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Development of a Dry Artificial Diet for *Nezara viridula* (L.) and *Euschistus heros* (Fabricius) (Heteroptera: Pentatomidae)

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Desenvolvimento de uma Dieta Artificial Seca para *Nezara viridula* (L.) e *Euschistus heros* (Fabricius) (Heteroptera: Pentatomidae)

RESUMO - Dietas artificiais preparadas à base de germe de trigo, proteína de soja, dextrosol, fécula de batata, sacarose, celulose, óleo de soja ou girassol, e solução vitamínica para a criação de *Nezara viridula* (L.) e *Euschistus heros* (Fabricius) foram testadas em condições controladas de temperatura (25 ± 1°C), UR (60 ± 10%), e fotofase (14h). Três dietas foram testadas e comparadas com a natural [sementes de soja e amendoim e fruto de *Ligustrum lucidum* Ait. (Oleaceae)] As três dietas artificiais permitiram o desenvolvimento completo. A dieta contendo óleo de girassol foi a mais adequada para *N. viridula* enquanto que, para *E. heros*, o melhor desenvolvimento foi na dieta contendo óleo de soja. Os resultados obtidos indicam que as dietas artificiais foram inferiores à natural. As dietas artificiais foram mais adequadas para *E. heros.*

PALAVRAS-CHAVE: Biologia, Hemiptera, criação massal, percevejo

ABSTRACT - Artificial diets prepared with wheat germ, soybean protein, dextrosol, potato starch, sucrose, cellulose, soybean or sunflower oil, and vitamin solution for rearing *Nezara viridula* (L.) and *Euschistus heros* (Fabricius) were tested under controlled temperature (25 ± 1°C), RH (60 ± 10%), and photophase (14h). Three diets were tested and compared with the natural diet privet [soybean and peanut seeds and privet *Ligustrum lucidum* Ait. fruit (Oleaceae)]. All three artificial diets allowed full development. The diet containing sunflower oil was the most suitable for *N. viridula* while *E. heros* developed better on a diet composed of soybean oil. Data indicated that the artificial diets were inferior to the natural diet. The artificial diets were more adequate for *E. heros*.

KEY WORDS: Biology, Hemiptera, mass rearing, stink bug

Egg parasitoids are important regulator agents of stink bug populations in soybean and their increasingly greater use in integrated pest management programs demands large-scale stink bug production (Corrêa-Ferreira 2002). The development and improvement of a host rearing methodology, as well as of an artificial diet, are essential for the large-scale production and release of parasitoids in soybean fields. Several attempts have been made to develop or adapt artificial diets for stink bug rearing with only partial success (Jensen & Gibbens 1973, Brewer & Jones 1985, Jones & Brewer 1987, Panizzi *et al*. 2000).

Meridic liquid and oligidic semiliquid diets have been developed for sap-feeding hemipterans that develop on plant tissues, such as *Lygus hesperus* Knight and L*ygus lineolaris* Palisot de Beauvois (Heteroptera: Miridae) (Debolt 1982).

In general, dry diets are used for omnivorous chewing insects, and are less contaminated by pathogens. However, Panizzi *et al*. (2000) used a dry artificial diet for *Nezara viridula* (L.) rearing, and obtained low nymph mortality (ca. 30%); however, developmental time was longer and adult body weight was lower compared with those reared on soybean pods. Noda & Kamano (2002) used a modified *Riptortus clavatus* Thunberg (Heteroptera: Coreidae) meridic diet to rear *N. viridula* and *Nezara antennata* Scott. The mean development time of *N. viridula* nymphs was also significantly longer than those reared on seeds, but survivorship was greater (ca. 87%) on the meridic diet.

Therefore, new and preferably artificial diets are sought, which would correct the indicated deficiencies and would demand less labor, since the process currently used requires a great deal of manipulation. The present work was carried out to improve the dry artificial diet based on Panizzi *et al*.

(2000), to rear *N. viridula* and *Euschistus heros* (Fabricius), aiming at obtaining a large number of insects with similar characteristics to those found in nature with reduced labor, and allowing natural enemies (especially those that attack eggs) to be reared for field releases.

Material and Methods

Obtaining and rearing *N. viridula* and *E. heros*. To establish laboratory stock colonies, *N. viridula* and *E. heros* were collected in soybean fields in Piracicaba, State of São Paulo, and reared as described by Corrêa-Ferreira (1985), under controlled conditions (25 ± 1°C, 60 ± 10% RH and 14 L:10D photoperiod). Egg masses were collected daily and placed in 9 cm diameter petri dishes, lined with filter paper and maintained in incubators, at the conditions previously described. Daily observations were made and nymphal hatching was recorded. Since 1st-instar nymphs do not feed, they were maintained in the petri dishes until the beginning of the 2nd instar, when they were transferred to the respective tested diet.

Artificial diet preparation. The dry ingredients were weighted, macerated in a crucible, and the liquid components were added and mixed until completely homogenized in different proportions to make up three alternative diets (Table 1). Diets were cut into small pieces (1 cm^2) and dried for 4h in an oven at 60°C.

Performance of *N. viridula* and *E. heros* on artificial diets. Second instars of the first laboratory generation (F1) of *N. viridula* and of *E. heros* obtained from different cohorts were individualized with a brush in 9-cm petri dishes and fed dry artificial diets; water was offered using a damped cotton wad. Nymphs were kept at constant temperature of 25 ± 1°C, RH 60 ± 10%, and 14h photophase. These nymphs were compared to nymphs fed natural diet [peanut and soybean seeds plus privet *Ligustrum lucidum* Ait. fruit (Oleaceae)]. The experiment consisted of four treatments [three dry artificial diets (Table 1) and one natural diet]. Each nymph was considered a replicate, the number of nymphs used varied (range of 172-191 for *N. viridula* and 172-188 for *E. heros*.).

To evaluate adult performance, 25 pairs of newly-emerged adults for each species studied were placed into 300 ml plastic cups containing artificial or natural diet. Sunflower seeds were offered as a food supplement for adults in all treatments and soybean leaves were placed in the containers as oviposition substrate. The following parameters were recorded daily and calculated: total duration and viability of the nymphal stage, adult weight 24h post-emergence, sex ratio, adult deformity, pre-oviposition and oviposition periods, longevity of males and females, percentage of females that laid eggs, number of eggs/female and, egg viability. Data were submitted to analysis of variance and compared using Tukey test ($P < 0.05$), in a completely randomized design. The number of eggs/female was transformed to $\sqrt{(x+8)}$, and the percentage of egg viability was transformed to arc sin $\sqrt{(x/100)}$.

In order to compare the diet quality, the fertility life table was calculated for both species by using the following indices: duration of one generation ($T = \frac{\sum mx.lx.x}{\sum mx.lx}$), net reproductive rate ($R_0 = \sum mx.lx$), intrinsic rate of increase ($rm = \frac{\ell n R_0}{T}$), and finite rate of increase ($\lambda = e^{rm}$) (Soutwood 1971).

Results and Discussion

Biology of *N. viridula* and *E. heros*. An elongation of the 2nd instar-adult period for both species was observed when

Table 1. Components of dry artificial diets tested for reearing *N. viridula* and *E. heros*.

Components	Diets		
	A	B	C
Wheat germ	12.5 g	17.9 g	12.5 g
Soybean protein	20.0 g	15.0 g	20.0 g
Dextrosol	5.0 g	7.5 g	5.0 g
Potato starch	5.0 g	7.5 g	5.0 g
Sucrose	5.0 g	2.5 g	5.0 g
Cellulose	7.5 g	12.5 g	7.5 g
Soybean oil	12.5 ml	10.0 ml	-
Sunflower oil	-	-	12.5 ml
Vitamin solution[1]	5.0 ml	5.0 ml	5.0 ml
Water	30.0 ml	30.0 ml	30.0 ml

[1]Vitamin solution composition = niacinamide 1 g, calcium pantothenate 1 g, thiamine 0.25 g, riboflavin 0.50 g, pyridoxine 0.25 g, folic acid 0.25 g, biotin 0.02 ml, vitamin B_{12} 1 g, added into 1000 ml distilled water.

Table 2. Duration (±SE) of the period from 2nd instar-adult and of each nymphal instar of *N. viridula* and *E. heros* reared on four diets. (25 ± 1°C; RH: 60 ± 10%; photophase: 14h).

Insect	Diets	2nd instar-adult (days)	Nymphal instars (day)			
			2nd	3rd	4th	5th
N. viridula	Natural	25.5 ± 0.22 c	5.8 ± 0.03 c	4.9 ± 0.06 b	6.4 ± 0.11 b	8.4 ± 0.10 c
	A	27.6 ± 0.18 ab	6.1 ± 0.04 b	5.7 ± 0.08 a	6.9 ± 0.09 a	8.9 ± 0.13 b
	B	28.4 ± 0.19 a	6.3 ± 0.05 a	5.5 ± 0.05 a	6.8 ± 0.07 ab	9.8 ± 0.13 a
	C	27.1 ± 0.16 b	6.4 ± 0.05 a	5.1 ± 0.08 b	6.9 ± 0.08 a	8.7 ± 0.09 b
E. heros	Natural	24.1 ± 0.16 c	5.5 ± 0.05 ab	4.8 ± 0.08 b	5.2 ± 0.08 b	8.6 ± 0.10 b
	A	26.0 ± 0.34 ab	5.4 ± 0.05 b	5.1 ± 0.07 a	6.1 ± 0.20 a	9.4 ± 0.11 a
	B	26.9 ± 0.14 a	5.6 ± 0.05 a	5.1 ± 0.51 a	6.3 ± 0.12 a	9.9 ± 0.13 a
	C	25.7 ± 0.28 b	5.3 ± 0.03 b	4.9 ± 0.06 ab	5.9 ± 0.13 a	9.6 ± 0.15 a

Means followed by the same letter in the same column are not different among themselves by Tukey test (P < 0.05).

nymphs were reared on all three artificial diets (Table 2). This has also been observed previously for other heteropterans reared on different artificial diets (Jensen & Gibbens 1973, Panizzi *et al*. 2000, Noda & Kamano 2002), and was shown to correlate with the inadequate nutritional quality of the diet (Panizzi & Rossini 1987). Although the elongation in time observed was small (never > 1.6 days for both species) it still gives an indication of the nutritional unsuitability of the tested diets (Panizzi 1991, Parra 1991).

For *N. viridula* the best diet was the sunflower oil-based diet (diet C), with a nymph developmental time of 27.1 days, slightly longer than that observed for nymphs raised on the natural diet (25.5 days) (Table 2). This value was similar to that reported by Panizzi *et al*. (2000) using a similar diet, but with different ingredients proportion. Noda & Kamano (2002) obtained a reduction in the time of nymph development when *N. viridula* was reared on an artificial diet containing casein, starch, dextrin, sucrose, soybean oil, and cellulose; this diet is similar to the one used to rear the alydid *R. clavatus* (Noda & Kamano 1983).

For *E. heros,* nymph developmental time on the diet containing sunflower oil (diet C) took 25.7days, similar to nymphs raised on diet A, which contained 12.5ml soybean oil (26.0 days) (Table 2). Diet B, with a smaller amount of soybean oil (10 ml), was the least suitable for both species of stink bugs. Elongation on time of development for *N. viridula* was constant for all instars, in *E. heros* this occurred in the last two instars (Table 2).

In previous studies, the viability of 2nd instar-adult, at least for one of the species studied, was ca. 75% when reared on artificial diets (Singh 1983, Panizzi *et al*. 2000); the duration of the immature stages and nymph mortality of *N. viridula* is variable according to the conditions in which the insect is reared (Corpuz 1969, Jones Jr. & Brewer 1987, Panizzi 1987). In the present study, the viability from 2nd instar to adult was similar on natural and on artificial diets, and

Table 3. Viability (±SE) of the period from 2nd instar-adult and of each nymphal instar of *N. viridula* and *E. heros* reared on four diets (25±1°C; RH: 60±10%; photophase: 14h).

Insect	Diets	Viability (%)				
		2nd instar-adult (n.s.)	2nd	3rd (n.s.)	4th (n.s.)	5th
N. viridula	Natural	74.2 ± 0.04	96.5 ± 1.65 a	91.8 ± 2.74	91.6 ± 3.02	91.4 ± 2.14 a
	A	69.6 ± 3.33	92.7 ± 1.41 a	95.9 ± 2.91	97.7 ± 1.69	80.1 ± 2.37 b
	B	71.3 ± 0.01	94.3 ± 1.50 a	96.7 ± 1.17	95.9 ± 1.43	81.6 ± 2.03 b
	C	76.0 ± 0.03	97.0 ± 0.87 a	96.3 ± 0.80	96.8 ± 1.69	84.1 ± 2.18 b
E. heros	Natural	81.6 ± 3.30	88.9 ± 3.88 b	97.3 ± 1.42	96.7 ± 1.08	97.6 ± 1.55 a
	A	77.8 ± 5.41	98.5 ± 0.76 a	91.9 ± 4.38	95.8 ± 1.03	89.6 ± 3.81 a
	B	64.1 ± 4.77	93.3 ± 2.35 ab	90.1 ± 3.43	91.9 ± 3.81	83.0 ± 5.58 a
	C	72.6 ± 5.10	96.1 ± 1.11 ab	94.3 ± 1.99	91.3 ± 3.34	87.8 ± 4.83 a

Means followed by the same letter in the same column are not different among themselves by Tukey test (P < 0.05). For analysis, the viability data were transformed by arc sin $\sqrt{(x/100)}$.

n.s. Non significant

Table 4. Sex ratio (±SE), male and female weight (±SE) (mg), and percentage of deformed adults of *N. viridula* and *E. heros* reared on four diets (25 ± 1°C; RH: 60 ± 10%; photophase: 14h).

Insect	Diets	[1]Sex ratio (SR)[n.s.]	Weight (mg)		Deformed adults (%)
			Male	Female	
N. viridula	Natural	0.48 ± 0.04	138.0 ± 2.74 a	187.0 ± 4.87 a	4.0
	A	0.42 ± 0.04	134.0 ± 1.88 a	166.0 ± 2.76 b	3.9
	B	0.47 ± 0.03	146.0 ± 13.83 a	156.0 ± 3.92 b	5.6
	C	0.44 ± 0.05	137.0 ± 2.09 a	167.0 ± 2.54 b	8.2
E. heros	Natural	0.56 ± 0.03	81.2 ± 2.11 a	84.3 ± 2.51 a	3.2
	A	0.61 ± 0.03	66.4 ±1.17 b	68.0 ± 1.79 bc	5.8
	B	0.42 ± 0.03	67.1 ± 1.35 b	68.1 ± 1.79 c	5.6
	C	0.55 ± 0.02	71.5 ± 1.43 b	78.4 ± 1.88 ab	7.6

[1]SR = female/(female+male)
Means followed by the same letter in the same column are not different among themselves by Tukey test (P < 0.05).
[n.s.]Non significant

was the same for each instar, except for a significantly lower viability for 5th instar *N. viridula* reared on each of the artificial diets (Table 3), stage that is considered critical for pentatomids (Ralph 1976, Panizzi 1991).

Fresh body weight of *N. viridula* females reared on artificial diets was always smaller than that of females reared on the natural diets (Table 4). Since there is a correlation between female weight and egg-laying capacity (Parra 1991), these lighter females should lay fewer eggs. For *E. heros*, females fed on diet C were heavier (78.4 mg) if compared to those reared on diets A and B (ca. 68.1 mg). The artificial diets did not affect the sex ratio and the percentage of deformed adults (Table 4).

The pre-oviposition period was significantly delayed for *E. heros* fed on diets B and C, while no change was detected for *N. viridula* (Table 5). The oviposition period, however, was dramatically reduced (ca. 4X), in relation to the natural diet for *N. viridula* while for *E. heros* it was reduced significantly only on diet C.

There was a reduction in the percentage of females that oviposited, for both *N. viridula* and *E. heros*, when reared on any of the artificial diets compared to the natural diet fed (Table 5). The same was observed for the number of eggs laid/female. However, the reduction in fecundity was more pronounced for *N. viridula* than for *E. heros*. This suggests that *E. heros* is either better adapted to feed on the artificial diets or has a less stringent nutritional requirement. *E. heros* reared on diet A (containing soybean oil), laid similar number of eggs in relation to insects reared on the natural diet (Table 5).

Viability of *E. heros* eggs from females reared on artificial diets was relatively high, and similar to that of eggs from females fed the natural diet (Table 6). However, for *N. viridula* egg viability was lower from females reared on

Table 5. Pre-oviposition, oviposition periods (±SE), percentage (±SE) of ovipositing females and number of eggs (±SE) produced by *N. viridula* and *E. heros* reared on four diets (25 ± 1°C; RH: 60 ± 10%; photophase: 14h).

Insect	Diets	Duration (days)		% of ovipositing females	Number of eggs/female
		Pre-ovipositon	Oviposition		
N. viridula	Natural	15.4 ± 0.90 a	37.2 ± 5.11 a	96.0 ± 4.00 a	470.0 ± 62.47 a
	A	20.3 ± 2.02 a	9.0 ± 2.37 b	44.0 ± 11.66 b	111.4 ± 35.56 b
	B	18.0 ± 1.83 a	9.0 ± 2.25 b	48.0 ± 12.00 b	104.6 ± 27.09 b
	C	15.3 ± 1.18 a	8.3 ± 2.25 b	48.0 ± 4.89 b	156.6 ± 36.24 b
E. heros	Natural	18.6 ± 1.26 b	65.4 ± 6.26 ab	100 a	266.2 ± 35.56 a
	A	17.3 ± 1.04 b	71.5 ± 8.48 a	90.0 ± 5.77 b	213.4 ± 32.10 ab
	B	32.2 ± 4.61 a	42.1 ± 6.64 b	80.0 ± 8.94 b	120.6 ± 23.39 b
	C	26.0 ± 3.55 ab	36.9 ± 5.15 c	80.0 ± 8.94 b	129.5 ± 21.39 b

Means followed by the same letter in the same column are not different among themselves by Tukey test (P < 0.05). For analysis, the data for number of eggs per female were transformed by $\sqrt{(x+8)}$.

Table 6. Egg viability (±SE), male and female longevity (±SE) of *N. viridula* and *E. heros* reared on four diets (25 ± 1ºC, RH: 60 ± 10%, photophase: 14h).

Insect	Diets	Egg viability (%)	Longevity (days)	
			Male	Female
N. viridula	Natural	89.7 ± 2.12 a	68.0 ± 4.54 a	75.0 ± 5.56 a
	A	55.9 ± 6.85 b	25.8 ± 3.63 b	42.7 ± 7.87 b
	B	63.7 ± 6.49 b	35.3 ± 4.32 b	40.4 ± 3.75 b
	C	65.7 ± 5.80 b	38.5 ± 5.10 b	38.9 ± 5.28 b
E. heros	Natural	83.5 ± 1.77 a	97.3 ± 6.89 a	98.0 ± 6.48 a
	A	66.4 ± 2.82 a	89.8 ± 6.66 a	87.6 ± 12.40 a
	B	70.0 ± 2.54 a	68.0 ± 8.41 a	83.2 ± 7.65 a
	C	65.8 ± 2.61 a	69.3 ± 3.43 a	69.7 ± 4.37 a

Means followed by the same letter in the same column are not different among themselves by Tukey test ($P < 0.05$). For analysis, the egg viability data were transformed by arc sin $\sqrt{(x/100)}$.

artificial diets than from females reared on the natural diet. Similarly, the longevity of *E. heros* males and females was similar on artificial diets and on the natural diet, but not for *N. viridula*, which adults lived longer on the natural diet (Table 6).

Fertility life table. The analysis of the fertility life table for *N. viridula*, indicated that the natural diet was more suitable than the artificial diets, showing higher Ro, rm and λ values. Among the artificial diets, diet C yielded greater R_0 (2.3 times greater than the value obtained for diet A), rm and λ values, although lower than the values recorded for the natural diet (Table 7).

For *E. heros*, the natural diet showed higher Ro, rm, and λ values when compared with the artificial diets. Among the artificial diets, diet A yielded greater Ro (2.8 times greater than the value obtained for diet B), rm and λ values, these last two close to the values recorded for the natural diet (Table 7).

A fertility life table is one way, among many, of evaluating the performance of a diet for insect rearing (Parra 2001). Thus, in the performance evaluation of the three dry artificial diets used, the fertility life table clearly showed that the dry artificial diets are still sub-optimal for rearing the stink bugs *N. viridula* and *E. heros*. It was observed that the pentatomids studied showed different nutritional requirements. Diet C, containing sunflower oil as a source of fatty acids, was the most suitable for *N. viridula*, because it yielded better fertility life table results in relation to the other dry artificial diets, and also resulted in a shorter duration of nymph development of *N. viridula*, higher nymph viability, and a higher fecundity. For *E. heros*, the life table indicated that diet A, containing soybean oil as a source of fatty acids, was the most suitable, since it showed higher nymphal viability, and a higher fecundity. The rearing of *E. heros* was much easier than *N. viridula*. This is the first report of an artificial diet for rearing *E. heros*.

We can conclude that the artificial diets allowed the complete development of *N. viridula* and *E. heros* and that these pentatomids have different nutritional requirements. These dry artificial diets proved promising for *N. viridula* and *E. heros* rearing, although they require adjustments in order to become suitable for mass rearing of the pentatomids.

Table 7. Duration of one generation (T), net reproductive rate (Ro), intrinsic rate of increase (rm), and finite rate of increase (λ) for *N. viridula* and *E. heros* reared on four diets (25 ± 1ºC; RH: 60 ± 10%; photophase: 14h).

Insect	Diets	T (days)	R_0	rm	λ
N. viridula	Natural	63.8	132.7	0.076	1.0796
	A	65.4	10.5	0.035	1.0366
	B	62.3	13.4	0.041	1.0416
	C	64.2	24.2	0.049	1.0502
E. heros	Natural	57.1	70.2	0.074	1.0772
	A	58.1	53.2	0.068	1.0708
	B	73.4	18.7	0.039	1.0406
	C	65.8	26.3	0.049	1.0502

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