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ORIGINAL RESEARCH PAPER

Effects of an alternative diet of *Artemia* cysts on the development and reproduction of *Nesidiocoris tenuis* (Hemiptera: Miridae)

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Abstract

The small green mirid *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) preys on pest insects such as whiteflies and serves as a biological control agent in many greenhouses. However, this mirid is limited in its application, because individuals tend to escape from agricultural fields and soon die due to a lack of available food sources. In addition, the food traditionally used to culture *N. tenuis* is expensive. Thus, identifying low-cost foods for *N. tenuis* would help to increase the species' further utilization. Brine shrimp (*Artemia* spp., Anostraca: Artemiidae) cysts are potentially useful as a low-cost alternative diet to sustain populations of predatory natural enemies. We evaluated the developmental and reproductive performance of *N. tenuis* when reared on *Artemia salina* L. cysts supplied in dry or wet form. The dry cysts showed a similar performance to that of Mediterranean flour moth (*Ephestia kuehniella* Zeller, Lepidoptera: Pyralidae) eggs, which are often used as a nutritional diet for mass rearing of *N. tenuis*. Although the wet cysts contributed to growth, compared to other diets, they were inferior in nymphal development time and longevity. These results suggest that relatively inexpensive *Artemia* dry cysts can be used to successfully breed *N. tenuis* and sustain populations in crop fields.

Keywords Alternative diet · *Artemia salina* · Biological control · Brine shrimp cysts · *Nesidiocoris tenuis*

Introduction

Biological control of insect pests has a long tradition in greenhouse crops. Many kinds of biological control agents (e.g., coccinellid beetles, lacewings, parasitic wasps, predatory heteropterans, and predatory mites) have become commercially available worldwide (Messelink et al. 2014; Pilkington et al. 2010; van Lenteren 2012). However, their uses are often limited. One of the obstacles to utilizing natural enemies appears to be an economic issue (Leppla and King 1996; Stinner 1977). Many commercial producers routinely use eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), as a food source for breeding biological control agents. Although these eggs contribute to good development and reproduction of predatory insects, the market price of the eggs is relatively high, resulting in higher prices of natural enemies (Arijs and De Clercq 2001; Bonte et al. 2010; De Clercq et al. 2005; Riddick et al. 2014; Urbaneja-Bernat et al. 2013). Thus, less-expensive alternative food sources could be a key element in broadening the use of natural enemies.

Another problem in using biological control agents is that released natural enemies establish and persist poorly in some crops (Portillo et al. 2012; Pumariño and Alomar 2012). The escape and death of natural enemies immediately after release often results from a temporary food shortage. Food supplementation is one solution for maintaining predators in crop fields (Lundgren 2009; Messelink et al. 2014; Vangansbeke et al. 2016; van Rijn et al. 2002; Wade et al. 2008).

Eggs of brine shrimp, *Artemia* spp. (Anostraca: Artemiidae), are potentially useful as an inexpensive alternative and supplementary food source for biological control agents. *Artemia* females produce embryos covered by a thick shell capsule that are deposited as long-term diapausing cysts. After being released from diapause by hydration, embryos emerge from the shell and soon hatch into swimming nauplii (van Stappen 1996). Various natural enemies, including ladybirds, anthocorid bugs, and predatory mites, can be bred on the dry form or wet form of *Artemia* cysts as an alternative diet (e.g., Arijs and De Clercq 2001; Bonte and

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De Clercq 2008; Castañé et al. 2006; Hongo and Obayashi 1997; Nguyen et al. 2014; Nishimori et al. 2016; Vandekerkhove et al. 2009; Vangansbeke et al. 2014). Furthermore, some studies suggested that the introduction of *Artemia* cysts into crop fields where predatory mirids or ladybirds were released is beneficial for their fecundity and survival, because the cysts complement available food resources (Messelink et al. 2015; Oveja et al. 2016; Seko et al. 2019).

The small green mirid *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is a cosmopolitan species that makes significant contributions to the control of greenhouse pests such as whiteflies, leafminers, thrips, and lepidopterans (Arzone et al. 1990; Calvo et al. 2009; Itou et al. 2013; Kajita 1978; Marcos and Rejesus 1992; Solsoloy et al. 1994; Urbaneja et al. 2009). These mirids are widely used in greenhouses and are sold as a biological control agent by some companies (De Puysseleyr et al. 2013).

Our objectives were to evaluate the developmental and reproductive performance of *N. tenuis* when fed on brine shrimp (*Artemia salina* L.) cysts supplied in two different forms. We compared development time, survival, fecundity, and hatching rate of *N. tenuis* when supplied with dry cysts, wet cysts, or Mediterranean flour moth eggs. Although dry cysts of *Artemia* was reported to have a positive effect on the reproduction of the mirids (Oveja et al. 2012), it is unknown whether the cysts are useful as a diet throughout the life of the mirids. We also calculated the intrinsic rate of increase of the three groups. Based on these results, we discuss the viability of using *Artemia* cysts for the culture of *N. tenuis* and the maintenance of populations in fields.

Materials and methods

Preparation of insects and their diets

In this study, we used a laboratory colony of the mirid *N. tenuis* provided by Agrisect Inc. (Tsukuba, Japan) that had been maintained on leaves of the jade plant [*Crassula ovata* (Miller)] and provided eggs of the moth *E. kuehniella* (Agrisect Inc.) as a food source for more than 3 years under laboratory conditions.

We tested two alternative diets: dry cysts of the brine shrimps *A. salina* (BS-dry) originating from the Great Salt Lake in Utah, USA (Japan Pet Design Co., Ltd.; Tokyo, Japan), and those that had been hydrated (BS-wet). As a control, *E. kuehniella* moth eggs (ME) were used. ME and BS-dry, ready-made diets that required no treatment, were, respectively, affixed to adhesive sheeting and supplied to *N. tenuis* as described below. BS-wet was prepared by placing the dry cysts on moistened cotton wool just prior to being given to the mirids. ME and BS-dry were stored at − 20 °C and 4 °C, respectively, until used.

Mirid culture

First-instar mirid nymphs (within 24 h after hatching) obtained from the laboratory colony were placed individually into a plastic cage (4 cm diameter, 3.5 cm high) containing Styrofoam netting (2 × 2 cm) and water-saturated cotton wool (1 × 1 cm). The top of the plastic cage was holed (1.5 cm diameter) and covered with nylon mesh. A piece of adhesive sheeting (1 × 2 cm) containing ca. 30 mg of ME or 20 mg of BS-dry or wet cotton wool containing ca. 20 mg of cysts (BS-wet) was placed in the cage with the nymphs and renewed every 2 days. We observed the mirids every day and recorded the survival and developmental stages.

A pair of adults that had emerged within 24 h was transferred into a new plastic cage under similar conditions as above, except we also placed a clean jade plant leaf as an oviposition substrate into the cage. The leaf was renewed every 24 h in all treatments. After removal, we counted the number of eggs laid by dissecting the leaf under a binocular microscope. When a male or female died during the experiment, another newly emerged mirid adult was introduced into the cage to remove the influence of solitariness on survival. The fecundity and survival of adults were recorded every day until both members of the original pair died. A piece of adhesive sheeting (1 × 1 cm) containing ca. 15 mg of ME or 10 mg of BS-dry or wet cotton wool containing ca. 10 mg of cysts (BS-wet) was provided to the mirid pairs. The diet was renewed every 2 days. In all treatments, the amounts of each diet were considered to be sufficient, because some food remained at the end of the experiment.

To evaluate the effect of the diets on egg hatching, four males and four females (7–10 days after adult eclosion) that had been individually reared on each diet were placed in a mesh-covered plastic cage (10 cm diameter, 5 cm high) with a jade plant leaf and the natal diet. The leaf and diet were exchanged every day. Leaves on which eggs were laid were transferred into a new cage, and we counted the number of hatched nymphs every 24 h. After 3 consecutive days without hatching, we counted the number of unhatched eggs by dissecting the leaf.

All the culture experiments were performed under laboratory conditions of 25 ± 1 °C, 70 ± 10% humidity, and a light:dark regime of 16:8 h.

Statistical analysis and reproductive parameters

To compare preoviposition period, nymphal development time, and total fecundity among mirids feeding on each diet, we used the Steel–Dwass multiple nonparametric comparison test, because these were ordinal variables. Nymphal survival rate and egg hatch rate were analyzed by using the Chi-squared test. The adult survival curve was compared among groups using the log-rank test with Bonferroni correction. Statistical analyses were conducted using the software R version 3.4.1 (R Core Team 2017).

Tables of the age-specific survival rate (l_x, survival rate of females at age x) and age-specific fecundity rate (m_x, number of female eggs produced by a female at age x) were constructed. The net reproductive rate (R_0), mean generation time (T), and intrinsic rate of increase (r_m) were calculated from the table following the methods of Birch (1948). The values of R_0 and T were estimated by calculating $\Sigma l_x m_x$ and $\Sigma x l_x m_x / \Sigma l_x m_x$, respectively, and r_m was estimated by selecting values of r that satisfied the expression $\Sigma e^{-rx} l_x m_x = 1$. The values of development time were based on the experiments.

Results

Nymphal survival rate and development time

Mirid nymphs developed successfully on each diet tested, and in each treatment, more than three-quarters of all nymphs reached adulthood (Table 1). No significant differences were found in nymphal survival rate among diet treatments (χ^2 test, $\chi^2 = 0.417$, $df = 2$, $p = 0.812$). Nymphal development time, however, was significantly affected by the diets. Nymphs feeding on ME developed more quickly than those feeding on BS-dry and BS-wet (Steel–Dwass test, ME vs. BS-dry, $t = 4.81$, $p < 0.05$; ME vs. BS-wet, $t = 5.72$, $p < 0.05$; Fig. 1). Nymphs feeding on BS-dry developed significantly faster than those feeding on BS-wet ($t = 4.26$, $p < 0.05$).

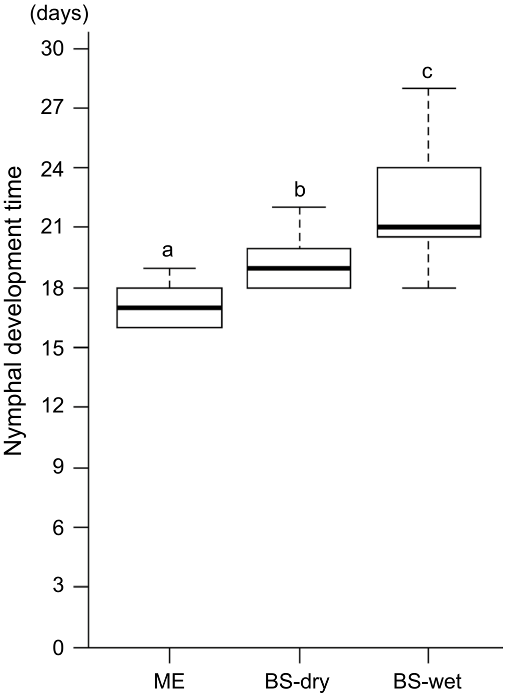

Fig. 1 Nymphal development time of *Nesidiocoris tenuis* reared on each diet: ME (eggs of the moth *Ephestia kuehniella*, $n = 24$), BS-dry (dry cysts of the brine shrimp *Artemia salina*, $n = 25$), or BS-wet (wet cysts of *A. salina*, $n = 23$). Same letters above each box plot indicate no significant differences according to the Steel–Dwass test ($p > 0.05$)

Adult longevity, preoviposition period, and fecundity

In both female and male adults, there were no significant differences in survival duration between the ME and BS-dry treatments (log-rank test, female, $\chi^2 = 1.1$, $df = 1$, $p = 0.296$; male, $\chi^2 = 1.8$, $df = 1$, $p = 0.175$ before Bonferroni correction; Fig. 2). On the other hand, the survival durations of both male and female mirids feeding on BS-wet were lower than those feeding on ME and BS-dry (ME vs. BS-wet, female, $\chi^2 = 9.7$, $df = 1$, $p < 0.01$; male, $\chi^2 = 15.8$, $df = 1$, $p < 0.001$; BS-dry vs. BS-wet, female, $\chi^2 = 6.9$, $df = 1$, $p < 0.05$; male, $\chi^2 = 6.7$, $df = 1$, $p < 0.05$ after Bonferroni correction). There were no significant differences in the preoviposition period of adults among the three treatments (Steel–Dwass test, ME vs. BS-dry, $t = 1.39$, $p = 0.348$; ME vs. BS-wet, $t = 1.80$, $p = 0.169$; BS-dry vs. BS-wet, $t = 0.433$, $p = 0.902$; Table 2) or in the number of eggs laid (Steel–Dwass test, ME vs. BS-dry, $t = 1.01$, $p = 0.568$; ME vs. BS-wet, $t = 2.30$, $p = 0.0552$; BS-dry vs. BS-wet, $t = 1.40$, $p = 0.340$; Fig. 3).

Table 1 Nymphal survival rate of *Nesidiocoris tenuis* reared on ME (eggs of the moth *Ephestia kuehniella*), BS-dry (dry cysts of the brine shrimp *Artemia salina*), or BS-wet (wet cysts of *A. salina*)

Diet	Nymphal survival rate (%)^x
ME ($n = 30$)	80.0 a
BS-dry ($n = 30$)	83.3 a
BS-wet ($n = 30$)	76.7 a

^{x}Values followed by the same letter in a column are not significantly different among diets by χ^2 test ($p > 0.05$)

Egg development time and hatch rate

The period from oviposition to hatch ranged from 8 to 9 days and was not affected by parental diet (Steel–Dwass test, ME vs. BS-dry, $t = 1.19$, $p = 0.462$; ME vs. BS-wet, $t = 1.10$, $p = 0.515$; BS-dry vs. BS-wet, $t = 0.122$, $p = 0.992$; Table 3). The egg hatch rates were greater than 95% regardless of the maternal diet, and there were no significant differences in the hatching rate among treatments (χ^2 test, $\chi^2 = 1.72$, $df = 2$, $p = 0.424$; Table 3).

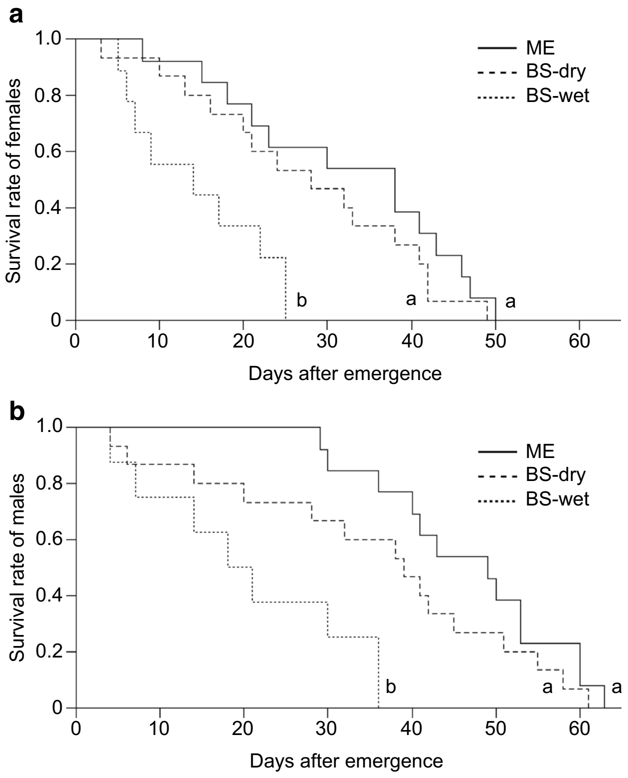

Fig. 2 Survival rate of *Nesidiocoris tenuis* **a** females reared on each diet: ME (eggs of the moth *Ephestia kuehniella*, $n = 13$), BS-dry (dry cysts of the brine shrimp *Artemia salina*, $n = 15$), or BS-wet (wet cysts of *A. salina*, $n = 9$) and **b** males reared on ME ($n = 13$), BS-dry ($n = 15$), or BS-wet ($n = 8$). Same letters indicate no significant differences according to the log-rank test with Bonferroni correction ($p > 0.05$)

Table 2 Preoviposition period of *Nesidiocoris tenuis* reared on ME (eggs of the moth *Ephestia kuehniella*), BS-dry (dry cysts of the brine shrimp *Artemia salina*), or BS-wet (wet cysts of *A. salina*)

Diet	Preoviposition period (days; mean ± SE)^x
ME ($n = 14$)	4.4 ± 0.2 a
BS-dry ($n = 14$)	4.1 ± 0.4 a
BS-wet ($n = 7$)	3.6 ± 0.4 a

^{x}Values followed by the same letter in a column are not significantly different among diets by Steel–Dwass test ($p > 0.05$)

Reproductive parameters

Population growth parameters of females showed some differences among diets (Table 4). The intrinsic rate of increase (r_m) of mirids feeding on ME, BS-dry, and BS-wet was 0.102, 0.093, and 0.079, respectively. Thus, r_m of ME was more than 1.1 times that of BS-dry and 1.3 times that of BS-wet. As we calculated the net reproductive rate (R_0) while

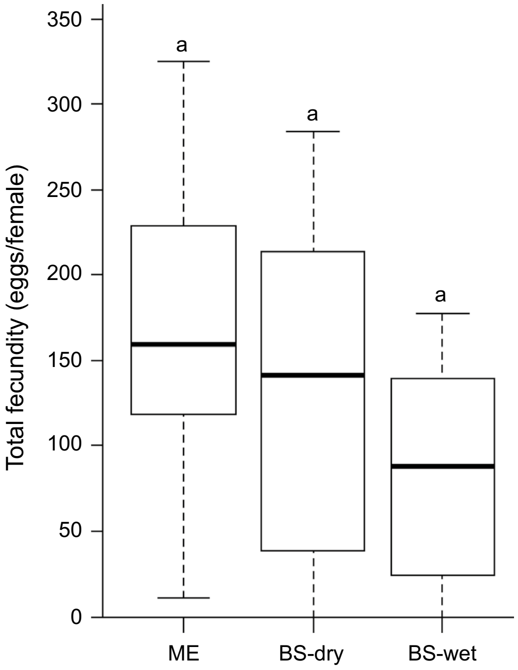

Fig. 3 Total number of eggs laid by *Nesidiocoris tenuis* females reared on each diet: ME (eggs of the moth *Ephestia kuehniella*, $n = 13$), BS-dry (dry cysts of the brine shrimp *Artemia salina*, $n = 15$), or BS-wet (wet cysts of *A. salina*, $n = 9$). Same letters above each box plot indicate no significant differences according to the Steel–Dwass test ($p > 0.05$)

Table 3 Egg development time and hatching rate of *Nesidiocoris tenuis* reared on ME (eggs of the moth *Ephestia kuehniella*), BS-dry (dry cysts of the brine shrimp *Artemia salina*), or BS-wet (wet cysts of *A. salina*)

Diet	Egg development time (days; mean ± SE)^x	Hatching rate (%)^y
ME	8.7 ± 0.1 a ($n = 25$)	96.6 a ($n = 26$)
BS-dry	8.9 ± 0.1 a ($n = 23$)	100 a ($n = 23$)
BS-wet	8.9 ± 0.2 a ($n = 21$)	100 a ($n = 21$)

Values followed by the same letter in a column are not significantly different among diets ($p > 0.05$)

^{x}Steel–Dwass test

$^y\chi^2$ test

Table 4 Population growth parameters—intrinsic rate of increase, r_m (females/female/day), net reproductive rate, R_0 (female eggs/female), mean generation time, T (days), and generation doubling time, DT (days)—of *Nesidiocoris tenuis* reared on each diet (ME: eggs of the moth *Ephestia kuehniella*; BS-dry: dry cysts of the brine shrimp *Artemia salina*; BS-wet: wet cysts of *A. salina*)

Diet	r_m	R_0	T	DT
ME	0.102	63.58	43.66	6.78
BS-dry	0.093	53.97	45.91	7.48
BS-wet	0.079	30.11	44.43	8.80

assuming an equal sex ratio, these parameters naturally followed the same order as total fecundity. That is, R_0 of mirids feeding on ME was highest, followed by BS-dry and then BS-wet, which was less than half that of ME. The mean generation time (T) showed similar values among treatments. The generation doubling time (DT) indicated that ME-fed mirids had the fastest rate of population growth, followed by BS-dry and then BS-wet. According to the *DT* values, a population of mirids feeding on ME increases 21.5 times, that feeding on BS-dry increases 16.1 times, and that feeding on BS-wet increases 10.6 times over 30 days.

Discussion

We evaluated the suitability of two alternative diets, dry cysts of the brine shrimp *A. salina* (BS-dry) and hydrated cysts (BS-wet), for *N. tenuis*. Mirids that fed on BS-wet developed more slowly and died faster than those that fed on *E. kuehniella* eggs (ME), which are the conventional alternative diet, and BS-dry (Figs. 1, 2). When comparing the mirids that fed on ME and BS-dry, nymphs feeding on BS-dry had a longer growth time (Fig. 1). In contrast, no differences were detected in nymphal survival rate, total fecundity, egg development time, and hatching rate among diets (Fig. 3 and Tables 1, 3). These results suggest that *Artemia* cysts can be used to successfully rear *N. tenuis*.

Sesame plants *Sesamum indicum* L. is also considered as one of the alternative foods for *N. tenuis*; Nakaishi et al. (2011) reported that *N. tenuis* can be reared on sesame plants without animal foods. Use of sesame for rearing is convenient because of their inexpensive price. However, the intrinsic rate of increase of *N. tenuis* on sesame was very low ($r_m = 0.0465$; Nakaishi et al. 2011) compared to that fed on BS-dry and BS-wet (Table 4), although it is difficult to directly compare the values because of the different methodology. These values suggest that *Artemia* cysts can be used to rear *N. tenuis* more effectively.

Mollá et al. (2014) reported the intrinsic rate of increase of *N. tenuis* as 0.112 ± 0.001 when feeding on ME on tomato crops, and the value is similar to our data for this mirid feeding on ME ($r_m = 0.102$). This similarity indicates sufficient reproducibility of our experiment and the strong performance of ME for increasing *N. tenuis* populations. *Nesidiocoris tenuis* populations also increased when fed BS-dry ($r_m = 0.093$) and BS-wet ($r_m = 0.079$). Although the performance when fed on BS-dry was similar to that of ME, the performance of the BS-wet diet was low. Thus, BS-dry is a suitable alternative diet substitute for ME for rearing *N. tenuis*.

One reason why BS-wet was less suitable than BS-dry may have been a decline of food quality and availability for *N. tenuis*. *Artemia* cysts become hydrated depending on the relative humidity of the atmosphere (Morris 1971) and hatch to nauplii when incubated in seawater (Vanhaecke and Sorgeloos 1982; van Stappen 1996). Although we hydrated cysts with fresh water, we observed some burst cysts and hatched nauplii on the cotton wool in the BS-wet treatment in our experiments. In contrast, we never observed hatched nauplii in the BS-dry treatment, despite the cysts being gradually hydrated by humidity in the atmosphere, as indicated by them changing from biconcave to spherical. The nauplii may not be adequate food for *N. tenuis*, resulting in the poor performance of BS-wet.

Although BS-dry and ME showed similar food source function for adult *N. tenuis*, the BS-dry diet resulted in slower development than the ME diet. Thus, depending on the developmental stage, it may be difficult for *N. tenuis* to consume cysts due to the hard chitin layer that covers them (van Stappen 1996). Prolongation of nymphal development time could affect frequency of cannibalism under mass rearing condition. Although cannibalism is not common in zoophytophagous insects such as *N. tenuis* under field conditions (Castañé et al. 2002; Lucas and Alomar 2002), adults of *N. tenuis* are demonstrated to occasionally prey on nymphs of them under laboratory condition without available food sources (Gervassio et al. 2017). It is necessary to investigate the frequency of cannibalism under mass rearing condition using BS-dry.

Producing *N. tenuis* by providing *Artemia* cysts is much less expensive than providing ME, the diet routinely used for the mass rearing of natural enemies including *N. tenuis* (Urbaneja-Bernat et al. 2013). In current rate, we can get *Artemia* cysts about one-tenth the price of ME. Other advantages of using *Artemia* cysts for rearing *N. tenuis* include the ease with which they can be obtained in the aquarium trade (Lavens and Sorgeloos 2000) and the fact that the cysts can be maintained for a long time due to their extended diapause (Morris 1971). Depending on the breeding environment, this latter advantage makes it possible to reduce the labor needed to rear mirids, such as the frequency of diet replacement, thus saving additional resources.

Spraying or dusting food resources onto crops can help to increase natural enemy populations (Wade et al. 2008). For example, pollen sprays serve as food for generalist predatory mites and enhance the biological control of thrips and whiteflies on cucumbers (Nomikou and Sabelis 2010; van Rijn et al. 2002). Recent studies also suggested the suitability of *Artemia* cysts as supplemental food for ladybirds (Seko et al. 2019), mirids (e.g., Messelink et al. 2015; Oveja et al. 2016), and predatory mites (e.g., Leman and Messelink 2015; Vangansbeke et al. 2016) in crop fields. Although the humidity fluctuations in the crop fields may influence the condition of *Artemia* cysts (Morris 1971), our results suggest that *N. tenuis* consumed both dry and wet cysts. However, future studies are needed to clarify the effect of the *Artemia* cysts on *N. tenuis* and pests when administered in crop fields.

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