets developed for rearing *S. exigua* support good insect growth, but have been ruled out as potential diets for mass rearing because of their high cost (Shorey and Hale 1965, Poitout and Bues 1974, Singh and Moore 1985). Considerable effort has been invested in the development of cost-effective diets based on the use of cheaper ingredients, or by reducing or eliminating nonessential ingredients and testing potential agar substitutes (Bell et al. 1981; Hunter-Fujita et al. 1998, Morimoto et al. 2004, Cappellozza et al. 2005).

The current study aimed to evaluate the effects of a simplified diet on the growth and reproduction of an established laboratory colony of *S. exigua.* The artificial diet developed by Hoffmann and Lawson (1964) for rearing tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), was compared with a slightly modified recipe developed for gypsy moth, *Lymantria dispar* (L.) (Lepidotpera: Lymantriidae) (Bell 1991). We then compared the production and insecticidal activity of viral OBs harvested from insects reared on each type of diet.

Materials and Method

Insects and Virus. A laboratory colony of *S. exigua* originally supplied by the Centre for Ecology and Hydrology (CEH) in Oxford, United Kingdom, was maintained continuously at 25 ± 2°C, 70 ± 5% humidity and 16L:8D photoperiod in the insectary facilities of the Universidad Pública de Navarra (Pamplona, Spain). Larvae were reared on an artificial diet adapted from Hoffman and Lawson (1964) (Table 1).

The wild-type isolate SeMNPV-SP2 used in this study originated from infected *S. exigua* larvae collected during an epizootic in vegetable greenhouses in El Ejido, southern Spain (Caballero et al. 1992). SeMNPV OBs were produced in fifth instar *S. exigua* larvae that were orally inoculated and reared on artificial diet until death. Virus-killed larvae were triturated and OBs were purified as described by Muñoz et al. (1997). OBs were resuspended in distilled water, counted in triplicate using a Neubauer improved chamber (Hawksley, Lancing, United Kingdom) under phase-contrast microscopy, and stored at 4°C before use.

Artificial Diets. Two artificial diets were tested (Table 1). The standard diet, widely used for rearing *S. exigua*, was modified from the tobacco hornworm diet of Hoffman and Lawson (1964). The simplified diet, with high wheatgerm content, was modified from that described by Bell et al. (1981) for mass rearing of *L. dispar.* The diets were prepared as follows: ingredients were added in a beaker in the order given in Table 1 and thoroughly mixed before being heat sterilized (121°C, 20 min). Antibiotics (streptomycin, chlortetracycline), a vitamin mixture, ascorbic acid and choline were added when the mixture had cooled to 50°C. The completed diets were blended with a kitchen mixer. Finally, each diet was poured into sterile containers of 400 ml (17 × 10 × 2.5 cm) and allowed to solidify at room temperature. The times taken by an

experienced technician to weigh the different ingredients for a 10-liter batch of diet, prepare and dispense the diet into containers were registered.

Rearing Procedure. Groups of 80 neonate larvae were reared individually in 25 ml plastic cups into which blocks of diet 2 × 2 × 1 cm had been placed. Diet blocks were replaced at 3 d intervals or until they showed signs of desiccation. The sex ratio and weight of the resulting pupae were recorded. At adult emergence, 30 females and an equal number of males from the same diet treatment were selected for mating in single pairs, transferred to plastic boxes (13 × 11 × 5 cm) that were wall-covered with filter paper to facilitate egg laying and which contained 10% honey-soaked cotton balls on the lid of a petri dish for adult feeding. The number of eggs laid by females was recorded during the first five days. The number of eggs that hatched and adult life span were also recorded at intervals of 24 h. Insects were maintained at 25 ± 2°C for the duration of the experiment. In summary, the following parameters were determined: (1) number of larvae that survived from egg to pupation (percentage of survival), (2) pupal sex ratio, (3) larval development time, (4) pupal weight, (5) duration of the pupal stage, (6) adult longevity, (7) number of eggs laid by females in a 5-d period (fecundity), and (8) proportion of eggs that hatched (fertility).

To determine diet consumption, 500 neonate larvae were placed in boxes (100 larvae/box) containing diet blocks of 9 × 3 × 1 cm (27 cm^3). The number of blocks that were entirely consumed was recorded during larval development. The experiment was performed six times for each type of diet. All results were analyzed in SPSS v.15.0. (SPSS Inc., Chicago, IL).

OB Production. The number of OBs produced in larvae fed with standard and simplified diets was determined by inoculating *S. exigua* fourth instars with a single concentration of SeMNPV-SP2 OBs previously estimated to result in 90% mortality (Murillo et al. 2003). Newly molted starved larvae were individually weighed and allowed to drink from aqueous suspensions containing 6.4×10^3 OBs/ml, 10% sucrose, and 0.001% (wt:vol) Fluorella Blue. Twenty-five larvae were transferred individually to standard or simplified diet and maintained at 25 ± 2°C. Larvae were examined daily for signs of nucleopolyhedrosis disease until death, at which time larvae were weighed and stored at −20°C. Cadavers were thawed, homogenized individually in 1 ml bidistilled water, filtered through a fine steel gauze to remove insect debris, and appropriately diluted for OB titration. The entire experiment was performed three times.

The number of OBs recovered from each larva was determined using a Neubauer improved counting chamber under phase contrast microscopy at 400× and calculated as the mean number of OBs counted in three replicate samples of each suspension. The relationship between OB production and larval weight (OB/mg) was estimated based on larval weight recorded 0–72 h postmortem, when the insect integument was still intact. Larval weights, numbers of OBs per larva and OBs per milligram of larval weight were normalized by logarithmic transformation and were subjected to statistical analysis in SPSS V.15.0 (SPSS Inc.).

Insecticidal Activity of OBs. The insecticidal activity of OBs was evaluated in *S. exigua* second instars using a modified droplet bioassay technique (Hughes and Wood 1981). Late first instars were starved at 25 ± 2°C and allowed to molt to the next instar over a period of 10 h. Groups of 30 larvae were allowed to feed on droplets of 10% sucrose, 0.001% Fluorella Blue food dye and one of a series of five concentrations of OBs in the range of 2.5×10^5 to 3.0×10^3 OBs/ml, previously calculated to result in mortalities between 10 and 90%. Control larvae were treated identically but fed on a solution of sucrose and food dye alone. The first 25 larvae that ingested the OB suspension within 10 min were placed individually in the cells of a 25 well tissue culture plate containing a cube of standard diet and incubated at 25 ± 2°C. Mortality was registered daily from 5 d postinoculation onwards. The bioassay was performed three times. Results were subjected to logit regression with the GLIM program with a binomial error distribution specified (Crawley 1993, Numerical Algorithms Group 1993). Minor overdispersion in the mortality results was taken into account by scaling the error distribution where necessary. OB pathogenicity was expressed as the 50% lethal dose based on an average ingested volume of 0.33 μl per larva in this instar (Chaufaux and Ferron 1986, Muñoz et al. 1997).

Results

Diet Preparation and Insect Rearing Cost Analysis. The time taken to produce a 10-liter batch of the standard diet was 93 min compared with 88 min for the simplified diet. This rather small difference was because of the shorter period required to weigh the ingredients of the simplified diet (45 min), compared with the more complex standard diet (50 min). Times taken to weigh and mix the thermolabile ingredients that were added after the autoclaved diet had cooled were identical for both diets (33 min), as was the time spent dispensing each type of diet into plastic containers (10 min), and time spent disinfecting and cleaning the working area (8 min).

In consequence, the labor costs for preparation of each type of diet were slightly lower for the simplified diet. However, the major saving for the simplified diet lay in the reduced cost of the ingredients, that represented a saving of approximately three-fold compared with the standard diet. This translated to a total cost of 23.81 € (equivalent to U.S. $32.14 at current rates of exchange) for a 10 liter batch of simplified diet compared with 51.15 € (U.S. $69.05) for the standard diet (Table 1). Moreover, because of differences in their physical properties, particularly their tendencies to dry out, larvae reared on standard diet consumed up to 0.13 ± 0.09 ml more diet during the entire larval development period, representing an 11% increase in consumption (Table 2), compared with insects reared on the simplified diet ($t = 2.300$; df = 10; $P = 0.044$).

Table 2. Mean diet consumption during *Spodoptera exigua* larval development and no. of larvae reared per liter of standard and simplified diet

Diet	Diet consumption per larvae (ml ± SEM)	Larvae reared per liter (means ± SEM)
Standard	1.28 ± 0.03a	780.33 ± 23.21a
Simplified	1.15 ± 0.04b	869.30 ± 32.40b

Diet consumption was estimated by determining the no. of 27 cm^3 blocks of diet that were consumed by groups of 500 larvae during the course of their development. Values followed by different letters within each column differ significantly (*t*-test, $P < 0.05$).

In consequence, a higher number of larvae could be reared on each liter of simplified diet compared with the standard diet ($t = 2.234$; df = 10; $P = 0.045$) (Table 2).

Effects of the Simplified Diet on Insect Development and Reproduction. Visual inspections of insects reared on the simplified diet suggested that they were as healthy, active and of similar size to insects reared on standard diet. The two diets were equally efficient in terms of larval development times from egg to pupa for both females ($t = 1.418$; df = 10; $P = 0.187$) and males ($t = 1.634$; df = 10; $P = 0.133$) (Table 3). The survival of larvae on simplified diet (90.4 ± 8.4%) was also similar to that of insects reared on standard diet (90.8 ± 6.2%), and no differences were observed in the sex ratio on either diet (1:1). The weight of male or female pupae did not differ according to diet (males: $t = 0.610$; df = 9; $P = 0.876$, females: $t = 0.377$; df = 9; $P = 0.715$), although female pupae tended to be heavier than males on both diets (Fig. 1A). The duration of the pupal stage was longer in males than females ($t = 3.505$; df = 9; $P = 0.03$), without significant diet-specific differences registered in insects of either sex (males: $t = 0.602$; df = 8; $P = 0.564$, females: $t = 1.063$; df = 8; $P = 0.319$) (Table 3). Adult longevity of males and females was greater for those reared on simplified diet compared with insects reared on standard diet (Fig. 1B). Thus, female moths from larvae reared on simplified diet lived 2.8 ± 0.6 d longer than females reared on standard diet ($t = 3.083$; df = 10; $P = 0.012$), whereas males lived 2.6 ± 0.8 d longer when reared on the simplified diet ($t = 2.188$; df = 10; $P = 0.041$). The average number of eggs laid by females reared on simplified diet (945.6 ± 125.7 eggs) was not significantly different ($t = 1.203$; df = 10; $P = 0.257$) from that of females reared on standard diet (1047.2 ± 112.4 eggs). Similarly, the proportion of eggs that hatched did not differ significantly between diet treatments at 93.3 ± 1.2 and 93.9 ± 0.6%, respectively (Mann-Whitney U = 13.0, $P = 0.423$).

Table 3. Developmental periods of larvae and pupae of *Spodoptera exigua* reared on standard and simplified diet

Diet	Larval development (days ± SEM)		Pupal development (days ± SEM)	
	♀	♂	♀	♂
Standard	13.0 ± 0.7a	12.9 ± 0.6a	7.3 ± 0.6a	8.1 ± 0.6a
Simplified	12.4 ± 0.6a	12.3 ± 0.6a	6.9 ± 0.3a	7.9 ± 0.4a

Means for each parameter followed by an identical letters are not significantly different for comparisons between treatments within each column (*t*-test, $P > 0.05$).

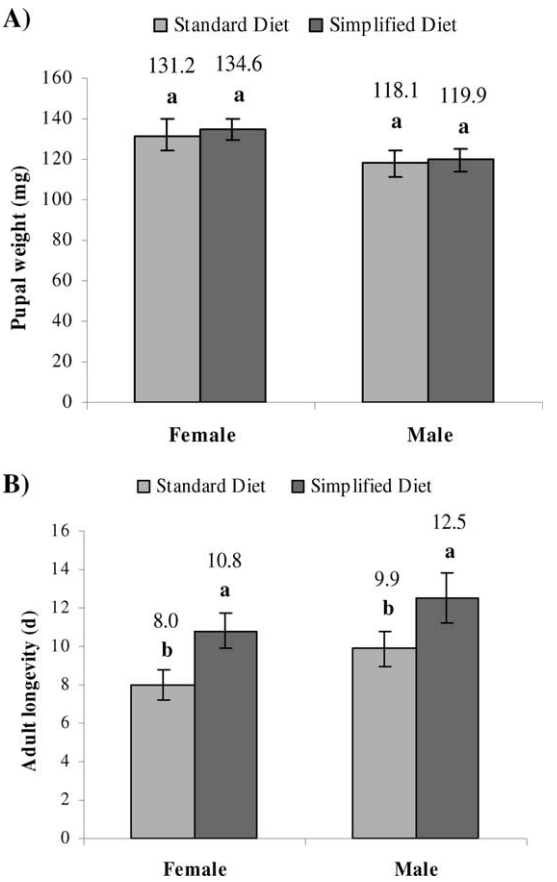

Fig. 1. (A) Pupal weight in mg (means ± SEM) of insects reared on standard and simplified diets. (B) Adult longevity in days (means ± SEM) from adult emergence to death of *Spodoptera exigua* moths obtained from larvae reared on standard and simplified diets. Columns headed by identical letters not differ significantly for comparisons of diet treatment within each sex (*t*-test, $P > 0.05$).

Effects of Diet on SeMNPV OB Production. Larval weight registered immediately before inoculation was similar for the standard and simplified diet treatments at 48.2 ± 1.2 and 54.5 ± 1.2 mg/larva, respectively ($t = 0.926$; df = 6; $P = 0.396$). Insects from both treatments were also of similar weight just after death at 141.1 ± 1.0 and 152.1 ± 1.1 mg/larva for the standard and simplified diets, respectively ($t = 1.336$, df = 6, $P = 0.230$). In line with these results on larval weight, the average yield of SeMNPV OBs recorded for larvae that fed on simplified diet (8.86×10^8 OBs/larva) was not significantly different from that observed in insects that fed on standard diet (8.71×10^8 OBs/larva) ($t = 0.386$; df = 6; $P = 0.715$). The yield of OBs per mg insect weight at death in insects that fed on simplified diet was 6.36×10^6 OBs/mg, compared with 6.09×10^6 OBs/mg for insects from the standard diet ($t = 0.454$; df = 6 $P = 0.666$).

Effects of Diet on Insecticidal Activity of SeMNPV OBs. The LD_{50} value of SeMNPV OBs produced in larvae reared on simplified diet was 9.2 OBs/larva in second instars which was similar to the corresponding value (10.2 OBs/larva) estimated for OBs from larvae reared on standard diet ($\chi^2 = 0.418$; df = 2; $P = 0.721$). Dose-mortality responses for OBs produced in both diets were fitted with a common slope of 0.665 ± 0.022 and intercepts of -1.477 ± 0.175 for the simplified diet and -1.547 ± 0.175 for the standard diet treatment. The dose*diet interaction was not significant ($\chi^2 = 0.015$; df = 1; $P = 0.900$) and overdispersion was not observed in datasets. No virus mortality was registered in the control.

Discussion

Development of promising pest control methods based on baculoviruses requires cost-effective rearing of healthy, vigorous insects in which baculovirus can be produced in a reasonably uniform manner. A number of techniques for small-scale rearing of *S. exigua* have been published (Shorey and Hale 1965, Poitout and Bues 1970, Singh and Moore 1985, Waldbauer et al. 1984). Studies are now being conducted to mechanize as far as possible the procedures involved in rearing the larval stage and to accommodate all other phases of the rearing process so that *S. exigua* can be mass-produced and maintained on a continuous basis.

The standard diet employed in this study has been used successfully for many years in the continuous rearing of *S. exigua* and other lepidopteran species at the Universidad Pública de Navarra, Spain. This diet includes a variety of ingredients such as casein, agar, cholesterol, or antibiotics that are expensive (Table 1). The diet must be exceptionally stable with regard to its nutritional and physical properties or else be replaced with fresh diet as necessary, which increases diet and labor costs and makes automated rearing technology difficult to apply. In contrast, the simplified diet described here was less expensive (53% cheaper), while allowing the rearing of nearly 100 more larvae per liter. This achievement may be attributed to the higher moisture content and the different hardness of the simplified diet, which remains in a suitable state for insect feeding for a longer period of time than the standard diet. The simplified diet also resulted in heavier pupae than the control diet and, as observed by other authors, the weight of pupae and female fecundity are often directly related (Leonard and Doane 1966, Miller et al. 1982). Insects reared on each type of media showed similar survival, developmental rates and adult size, indicating that the simplified diet provides a nearly optimal balance of nutrients. Moreover, the sex ratio and larval development times of insects reared on simplified diet did not deviate substantially from the values for *S. exigua* reported by others that employed more complex diet recipes (Poitout and Bues 1970, Griswold and Trumble 1985, Singh and Moore 1985, Chu and Wu 1992, Aiping et al. 2005).

Rearing insects for virus production requires a less complex diet than that used for sustained maintenance of a colony, because the prime objectives are vigorous larval growth and good survival rates (Shapiro 1986). For this reason, the simplified diet described here was prepared without cholesterol, linseed oil, brewer's yeast, and some antibiotics, such as streptomycin and chlortetracycline (aureomycin), all of which were present in the standard diet. In this study, we observed that larvae reared on simplified diet were similar in weight to larvae reared on standard diet at the moment of inoculation and at the moment of virus-induced death when OBs were collected. Larval weight at the time of infection has been demonstrated to be an important factor in determining overall OB production (Sherman 1985, Shapiro 1986, Cherry et al. 1997). Similarly, total OB yields per insect (8.86×10^8 OBs/larva) and OB production per mg larval weight were similar for insects reared on each type of diet.

Lack of some of the components present in the standard diet was compensated by increased use of wheatgerm, which likely provided some additional sterols and essential fatty acids. Wheatgerm has been used for years as a major ingredient and source of energy, minerals, and lipids, and has the additional advantage of containing substances that stimulate the feeding response in insects (Vanderzant et al. 1962). Dietary protein quality is also an important determinant for insect rearing and influences not only survival and growth rates, but also cuticle melanization and immune system function (Lee et al. 2008). Casein, which has been used frequently as a primary protein supply was substituted in the simplified diet by soy protein, an inexpensive source of protein that supplies all the essential amino acids without affecting physiological functions. Vitamins and micronutrients are also essential for insects (Shapiro et al. 1981, Popham and Shelby 2006). The most important vitamins required for insect growth and development are within the B vitamin group, which act as coenzymes in the metabolism of proteins, carbohydrates, and fat. Ascorbic acid also plays a key role in reproduction and larval physiology. However, this role changes depending on the developmental stage. Absence of ascorbate in the diet, particularly during the first and last instars, can have beneficial effects on pupal development without affecting the survival rate or larval development times (Cappellozza et al. 2005). However, in *Heliothis virescens* (F.), very low levels of dietary ascorbate are correlated with a significant increase in susceptibility to NPV infection and marked changes in the dynamics of infection within insects reared on ascorbate reduced diet (Popham and Brandt 2006). In contrast, Shapiro et al. (1981) observed that an increase in dietary vitamin concentration resulted in increased virus yields during mass production of the gypsy moth NPV (LdMNPV), although the additional cost did not seem to justify the extra amount of vitamin mixture.

Because of their nutrient and moisture content, insect diets can be highly susceptible to contamination by microorganisms, even more so when incubated at warm temperatures (≈25°C). For this reason, antibi-

otics and antimicrobial agents are often necessary to suppress microbial growth in diets. An ideal antimicrobial dietary additive should check opportunistic microbial growth without harming the insect. Tests of the effectiveness of different antimicrobial agents have identified benzoic and sorbic acids the most efficient compounds, with little or no detrimental effects on insects (Alverson 2003). In this study, we decided to eliminate the costly antibiotics streptomycin and chlortetracycline, and control microbial contaminants in the simplified diet by increasing the concentration of sorbic acid to 4000 mg/kg, which proved to be highly effective as a diet preservative without undue effects on insect development (Bell et al. 1981). Nevertheless, "clean" techniques must be strictly adhered to during diet preparation and handling, especially when working in mass production conditions, where periods between diet replacements can exceed 10 d, increasing the risk of microbial growth.

Agar is usually the most expensive ingredient in insect diets and frequently accounts for 30–50% of total control diet cost. We substituted agar for carrageenan, an alternative gelling agent that has been used successfully for rearing lepidopterans such as *L. dispar* (Bell and Romine 1986) and *Trichoplusia ni* (Hübner) (Vail et al. 1973). The physiochemical properties of carrageenan are different from those of agar, particularly in terms of the gelling temperatures that are much higher for carrageenan. Frequent stirring during diet dispensation and controlling the exposure of the mixture to air are required to prevent premature solidification of carrageenan-containing diets. Moreover, a positive correlation between diet gel strength and viral biological activity has been reported, which was thought to be affected by the capacity of the gelling agent to bind water molecules (Shapiro 1986). It is hypothesized that water release was accompanied by the release of ions or water-soluble materials that could be detrimental to OB activity. However, no changes in OB pathogenicity were observed in the current study in virus extracted from larvae reared on simplified diet with carrageenan compared with OBs produced in larvae reared on the agar-containing control diet, or to those registered by Lasa et al. (2007) with the same virus strain and diet (SeMNPV-SP2).

The cost of ingredients and diet processing (e.g., weighing, blending, and dispensing of ingredients that are handled manually) must be taken into account to evaluate the efficiency of the rearing system (Shapiro 1986). Overall, ingredients in the simplified diet were nearly four-fold cheaper than those of the standard diet (Table 1). Labor costs were only slightly reduced for the simplified diet, but the difference was small because most operations involved in diet preparation are common to both diets. Our attention is now being directed to the development of automation of a large part of this process. However, several features, including the ability to accommodate a wide variety of batch sizes and rapid and thorough mixing of the ingredients are some of the limitations that still hamper the development of this technology (Bell 1991).

Before a diet is considered suitable for insect mass rearing, it should be evaluated for several generations to determine whether it is capable of maintaining vital biological parameters for insect survival, reproduction and normal behavior (Cohen 2001). The simplified diet presented here has now been used successfully for over eight generations of *S. exigua* rearing in the insectary of the Universidad Pública de Navarra without any adverse effects observed on the health or vigor of the colony, although quantative data are not available. In addition, it appears to provide all the essential nutrients in the proportions needed for normal growth, development and reproduction of *S. exigua.* Moreover, the simplified diet offers considerable promise for mass production because more larvae can be reared compared with the same volume of standard diet. The simplified diet was also found to be suitable for SeMNPV production, with no reduction in OB insecticidal activity and at significantly lower cost compared with the standard diet.

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