Demographic Parameters of *Nezara viridula* (Heteroptera: Pentatomidae) Reared on Two Diets Developed for *Lygus* spp.

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ABSTRACT. Two artificial diets developed for rearing *Lygus* spp., a fresh yolk chicken egg based-diet (FYD) and a dry yolk chicken egg based-diet (DYD), were evaluated as an alternative food source for rearing the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). Survival to adult was 97.3 and 74.67%, respectively, on the fresh and dry yolk diets. Insects fed FYD had 100% survival of nymphs from first through fourth instars. Adult development was significantly shorter on FYD (30.37 ± SE 0.30 d) as compared with DYD (32.77 ± SE 0.16 d). Increased male and female longevity, higher fecundity, and larger egg mass sizes were also observed with *N. viridula*-fed FYD. However, fertility and hatchability was higher on DYD. A complete cohort life table was constructed to describe the development of *N. viridula* on both diets.

Key Words: *Nezara viridula*, demographic parameter, *Lygus* diet, rearing, life tables

The southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), is a highly polyphagous pest that feeds on >150 species of plants from over 30 families (Oho and Kiritani 1960, Todd 1989, Panizzi et al. 2000, Noda and Kamano 2002). This insect is considered as one of the most important agricultural pests in the world and is distributed throughout most of the warmer regions, primarily ecosystems within the 35° northern and southern latitudes (Todd 1989). *N. viridula* is a key pest of cotton and soybean in the southeastern and mid-south ern US (Huang and Toews 2012, Zavala et al. 2015). In 2004, stink bugs were responsible for an estimated $6.5 million dollars of crop loss and control cost on soybean in the US (Tillman 2006). A survey conducted in Mississippi in 2008 indicated that the number of acres scouted and the use of insecticide on soybean doubled as compared with estimates 4 yr earlier. The increase in insecticide use (58%) was attributed largely to increased problems with stink bugs (Musser et al. 2011). egg parasitoids are important biological control agents of stink bug populations in soybean. Potential augmentative use of these parasitoids in integrated pest management programs demands mass rearing large quantities of host stink bugs (Correa-Ferreira and Azevedo 2002, Fortes et al. 2006). As discussed by Brewer and Jones Jr. (1985), there has been limited improvement in rearing *N. viridula* in the laboratory since Menusan Jr. (1943) demonstrated methods to use fresh green beans, *Phaseolus vulgaris* (L.), as a food source. Since then, most laboratory rearing and studies of *N. viridula* biology has been done using Menusan’s green bean method (Corpuz 1969, Harris and Todd 1980, Egwuatu 1981, Begon et al. 1988, Velasco and Walter 1993, Panizzi and Mourao 1999, Panizzi et al. 2000, Gonzales and Ferrero 2008, Silva et al. 2011). Only a few studies have utilized artificial diets (Jensen and Gibbens 1973, Brewer and Jones Jr. 1985, Noda and Kamano 2002, Fortes et al. 2006). Rearing methods utilizing natural hosts can produce consecutive generations under laboratory conditions (Harris and Todd 1980, 1981; Brewer and Jones Jr. 1985, Jones Jr. and Brewer 1987), but the production of large numbers of individuals under laboratory conditions has not been achieved. Based on the work done by Brewer and Jones Jr. (1985), who reported that *N. viridula* could be cultured successfully on a liquid meritic diet developed for *Lygus hesperus* Knight (Debolt 1982), this study was initiated to evaluate two additional lygus diets: (a) a fresh yolk chicken egg based-diet (FYD) and (b) a dry yolk chicken egg based-diet (DYD). These diets were originally developed for mass rearing of *L. hesperus* and *Lygus lineolaris* (Palisot de Beauvois) (Portilla et al. 2011). This article reports biological and demographic parameters observed for *N. viridula* successfully reared on the two diets.

Materials and Methods

**Insects.** All studies were conducted at the USDA-ARS National Biological Control Laboratory, Stoneville, MS, in an environmental room with a temperature of 27 ± 2°C, 65 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Egg masses from a laboratory colony of *N. viridula* were obtained from USDA-ARS Crop Protection and Management Research Unit, Tifton, GA. A total of 60 first instar nymphs from the same cohort were selected as test groups to be reared on each diet. For both diets, insects ranging from first through fourth instar were placed in plastic containers (4 cm depth by 15 cm diameter). The lid of the container was modified with three screened openings (3-cm diameter) (Pioneer Plastics 175-C) and the base was covered with white paper towel (Portilla 1999, Portilla et al. 2013). After hatching, nymphs were fed using a cotton ball soaked in 1.0% benzoic acid—reverse-osmosis (RO) water solution impregnated with each of the respective diets (1.5 g/cotton ball of either FYD or DYD) (Fig. 1A). Nymphs were fed and held in the rearing container until they molted to the fourth instar (Fig. 1B). Fifth instars were transferred to Pioneer Plastic 250-C cages (Portland, OR, USA) (12 cm depth by 25 cm diameter). The lid of the cage was also modified with a screened opening of 7-cm diameter, and the base was covered with a white paper towel. Fifth instar nymphs were fed using the same methods described for earlier instars until they reached adulthood (Fig. 1C). Adults from each test group were removed and placed in a new Pioneer Plastic 250-C cage. There were three groups of nine pairs (females and males) for FYD and three groups of seven, seven, and eight pairs (females and males) for DYD. Three pieces (5 by 5 cm) of white half-folded paper were placed in each cage as a substrate for adults to walk on and as a site for oviposition (Fig. 1D). Each group was provided 1% benzoic acid—RO water and diet (FYD or DYD) impregnated cotton balls. Adults were maintained in an environmental room following procedures and conditions described above until all insects were dead.

**Composition and preparation of the diet.** The FYD and DYD were prepared according to procedures described by Portilla et al. (2011). Modifications included an increase in the amount of benzoic
acid (Part B) and not autoclaving the mixture of all Part B ingredients of both diets (Table 1). To prepare the semisolid FYD and DYD diets, a mixture of toasted wheat germ, coarsely ground lima bean meal, soy flour, and fresh yolk chicken egg (for FYD) or dry yolk chicken egg (for DYD) were weighted, mixed, and blended with boiled water for about 5 min. After the blended mixture cooled to $24 \pm 5^\circ C$, all ingredients of Part B were added and blended for 2 min. The prepared diets were poured into 500 cc plastic dispensing bottles and stored at $15 \pm 1^\circ C$.

**Biological parameters of *N. viridula***. The total number of individuals surviving from first instar through adult was recorded daily for each diet. Nymphal development time, immature mortality, adult emergence, and sex ratio were recorded on a daily basis. Cages (Pioneer Plastic 250-C) (replicates) each with pairs of 2-d-old adults of *N. viridula* reared with FYD and DYD were used to evaluate population growth rates and construct life tables. Insects in the six rearing cages were fed every 2–3-d with the respective diet (FYD or DYD) for ~90 d until the last adult was dead. Total mating population and total egg masses per cage were recorded daily. Total egg masses per female, number of eggs per egg mass, number of fertile and unfertile eggs, and eclosion of nymphs were calculated from the total egg masses obtained in each cage. Image Pro Plus Software (Media Cybernetics Version 7.0) was used to count individual eggs. Fifteen egg masses obtained from insects fed each treatment diet (FYD and DYD) were used to determine egg developmental time.

**Demographic parameters of *N. viridula***. Data obtained for the number of individuals alive each day at each age ($x$), from egg to adult, and measured number of eggs oviposited each day were used to calculate life tables and fertility tables. Formulae and definitions for each demographic parameter are listed as follow:

- **Gross fecundity ($M_x$)** is defined as the average number of total offspring per female in the interval $x$.

$$M_x = m_x \times l_x.$$

- **Net reproductive rate or net fecundity ($m_x$)** represented the mean number of female progeny produced by female of age $x$, which is obtained by multiplying gross fecundity by the sex ratio (proper for females). The maternity function is the product of net fecundity and survival ($l_x$). Net reproductive rate ($R_0$) is the mean female progeny produced by a female during its life span and is calculated as the sum of maternity functions over all age ($x$).

$$R_0 = \sum_{x=2}^{\infty} l_x m_x.$$

The intrinsic rate of increase ($r_m$) is obtained by iterating and changing the value of $r_m$ until the sum produced the value of 1. Intrinsic

![Fig. 1. Feeding method for rearing *N. viridula* using a cotton ball soaked in 1.0% benzoic acid—RO solution impregnated with fresh yolk chicken egg-based diet (*Lygus* spp. diet). (A) Second instar nymphs of *N. viridula* feeding on diet. (B) Third through fifth instar nymphs of *N. viridula* feeding on diet. (C) Adults of *N. viridula* feeding on diet. (D) *N. viridula* adult female depositing an egg mass on its site for oviposition.]

**Table 1. Composition of the *Lygus* spp. diets for rearing *N. viridula* (Portilla et al. 2001)**

| Components                      | FYD       | DYD       |
|--------------------------------|-----------|-----------|
| **Group A**                    |           |           |
| Toasted wheat germ             | 100 g     | 100 g     |
| Coarsely ground lima bean meal | 150 g     | 150 g     |
| Soy Flour                      | 25 g      | 25 g      |
| Yolk egg chicken               | 9 yolks   |           |
| Dry yolk chicken egg           |           | 40 g      |
| Water                          | 1000 ml   | 1000 ml   |
| **Group B**                    |           |           |
| Torula Yeast                   | 3 g       | 3 g       |
| Soy lecithin with oil          | 5 g       | 5 g       |
| Vanderzant vitamin mixture     | 4 g       | 4 g       |
| Streptomycin                   | 0.025 g   | 0.025 g   |
| Sucrose                        | 16 g      | 16 g      |
| Honey                          | 7.5 g     | 7.5 g     |
| Benzoic acid                   | 1 g       | 1 g       |
| **Total price per liter of diet** | $\$3.34^a$ | $\$3.45^a$ |

*Price of these ingredients obtained in Mississippi supermarket and Sigma catalog in 2015.*
capacity for increase, $r_m$ is determined by Lotka’s equation (Carey 1993, Krebs 2001),

$$r_m = \sum_{t=0}^{\infty} e^{-r(t+0.5)} I_t m_x = 1.\,$$

Finite rate of increase ($\lambda$) is the factor by which a population will increase each day.

$$\lambda = e^{r_x}.\,$$

Mean generation time ($T$) is the mean age of reproduction, which characterizes $T$ as the mean interval of time (days) separating the birth of one generation from those of the subsequent generation.

$$T = \frac{\sum_{t=0}^{\infty} I_t m_x}{\sum_{t=0}^{\infty} x_l I_t m_x}.\,$$

Intrinsic capacity for increase was then used to calculate the finite rate of increase ($\lambda$), denoted as the population increment.

$$DT = \ln(2)/r_m.\,$$

**Statistical analysis.** Demographic parameters, included total percentage of population mating, total egg masses per female, number of eggs per mass, number of unfertile eggs, and percentage survival from eclosion to nymph, were analyzed in a randomized complete block design using a one-way analysis of variance (ANOVA) by the general linear model (GLM) procedure (SAS Institute 2013). Differences between least square means for all variables for each diet were evaluated by the $t$-test. Proc LIFETEST non-parametric tests were used to compare estimates of the different survival functions.

**Results**

**Biological parameters of N. viridula.** Nezara viridula successfully matured from egg to adult on both FYD and DYD (Fig. 1). The mean
developmental times for eggs and first instar nymphs were not statistically different between diets (Fig. 2); however, second instar nymphs (Fig. 3). The first egg masses were present 2 d after the first copulation occurred in N. viridula reared on FYD (female 13-d-old), and 10 d after the first copulation occurred in N. viridula reared on DYD (female 20-d-old). Thereafter, total eggs/day/total population for both groups was dependent on the frequency of mating (Fig. 4). Cumulative egg mass production/female/day indicated that more egg masses were obtained for insects reared on FYD. Longevity of females and egg masses was found to be the same on both diets with no significant differences between diet or sex (Fig. 6; Table 3). Although reproduction of stink bugs on the FYD diet was higher than that of the DYD group, fertility, and hatching rates were significantly higher for insects fed FYD.

**Demographic parameters of N. viridula.** Gross fecundity (\(F_x\)) and net fecundity (\(m_x\)) were calculated assuming a 1:1 sex ratio for insects reared on both diets. All demographic measurement for insects reared on FYD were significant higher than those for insects fed DYD, except for generation time (\(T\)) which was shortened almost a week for insects fed DYD (Table 4). A higher gross fecundity of 395.8 ± 28.7 females and males eggs/female was obtained for females reared on FYD. Those reared on DYD produced 140.3 ± 51 eggs/female. The highest finite rate of increase was found in N. viridula reared on FYD. Net reproductive rate for females reared on FYD was 130.8 ± 33.9 SE females per new born female. This resulted in a mean generation time of 66.7 ± 3.1 SE days. The daily rate of increase was 1.04 females per female, with a doubling time of 9.3 ± 0.23 SE days. Net reproductive rate for females reared on FYD was 22.6 ± 7.01 SE females per female with a mean generation time of 59.2 ± 13.12 SE days and a daily rate of increase of 1.07 females per female. Doubling time was 13.7 ± 1.21 SE days for insects reared on FYD. Differences among these population parameters for insects reared on the two diets values are shown in Table 4.

**Discussion**

This study demonstrated that N. viridula can successfully survive and mature from egg to adult on two diets previously developed for L. lineolaris and L. hesperus (Portilla et al. 2011) (Fig. 1A–D; Table 2). Among the diets tested, FYD was the most suitable for nymphal development and survival. DYD showed better performance when fecundity and egg hatchability were the parameters of interest (Table 2). Chicken egg yolk is the main component of both diets and appears to be an important influence in both fertility and reproduction. A trade-off mechanism was clearly observed on insects fed DYD, where fertility and hatchability tended to be higher and longevity tended to be shorter.
Remolina and Hughes (2008) mentioned that longevity and fecundity typically are negatively correlated and the extent of this trade-off mechanism varies within among species. A reasonable interpretation of this mechanism could be that the egg protein may gave the diets greater elasticity or other texture features that may be either desirable or detrimental, depending on the circumstances. In this case, the DYD aqueous texture seems to affect the consumption of all nutritional value of the diet affecting the life expectancy of the adults but some how compensating fertility. However, even with higher fertility, insects fed with DYD did not have greater fecundity than those insects fed FYD. Cohen (2004) indicated that animal protein from fresh chicken eggs contains all the essential amino acids in sufficient quantities and well-balanced proportions for insect development. This may explain why insects fed FYD had longer longevity, higher fecundity, and reproductive activity measure by number of mating insects. Larson (1989), Evans (1982), and Kawada and Kitamura (1983) reported that the fecundity in the Pentatomidae bugs depends on number and quality of mating events. Egg fertility and hatchability in the stink bugs fed DYD was similar to that for *L. hesperus* as reported by Portilla et al. (2011). Higher mortality of first instar nymphs was observed in the *L. hesperus* study at the time of hatching.

Nymphs and adults appeared to feed and develop normally on both FYD and DYD (Fig. 1A, B, and D). Mean developmental time for *N. viridula* from egg to adult was 33.2 ± 0.12 SE and 34.20 ± 0.18 SE days for FYD and DYD, respectively. Greater survival rate of insects was obtained on FYD (97.37%) as compared with DYD (74.57%) (Fig. 6; Table 2). Mean developmental times of nymphs were 26 and 27 d for FYD and DYD, respectively. These results did not differ greatly from those reported in other studies (27.3–28.9) that used *Lygus* diet to rear *N. viridula* (Brewer and Jones Jr. 1985, Jones Jr. and Brewer 1987). Mean generation time for nymphs of *N. viridula* reared on *Lygus* diet were also similar to those reported for two semi-solid diets developed for *N. viridula* (Noda and Kamano 2002). They reported 27.6–28.2 d for developed females and 26.8–27.5 d for developed males. Panizzi et al. (2000) reported 29 d for stink bugs reared on solid diet developed for *Riptortus clavatus* Thunberg. Fortes et al. (2006) reported 27.6, 27.1, and 28.4 d for *N. viridula* reared on three solid diets. Survival of nymphs on FYD (97.37%) in this study was greater
than that reported from all previous cited studies where the survival ranged from 55 to 87%. The highest survival (87%) previously reported was obtained with *N. viridula* fed a *Lygus* diet (Brewer and Jones Jr. 1985). There have been many reports on rearing *N. viridula* from second instar to adult on seed, different host plants, and combinations of seeds and plant tissue. The nymphal period seemed to be shorter when *N. viridula* was reared on the combination of seeds and plant tissue. Harris and Todd (1980), Noda and Kamano (2002), and Jones Jr. and Brewer (1987) reported nymphal durations of 28.0, 26.4, and 22.7 d with *N. viridula* fed on green snap beans and raw-shelled peanuts. Panizzi and Saraiva (1993) reported 39.3 d from second instar to adult for *N. viridula* reared on immature radish fruits and Gonzales and Ferrero (2008) reported 90.6 d from egg to adult for *N. viridula* reared on immature soybean pods.

The novel method developed for rearing *N. viridula* in this study facilitated the collection of information necessary to construct life tables and estimate the demographic parameters. Morris and Miller (1954) indicated that life tables are fundamental tools to understand critical life stages during arthropods development, and their influence on the overall population structure. Mining natural population development would be a positive attribute of insect rearing systems. In this study, growth rates were higher for *N. viridula* reared on FYD than those reared on DYD, where the $R_0$ (130.8) of FYD was 5.9-fold higher than DYD (22.61) (Table 4). Results obtained in this investigation differed from those reported by Fortes et al. (2006), who reported $R_0$ values of 10.5, 13.4, and 24.2 on the three solid diets developed for rearing *N. viridula*. However, they reported $R_0$, $r_m$, and $\lambda$ values of 132.7, 0.076, and 1.08, respectively on *N. viridula* reared on green beans and peanuts seeds, which are similar for those found in this study for *N. viridula* reared on FYD (130.8, 0.074, 1.07, respectively). Gonzales and Ferrero (2008) reported lower $R_0$ values on soybeans (5.60). The demographic parameters obtained in this investigation indicated that DYD did not have the qualities that can be taken as key requisites for a highly useful diet.

Harris and Todd (1980) indicated that reproductive behavior of *N. viridula* was a definite need for biological studies aimed at developing life tables, population models and biological control measures. The mating population trends in this study were similar for both diets (Figs. 2–4). First mating and pre-oviposition periods were directly correlated as well as the frequency of mating with the number of ovipositions for insects fed both FYD and DYD (Fig. 4). Harris and Todd (1980) observed 57 ovipositions and reported that 90% of the observed population deposited an egg mass within 72 hr after their first mating. Observations ranged from 6 hr to $>3$ d. Similar results were obtained in

![Fig. 5. Product-limit survival estimates for females and males of *N. viridula* reared on FYD and on DYD ($P = 0.05$, LIFETEST of Equality over Strata)](image)

Table 3. Test of equality over Strata LIFETEST for *N. viridula* fed on two *Lygus* diets

| Test        | $\subseteq$ FYD | $\subseteq$ fed DYD | $\subseteq$ fed FYD-$\subseteq$ fed DYD |
|-------------|----------------|---------------------|---------------------------------------|
|             | $X^2$ | DF | $P > X^2$ | $X^2$ | DF | $P > X^2$ | $X^2$ | DF | $P > X^2$ |
| Log-Rank    | 0.0396 | 1 | 0.8423 | 0.8099 | 1 | 0.3685 | 19.2716 | 1 | <0.0001 |
| Wilcoxon    | 0.0054 | 1 | 0.9416 | 0.0003 | 1 | 0.9852 | 16.6599 | 1 | <0.0001 |
| (LR)-Zlog   | 0.0054 | 1 | 0.9415 | 0.2729 | 1 | 0.6014 | 5.9978 | 1 | <0.0143 |

*Homogeneity Tests of survival curves ($P \leq 0.0001$)
the current investigation, where egg masses were found 2 d after first mating on insects reared on FYD. It is well known that factors such as resonance and sex pheromones may affect the temporal and numerical patterns of *N. viridula* mating (Harris et al. 1982, Gogala 1984, Borges et al. 1987, Kon et al. 1988, Stritih et al. 2000, Spezia et al. 2008).

Another important component that affects male and female reproduction is dietary requirements. Natural or artificial food should contain all essential nutrients in appropriate amounts for feeding stimuli. Cohen (2004) indicated that an adequate amount of beneficial components could have a positive effect on fecund progeny to support continuous generations of insects in a rearing system. It was observed in this study that females of *N. viridula* fed on both diet did not have refractory periods prior to or following oviposition. Therefore, its mating behavior was highly variable throughout the insect survival period. More mating pairs were found for *N. viridula* fed FYD. Panizzi and Mourao (1999) indicated that the number of *N. viridula* mating pairs was constantly greater on privet (*Ligustrum vulgare* L.) as compared with those reared on soybean pods.

The requirement for plant material to rear *N. viridula* is expensive and labor intensive. Reducing cost and increasing reliability and simplicity of its production would greatly enhance biological research and possibly facilitate the production of biological control agents. The biological and demographic parameters obtained in this investigation confirmed that the *Lygus* FYD has the potential to rear *N. viridula*.

Stink bugs are strictly liquid feeders capable of ingesting only dissolved material. Therefore, the use of raw yolk chicken egg in FYD as a part of a semisolid diet appears to satisfy the apparent requirement for protein. The growth rates values obtained with FYD showed that diet support robust feeding, development, growth, and reproduction. However, additional investigations are needed that examine the potential of this diet for unlimited numbers of continuous rearing, mass production and sustain population that rival those taken from the field in healthy

![Graph](image_url)

**Fig. 6.** Cumulative egg mass production/female/day of *N. viridula* reared on two artificial diets: FYD (9 pairs/cage) and DYD (7, 7, and 8 pairs/cage) (*t*-test *P* ≤ 0.0001)

| Demographic rates of increase Diets (mean ± SE) |   |
|-----------------------------------------------|---|
| Gross fecundity (total progeny/female) (*M*<sub>x</sub>) | 395.84 ± 28.69 a  |
| Net fecundity (progeny females/female) (*m*<sub>x</sub>) | 197.92 ± 14.34 a |
| Net reproductive rate (females/female/generation) (*R*<sub>0</sub>) | 130.80 ± 33.85 a |
| Mean generation time (*T*) | 66.65 ± 3.14 a |
| Doubling time (*DT*) | 9.29 ± 0.23 a |
| Intrinsic rate of increase (*r*<sub>m</sub>) | 0.074 ± 0.0018 a |
| Finite rate of increase (*λ*) | 1.08 ± 0.0032 a |

Means (± SE) in the same row followed by the same letter were not significantly different at *P* > 0.05

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behavior and physiology. Colonies of *L. hesperus* and *L. lineolaris* have been maintained on FYD for over 10 yr with no detrimental changes of its reproduction (Cohen 2000, Portilla et al. 2011, 2014). Perhaps *N. viridula* can be similarly reared on FYD.

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