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Artificial Diets for Life Tables Bioassays of Tarnished Plant Bug (*Lygus lineolaris*) in Mississippi

Report

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Abstract: Two artificial diets for mass rearing and bioassay of the tarnished plant bug, (TPB), *Lygus lineolaris* Palisot de Beauvois, (Hemiptera: Miridae) were modified and developed, respectively. The first diet is a modification of a semisolid artificial diet (NI diet), which permits large scale rearing of omnivore insects such as TPB and the western tarnished plant bug, (WTPB), *Lygus hesperus* Knight. Both species can be produced with higher biological fitness values than those reported for both species using the existing standard NI diet. The modified artificial diet cut the coking component (NI carnivore diet), avoided antibiotics, acids, and formaldehyde, and changed other ingredients such as preservatives and minerals. These modifications made the modified NI artificial 20% less expensive, 75% less laborious and completely harmless to humans in its preparation. The second diet is a non-autoclaved solid artificial diet for rearing *Lygus spp*. and beneficial insects. Diet's ingredients include soybean, lima bean, wheat germ, chicken egg yolk, agar and inhibitors. The diet can be prepared in one step by blending the ingredients in a heated aqueous solution. Insects maintained with the solid diet can be used to measure virulence of fungal entomopathogens, the efficacy of insect growth regulators, or resistance to insecticides. Solid artificial diet allows for evaluation of large scale bioassays of late instars and adults of *Lygus spp*. and several omnivore insects. Both diets could have a large impact on agriculture by enabling researchers to investigate control of TPB, protection of beneficial organisms, development of new and improved pest control technologies, integration of component technologies in an IPM system and development of area wide suppression programs for TPB.

Keywords: Lygus diet, mass rearing, quality control, fitness estimate

Introduction

Tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois, (Hemiptera: Miridae) is found in all agricultural regions of North America and probably has the broadest feeding niche of any known arthropod (Robbins et al. 2000). Young (1986) reported more than 385 plant species in 55 families across North America and Snodgrass et al. (1984) reported for Mississippi 169 host plants species in 36 families. The impact of TPB is amplified by its remarkable ability to become resistant to pesticides (Snodgrass 1996, Snodgrass and Scott 2002, and Snodgrass et al. 2009). Development of management programs based on biological alternatives such as biological control, bio-rational chemicals, plant breeding, sterile insect release, and genetic engineering of target crops are very important and all these approaches depend on a mass rearing system (Cohen 2000).

Rearing of Lygus on an artificial medium began when Auclair and Raulston (1966) reported limited success rearing *Lygus spp.* on a chemically defined diet. Vanderzant (1967) and Frank and Krutwagen (1969) developed diets that supported growth and development of WTPB and TPB than were comparable to rearing them on fresh green beans. The Debolt diet (Debolt 1982) and more recently the NI diet (Cohen 2000) have been the only two diets that successfully supported multiple generations. However, the NI diet is the only successful diet currently used for mass rearing of *Lygus spp*. For many years, several commercial insectaries and research agencies have used the NI diet for the continuous production of TPB and WTPB. However, because preparation of the diet is time consuming, use of the diet is not as widespread as it could be. The use of green beans or other natural hosts is more convenient and they are frequently used to rear Lygus.

Current approaches to the evaluation of the entomopathogenic fungus *Beauveria bassiana*, the insect growth regulator novaluron (Diamond 0.83 EC), and many other control options (pesticides, sterile insect, genetic engineering target crops, biological agents), for activity against TPB often rely on field experiments and bioassays using green beans, broccoli or other materials such florist wet foam (Leland and Snodgrass 2005, Leland 2005, Leland et al. 2005, McGuire et al. 2006). Detailed life-tables studies or more quantitative estimates of the impact of control agents on TPB life history require a bioassay option to study the impact of prolonged exposure for weeks following contact with the control agent. This is difficult with plant tissues or florist wet foam that must be routinely replaced over the period of the study. Tables 1 and 2 present a brief protocol of diet preparation and a list of ingredients for both semi-solid and solid Lygus diets. The results presented in this paper summarize the impact of both diets on TPB mass production and control.

Semi-Solid Lygus Diet for TPB Mass Production

This study was conducted at the USDA, ARS, National Biological Control Laboratory and Southern Insect Management Research Unit in Stoneville, MS. Tarnished Plant Bugs were reared according to method described by Cohen (2000). Survival to adult, sex ratio and fourth-fifth instar nymphal mortality were not different between diets (Table 3). Daily egg production was significantly higher using the Modified NI diet with 6806.91 ± 302 versus 4953.32 ± 316 eggs per day/ cage for the NI Diet. Each modified Ni diet cage produced about 2,000 - 8,000 eggs per day between first and tenth day of oviposition. This was significantly greater than the NI diet egg production per day which ranged from 800 - 6,000 eggs per day / cage. The cumulative egg production of TPB was significantly higher in the Modified NI Diet (over 67,000 eggs produced in 10 days) compared to cumulative egg production observed for the NI diet (49,000). The percentage of no-embryo eggs produced per day was lower for the Modified NI diet, and the fertility and hatchability rate was higher than in the NI diet. The greater egg production, lower fourth-fifth instar nymphal mortality, and higher hatchability rate obtained in the modified NI Diet resulted in a greater biomass accumulation.

Slight differences between diet components can have an effect on feeding preferences (Singh and Moore 1985) and can affect the required criteria for evaluating the nutritional adequacy of a diet used for mass rearing. Our results indicated that the modified NI Lygus diet was adequate for development and mass production of TPB and was superior in biomass accumulation and fecundity to the regular NI diet (control). The biomass accumulation obtained in the NI diet (control) was similar to Cohen (2000), who obtained roughly 1000 adults per standard cage (500 females) of TPB from a gel pack of 5000 eggs.

One large factor in the extremely low survival to adult obtained in this study and in studies by Cohen (2000) and Portilla et al. (2011) was cannibalism during nymphal and adult stages, especially at high rearing densities. TPB, like other *Lygus spp*., is an omnivorous, and consumes both plant-based foods and arthropod prey (Rosenheim et al. 2004). Omnivory in TPB has been documented in both the laboratory and field (Wheeler 1976, 2001, Cohen 1996). Therefore, the use of cooked or raw eggs seemed to be essential to the nutritional and phagostimulatory quality of the complete diet and could decrease cannibalism as it was discussed by Cohen and Smith (1998) and Portilla et al. (2011). The modified NI artificial diet allows for the production of insects with specific and similar ages with higher biological fitness values than those reported for the existing standard NI diet.

Non Autoclaved Solid Lygus Diet for TPB Bioassays

The study was conducted at the USDA-ARS, South Insect Management Research Unit in Stoneville, MS. A non-autoclaved solid diet and a novel bioassay were specifically developed for conducting laboratory experiments to evaluate the susceptibility of TPB to *Beauveria bassiana* (NI8 native strain) and the insect growth regulator novaluron (Diamond 0.83EC) as the model fungus and insecticide, respectively. The assay technique can efficiently keep alive immature TPB (second to fourth) over 10 days and fifth instar and adults over 30 days. Portilla et al. 2014 (In press) obtained differences in mortality, longevity, fungal infection, growth inhibition, and sporulation that indicated that the novel bioassay for TPB on solid Lygus diet was effective in determining the activity of *B. bassiana* and novaluron against all developmental stages of the tarnished plant bug. Fungal sporulation and growth disruption in TPB held on solid artificial diet at 27°C occurred in a period of time that ranged from two to ten days when a concentration of 492 ± 71 spores per mm^2 of *B. bassiana* was used. The fungal incubation period ranged from 10.78 (8.40 ± 0.8 days to death and 2.38 ± 0.12 days to sporulation) to 5.77 (3.80 ± 0.4 days to death and 1.97 ± 0.11 days to sporulation) days depending on insect developmental time. Previous laboratory studies reported most mortality for TPB as three days or longer after inoculation at 28°C (Leland and Behle 2005, McGuire et al. 2005, McGuire et al. 2006, Spurgeon 2010). Determining the time needed for entomopathogenic fungus to work is important in conducting a bioassay. This time period can be more than 20 days under low temperature (12.8°C) and low concentration ($1x10^6$ conidia/ml) (Spurgeon 2010) which may affect control mortality due to excessive insect handling when feeding the insects. For example, Lund et al. (2006) reported a control mortality of 82.8% for TPB nymphs and 56.4% for adults. Portilla et al. 2014 (In press) demonstrated that insects bioassayed on solid Lygus diet had low mortality in the control. The solid diet used in the novel bioassay technic is not changed from the day of insect inoculation until the end of the bioassay. This avoids the 3 times weekly food changes that are commonly required in the standard methods where fresh green beans or florist wet foam are provided to the remaining alive insects until the end of the assay (Steinkraus and Tugwell 1997, Liu et al. 2002; Leland et al. 2005, McGuire et al. 2005, McGuire et al. 2006, Spurgeon 2010). Avoiding insect handling can also minimize contamination and handling death. The inhibitors in the solid diet can last for about 20 days, after that, *B. bassiana* from infected insects can slowly grow on the diet.
The technique was developed for TPB bioassays; however, it could also be used to evaluate susceptibility to insecticides or other entomopathogenic fungi in different species of insects and control agents such as WTPB, brown marmorated stink bug, *Halymorpha halys* Stal (Hemiptera: Pentatomidae), green stink bug, *Acrosternum Hilary* Say (Hemiptera: Pentatomidae), southern green stink bug, *Nezara viridula Linnae* (Hemiptera: Pentatomidae), fall army worm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), beet army worm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae), corn ear worm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), tobacco budworm, *Heliothis virescens* Fabricious (Lepidoptera: Noctuidae), multicolored Asian ladybug, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), convergent ladybug, *Hippodamia convergens* Guerin-Meneville, green lacewings, *Chrysopa spp., Crysoperla spp*., minute pirate bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), jumping spiders (Araneae: Salticidae), and crab spiders (Aranea: Thomisidae) (Portilla et al. 2013, Portilla et al. 2014 in press, MP unpublished data). The solid artificial diet for *Lygus spp.* provides improved research capacity for studying the ecology and susceptibility of insects to a number of different control agents including beneficial organisms, insect pathogens and insecticidal toxins being developed for transgenic technologies.

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Table 1. Diet components of Modified Semi solid NI artificial diet and the current NI diet for mass rearing TPB (small batch preparation**: approximately 2.5 L of diet)

Components	Diets		
	Current NI Diet amount	**Modified NI Diet amount**	**Sources**
Group A (Carnivore Diet)			
Water	40 ml		
Sugar	33 g		Local Grocery Store
Honey	15 g		Local Grocery Store
Acetic acid	2 ml		MP Biomedical
Brewer's yeast	6 g		MP Biomedical
Whole chicken egg	180 g (3 eggs)		Local Grocery Store
Group B (Autoclave)			
Toasted wheat germ	200 g	200 g	Nutritional Designs Inc.
Coarsely ground lima bean meal	300 g	300 g	Bio-Serv
Soy Flour	50 g	50 g	Arrowhead Mills
Yolk egg chicken	300 ml (18 yolks)	18 yolks	Local Grocery Store
Water	900 ml	1000 ml	
Group C			
Torula Yeast		6 g	MP Biomedical
Soy lecithin with oil	10 g	10 g	MP Biomedical
Vanderzant vitamin mixture	8 g	8 g	Sigma
Propionic acid	1 ml		Sigma
Chlortetracycline	0.05 g		Sigma
Streptomycin	0.05 g		Sigma
Formaldehyde	1 ml		Sigma
Sugar		33 g	Local Grocery Store
Honey		15 g	Local Grocery Store
Benzoic acid		2 g	Sigma
Water	700 ml	1000 ml	
Total Price per L of diet	$ 4.07 *	$ 3.34*	

***** Price of these ingredients obtained in Mississippi supermarket and sigma catalog in Portilla et al., 2011.

** The Current NI diet consists of 3 (A, B and C) groups; while, the modified diet consists of 2 groups (B and C) (see above). The Current NI diet is prepared according to the procedure of Cohen (2000) as follow: the carnivore diet is prepared by mixing ingredients of group A and bringing the mixture to a rapid boil, at which time whole chicken eggs were stirred and heated until the mixture had scrambled egg consistency. The Group B components are mix and autoclave for 20 min at 120ºC and 15 lb/m². Group C components are weighted, mixed and blended for about 4 min with groups A and B when they had cooled to about 50ºC. Modified NI diet is prepared following the procedures of NI Groups B and C.

Table 2. Diet components of the new non-autoclaved solid artificial diet for TPB to evaluate the potential of *Beauveria bassiana* and novaluron (batch preparation: approximately 1 Gallon of diet*) Portilla et al., 2014 (In press)

Ingredients	Amount	Sources
Boiled Water	3000 ml	
Gelcarin GP812	70 g	FMC
Toasted wheat germ 510-16	100 g	Nutritional Designs Inc.
Coarsely ground lima bean meal G1305	150 g	Bio-Serv
Soy Flour	25 g	Arrowhead Mills
Sugar	156 g	Local Grocery Store
Yolk egg chicken	12 yolks	Local Grocery Store
Vanderzant vitamin mixture V-1007	36 g	Sigma
Propionic acid P1386	4.0 ml	Sigma
Phosphoric acid 696017-2	0.5 ml	Sigma
Methyl Paraben 10234	3.8 g	MP Biomedical
Aureomycin	3.8 g	Sigma
Sorbic acid 102937	3.8 g	MP Biomedical
Benzoic acid B9300-500	3.0 g	Sigma

* The non-autoclaved solid diet consists of 13 ingredients. It is prepared by mixing the weighted components and blending them in boiling water and yolk from chicken eggs for about 4 min. The final mix (5 ml of diet/ cup) was poured into individual 37 ml plastic cups (T-125, Solo Cup Company, www.solocup.com) and kept at room temperature to cool for solidification before use.

Table 3. Mean fitness parameters of *L. lineolaris* reared on NI diet modifications

	Diets (Mean ± SE)	
Fitness Parameters	Modified NI Diet	NI Diet
Survival to Adult (%)	80.94±1.91a	80.89±2.29 a
Sex Ratio (F:M)	1.06:1a	1.09:1a
First–Third Instar Nymphal Mortality (%)	12.93±1.71b	14.23±2.88a
Fourth-Fifth Instar Nymphal Mortality (%)	6.12±0.71a	6.86±1.07a
Total Biomass Weight (g)	12.93±1.62a	11.54±1.09b
Total Eggs / Day / Cage	6806.91±302a	4953.32±316b
No-embryo egg production (%)	20.13±0.82b	25.99±1.53a
Hatchability Rate (%)	53.52+0.72b	43.70+1.16a