



Nutritional Ecology of the Southern Green Stink Bug *Nezara viridula* (Hemiptera: Pentatomidae) on Selected Varieties of Cowpea and Tomato

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Abstract: Cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae), and tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae), are two economically important crops in the southern United States. The southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is a highly polyphagous pest that attacks a wide range of crops including cowpea and tomato causing a reduction in yield and produce quality. Considering the damage and the associated losses in cowpea and tomato production by this and other pests, as well as the demand for reduced use of pesticides, there is a need to cultivate varieties that are resistant to *N. viridula*. One key indicator of resistance is host nutritional availability and adequacy. This study evaluated the effect of two selected varieties of cowpea and tomato on the growth and development of *N. viridula* under laboratory conditions as a first step towards the assessment of nutritional adequacy of both crops. We carried out feeding experiments to compare food utilization and suitability by various life stages of *N. viridula*. The food substrates tested included fresh immature cowpea pods, fresh cowpea seeds, dry cowpea seeds and newly ripened tomato fruits. Overall, mean nymphal mortality was less when reared on cowpea ($63.7 \pm 2.9\%$) than tomato ($83.0 \pm 1.8\%$). Nymphs required less time to complete development on cowpea (27.8 ± 2.8 d) than on tomato (45.0 ± 3.9 d). Fresh body weight at adult emergence was greater on cowpea (132.3 ± 12.1 mg) than tomato (83.9 ± 1.5 mg). Consumption index (CI) and growth rate (GR) were higher on cowpea (CI: 22.0 ± 1.3 ; GR: 2.1 ± 0.3) than tomato (CI: 8.7 ± 1.4 ; GR: 0.9 ± 0.1). Collectively, these results suggest that cowpea provided a more suitable nutritional substrate for nymphal development. Many small vegetable growers cultivate both crops.

Keywords: Nutritional Index, Cowpea, *Nezara viridula*, Tomato

1. Introduction

Southern green stink bug, *Nezara viridula* (L.), is one of several polyphagous pentatomid insect pests that attacks a wide variety of crops that include cowpea and tomato [1-6]. In the United States the southern green stink bug is an important pest that feeds primarily on seeds, fresh pods and immature fruits and sporadically on the tender shoots of several crop species [7-10]. Feeding damage often results in deterioration of fruit and seed quality and significant yield loss in many crops [11-13]. In the United States, damage by

N. viridula and other stinkbugs in cotton alone is estimated to cause losses of \$9.4 million annually [14].

Nymphs and adults of stink bugs feed by puncturing plant tissues with their piercing sucking mouthparts (stylets) removing the cell contents [4, 5]. Feeding results in seed damage that may lead to distorted development of pods [15]. Feeding may also result in premature fruit drop, delay in crop maturity and reduced seed quality or quantity [15]. Secondary damage may also occur when many toxicogenic fungi and bacteria are transmitted during feeding [15, 16]. Damaged fruits and pods typically have hard brownish or

black spots.

Stink bugs are generally multivoltine and highly polyphagous; they attack a wide variety of important food crops [17]. Host switching is common in *N. viridula*, which potentially benefits them by helping meet the nutritional requirements of the developmental stages [18, 5, 19]. Consumption of a diet by insects and their growth, development and survival on a particular diet is affected by factors that may include morphological attributes and chemical composition of the diet [20-23]. There are many reports on the growth and development of *N. viridula* on soybean (8) and on other crop hosts (21). The performance of *N. viridula* on soybean varies on different phenological stages and plant parts (especially on seeds and pods) on which they are feed [24]. Apparently, *N. viridula* has higher survival, fitness and fecundity when fed on pods from plants at full seed stage [24]. However, to the best of our knowledge, there are no studies on the utilization and performance of *N. viridula* on cowpea and tomato, two important hosts of the pest. Changes in nutrient composition of the plant plays a vital role in food choice and performance responses by phytophagous insects [25-27]. In this study, we investigated the nutritional ecology of *N. viridula* on cowpea and tomato.

Nutritional ecology underscores the importance of nutritional factors to our understanding of the behavioral strategies of herbivorous insects directed toward optimization of nutrient acquisition, utilization and allocation [28]. As noted by Scriber and Slansky [29], nutritional ecology can be a valuable tool that may be used to understand feeding, habitat selection and reproduction by an insect that would potentially help in the selection of insect resistant crop varieties. Nutritional ecology utilizes quantitative nutritional approaches which measure the amount of food consumed, digested, assimilated, excreted, metabolized and converted into biomass. Analysis of these metrics reveals the responses of arthropods to different foods, including the effect on growth [30]. Accurate measurements on consumption, utilization, and allocation of food using gravimetric methods reinforce this [31]. Despite their importance, to date no quantitative food utilization studies are available for the two crops in this study, nor generally among most Pentatomidae [30]. In this study, we compared food utilization indices for *N. viridula* on cowpea seeds and tomato fruit as an index of food suitability which will serve as a prelude to making decisions on their resistance to this and other stink bugs. The use of resistant varieties is a key component of many integrated pest management programs and has proved to be a reliable alternative or companion to insecticide use on many crops thus reducing their frequency or mitigating the negative environmental impact associated with frequent pesticide application.

2. Materials and Methods

2.1. Source of Insects

Feral *N. viridula* adults were collected from the Research farm at Tuskegee University to start a culture that served as

the source of insects for the laboratory experiments. The culture was maintained in mesh cages measuring 30.2cm x 30.2cm x 30.2cm (BioQuip Product, Rancho Dominguez, California). Paper towels were suspended from the roof along the inner side of the cages to serve as oviposition substrates and moist cotton balls were placed in Petri-dishes on the floor of the cage as a source of water, especially for the first and second nymphal stages that hardly or barely feed. Egg masses were collected daily and held in 500 ml cups to hatch under laboratory conditions (27.5±1°C, 60 ± 10% RH 12L: 12D photoperiod). Lightly moistened cotton wool was provided in the cups with egg masses to maintain high humidity. The adults and their progeny were reared on a mixture of fresh green beans, peanuts and other fruits as described by [32]. The food source was replaced every 2 days or sooner if it became moldy or desiccated.

2.2. Test Varieties

Two varieties of cowpea (Mississippi Silver [MS] and Pinkeye Purple Hull [PPH]), and two tomato varieties (Mariana [MAR] and German Johnson [GJ]) were used in this study. These varieties are among the most popular varieties in many southern states. MS is an erect cowpea variety with a brown and smooth seed coat with silvery-green pods. It is resistant to fusarium wilt and root knot nematodes [33]. Pink Purple Hull (PPH) is a semi-spreading variety with a wrinkled cream-colored seed coat and a distinctively purple-colored pod hull. MAR has a uniform smooth shape with large internal locules. It is resistant to the fungi *Verticillium dahliae* Kleb and *Fusarium oxysporum* Schltdl [34]. German Johnson (GJ) is an heirloom tomato variety with an indeterminate habit and large fruits that have a rough and undulating surface; it is slightly disease resistant [34]. The seeds of each variety were planted in the greenhouse in 5-gallon pots filled with Metro-Mix (potting soil). We watered the plants daily, and labelled newly formed fruits with the dates of their formation. Young cowpea pods and newly ripened tomato fruits were the food substrates used in the study. The insects did not feed on unripe tomato fruits.

2.3. Food Consumption and Utilization

Food consumption and utilization by *N. viridula* were determined using third instar (N3) nymphs from the laboratory culture, immature pods (10-12d) of the two cowpea varieties and ripened fruits (N3 did not feed on unripe tomato fruits) of the two tomato varieties. The first nymphal stage (N1) does not feed, and the second nymphal stage (N2) does not feed as actively as the third nymphal stage. In addition, handling the N1 and N2 generally lead to high mortality; this advised our decision to exclude these instars from the tests. Twenty N3 nymphs were used for each variety. A weighing scale (Mettler ME54TE, Toledo, OH, USA) with sensitivity 0.0001g was used to weigh the insects and the food substrates daily. Each food substrate and a set of 40 insects were weighed and dried at 75°C to constant weight. Food consumption was calculated on both dry food (using

dry weight of food eaten and fresh weight of insects) and a fresh food basis (using fresh weight of food eaten and fresh weight of insect) using the method described by Waldbauer [31] and modified by Baker et al. [35].

The following indices were calculated:

- Consumption index (CI), the consumption rate corrected for final body weight: $CI = F/TA$, where F is dry weight of food ingested, T is duration of feeding period (in days) and A is the mean dry weight of insect;
- Growth Rate (GR), biomass gained per day = WT/TA , where WT = dry weight gained;
- Efficiency of conversion of ingested food to body mass or Conversion of Ingested food (ECI), a measure of the ability to convert ingested food into biomass: $ECI = (WT/F) \times 100$;
- Relative growth rate (RGR), the amount of growth attained (mg dry matter) per unit body weight (mg dry matter) per unit time = $(\text{Insect wet weight gain}) / (\text{Insect wet weight at the beginning of the trail}) \times (\text{Time})$;
- Relative Consumption rate (RCR), food ingested per unit nymphal mass per day: $(\text{Dry weight of food eaten}) / (\text{Insect weight at the beginning of the trail}) \times (\text{Time})$;
- Growth Index (GI) = no surviving nymphs/initial no of nymphs [36].

2.4. Nymphal Development on Cowpea Seeds, Pods and Tomato Fruit

Egg masses were collected on the day of oviposition and placed in plastic containers as described in the previous section. First instars (N1) were meticulously removed and placed individually in Petri-dishes (9.0 × 1.5cm) with paper

towels and moisten cotton balls. Fresh cowpea pods (12-day old after bloom initiation) were placed individually in the Petri-dishes. Each insect was caged separately, as a replication, and forty insects were used for each food substrate or variety. The insects were weighed after each molt until they molted to adult; other data was collected on survival as well as development time. Petri-dishes were cleaned when necessary and the food source replenished *ad libitum*. A similar experiment was conducted using fresh seeds, dry seeds and ripened tomato fruits.

2.5. Statistical Analysis

Differences in fresh body weight and developmental time were compared by t-test. All data were analyzed using SAS 9.4 at a 0.05 level of significance. Tukey HSD was performed to separate means if significant effects existed.

3. Results

3.1. Development of *Nezara viridula* on Cowpea

The total developmental time (TDT) of *N. viridula* was not significantly ($t=0.376$; $df=5$; $P>0.05$) longer in females (22.0-32.0d) than males (19.3-27.6d) regardless of the cowpea variety. On fresh seeds the TDT of nymphs that fed on Mississippi Silver (MS) was 21.8-23.3d which was not significantly longer ($t=1.19$; $df=2$; $P>0.05$) than that on Pinkeye Purple Hull (PPH) (19.3-22.0d) (Table 1). On all the varieties, nymphal mortality was greatest in the fourth instar. On fresh seeds only nymphal mortality was significantly higher on PPH (ca. 70%) than MS (ca. 40%) (Table 1).

Table 1. Mean (\pm SE) developmental time and mortality of *Nezara viridula* fed on 12d old seeds of cowpea in the laboratory.

Cowpea variety	Stadium duration, d				Total Developmental Time		GI	TM (%)
	2nd	3rd	4th	5th	Male	Female		
MS	5.3 \pm 0.18 ^a (36)	4.8 \pm 0.16 ^a (32)	3.9 \pm 0.39 ^a (32)	9.1 \pm 0.29 ^a (24)	21.8 \pm 0.43 ^a (12)	23.3 \pm 0.37 ^a (12)	0.6 \pm 0.3 ^a	40 \pm 5.7 ^a
PPH	5.9 \pm 0.2 ^a (40)	3.7 \pm 0.33 ^a (26)	3.9 \pm 0.42 ^a (22)	7.5 \pm 1.15 ^a (12)	19.3 \pm 1.2 ^a (6)	22.0 \pm 3.0 ^a (6)	0.3 \pm 0.05 ^a	70 \pm 5.8 ^b

Means in each column followed by the same letter are not significantly different ($P>0.05$; t-test). Initial number of nymphs n=40 on each food; MS=Mississippi silver; PPH=Pinkeye purple hull; TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

Weight gained by females (n=12) that fed on MS (158 mg) was not significantly greater ($t=0.79$; $df=16$; $P>0.05$) than those that fed on PPH (n=6) (155.7mg) (Table 2).

Table 2. Mean (\pm SE) body weight of *Nezara viridula* fed on 12d old cowpea seeds in the laboratory.

Cowpea variety	Nymphal weight				Adult weights		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MS	0.5 \pm 0.04 (36 ^a)	6.7 \pm 0.54 ^a (32)	27.7 \pm 2.6 ^a (32)	67.3 \pm 5.9 ^a (24)	129.0 \pm 10.85 ^a (12)	158.7 \pm 10.0 ^a (12)	0.60 \pm 0.08 ^a	40 \pm 2.9 ^a
PPH	0.5 \pm 0.04 ^a (40)	8.3 \pm 0.72 ^a (26)	22.9 \pm 3.6 ^a (22)	56.6 \pm 5.8 ^a (12)	69.3 \pm 9.23 ^a (6)	155.7 \pm 27.4 ^a (6)	0.30 \pm 0.04 ^b	70 \pm 1.2 ^b

Means in each column followed by the same letter are not significantly different ($P>0.05$; t-test). Initial number of nymphs n=40 on each food; MS=Mississippi silver; PPH=Pinkeye purple hull; TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

However, weight gained by males (n=12) was significantly greater ($P<0.05$) on MS (129.0mg) than males (n=4) on PPH (69.3mg). On fresh pods females (n=8) that feed on PPH

(173 mg) significantly ($t<0.0001$, $df=13$, $P<0.05$) gained more weight (173mg) than those (n=4) that fed on MS (115 mg) (Table 3).

Table 3. Mean (\pm SE) body weight of *Nezara viridula* fed on 12d cowpea pod in the laboratory.

Cowpea variety	Nymphal weight				Adult weights		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MS	0.5 \pm 0.01 ^a (18)	4.1 \pm 0.9 ^a (10)	36.4 \pm 4.7 ^a (12)	58.8 \pm 3.5 ^a (8)	110.0 \pm 0.51 ^a (4)	115.5 \pm 0.51 ^a (4)	0.2 \pm 0.08 ^s	90 \pm 1.8 ^a
PPH	0.5 \pm 0.03 ^a (40)	2.4 \pm 0.2 ^a (40)	18.2 \pm 1.52 ^a (40)	67.6 \pm 27.3 ^a (28)	102.3 \pm 8.25 ^a (14)	173.5 \pm 47.8 ^b (8)	0.7 \pm 0.06 ^b	45 \pm 0.7 ^b

Note. Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MS=Mississippi silver; PPH=Pinkeye purple hull. TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

However, males ($n=4$) that feed on MS (110 mg) significantly ($t<0.0001$, $df=17$, $P<0.05$) gained more weight than males ($n=14$) than fed on PPH (102 mg) (Table 3). The total developmental time (TDT) on dry seeds (Table 4) was similar to that fresh seeds (Table 1). Regardless of the cowpea variety the TDT of females ($n=10$) on dry seeds was not significantly ($t=1.32$, $df=22$, $P>0.05$) longer (27.1-28.1d) than those of males ($n=6$) (26.0d) (Table 4).

Table 4. Mean (\pm SE) developmental time and mortality of *Nezara viridula* fed on dry seeds of cowpea in the laboratory.

Cowpea variety	Stadium duration, d				Total Developmental Time		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MS	4.8 \pm 0.23 ^a (32)	5.5 \pm 0.35 ^a (26)	6.3 \pm 0.53 ^a (19)	10.9 \pm 0.5 ^a (18)	26.0 \pm 0.70 ^a (6)	28.1 \pm 1.0 ^a (10)	0.4 \pm 0.05 ^a	60 \pm 2.9 ^a
PPH	4.6 \pm 0.24 ^a (30)	5.0 \pm 0.28 ^a (24)	5.9 \pm 0.38 ^a (19)	11.2 \pm 1.1 ^a (9)	26.0 \pm 4.1 ^a (3)	27.1 \pm 1.72 ^a (6)	0.2 \pm 0.06 ^a	77.5 \pm 1.5 ^b

Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MS=Mississippi silver; PPH=Pinkeye purple hull; TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

TDT of nymphs was generally similar ($t=0.42$, $df=2$, $P>0.05$) between the two varieties on MS (26.0-28.1d), and on PPH (26.0-27.1d). In addition, TDT of nymphs was shorter on fresh seeds than on dry seeds (Tables 1 and 4). Highest mortality of nymphs was recorded during the fourth instar regardless of variety. Mortality was significantly ($t=24$, $df=2$, $P<0.05$) lower on MS (ca. 60%) than on PPH (ca. 78%) (Table 3), as was the case on fresh seeds compared to dry seeds (Table 1 and 4). Females gained significantly ($t=3.20$, $df=14$, $P<0.05$) more weight on MS than on PPH (Table 5). However, weight gain among males on MS (136.0 mg) and on PPH (131.8 mg) did not differ significantly ($t=2.96$, $df=6$, $P>0.05$) (Table 5).

Table 5. Mean (\pm SE) body weight of *Nezara viridula* fed on dry cowpea seeds in the laboratory.

Cowpea variety	Nymphal weight				Adult weights		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MS	0.5 (32)	6.6 \pm 0.46 ^a (26)	18.5 \pm 1.7 ^a (19)	66.3 \pm 4.1 ^a (18)	136.0 \pm 4.38 ^a (6)	157.9 \pm 5.87 ^a (10)	0.4 \pm 0.06 ^a	60 \pm 5.3 ^a
PPH	0.5 (30)	7.3 \pm 1.29 ^a (24)	21.5 \pm 2.3 ^a (19)	83.6 \pm 14.0 ^a (9)	131.8 \pm 10.0 ^a (3)	149.8 \pm 10.0 ^b (6)	0.2 \pm 0.05 ^a	77.5 \pm 3.8 ^a

Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MS=Mississippi silver; PPH=Pinkeye purple hull. TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

Nymphs took significantly ($t=2.4$, $df=10$, $P<0.05$) longer to develop on the pods (27.3-32.0 d) than on both fresh and dry seeds (19.3-28.1d) (Tables 1, 4, and 6). On fresh pods males and females that fed on MS required a slightly longer time (29-30d) to develop compared to those that fed on PPH (27.6-27.3d) (Table 6), although the difference was not significant ($t=2.0$, $df=2$, $P>0.05$). Nymphal mortality was greatest in the third instar and fifth instar on the pods of MS and PPH, respectively (Table 6). In contrast to the seeds, mortality was significantly ($t=63.6$, $df=2$, $P<0.05$) greater on MS (ca. 90%) than PPH (ca. 45%) (Table 6). Growth index (GI), which measures survival of *N. viridula* on each food substrate was generally higher on fresh seeds (0.6) than dry seeds (0.4) and pods (0.4) (Tables 1, 4, and 6). Higher values were recorded on MS (0.4-0.6) than PPH (0.2-0.3) (Tables 1, 4, and 6).

Table 6. Mean (\pm SE) developmental time and mortality of *Nezara viridula* fed on 12 d cowpea pods in the laboratory.

Cowpea variety	Stadium duration, d				Total Developmental time		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MS	4.2 \pm 0.70 ^a (18)	6.4 \pm 0.82 ^a (10)	6.8 \pm 0.79 ^a (12)	13.0 \pm 1.7 ^a (8)	29.0 \pm 7.0 ^a (4)	32.0 \pm 6.31 ^a (4)	0.2 \pm 0.06 ^a	90 \pm 0.8 ^a
PPH	2.9 \pm 0.07 ^b (40)	6.3 \pm 0.32 ^a (40)	5.7 \pm 0.32 ^a (40)	11.9 \pm 0.4 ^a (28)	27.6 \pm 0.98 ^a (14)	27.3 \pm 1.95 ^a (8)	0.7 \pm 0.05 ^b	45 \pm 1.8 ^b

Note. Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MS=Mississippi silver; PPH=Pinkeye purple hull. TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

3.2. Development of *Nezara viridula* on Two Tomato Cultivars

The total developmental time (TDT) was generally significantly ($t=9.7$, $df=14$, $P<0.05$) longer on ripened tomato (41.3-44.3d) (Table 7) compared to cowpea (29-32d)

(Tables 1, 4, and 6). The total developmental time (TDT) observed on German Johnson (GJ) (46.7-47.7d) was not significantly ($t=2.8$, $df=2$, $P>0.05$) greater than on Mariama (MAR) (41.3-44.3d) (Table 7).

Table 7. Mean (\pm SE) developmental time and mortality of *Nezara viridula* fed on freshly ripened tomato fruit in the laboratory.

Tomato variety	Stadium duration, d				Total Developmental time		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MAR	9.2 \pm 0.72 ^a (11)	9.2 \pm 0.54 ^a (11)	8.7 \pm 0.81 ^a (6)	16.1 \pm 0.89 ^a (6)	44.3 \pm 7.0 ^a (3)	41.3 \pm 6.31 ^a (3)	0.2 \pm 0.08 ^a	85 \pm 1.0 ^a
GJ	8.9 \pm 0.53 ^a (9)	8.9 \pm 0.96 ^a (8)	7.3 \pm 0.65 ^a (7)	14.2 \pm 1.1 ^a (7)	47.7 \pm 1.3 ^a (3)	46.7 \pm 0.88 ^a (4)	0.2 \pm 0.03 ^a	82.5 \pm 2.3 ^a

Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MAR=Mariana; GJ=German Johnson. TM=Total mortality (%); Numbers surviving each stadium are given in parentheses.

Males generally took longer (44.3-47.7d) to develop as compared to females (41.3-46.7d). The difference, however, was not significant ($t=0.62$, $df=2$, $P>0.05$). Between the varieties, the TDT of adult *N. viridula* on German Johnson (GJ) was not significantly ($t=2.8$, $df=2$, $P>0.05$) from that on Mariana (MAR) (Table 7). High nymphal mortality was observed as early as the second instar and was only slightly

and non-significantly ($t=3.1$, $df=2$, $P>0.05$) greater on MAR (ca. 85%) than GJ (ca. 82.5%). Growth index (GI) was not significantly ($P<0.05$) different between GJ (0.2) and MAR (0.2) (Tables 7). The weight of newly emerged adult females ($n=4$) that fed on GJ was significantly ($t=22.5$, $df=2$, $P<0.05$) greater (62.0mg) than females ($n=3$) that fed on MAR (56.8mg) (Table 8).

Table 8. Mean (\pm SE) body weight of *Nezara viridula* fed on freshly ripened tomato fruit in the laboratory.

Tomato Variety	Nymphal weight				Adult weights		GI	TM
	2nd	3rd	4th	5th	Male	Female		
MAR	0.5 \pm 0.03 ^a (11)	3.1 \pm 0.63 ^a (11)	21.8 \pm 0.55 ^a (6)	34.0 \pm 1.44 ^a (6)	55.3 \pm 1.75 ^a (3)	56.8 \pm 3.28 ^a (3)	0.2 \pm 0.08 ^a	82.5 \pm 1.9 ^a
GJ	0.5 \pm 0.05 ^a (9)	2.8 \pm 0.31 ^a (8)	22.1 \pm 0.71 ^a (7)	58.8 \pm 3.5 ^a (7)	110.0 \pm 0.5 ^a (3)	115.5 \pm 0.51 ^b (4)	0.2 \pm 0.03 ^a	82.5 \pm 2.1 ^a

Means in each column followed by the same letter are not significantly different ($P>0.05$; t -test). Initial number of nymphs $n=40$ on each food; MAR=Mariana; GJ=German Johnson. TM=Total mortality (%); GI=Growth index. Numbers surviving each stadium are given in parentheses.

3.3. Measurement of Nutritional Indices

Nutritional indices are employed to assess food suitability. Indices measured typically include Consumption index (CI) which gives an idea of consumption rate; efficiency of conversion of ingested food (ECI) measures the ability to convert ingested food into biomass [37]; growth rate (GR) measures biomass gained per day; relative growth rate (RGR) measures the amount of growth attained per unit body and relative consumption rate (RCR) which measures food ingested per unit nymphal mass per day. CI was not significantly ($t=3.1$, $df=2$, $P>0.05$) higher on cowpea (12.4-32.7) compared to tomato (7.1-9.9) (Table 9). A significantly ($t<0.0001$, $df=22$, $P<0.05$) higher CI value was recorded on

PPH (32.7) than MS (12.4). Between tomato varieties, the CI value was not significantly ($t=0.1$, $df=23$, $P>0.05$) higher on MAR (8.9) than GJ (8.5) (Table 9). ECI values suggest that nymphs digested cowpea (14.25-24.55) significantly ($t=1.5$, $df=2$, $P<0.05$) more efficiently than tomato (5.7-13.2) (Table 9). Growth rate (GR) was generally not significantly ($t=1.5$, $df=2$, $P>0.05$) higher on cowpea (1.5-2.0) than tomato (0.4-1.4). The GR recorded on PPH and MS was similar ($t=0.83$, $df=22$, $P>0.05$) (Table 9). The RGR was significantly ($t=5.1$, $df=2$, $P<0.05$) higher on cowpea (62.4-85.1) than tomato (13.4-17.3) (Table 9). Pink eyed Purple Hull (PPH) (85.1) recorded a significantly higher RGR ($t<0.0001$, $df=22$, $P<0.05$) than MS (62.4) (Table 9).

Table 9. Consumption indices of third instar *Nezara viridula* on cowpea and tomato.

Crop variety	CI	ECI	GR	RGR
MS	12.4 \pm 1.2 ^a	14.25 \pm 1.2 ^a	1.5 \pm 0.4 ^a	62.4 \pm 0.9 ^a
PPH	31.5 \pm 1.4 ^b	24.55 \pm 1.6 ^b	2.0 \pm 0.1 ^b	85.1 \pm 6.7 ^b
MAR	8.9 \pm 1.2 ^c	13.2 \pm 1.4 ^c	1.4 \pm 0.1 ^c	13.4 \pm 1.2 ^c
GJ	8.5 \pm 1.5 ^c	5.7 \pm 0.4 ^d	0.4 \pm 0.1 ^d	17.3 \pm 0.9 ^d

Means in each column followed by the same letter are not significantly different according to Tukey test ($P>0.05$). CI=consumption index, ECI=efficiency of conversion of ingested food, GR=growth rate, RGR=relative growth rate. MAR=Mariana; GJ=German Johnson; MS=Mississippi silver; PPH=Pinkeye purple hull. The indices were not replicated.

4. Discussion

Nutritional ecology underscores the importance of nutritional factors in the behavioral patterns of insect herbivores [28] that are always seeking to optimize food utilization to enhance survival, growth, and reproduction. The quantification of food utilization by insects therefore provides us an insight on the suitability of various foods.

Several indices are used to understand this. Development time and mortality are used to compute the growth index which provides a good estimate of food suitability and its conversion into energy by the insect. Growth Index (GI) was low for both tomato varieties and Mississippi Silver (MS) cowpea possibly due to high mortalities experienced on these substrates, the same varieties that had relatively longer development rates. Development on MS cowpea and both tomato varieties was poor with high mortalities on all

food that was tested except the pods of Pink Purple Hull (PPH). This is a clear indication that only PPH pods are suitable food substrate. Longer nymphal developmental time, lower body weight at adult emergence, higher nymphal mortality and lower growth index on tomato indicate that tomato suggest a less suitable food source compared to cowpea.

Extended juvenile development time is usually indicative of a suboptimal food substrate, as is low female weight gain especially in the adult. Only the fresh pods of PPH gave relatively high body weight (Table 2). To better understand how growth and development are impacted by the nutritional status of each food substrate we need to examine the results of the various nutritional indices of each tested food substrate. All of these indices culminate into producing the relative growth rate, or RGR. The highest CI value was obtained on green cowpea pods of PPH which would be indicative of the ability of the insect to convert much of the food it consumed to biomass. This produced the highest weight and survival, predictably leading to a higher state of fitness.

With herbivores, Mulatu *et al.* [38] found that the presence of allelochemicals in tomato slows the growth of potato tuber moth, *Phthorimaea operculella*. We also known that host plant properties reportedly influence growth, development and survival of juveniles with direct implication on adult fitness [39, 40]. Steroidal glycoalkaloids α -tomatine, rutin, chlorogenic acid and tomatin are the major constitutive allelochemicals in tomatoes that interfere with growth and development of insect pests such as *Heliothis zea* [41]. The presence of these chemicals may account for the low consumption index (consumption rate corrected for final body weight of these insects) on the two varieties of tomato in our current study.

In the current study, fifth instars required a longer time to complete development on both tomato and cowpea. Previous studies have demonstrated hemipterans require longer duration to complete fifth instars compared to earlier stages [42, 43]. The longer time to complete development suggests that the insects must feed for a longer time to have enough energy resources to develop structures and maximum reproductive potential. Fifth instars of southern green stink bug have previously been reported to cause a greater damage to seeds than any other stage [44]. Females required a longer developmental time probably because they need greater amount and higher quality of nutrients for reproduction than do males [45, 46]. Third and fourth instars that fed on pods of MS required a longer developmental time, a phenomenon usually associated with sub-optimal diets. This may be the reason for the low consumption index (CI) (cf. PPH). On other hand, it has also been suggested that an increase in consumption rate reduces immature development [47]. The results from both cowpea varieties would seem to indicate that they may also not be the best cowpea varieties for this insect given the relatively high nymphal mortalities that occurred on each of them. More susceptible varieties are known, including “Early Scarlet” which only has recently

received research interest.

In addition, an increase in consumption rate of the nymphs that fed PPH might account for their high relative growth rate. MS recorded a higher efficiency of conversion of ingested (ECI) food indicating that the nymphs were more efficient at the conversion of ingested food to biomass. Studies have shown that high ECI is associated with low food conversion [48]. Two explanations have been proposed for this correlation [49]. First, when juvenile consume less, the food slowly passes through the digestive system and it is completely converted and used, and secondly insects eat less because they can convert it easily. In contrast, MAR tomato variety displayed a relatively high CI and ECI compared to GJ suggesting that the insects were able to convert MAR (as cf. to GJ) more efficiently into biomass.

Nymphs on fresh cowpea seeds required less time to develop compared to those on dry seeds or immature pods. This is an indication of the higher nutritional suitability of fresh seeds over dry seeds for growth and development. Panizzi and Slansky (unpublished data) indicated that development of *N. viridula* was shorter on immature seeds compared to pods and dry seeds of soybeans. Other studies have shown that the pod walls contain sclerotic cells in addition to parenchyma and other plant tissues that hinder the nymphs of herbivorous insects from having a normal feeding activity [50].

A similar study showed that the mortality of young nymphs of *N. viridula* was high on pods of the legume *Sesbania vesicaria* Jacq. but most nymphs survived on exposed seeds. The high mortality was probably because young nymphs find it difficult to reach the seeds in the pods because of air space that separates the seeds from the pod wall (Panizzi and Slansky, unpublished data). Stink bugs that feed on seeds of leguminous crops normally insert their stylets through the pod wall to have access to the seeds which are packages of highly concentrated nutrients [51]. Longer developmental time was required on dry seeds than fresh seeds most probably because the insects took a longer time to digest dry seeds than is required for fresh seeds. This might explain why mortality on dry seeds was higher compared to fresh seeds, especially since this insect is not known to be a dry seed feeder in nature.

The differences in developmental time, mortality and weight gain by nymphs on the two cowpea varieties suggest differences in the level and availability of physical plant traits that make the food substrate unsuitable. It appears that seeds of PPH meet these criteria more than MS seeds. In contrast, there was a high mortality on the pods of MS compared to PPH. PPH pod walls are thin and can be penetrated more easily. It has been suggested that tissue hardness could hinder sucking insects by preventing easy access to feeding sites [52]. The pod walls of MS are thicker than those of PPH thus making it more difficult to penetrate [51]. Biochemical analyses are needed to determine the major factors that may be impeding the development of *N. viridula* in these varieties.

5. Conclusion

These results indicate that cowpea is more suitable host plant for *Nezara viridula* than is tomato as indicated by the various metrics examined in this study. The nymphs of *N. viridula* required less time to develop and performed better on cowpea than tomato. In fact, consumption indices and growth rates were significantly higher on cowpea. So while the Pentatomidae are known for their wide diet breadth that contributes to their success and adaptive radiation, we are reminded that “all plants are not created equal” and this has direct application to food suitability. From a more applied perspective, one can see the possible use of cowpea as a trap crop to protect tomato from damage by *N. viridula*. It is unclear whether the reverse situation (to deter infestation of cowpea by inter-planting it with tomato) is even possible, or desirable, especially given that tomato is considered of higher value than cowpea that is generally seen as a crop of the poor. An intercropping study of both crops would probably provide some insight into these questions. Biochemical analyses would also help to determine the range of factors in these varieties that are responsible for the differences observed in various indices measured in this study.

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References

- [1] Esquivel, J. F., D. L. Musolin, W. A. Jones, W. Rabitsch, J. K. Greene, M. D. Toews, and R. M. McPherson (2018) *Nezara viridula* (L.). Invasive stink bugs and related species (Pentatomidae): Biology, higher systematics, semiochemistry, and management. CRC Press, Boca Raton, FL, 351-423.
- [2] Cantón, P. E. and B. C. Bonning (2019) Proteases and nucleases across midgut tissues of *Nezara viridula* (Hemiptera: Pentatomidae) display distinct activity profiles that are conserved through life stages. *Journal of insect physiology*, 119, 103965.
- [3] Panizzi, A. R. and T. Lucini (2016) What happened to *Nezara viridula* (L.) in the Americas? Possible reasons to explain populations decline. *Neotropical entomology*, 45 (6), 619-628.
- [4] Grozea, I., A. M. Virteiu, R. Stef, A. Carabet, L. Molnar, V. Marcu, and D. Draga (2016) The Spread of *Nezara viridula* (Hemiptera: Pentatomidae) Species from its First Occurrence in Romania. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Horticulture*, 73 (2).
- [5] Lucini T, and A. R. Panizzi (2018) Electropenetrography (EPG): a breakthrough tool unveiling stink bug (Pentatomidae) feeding on plants. *Neotropical entomology*, 47 (1), 6-18.
- [6] Ewunkem, J. A., L. E. N. Jackai, H. Osofuhene Sintim, and B. N. Dingha (2014) Comparing the Impact of a Neonicotinoid and Biorational Agroneem? on Herbivorous and Beneficial Arthropods on Cowpea and Tomato. *Journal of Agricultural Science and Technology. A*, 4 (7A).
- [7] Clower, D. F (1958) Damage to corn by the southern green stink bug. *Journal of Economic Entomology*, 51 (4), 471-473.
- [8] Todd, J. W and D. C. Herzog (1980) Sampling phytophagous Pentatomidae on soybean. In *Sampling methods in soybean entomology* Springer New York. pp. 438-478.
- [9] Greene, J. K, S. G. Turnipseed, M. J. Sullivan, and O. L. May (2001) Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. *Journal of Economic Entomology*, 94 (2), 403-409.
- [10] Pedigo, L. P (2002) *Entomology and pest management*. 4th Ed. Prentice Hall, New Jersey.
- [11] Reeves, R. B, J. K. Greene, F. P. F. Reay-Jones, M. D. Toews and P. D. Gerard (2010) Effects of adjacent habitat on populations of stink bugs (Heteroptera: Pentatomidae) in cotton as part of a variable agricultural landscape in South Carolina. *Environmental entomology*, 39 (5), 1420-1427.
- [12] Tillman, P. G (2011) Influence of corn on stink bugs (Heteroptera: Pentatomidae) in subsequent crops. *Environmental entomology*, 40 (5), 1159-1176.
- [13] Temple, J. H., J. A. Davis, S. Micinski, J. T. Hardke, P. Price, B. R. Leonard (2013) Species composition and seasonal abundance of stink bugs (Hemiptera: Pentatomidae) in Louisiana soybean. *Environmental entomology*, 42 (4), 648-657.
- [14] Williams, M. R (2015). Cotton insect losses-2014. (<http://www.entomology.msstate.edu/resources/croplosses/2014loss.asp>).
- [15] Payne, J. A. and J. M. Wells (1984) Toxic penicillia isolated from lesions of kernel-spotted pecans. *Environmental entomology*, 13 (6), 1609-1612.
- [16] Medrano, E. G., J. Esquivel, A. Bell, J. Greene, P. Roberts, J. Bacheler, and J. Lopez (2009) Potential for *Nezara viridula* (Hemiptera: Pentatomidae) to Transmit Bacterial and Fungal Pathogens into Cotton Bolls. *Current microbiology*, 59 (4), 405-412.
- [17] Wiman, N. G., V. M. Walton, P. W. Shearer, and S. I. Rondon (2014) Electronically monitored labial dabbing and stylet ‘probing’ behaviors of brown marmorated stink bug, *Halyomorpha halys*, in simulated environments. *PloS one*, 9 (12), e113514.
- [18] Simpson, S. J (1990) The mechanisms of nutritional compensation by phytophagous insects. *Insect-plant interactions*, 2, 111-160.
- [19] Tillman, P. G., T. D. Northfield, R. F. Mizell, and T. C. Riddle (2009) Spatiotemporal patterns and dispersal of stink bugs (Heteroptera: Pentatomidae) in peanut-cotton farm scapes. *Environmental Entomology*, 38 (4), 1038-1052.

- [20] Blumberg, D. and E. Swirski (1974) Prey consumption and preying ability of three species of Cybocephalus (Coleoptera: Cybocephalidae). *Phytoparasitica*, 2 (1), 3-11.
- [21] Panizzi, A. R. and A. M. Meneguim (1989) Performance of nymphal and adult *Nezara viridula* on selected alternate host plants. *Entomologia experimentalis et applicata*, 50 (3), 215-223.
- [22] Ashfaq, M., K. J. Ahmad, and A. Ali (2003) Morphophysical factors affecting consumption and coefficient of utilization of *Helicoverpa armigera* (Hübner). *Pakistan Journal of Applied Sciences*, 3 (4), 225-230.
- [23] Gimnig, J. E., M. Ombok, S. Otieno, M. G. Kaufman, J. M. Vulule, and E. D. Walker (2002) Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *Journal of Medical Entomology*, 39 (1), 162-172.
- [24] Panizzi, A. R. and R. M. Alves (1993) Performance of nymphs and adults of the southern green stink bug (Heteroptera: Pentatomidae) exposed to soybean pods at different phenological stages of development. *Journal of Economic Entomology*, 86 (4), 1088-1093.
- [25] Raubenheimer, D., S. J. Simpson, and D. Mayntz (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology*, 23 (1), 4-16.
- [26] Hafsi, A., Facon, B., Ravigné, V., Chiroleu, F. Quilici, S. Chermiti, and Duyck, P. F (2016) Host plant range of a fruit fly community (Diptera: Tephritidae): does fruit composition influence larval performance?. *BMC ecology*, 16 (1), 40.
- [27] de la Masselière, M. C., B. Facon, A. Hafsi, and P. F. Duyck (2017) Diet breadth modulates preference-performance relationships in a phytophagous insect community. *Scientific reports*, 7 (1), 16934.
- [28] van Loon, J. J (2005) Nutritional ecology of insect-plant interactions: persistent handicaps and the need for innovative approaches. *Oikos*, 108 (1), 194-201.
- [29] Scriber, J. M and F. Slansky Jr (1981) The nutritional ecology of immature insects. *Annual review of entomology*, 26 (1), 183-211.
- [30] Stansky Jr, F. and A. R. Panizzi (1987) Nutritional ecology of seed-sucking insects. *Nutritional ecology of insects, mites, spiders, and related invertebrates*, J. Wiley, New York, USA, 283-320.
- [31] Waldbauer, G. P (1968) The consumption and utilization of food by insects. *Advances in insect physiology*, 5, 229-28.
- [32] Harris, V E. and J. W. Todd (1981) Rearing the southern green stink bug, *Nezara viridula*, with relevant aspects of its biology. *Journal of the Georgia Entomological Society*.
- [33] Thomason, I. J. and H. E. McKinney (1960) Reaction of cowpeas, *Vigna sinensis* to root-knot nematodes, *Meloidogyne spp.* *Plant Disease Reporter*, 44 (1), 51-53.
- [34] Sakata (2010) Mariana F1 hybrid determined saladette tomato. Technical bulletin. <http://www.sakatavegetables.com/>.
- [35] Baker, S. C., J. A. Elek, and S. G. Candy (2002) Comparison of feeding efficiency, development time and survival of Tasmanian eucalyptus leaf beetle larvae *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae) on two hosts. *Austral Entomology*, 41 (2), 174-181.
- [36] Céspedes, C. L., J. R. Salazar, M. Martínez, and E. Aranda (2005) Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*. *Phytochemistry*, 66 (20), 2481-2493.
- [37] Nathan, S. S., P. G. Chung, and Murugan, K (2005) Effect of biopesticides applied separately or together on nutritional indices of the rice leafhopper *Cnaphalocrocis medinalis*. *Phytoparasitica*, 33 (2), 187-195.
- [38] Mulatu, B., S. W. Applebaum, Z. Kerem, and M. Coll (2006) Tomato fruit size, maturity and α -tomatine content influence the performance of larvae of potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Bulletin of entomological research*, 96 (2), 173-178.
- [39] Coll, M. and B. Yuval (2004) Larval food plants affect flight and reproduction in an oligophagous insect herbivore. *Environmental Entomology*, 33 (5), 1471-1476.
- [40] Silva, D. B., V. H. Bueno, F. C. Montes, and J. C. van Lenteren (2016) Population growth of three mirid predatory bugs feeding on eggs and larvae of *Tuta absoluta* on tomato. *BioControl*, 61 (5), 545-553.
- [41] Isman, M. B. and S. S. Duffey (1982) Toxicity of tomato phenolic compounds to the fruitworm, *Heliothis zea*. *Entomologia Experimentalis et Applicata*, 31 (4), 370-376.
- [42] Wu, H., X. Li, and H. Liu (2016) Starvation resistance of invasive lace Bug *Corythucha ciliata* (Hemiptera: Tingidae) in China. *Entomol Fenn*, 27.
- [43] Aldana, E., P. Medone, D. Pineda, F. Menu, and J. Rabinovich (2017) Development time and fitness: is there an adaptive development delay in the *Rhodnius prolixus* fifth nymphal stage? *Entomologia Experimentalis et Applicata*, 163 (1), 1-8.
- [44] Yeargan, K. V (1977) Effects of Green Stink Bug Damage on Yield and Quality of Soybeans 1 2. *Journal of Economic Entomology*, 70 (5), 619-62.
- [45] Söber, V., S. L. Sandre, T. Esperk, T. Teder, and T. Tammaru (2019). Ontogeny of sexual size dimorphism revisited: Females grow for a longer time and also faster. *PloS one*, 14 (4), e0215317.
- [46] Harari, A. R., A. M. Handler, and P. J. Landolt (1999) Size-assortative mating, male choice and female choice in the curculionid beetle *Diaprepes abbreviatus*. *Animal Behaviour*, 58 (6), 1191-1200.
- [47] Amalraj, D. D., N. Sivagnaname, and P. K. Das (2005) Effect of food on immature development, consumption rate, and relative growth rate of *Toxorhynchites splendens* (Diptera: Culicidae), a predator of container breeding mosquitoes. *Memórias do Instituto Oswaldo Cruz*, 100 (8), 893-902.
- [48] Simpson, S. J (1982). Changes in the efficiency of utilisation of food throughout the fifth - instar nymphs of *locusta migratoria*. *Entomologia Experimentalis et Applicata*, 31 (2 - 3), 265-275.
- [49] Hoo, C. S and G. Fraenkel (1966) The consumption, digestion, and utilization of food plants by a polyphagous insect, *Prodenia eridania* (Cramer). *Journal of insect physiology*, 12 (6), 711-730.

- [50] Oghiakhe, S., L. E. N. Jackai, and W. A. Makanjuola (1991) Cowpea Plant Architecture in Relation to Infestation and Damage by the Legume Pod Borer, *Maruca Testulalis* Geyer (Lepidoptera: Pyralidae)—I. Effect of Canopy Structure and Pod Position. International Journal of Tropical Insect Science, 12 (1-2-3), 193-199.
- [51] Slanksy, F. and F. M (1985) Food consumption and the utilization G. A. Kerkut and L. I. Gilbert (Eds.), Comprehensive insect physiology, biochemistry and pharmacology. vol 4. Regulation, digestion, nutrition, excretion. Oxford, U.K. Pergamon Press. pp 639.
- [52] Pollard, D. G (1973) Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bulletin of Entomological Research, 62 (4), 631-714.