New Oligidic Production Diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois)[1,2,3]

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Abstract A new oligidic (undefined) diet for rearing *Lygus hesperus* Knight (Heteroptera: Miridae) and *L. lineolaris* (Palisot de Beauvois) is described. The diet (referred to as NI diet) is a semisolid slurry that accommodates the solid-to-liquid feeding habits of *Lygus* spp. The NI diet consists of an "entomophage component" (cooked, whole chicken eggs, chicken egg yolks, sugar, and yeast) combined with plant components (soy bean flour, wheat germ, lima bean meal, and soy lecithin). Biological fitness estimates for *L. hesperus* indicated that mean biomass production per cage, adult wet and dry weights, survival to the adult stage, and egg production were significantly greater for the NI diet than for the existing standard, Debolt (1982) diet. The ingredients in the NI diet cost about ⅛ those in the Debolt diet, and preparation requires less than ½ of the labor. The cost of diet for production per 1000 eggs was approximately $0.004 compared to $0.04 for an equal number of eggs from Debolt diet. Recent work, started after the currently reported bioassays with *L. hesperus,* indicates that the NI diet also supports development and reproduction in the tarnished plant bug, *L. lineolaris.* This diet was used to rear and 15 generations of *L. hesperus* and is currently being used to support production colonies; it has also been used to rear *L. lineolaris* for 5 generations, thus far. The *L. lineolaris* colony started from field collected populations has been reared continuously and exclusively on the NI diet and is currently in a log phase of population growth. This diet should be beneficial in providing a great reduction of *Lygus* production costs while producing a high quality, vigorously reproducing insect.

Key Words Mass rearing, quality control, Miridae, Hemiptera/Heteroptera

Western tarnished plant bugs, *Lygus hesperus* Knight (Hemiptera: Miridae), and tarnished plant bugs, *L. lineolaris* (Palisot de Beauvois), are very destructive pests, and their economic impact spans several cropping systems in North America (Hedlund and Graham 1987). Their impact is amplified by their remarkable ability to become resistant to pesticides and by their extremely broad host range (Hedlund and Graham 1987). Therefore, biologically-based alternatives to conventional pesticides to control this pest have become very important. Such alternatives include development of biological control, biorational chemicals, plant breeding, sterile insect release, and genetic engineering of target crops. Development of management strategies based on these approaches would depend upon rearing systems that permit medium-

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[2]Mention of a trade name does not constitute endorsement by the USDA.
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to-large scale rearing of *Lygus* bugs. A major component necessary for such rearing includes an inexpensive, high quality artificial diet that can be used to rear thousands to millions of the targeted pest (Cohen et al. 2000, Nordlund 1996, Nordlund and Greenberg 1994).

Debolt (1982) reported the development of a high quality artificial diet for *L. hesperus.* That diet had been used successfully in rearing *L. hesperus* that were used for rearing both egg and nymphal parasitoids (Debolt 1987, 1989). Rearing systems based on artificial diets for pests are also useful for research in genetics, production and testing of microbial pest control agents, and many other functions (Singh and Moore 1985). Therefore, the availability of good artificial diets for insects such as the *Lygus* complex remains an important component of emerging systems of managing these pests. Although the Debolt diet, which is meridic and contains many expensive, defined ingredients, is an excellent growth medium for *L. hesperus* in terms of biological fitness, its complexity, the expense of its ingredients (>$8/kg), and the labor intensity of its production demand less expensive alternative diets. This need prompted efforts to develop a less expensive diet and one that would also support mass production of *L. lineolaris*. These efforts resulted in the development of a diet (herein described as the "C diet") that had been used to rear *L. hesperus* in the Gast facility (USDA, ARS, MSA, BCMRRU) for 1.5 yrs (approximately 15 to 18 continuous generations). The C diet contains undefined plant components and an entomophagous insect diet (Cohen and Smith 1998, Cohen 1998b) to satisfy the facultative zoophagy observed in *Lygus* spp. and is void of the many defined components included in the Debolt diet. The cost of materials in the C diet is ⅛ that of the Debolt diet. However, preliminary comparisons showed that while there was success in using an oligidic diet for rearing *L. hesperus* in continuous generations, the C diet did not support the same level of biological fitness as did the Debolt diet. Fecundity, adult survival, and adult body weights were lower on the C diet than they were with the Debolt diet (Cohen, unpub. data). This intermediate success prompted the current effort to develop a diet that would support the biological quality afforded by the Debolt diet but would resemble the C diet in simplicity and cost. Using the base of knowledge of *L. hesperus* feeding biology summarized by Cohen (2000), such a diet was developed and compared initially with the C diet and ultimately with the Debolt diet. Tests were conducted to assess rearing of *L. hesperus* under communal rather than individual conditions because the intended purpose of the new artificial diet is use in a mass rearing facility. Another intention of this work was to develop a diet that would support robust production of *L. lineolaris,* a goal that up to now has been elusive.

Materials and Methods

Insects. The *L. hesperus* used in these studies were derived from a colony from Biotactics, Inc. (Riverside, CA). They had been colonized on the C diet at the Gast (USDA, ARS, BCMRRU) facility for 1.5 yrs (Cohen unpub. data). The C diet is a combination of soy flour, wheat germ, lima bean meal, yeast, and vitamins mixed with the Cohen (1998b) entomophage diet. The *L. lineolaris* were collected from weeds in Chickasaw Co., MS. Voucher specimens from each colony have been placed in the Mississippi State University Entomology Museum.

Production set-up. The standard cages used for all life stages were Rubbermaid® 8.3 L rectangular storage boxes, with openings cut into the tops and replaced with 0.4-mm organdy cloth for smaller nymphs or 1.0 mm mesh fiberglass screen for larger

nymphs and adults. Rearing room conditions were light:dark cycle of 16:8 h; temperature of 27°C (±1.5°C), and 50 to 60% RH. Cages were placed on racks to allow air circulation and light to reach each cage. The production colony was treated according to the recommendations of Debolt and Patana (1985) except for the modifications specified here. Egg packets were placed intact into cages, rather than being separated from the gel. Egg packets were placed inside cages with shredded paper (0.6 × 28.4 cm) rather than loosely wadded paper towels to reduce cannibalism. The first feeding packet provided to newly-eclosed nymphs was stretched to facilitate feeding. The colony was kept at 27°C, rather than 26°C. The cages were topped with an organdy cloth held tight by the box's snap-on top that had a 21 × 30 cm opening. In contrast with the procedures of Debolt and Patana (1985), feeding units were only the cages made from 8.3 L rectangular storage boxes rather than a mixture of cardboard cartons for nymphs and larger feeding units for adults. Using only a single cage reduced the labor and mortality inherent in the extra handling involved in transfer of insects. Finally, a 2%, rather than a 1.2%, gel was used for oviposition. Previous work (Cohen, unpub. data) indicated that the higher percentage gel seemed to increase egg hatch.

The NI diet. The diet consisted of 3 groups of ingredients. Group (A) (carnivore diet) was pre-made and is stored refrigerated in 250 g aliquots of the following mixture: whole, blended chicken eggs (900 g), water (200 ml), sucrose (165 g), brewers yeast (30 g), 50% honey solution (150 g), 10% acetic acid solution (30 ml). Group A was made by mixing water, sucrose, honey and acetic acid and bringing to a rapid boil, then stirring in the eggs and heating until they were a sticky, scrambled egg consistency. Group (B) contained toasted wheat germ (200 g), coarsely ground lima bean meal (300 g), soy flour (50 g), tap water (900 g), egg yolk (300 g). These materials were mixed and autoclaved for 20 min. Group (C) consisted of tap water (700 ml), formalin (1 ml), soy lecithin with oil (10 g), Vanderzant vitamin mixture (8 g), 1 ml propionic acid, chlortetracycline (0.05 g), streptomycin sulfate (0.05 g). After the ingredients from group B were cooled to about 50°C, the 250 g aliquot of group A were added, and group C was then added and mixed in a blender for 4 min at medium speed. The completed diet was still warm enough (approximately 45°C) to pour as a thick slurry into either storage containers or feeding packets. This formulation results in production of a 2.5 kg batch that is stored refrigerated for no longer than 2 wks or is freeze dried and stored in sealed containers for no more than 2 months.

Experimental design. To compare the relative effectiveness of each diet, 3 replicates were set up on Debolt diet (1982) and NI diet. In preliminary work, the C diet was tested in comparison with Debolt diet. Earlier tests with the C diet (Cohen, unpub. data) indicated that the defined ingredients in the Debolt diet were not necessary for production of *L. hesperus*. However, observations of superior biological fitness of *L. hesperus* reared on the Debolt diet compared with those reared on the C diet (Cohen, unpub. data) prompted the current study, aimed at improvement of the oligidic C diet.

To begin all tests, rearing units were set up consisting of a cage, described above, and inoculated with Parafilm® packet containing approximately 5000 eggs. The packet (about 72 cm^2) containing 2.0% Gelcarin® gel (FMC-Food Ingredients Division, Rockland, ME) was placed in a standard feeding cage (described above). The following parameters were measured for each treatment group: (1) the weight of sexually mature adults, (2) the number and percent of eggs that became adults, (3) the survival of adults 3 wk after adult eclosion, (4) the mean biomass (dry weight) accumulated per cage over the total development period and 15 d post adult eclosion,

including adults, eggs, and deceased nymphs, (5) the mean number of eggs produced by each cage of adults. For egg counts, an Image Pro® Plus image analysis system (Media Cybernetics, Silver Spring, MD) was used. Means were compared by Student t tests (unpaired or paired, when appropriate). First-instar *L. hesperus* were provided with appropriate diet from a packet made of stretched Parafilm and placed within the cage. After the first feeding (after 2 d), the standard diet packets were placed on top of the cages every Monday, Wednesday, and Friday. These packets were made with heat sealed, unstretched Parafilm and contained about 10 to 15 g aliquots of appropriate diet (Debolt and Patana 1985). To estimate egg production per cage, adults from each feeding regimen were provided with oviposition packets (Patana 1982) daily. Egg packets were made with 2.0% Gelcarin in tap water and were placed on top of cages as were feeding packets. Oviposition packets were collected every day, and egg numbers were estimated by counting with the image analysis system mentioned above. The mean number of eggs in 3 randomly selected 3-cm^2 areas was multiplied by 24 to correct for the area of the whole packet (72 cm^2). Egg packets were removed and counted daily over a 10-d period. Estimates of egg biomass were made by multiplying the numbers of eggs by 0.018 mg, the mean dry weight of individual *L. hesperus* eggs.

Adult weights were measured 4 wk after adult eclosion. Samples of 5 adults of each sex from each cage (i.e., 15 males and 15 females per treatment) were weighed to determine the fresh weights. Fifteen adult males and 15 females were dried for 48 h at 60°C and were used to determine the mean dry weights of individuals from each sex and each treatment. Numbers of adults produced in each cage and survival numbers were estimated from dry weight biomass of surviving and dead individuals harvested at the end of the experimental period. Estimates were made by determination of dry weights of adults (15 from each sex from each treatment). These measurements were made 20 d after adult eclosion.

Results

Mean biomass production (in dry weight) per cage was significantly greater on the NI diet than it was on the Debolt diet with means of 11.2 (±0.91) and 7.8 (±0.62) g of adults and eggs, respectively, for the 2 diets ($t = 3.10$, $P = 0.036$, df = 4). The biomass accumulation resulted from approximately 0.09 g of eggs in each cage (approximately 5000 eggs/cage) which yielded a mean of 7.4 (±0.65) g of adults for the NI diet and 4.6 (±0.46) g of adults for the Debolt diet ($t = 4.09$, $P = 0.05$, df = 2, paired t test); this represented increases of 82 and 51 fold, respectively. On a per cage basis, the mean number of individuals that survived until 20 d post adult eclosion (=15 d post onset of oviposition) was 1076 (±102.5) for the NI diet and 625 (±133.3) for the Debolt diet ($t = 22.3$, $P = 0.002$, df = 2). For NI and Debolt diet reared *L. hesperus,* respectively, mean (±SE) fresh weights of 1 wk post-adult eclosion females were 11.7 (±0.26) and 11.4 (±0.30) mg, and 4-wk post-adult eclosion females weights were 11.2 (±0.15) mg and 10.6 (±0.21). Corresponding weights of 1 wk adult males were 8.2 (±0.22) and 7.75 (±0.23) mg, and 4-wk post-adult eclosion males were 7.9 (±0.21) and 6.7 (±0.18). Dry weights were significantly greater both for females and males on the NI diet, 4.40 (±0.12) and 3.93 (±0.12) for females ($t = 2.73$, $P = 0.01$, df = 28) and 3.09 (±0.08) and 2.57 (±0.09) for males ($t = 4.51$, $P = 0.0001$, df = 28), respectively, for the NI and Debolt diet treatments. Mean survival from the egg to the adult stage was 20.5% for the Debolt diet individuals and 29.0% for the NI diet. A mean of 58.9 (±SE

2.80) Debolt adults remained alive 4 wk after adult eclosion compared to 74.4% (±SE 4.16) NI diet adults. The ingredients in the NI cost less than ⅛ those used in the Debolt diet, and it demanded less than ½ of the time required for that diet's preparation. Fig. 1 shows a comparison of the number of eggs produced per d over a 10-d period.

Production set-up. The NI has supported 15 continuous generations of *L. hesperus* between March 1999 and January 2000. Over the past several generations, the populations have grown so robustly that at least half of the eggs must be routinely discarded because of the overproduction inherent in this rearing system. A cage containing approximately 1000 adults can produce 8000 to 12000 eggs per day. A technician working 20 h per wk on the colony can maintain about 60 cages (30 being adult cages), which would allow production of 240,000 to 360,000 eggs per day.

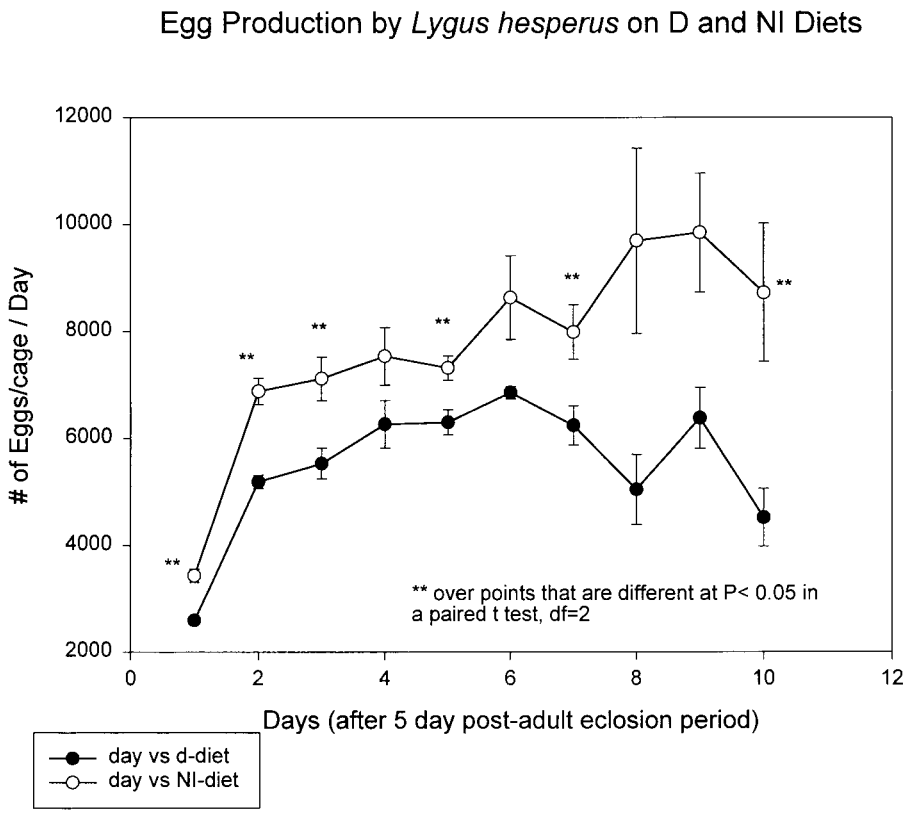

Fig. 1. Egg production by *Lygus hesperus* on Debolt (D) versus NI diet. Circles represent the mean number of eggs per cage on each treatment (±SE). The ** symbols represent significant differences in means for a given day in a paired *t* test, $P = 0.05$, df = 2.

Discussion

Performance of *L. hesperus* reared on the NI diet was superior to that of subjects reared on the existing standard, Debolt diet, in all biological fitness characteristics measured. This includes biomass accumulation per standard cage (including adults and eggs produced over the life span of a generation). Each cage, started with about 5000 eggs (approximately 90 mg biomass dry weight), contained roughly 1000 *L. hesperus* adults. By the onset of full reproductive maturity (approximately 5 d post-adult eclosion), each cage produced between 2000 and 6000 eggs per day with the Debolt diet treatments and between 3000 and 10,000 eggs per day in the NI diet treatments (Fig. 1) This resulted in a 51-fold per cage increase biomass accumulation with the Debolt diet treatment and 82 fold for the NI treatment. This biomass estimate includes growth from eggs to adults and the eggs produced per cage over 10 d of oviposition.

The greater biomass accumulation in the NI treatment resulted from several factors including a significantly greater egg production, greater weight of individuals, greater percentage of individuals that became adults, and longer survival past the onset of reproductive period (contributing to greater production of eggs). It is important to note that both wet weights and dry weights were greater for NI individuals than for Debolt diet treated *L. hesperus* and that the males were especially impacted by the NI diet treatment.

In mass rearing, two of the most important indicators of quality are biomass accumulation and fecundity. These related factors inherently relate to the major purpose of rearing programs: efficient production of high quality arthropods. Therefore in research on development of rearing systems (including artificial diets, preferred temperature regimens, improved cages, oviposition substrates, etc.), both the most immediate and also far reaching questions of quality would be most directly answered by comparisons of biomass accumulation per unit of time. Assessment of accumulated biomass would quickly and sensitively indicate that a diet was not adequate or that cannibalism was taking place and obscuring failings in a newly-developed diet (Debolt 1982, Cohen 1985).

The objective of this study, to develop for *L. hesperus* and *L. lineolaris* an inexpensive diet that requires little labor to produce, was met successfully. The NI diet thus far has supported 15 continuous generations of apparently healthy *L. hesperus* and 5 generations of *L. lineolaris* with no recourse to whole, fresh plant parts or insect prey. Unlike previous diets developed for *Lygus* species, it has the consistency of a thick slurry imbued with solid debris. The nutrient composition is roughly 6% protein, 2% lipid, 17% carbohydrate, and 75% water. Its consistency serves the feeding biology of *L. hesperus* and *L. lineolaris,* which use extra-oral digestion (Strong 1970, Cohen 1990, Agustí and Cohen 1998) to macerate tissues of its host plants and prey. The cost of the diet ingredients for the NI diet is about and ⅛ that of the ingredients in the Debolt diet, and the time required to prepare the NI or the C diet is less than ½ that required to prepare the Debolt diet.

Rationale for the diet and the specific ingredients. The Debolt diet works well for rearing *L. hesperus;* therefore, an explanation is in order to explain why efforts were made to improve upon it. The Debolt diet had a pioneering impact on *Lygus* rearing. It has been used as the basis for *L. hesperus* production in small to moderate scale for biological control (DeGrandi-Hoffman et al. 1994) and for several studies of the biology of *L. hesperus* and its parasitoids. The diet supported rearing of continu-

ous generations of biologically fit *L. hesperus* where other diets developed over 2 decades prior to its development failed. The drawbacks of the Debolt diet are its complexity, making it labor intensive and difficult to produce, and the expense of using highly purified ingredients such as salt mixtures, cholesterol, casein, RNA, and linoleic acid (Tables 1, 2).

A major reason why the Debolt diet and the NI diet proved to be excellent nutrient bases for *L. hesperus* is that they satisfy the needs of this species in terms of state of the nutrients (solids) and the concentrations of nutrients. Cohen (2000) reviewed the assumptions behind earlier attempts to develop diets for *Lygus* spp. and he pointed out that underlying all these efforts was the idea that these insects were strictly liquid feeders that were capable of using only dissolved materials (e.g., Strong and Kruitwagen 1969, Strong and Landes 1965, Raulston and Auclair 1968, Vanderzant 1967). Although these studies made basic contributions regarding *Lygus* nutrition, they did not result in development of a practical diet, possibly because of a failure to recognize the importance of extra-oral digestion and solid-to-liquid feeding by these mirids (as well as in many other heteropterans) (Cohen 1998a).

The addition of chicken eggs to both the Debolt diet and cooked chicken eggs (enriched with extra yolks) in the NI diet satisfies the apparent requirement that *L. hesperus* (and evidently *L. lineolaris,* also) has for animal derived materials. The efficacy of using cooked eggs (NI diet) rather than raw eggs (Debolt diet) was dis-

Table 1. Major diet components (1 g or more per kg) in the NI diet and the Debolt diet in g of material/kg (cost in U.S. $)

Component	NI Diet	Debolt Diet for *Lygus hesperus*
chicken egg yolks	120 (0.12)	0
whole chicken eggs	55 (0.04)	144 (0.10)
wheat germ (toasted)	80 (0.22)	36 (0.10)
lima bean meal	120 (0.17)	36 (0.05)
soy flour (toasted)	20 (0.06)	0
sucrose	10 (0.12)	22 (0.22)
lecithin	4 (0.27)	0.04 (0.03)
vitamins (Vanderzant)	3.2 (0.38)	7.2 (0.86)
brewer's yeast	1.8 (0.05)	0
honey solution (50%)	8.9 (0.03)	0
salt mixture	0	2.3 (0.50)
casein hydrolyzate	0	14.3 (0.96)
Gelcarin	0	2.2 (0.22)
water	573	730
acetic acid (10%:90% water)	3 (0.01)	0

Table 2. Other diet components (<1 g or more per kg) in the NI diet and the Debolt diet in g of material/kg (cost in U.S. $)

Component	NI Diet	Debolt Diet for *Lygus hesperus*
chlortetracycline	0.01 (0.04)	0.4 (1.84)
streptomycin sulfate	0.01 (0.05)	0.1 (0.50)
formalin	0.4 (0.01)	0.4 (0.01)
niacin	0	0.8 (0.91)
p-aminobenzoic acid	0	0.8 (0.90)
cholesterol	0	0.4 (0.25)
RNA	0	0.4 (0.31)
Tween 80®	0	0.7 (0.05)
linoleic acid	0	0.14 (1.23)
propionic acid	0.3 (0.01)	0
Total Cost of Materials	$1.46	$9.04

Prices based on local (Starkville, MS) supermarket prices of liver, high fat ground beef, fresh eggs, baby lima beans, wheat germ, and soy flour. The other prices are based on a 1998 Sigma catalog.

cussed by Cohen and Smith (1998), and Cohen (1998b). The use of egg yolks, blended and autoclaved with the plant materials (in component group B), proved to be essential to the nutritional and phagostimulatory quality of the complete diet (Cohen, unpub. data). A major function of the eggs in both diets is the satisfaction of a need for animal products such as high protein concentration. The nature of facultative carnivory of *Lygus* was discussed by Bryan et al. (1976) and by Wheeler (1976). The presentation of wheat germ and lima bean meal, both of these being highly concentrated with proteins, lipids, vitamins, etc., characteristic of seeds, allows the *Lygus* bugs access to nutrients very much like those in the reproductive structures in plants that these insects attack. *Lygus* bugs have an elaborate complex of salivary digestive enzymes (Hori 1970a, 1970b, 1971, Cohen 1990, Agustí and Cohen 1998) that they inject both into normal host plants and into the artificial diet to macerate solid structures, allowing the removal of nutrients as described by Cohen (1998a) in a process of solid-to-liquid feeding or extra-oral digestion.

A final point must be made about the use of the NI diet for *L. lineolaris:* the colony of this species now being reared at the Gast facility was started in October 1999 from about 200 field-collected adults that were apparently in reproductive diapause. After about 2 to 3 wk on the NI diet and at the above specified thermal, humidity, and light regime, they began to lay eggs in oviposition packets, mentioned above. It was found that using a 4% gel with *L. lineolaris* instead of 2% (as described above for *L. hesperus*) greatly increased the hatch rate (Cohen, unpub. data). These insects have now proceeded through a lag phase of production (linear increase) and are now in a log (exponential) phase of population growth. Rearing units of about 2000 adults per cage produce roughly 5000 to 7000 eggs per day in a single egg packet. These packets are being used to start new cages and will be the basis of a planned ex-

panded colony of this species that has not previously been reported to be produced on artificial diet. The cost of diet per 1000 eggs for this species and for the *L. hesperus* is approximately $0.004 (compared to about $0.04 estimated for the Debolt diet, used for producing only *L. hesperus*). Therefore, it appears that the NI diet represents an important breakthrough in rearing species of *Lygus.*

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