ISSN 1727-1320 (Print),
ISSN 2308-6459 (Online)

ВЕСТНИК
ЗАЩИТЫ РАСТЕНИЙ

PLANT PROTECTION NEWS

2023 ТОМ VOLUME 106 ВЫПУСК ISSUE 2

Санкт-Петербург
St. Petersburg, Russia

Для оформления обложки использована фотография клопа *Nesidiocoris tenuis*
к статье Пазюк И.М. (стр. 100–104) (© С.Г. Удалов, ВИЗР)

For the cover design, a photo of the *Nesidiocoris tenuis* bug
for the article by I.M. Pazyuk (p. 100–104) was used (© S.G Udalov, VIZR)

OECD+WoS: 1.06+IY (Entomology) https://doi.org/10.31993/2308-6459-2023-106-2-15517

Short communication

EVALUATION OF DIETS FOR MASS REARING OF THE PREDATORY BUG *NESIDIOCORIS TENUIS* (HEMIPTERA, HETEROPTERA, MIRIDAE)

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Nesidiocoris tenuis (Hemiptera, Miridae) is a predatory bug which is widely used in biocontrol. This bug can consume both plant and animal matter. In the present paper, three alternative diets were evaluated in mass rearing of nymphs of *N. tenuis* maintained in cages on tobacco plants: eggs of the grain moth *Sitotroga cerealella*, cysts of *Artemia salina*, and bee-collected pollen. Adults of *N. tenuis* were fed a mixture of grain moth eggs and pollen during oviposition. The type of diet did not effect the survival of nymph. Output of adults was higher when nymphs were fed grain moth eggs than *Artemia* cysts while the body weight of adult bugs did not differ.

Keywords: biocontrol, entomophagous insect, grain moth, *Artemia*, pollen

Submitted: 06.11.2022 *Accepted: 27.06.2023*

Introduction

Nesidiocoris tenuis Reuter (Hemiptera, Miridae) is a predatory bug widely used in biocontrol alongside with the other representatives of the family, belonging to the genera of *Macrolophus* and *Dicyphus*. This bug can consume both plant and animal matter (Wheeler 2001). It feeds on whiteflies, aphids, thrips, spider mites, and young caterpillars and eggs of moths (Yano et al., 2020; Dhanapal et al., 2021; Nakano et al., 2021). However, plant-only diets are less nutrient-dense than carnivorous diets (Urbaneja et al., 2005; Sanchez 2008; Pazyuk, Vasiliev, 2013). This species was successfully used on tomato plants in greenhouses (Calvo et al., 2012) and in botanical gardens (Varfolomeeva, Pazyuk, 2017). Mass rearing of *N. tenuis* requires a nutrient-dense, advanced, and cost-effective feed. Eggs of moths such as *Sitotroga cerealella* Olivier (Lepidoptera, Gelehiidae) and *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae) have been used as an alternative feed for laboratory-reared entomophagous insects since the 20th century (van Lenteren, 2003). Since that time, *Ephestia* eggs have been tested in Europe as a feed for *Orius*, *Macrolophus*, and *Nesidiocoris* bugs. According to Fauvel et al. (1987) and Cocuzza et al. (1997), *Macrolophus caliginosus* Wagner (Hemiptera, Miridae) and *Orius laevigatus* Fieber (Hemiptera, Anthocoridae) reared on *E. kuehniella* eggs possessed shorter nymph development time compared to the predators reared on whiteflies and thrips respectively. However, the high cost of these diets is a disadvantage. According to European researchers, the price of *E. kuehniella* eggs ranged from 500 to 600 euros per kg (Vandekerkhove et al., 2009).

The first reports on using cysts of *Artemia franciscana* Kellogg (Branquiopoda, Artemiidae) for mass rearing of *Orius* species were published in Europe in the 2000s (Arijs, De Clercq, 2001). Belgian researchers discovered that decapsulated cysts soaked in water for two hours before feeding bugs, can be successfully used for their rearing. Similar studies were performed with *Macrolophus* spp. by Spanish researchers who demonstrated the ability of the bug *M. caliginosus* to give 8 generations when fed on dry or hydrated *Artemia* cysts from two geographical populations (Castane et al., 2006). Later, researchers from Belgium and France emphasized the importance of *Artemia* cyst decapsulation before their use as a feed for the bug *Macrolophus pygmaeus* Rambur. They demonstrated that decapsulated *Artemia* cysts are a cost-effective alternative food source for mass rearing of this species (Vandekerkhove et al., 2009). Later, it was shown that decapsulated cysts are also suitable for feeding nymphs and adults of *N. tenuis* in laboratory (Owashi et al., 2020) and on tomato plants in greenhouses (Valiente, 2014). In Europe, one kilogram of *Artemia* cysts costs about 200 euros, which is much cheaper than one kilogram of *E. kuehniella* eggs (Valiente, 2014).

Alongside animal food, the predatory mirids, including *N. tenuis,* can feed on plant food such as: plant juices, nectar, and pollen (Wheeler, 2001). In this sense, pollen can be a good nutrient source for these bugs because it is rich in amino acids and proteins (Ioyrish 1976, Wackers et al., 2005). In nature, depending on the predators, pollen can be either primary or secondary food source for various entomophagous arthropods: Phytoseiidae, Carabidae, Caccinellidae, Chrysopidae, Syrphidae, Anthocoridae, Nabidae, Reduviidae, etc. (Zaher et al.,1969; Gilbert, 1981; Kiman, Yeargan, 1985; Larochelle, 1990; Villenave et al., 2005; Berkvens et al., 2007; Lundgran, 2009). As for mirids, the majority of *M. pygmaemus* nymphs when fed only on bee pollen or pollen and eggplant leaves, completed their development slightly later than those fed on animal diet (Perdikis, Lykouressis, 2000). In another study, the survival rate of *M. pygmaemus* nymphs fed on only pollen was 80 % less, and their oocyte weighted sum was 32 % lower as compared to nymphs fed on *E. kuehniella* eggs. However, when given a small amount of *E. kuehniella* eggs (10 eggs for 3 days) together with pollen, 87 % of adults emerged, and the number of oocytes in females was the same as when nymphs were fed only *Ephestia* eggs (Vandekerkhove, De Clercq, 2010). *N. tenuis* is able to develop and reproduce feeding only on bee pollen (Pazyuk, Vasilyev, 2013) but the time of its development is longer, and fecundity is less than if it feeds on animal food (eggs of *Sitotroga cerealella*). When it comes to specific plants, two plant species, *Verbena hybrida* Voss (Lamiales: Verbenaceae) cv. Tapien and *Scaevola aemula* R. Br. (Asterales: Goodeniaceae) with flowers, are suitable for development, survival, and oviposition of *N. tenuis* (Kandori et al., 2022).

This predatory bug can be reared without plants (Puysseleyr et al., 2013) or using the following plant species: tomato *Solanum lycopersicum* L. (Solanaceae) (Urbaneja-Bernat et al., 2013), tobacco *Nicotiana tabacum* L. (Solanaceae) (Pazyuk, 2010), *Dittrichia viscosa* L. (Asteraceae), *Sesamum indicum* (L.) (Pedaliaceae) (Biondi et al., 2016), and common bean *Phaseolus vulgaris* L. (Fabaceae) (Martinez-Garcia et al., 2016).

The goal of the present paper was to assess effect of diets on mass rearing of the predatory bug *N. tenuis*. Nymphs of *N. tenuis*, kept on tobacco plants, were fed three types of diet: 1) eggs of *S. cerealella*, 2) *Artemia* cysts, and 3) bee-collected pollen. We used dry, decapsulated cysts for the experiments because wet, hydrated cysts are shown to cause mold in rearing cages (Vandekerkhove et al., 2009).

Materials and Methods

Insect rearing

A stock culture of the bug *Nesidiocoris tenuis*, was obtained from Sesil Corporation (Nonsan, Republic of Korea) in the year 2010. Three types of diet were compared: 1) fresh eggs of *S. cerealella*, dry decapsulated cysts of *Artemia salina*, and ground pollen collected by bees from maple, coltsfoot, and willow (Oryol Region, Russia).

Experimental design

Bugs were reared at 24±0.2 °C in organza-covered cages measuring 60×40×40 cm, as previously described (Pazyuk, 2010): Eight tobacco plants with 6–8 leaves (tobacco seeds were obtained from the All-Russian Scientific Research Institute of Tobacco, Makhorka and Tobacco Products, Krasnodar, Russia) and 250 mature adults of *N. tenuis* were placed in each cage and kept there for a week. During oviposition, adult insects were supplied with the same diet: a mixture of grain moth eggs and pollen, three times per week. The diet was applied on top of the leaves. During this week, females laid eggs in tobacco plant tissues. The tobacco plants with eggs of *N. tenuis* were removed from the cages and transferred to new cages for hatching and further development of nymphs.

After nymphs hatched from eggs, they were fed one of three diets: 1) grain moth eggs, 2) *Artemia* cysts, and 3) ground pollen. The amount of food was 2.5 g per cage for all diets. Nymphs were counted on the 23rd day after oviposition started. By that time, nymphs of the first, second, and third instar were present. The emerged adults were collected with electric exhauster and counted on the 40th day. Ten females and ten males were taken from each cage and individually weighed on a balance Vibra HT Shinko Denshi, Japan. There were eight replications for each treatment. Overall, 1300 adults of maternal generation were tested in this study.

Statistical analysis

One-way analysis of variance (ANOVA) was used to access the effect of 3 diets (1) *Sitotroga* eggs; (2) *Artemia* cysts and (3) pollen, on the mean values of the following variables: (1) the number of nymphs of offspring (F1); the number of adults successfully developed from these nymphs;(3) the adult to nymph ratio, (4) the weight of F1 males and females.

Tukey's parametric test was used to assess differences between experimental treatments. All calculations were made with SYSTAT 12.00.08.

Results and Discussion

The obtained results showed that the three types of diet (grain moth eggs, *Artemia* cysts and bee pollen) did not affect number of nymphs, but number of adults was higher in the case of *Sitotroga* eggs while the percentage of emerged adults was less in the case of *Artemia* cysts.

One-way ANOVA showed that the number of nymphs per cage did not significantly differ in all three experimental groups ($p=0.124$) (see Table). The Tukey's test revealed that the number of adults (offspring) in the case of grain moth eggs was significantly higher than in the case of *Artemia* cysts (Tukey's test, $p<0.05$) with the case of pollen in between.

Comparison of the three diets based on the percentage of emerged adults showed no differences between grain moth eggs and honeybee pollen while significantly fewer adults emerged in the case of the *Artemia* cysts diet (Tukey's test, $p<0.05$). Feeding on *Artemia* cysts and pollen yielded 27 % and 17 % less adults of *N. tenuis*, respectively, compared to feeding on grain moth eggs (taken as 100 %).

At the same time, the weight of females and males did not statistically differ between experimental groups (see Table 1) probably due to cannibalism.

Table 1. Effect of diets on F1 generation of *Nesidiocoris tenuis* in mass rearing

Feed type	Number of offspring nymphs per cage (instars 1–3, offspring generation) (mean±SE)	Number of adults per cage (offspring generation) (mean±SE)	Share of emerged adults (% of nymphs) (mean±SE)	Weight of females, (mean±SE), mg	Weight of males, (mean±SE), mg
Sitotroga cerealella eggs	2,705.5±144.1a	1,909.1±80.2a	71±4a	1.1±0.07a	0.82±0.06a
Artemia cysts	2,504.5±208.7a	1,394.9±150.9b	56±4b	1.03±0.06a	0.73±0.06a
Bee pollen	2,141.4±204.3a	1,589±181.2ab	74±3a	1.02±0.11a	0.82±0.12a

Different letters (a, b) indicate values which are significantly different at $p<0.05$ (Tukey's test).

Таблица 1. Влияние корма на F1 поколение *Nesidiocoris tenuis* при массовом разведении

Тип пищи	Число нимф на садок (1–3 возрастов) (потомство)	Число взрослых особей на садок (потомство)	Доля вышедших имаго (% от нимф)	Вес самок, мг	Вес самцов, мг
яйца *Sitotroga cerealella*	2,705.5±144.1a	1,909.1±80.2a	71±4a	1.1±0.07a	0.82±0.06a
цисты *Artemia*	2,504.5±208.7a	1,394.9±150.9b	56±4b	1.03±0.06a	0.73±0.06a
Пчелиная пыльца	2,141.4±204.3a	1,589±181.2ab	74±3a	1.02±0.11a	0.82±0.12a

Различными буквами (a, b) в столбцах отмечены различия между вариантами при $p<0.05$ (тест Тьюки).

Thus, the experimental data showed significant differences in the three tested diets used for rearing of the predatory bug *N. tenuis* at the nymphal stage. Cannibalism appeared to occur during the mass rearing of *N. tenuis* in all experimental groups but was more prevalent with unfavorable diets. Cannibalism was also observed in all diets of the predatory bug *M. pygmaeus* but decreased when the bug was fed a more favorable diet (*E. kuehniella* eggs) (Hamdi et al., 2013). In similar experiments with the predatory bug *M. pygmaeus*, the authors demonstrated that two foods, *Artemia* cysts and pollen, were worse in nutritional value than grain moth eggs (Kozlova, Khodzhash, 2019), the share of emerging adults dropped to 33.6% and 43.7%, respectively, comparing to grain moth eggs (taken as 100%). More significant fall in the percentage of emerged adults in the case of *M. pygmaeus* than in *N. tenuis* suggests that the latter species has less cannibalism.

When the diet is marginally worse in nutritional value but is much cheaper it may be the best option, since prices for natural enemies have to compete with chemicals and production of entomophagous insects must be economically efficient and still managing their optimal quality (Arjis, De Clercq, 2001, De Clercq et al., 2005). In Russia, the average market prices for 100 grams of grain moth eggs, *Artemia* cysts, and pollen are around 10,000 rubles, 1,150 rubles, and 260 rubles, respectively. Therefore, less nutritious but more affordable diet can be used for mass rearing of *N. tenuis* nymphs. Lu et al. (2011) came to the same conclusion, showing that the predatory bug *Orius strigicollis* Poppius (Hemiptera, Anthocoridae) is rationally maintained at the nymphal stage when feeding on cysts, and at the adult stage when feeding on eggs of the moth *Cadra cautella* Walker (Lepidoptera, Pyralidae). Our investigation suggests that the tested diets (*Artemia* cysts and pollen) can be used for mass rearing of *N. tenuis* at nymphal stage. But whether it is possible to use these diets on the constant basis and commercially produce predatory bugs of high quality, requires additional research.

Acknowledgements

The author wishes to thank N.V. Binitskaya for her assistance with the experiment.
This study was supported by Russian Science Foundation Project No. 20-66-47010.

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Вестник защиты растений, 2023, 106(2), с. 100–104

OECD+WoS: 1.06+IY (Entomology) https://doi.org/10.31993/2308-6459-2023-106-2-15517

Краткое сообщение

ОЦЕНКА КОРМОВ ДЛЯ МАССОВОГО РАЗВЕДЕНИЯ ХИЩНОГО КЛОПА *NESIDIOCORIS TENUIS* (HEMIPTERA, HETEROPTERA, MIRIDAE)

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Nesidiocoris tenuis (Hemiptera, Miridae) – хищный клоп, широко используемый в защите растений. Он способен употреблять в пищу материалы как растительного, так и животного происхождения. В настоящей работе была проведена оценка различных вариантов корма для массового содержания нимф *N. tenuis* в садках на растениях табака. При откладке яиц имаго *N. tenuis* питались смесью яиц зерновой моли и пчелиной пыльцы. Варианты корма для нимф включали яйца зерновой моли *Sitotroga cerealella,* цисты *Artemia salina* и пчелиную пыльцу. Состав корма не влиял на выживаемость нимф. Выход имаго был выше, когда нимфы питались яйцами зерновой моли, чем при питании цистами артемии, хотя вес имаго не различался.

Ключевые слова: биометод, энтомофаги, зерновая моль, артемия, пыльца

Поступила в редакцию: 06.11.2022 *Принята к печати: 27.06.2023*