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Effects of dietary salts and RNA on the total adult and female emergence of *Pimpla turionellae* (Hymenoptera: Ichneumonidae)

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Abstract

The present study investigated the effects of dietary salts and RNA on the sex ratio of emerging *Pimpla turionellae* (L.). The tested diets contained different concentrations of a salt mixture or RNA (0.0000, 0.0375, 0.0750 (control), and 0.1125 mg per 100 ml diet for each). To determine the sex ratio, 10 female and five males that matured on the same day were transferred to each experimental cage. There was no clear effect of the concentration of salt mixtures on total adult emergence. However, maximum female emergence (66.57%) occurred when 50% of the salt content of the control diet was removed. In terms of RNA, maximum adult emergence (81.85%) occurred when the amount in the control diet was increased by 50%, which corresponded to 0.1125 g RNA in the diet. By contrast, minimum female emergence (50.73%) occurred when there was 0.0750 g RNA in the diet.

Keywords: *Pimpla turionellae*, sex ratio, synthetic diet, salt mixture, adult emergence

1. Introduction

The use of chemical insecticides against economically harmful insects has resulted in environmental pollution and disruption of the ecological balance. This has led to an increase in the number of studies examining the impacts of pesticides on the environment and their effects on non-target organisms in recent years [1, 2], as well as an increase in the use of biological control agents.

Biological control can be defined as the use of natural enemies to regulate the population of a pest below the level of economic damage. The mass production of parasitic insects is an important prerequisite for the success of any biological control program [3]. To achieve this, we require information about the nutritional requirements for the rearing and production of insects in the laboratory. Although much work has already been completed in this research area, essential improvements are still required to improve the reproductive performance, egg efficiency, and sex ratios of these insects [4].

Few studies have investigated the effects of inorganic salts on insects compared with other nutrients [5, 6]. It is known that salts play a significant role in the reproductive activities of parasitic hymenoptera [7, 8, 9]. However, there is also great variation between species. For example, females of *Exeristes comstockii* (Cress.) that were fed a diet that only contained potassium as an inorganic salt had reduced egg productivity [10], whereas this remained unaffected in adult females of *Pimpla turionellae* (L.) that were fed a salt-free diet [9].

Ribonucleic acids (RNA) are sometimes incorporated into insect diets, but the requirement for these is somewhat questionable. RNA may increase survival or promote growth, and nucleic acids are particularly needed for the production of DNA and RNA [6, 11]. However, insects, like other animals, are able to synthesize nucleotides, albeit slowly in some dipterans. Furthermore, some larvae are able to complete their development in the absence of RNA, although this does take a long time. Therefore, the effect of dietary RNA on insects remains unclear [12].

One of the main concerns in the mass production of parasitic hymenopteran species for use in biological control programs is the development of techniques that result in the maximum number of productive females under laboratory conditions [13, 14]. Therefore, this experiment was designed to test the effect of different concentrations of a salt mixture and RNA on the sex ratio of emerging adult *P. turionellae* (Hymenoptera: Ichneumonidae).

2. Materials and Methods

The *P. turionellae* adults used in the experiments were reared in the laboratory on the pupae of greater wax moths, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). The synthetic diet] was used as a control diet [15], which consisted of amino acids, lipids, vitamins, inorganic salts, sucrose, and other nutrients. The preparation of the synthetic diet has been described in detail previously [16]. The test diets were prepared by changing the concentrations of the salt mixture or RNA (0.0000, 0.0375, 0.0750 (control), and 0.1125 mg per 100 ml diet for each). To determine the effect of diet composition on the sex ratio of emerging adults, 10 female and 5 male *P. turionellae* that had matured on the same day were transferred to each experimental cage. At the same time, 10 *G. mellonella* pupae were exposed to the *P. turionellae* for 1 h for parasitization. The insects parasitized the host pupae 10 days after they were placed in the cages, and this was repeated every 3 days over the 34-day-long experiment. Following egg laying, the parasitized pupae were removed from the cages and placed in a cup. They were then kept under laboratory conditions until adult emergence, at which time the number of male and female wasps that emerged was recorded. The methods used have been described in detail previously [14].

The adult emergence ratio for each diet was determined by calculating the ratio of the number of emerging individuals to the total number of pupae placed in the cages to be parasite [14]. The female emergence ratio was determined by calculating the proportion of females that emerged among the total number of emerging adults [14].

The test individuals were newly matured females of *P. turionellae* that had not been fed or mated. The insects were fed the liquid synthetic diets in the experimental cage for 1 h per day. All of the experiments were repeated three times under laboratory conditions at 24 ± 2°C, 75 ± 5% humidity, and a 12-h photoperiod.

2.1 Statistical analysis

Statistical analysis was performed with the Student-Newman-Keuls test, using SPSS version 16.00 for Windows. Differences between groups were considered to be significant at a probability level of 0.05%.

3. Results

Table 1 show the effects of different salt mixtures on the sex ratio of *P. turionellae*. There was no clear trend in the effect of salt concentration on total adult emergence. The control nutrient, which contained 0.0750 g of salt, resulted in an adult emergence rate of 71.48%. By contrast, when the amount of salt in the synthetic diet was decreased to 0.0375 g or increased to 0.1125 g of salt, adult emergence significantly decreased (65.92% and 57.03%, respectively). The diet that contained no salt mixture had no significant effect on total adult emergence (72.85%) compared with the control.

Table 1: Effects of different concentrations of salt mixture on the adult emergence of *Pimpla turionellae*

	Adult Emergence (%)^{a}	
Salt Mixture	**Total**	**Female**
(g/100 ml)	Mean ± SD	Mean ± SD
0.0000	72.85 ± 2.35 a	62.83 ± 0.88 a
0.0375	65.92 ± 1.33 b	66.57 ± 1.35 b
0.0750^{b}	71.48 ± 0,98 a	57.41 ± 0.30 c
0.1125	57.03 ± 1.48 c	50.50 ± 0.79 d

Student-Newman-Keuls test: Different lower-case letters indicate significant differences between the concentrations ($P < 0.05$)
^{a} Mean ± standard error; ^{b} Control

When the salt mixture content of the diet was increased by 50%, the emergence rate for mature females fell to 50.50%, which was the lowest value obtained in this series of experiments. By contrast, when the salt mixture was completely removed or reduced by 50%, there was a significantly higher rate of female emergence compared with the control, with maximum values (66.57%) occurring with a salt content of 0.0375 g (Table 1).

Table 2 shows the effects of different amounts of RNA in the diet on the total and female emergence of *P. turionellae*.

Maximum adult emergence occurred when the amount of RNA in the control diet was increased by 50%, corresponding to 0.1125 g per 100 ml of diet, which resulted in an increase from 70.00% to 81.85%. By contrast, a 50% reduction in the amount of RNA or its total removal from the diet was found to have no effect on total adult emergence compared with the control group. There was, however, an extremely large difference in emergence between the diet that contained no RNA and that containing 50% more RNA.

Minimum female emergence (50.73%) occurred when there was 0.0750 g RNA in the diet, whereas maximum female emergence (77.33%) occurred when there was no RNA in the diet. The difference between the minimum and maximum female emergence rates were statistically more significant than between any other nutrient combinations tested (Table 2).

Table 2 Effects of different concentrations of RNA in the diet on the adult emergence of *Pimpla turionellae*

	Adult Emergence (%)^{a}	
RNA	**Total**	**Female**
(g/100 ml)	Mean ± SD	Mean ± SD
0.0000	64.44 ± 2.31 a	77.33 ± 0.78 a
0.0375	74.07 ± 1.48 b	66.59 ± 0.68 b
0.0750^{b}	70.00 ± 2.22 ab	50.73 ± 0.89 d
0.1125	81.85 ± 0.98 c	55.43 ± 0.72 c

Student-Newman-Keuls test: Different lower-case letters indicate significant differences between the concentrations ($P < 0.05$)
^{a} Mean ± standard error; ^{b} Control

4. Discussion

This study investigated the effects of changing the concentrations of a salt mixture and RNA in a meridic diet of known chemical composition on the sex ratio of emerging adults of the parasitoid *P. turionellae*.

It is well known that all animals require inorganic salts, albeit in small amounts in some instances. These substances are known to play a role in various metabolic activities, as well as serving as a cofactor to some enzymes, and among these sodium, potassium, and calcium in particular play significant roles in many physiological activities [17]. It is hard to remove salt completely from the diets of insects, making it difficult to clarify the need for these substances for each species. However, a small number of studies have successfully determined the effects of salts on insect physiology through the use of synthetic nutrients. For example, it has been shown that *Agria housei* require 2% salt in their diet for development and reproduction [18] the total exclusion of salt from the diet results in a significant reduction in the number of eggs produced by *Exeristes comstockii* [19, 20, 10], the salt mixture used significantly affects the mature insect output of *Hylobius transversovittatus* [21]; and salts affect the development of *Ceratitis capitata* larvae [22].

An absence of salt from the diet had no effect on the total adult emergence of *P. turionellae*, but increased female emergence. This supports the previous finding that inorganic salts have no significant effects on the number of eggs or the number of hatched eggs in this species [9], suggesting that this species may not require inorganic salts to perform its reproductive functions. However, the decrease in female emergence with increasing concentrations of salt in the diet indicates that salt is likely to be used in the development of female insects. Moreover, the negative effect on adult emergence at both lower and higher concentrations than the control diet clearly indicates that the amount of this element may be of critical significance.

Nucleic acids are particularly important for the production of DNA and RNA. However, as is the case for other highly organized animal groups, insects are able to synthesize nucleotides [23]. Few studies have investigated the effects of dietary RNA on insects. In the present study, the exclusion or addition of small amounts of RNA to the diet had no effect on total adult emergence in *P. turionellae*. However, an increased concentration of RNA in the diet led to a significant increase in adult emergence. By contrast, there was an inverse relationship between the amount of RNA in the diet and female emergence, with an absence of RNA resulting in a significant increase in female emergence in *P. turionellae*. Similar results have been reported for different nutrient studies, in the absence of honey, the progeny sex ratio is always female biased but with an increase of male percentage, while total progeny production is significantly lower [24]. Moreover, the *Acerophagus papayae* sex ratio is not influenced by the presence or the kind of food sources, although the fecundity is increased [25].

Commonly, optimum fed female parasitoids alter the proportion of female off spring [26] *Pachycrepoideus vindemmiae* (Rondani) females also exploit diet by producing a higher proportion of female offspring [24]. These findings indicate that the effect of RNA on the sex ratio results from its effects on males rather than females.

Some nutrients have a significant effect on the sex ratio of *P. turionellae*, while others have no effect, despite being important for other species of insects. It is known that many factors affect the sex ratio of insects, including the host and parasitoid density [27], sperm quality [28, 29], environmental factors [30], host size [31], temperature [32], nutrients [33], and age of the parasitoids [34].

This study further reinforces the fact that nutrients can affect the sex ratio of insects, with some of the tested nutrients having a positive effect on female emergence. This has important implications for biological control operations, in which females are frequently used.

5. Conclusion

In this study, eliminating salt and RNA totally from the diet did not significantly affect adult emergence. For an optimal food it is not adequate to take all mixtures that are required. These compounds should be in balance for the metabolism. Dietary balance is a key for beetle in both the larval and mature stages. Therefore, differences in sex ratio of *P. turionellae* are important as they show that insects can be significantly affected by the quality of their diet.

6. Acknowledgments

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