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Optimal rearing medium for the population growth of the predatory mite, *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae)

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Abstract

Background: The successful rearing of predators or parasitoids is one of the most important elements in biological control programs. The dried fruit mite, *Carpoglyphus lactis* (L.), can be used as an alternative prey for the phytoseiid predatory mite, *Amblyseius swirskii* (Athias-Henriot).

Main body: This study aimed to evaluate the suitability of 3 different diets for *C. lactis* when rearing *A. swirskii*: brown sugar, baker's yeast, and the mixture of brown sugar and baker's yeast along with 3 initial predator:prey ratios (1:7, 1:12, and 1:20). After 30 days, both the baker's yeast and the mixture of brown sugar + baker's yeast diets resulted in the highest predator densities (40.5 times increase at an initial predator:prey ratio of 1:20, with the baker's yeast diet), whereas the mixture diet produced the highest density of prey. The brown sugar resulted in the lowest number of predator and prey mites. This may be due to lower feeding and predation rates of the prey and the predator mites on the brown sugar medium since its relative moisture content and adhesiveness inhibits mite movement. The final predator:prey ratio was also highest on the baker's yeast diet.

Conclusions: This study demonstrated that the population growth of *A. swirskii* was the highest when *C. lactis* was reared on baker's yeast.

Keywords: *Amblyseius swirskii*, *Carpoglyphus lactis*, Baker's yeast, Brown sugar, Diet, Effect on population increase

Background

Phytoseiid mites are used in biological control programs to suppress pests of greenhouse plants and field crops throughout the world. Among phytoseiid mites, *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) is currently applied as a highly efficient biological control agent of thrips, whiteflies, and spider mites on ornamental plants and vegetable crops (Calvo et al. 2011). These pests quickly reproduce and are able to establish large populations often resistant to chemical pesticides, making them difficult to control (Herron and James 2007;

Naveen et al. 2017). Biological control offers economic and environmental benefits by using natural enemies as an alternative strategy of suppressing target pests, besides reducing the negative environmental effects of pesticides.

While the efficiency of predatory mites against target pests is important, rearing protocols also need to be simple and cost-effective. The development of a practical rearing method is a fundamental requirement for controlling serious pests by ensuring a continuous supply of biological control agents (De Clercq 2004). In practice, the production of natural enemies on natural prey (or hosts) is both complicated and expensive due to the logistical requirements of rearing facilities and the need to rear a sufficient amount of prey with a suitable food source. However, because many generalist predators and parasitoids can successfully develop on other species,

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rearing cost and complexity might be significantly reduced by substituting natural prey items with alternative prey or factitious hosts (Riddick 2009).

Alternative prey has been used to develop successful rearing systems for phytoseiid mites. For example, *Neoseiulus baraki* (Athias-Henriot), an important predator of coconut pests, can easily be reared on alternative prey such as the mold mite *Tyrophagus putrescentiae* (Schrank) in a medium of rice bran and wheat flour (Fernando et al. 2004). Similarly, *A. swirskii* can be reared on the astigmatid dried fruit mite, *Carpoglyphus lactis* (Linnaeus) (Acari: Carpo-glyphidae) (Fidgett et al. 2010). Astigmatid mites have proven to be suitable alternative prey for *A. swirskii*, which can feed on multiple life stages of these mites. Many species of astigmatid mites have been successfully reared on wheat bran and yeast and used as factitious prey in the mass rearing of predatory mites (Barbosa and de Moraes 2015). However, a predator's performance is generally affected by the nutritional quality of prey, which, in turn, is primarily influenced by the prey's diet. For example, the larval stages of the prey mite, *T. putrescentiae*, reared on a protein-rich and fat-rich diet (dog food), resulted in a high oviposition rate of *A. swirskii* (Pirayeshfar et al. 2020). However, since *T. putrescentiae* could feed on soft plant tissues and damage crops (Oliveira et al. 2007), it is unsuitable as alternative prey when inoculating the predatory mite in greenhouses or agricultural fields.

Populations of *C. lactis* increased at higher rates when provided with a diet of white sugar mixed with dry brewer's yeast compared to each diet alone (Matsumoto 1964). While *A. swirskii* was reared on *C. lactis* fed on dry yeast (Ji et al. 2015), the optimal diet for *C. lactis* when rearing *A. swirskii* is unknown.

The purpose of this study was to identify a rearing medium that promotes the highest rate of population growth of the predatory mite *A. swirskii*, feeding on its alternative prey *C. lactis*.

Materials and methods

Stock culture of mites

A culture of the predatory mite *A. swirskii* (Israeli strain) with *C. lactis* as prey was obtained from Arysta Life-Science Cooperation (Tokyo, Japan). The predatory mite was maintained on *C. lactis* with wheat bran and sugar in a plastic container (9 × 4 cm). Prior to the experiment, a colony of *C. lactis* alone was established in another plastic container with a mixture of wheat bran and sugar. Fresh diet was added to each container depending on the population growth of *C. lactis*. If fungi developed in the media, the mites were transferred to a clean container with a fresh diet. The lids of the containers were sealed with parafilm to prevent mites from escaping and contamination by other mites. The *A. swirskii* cultures

were provided with *C. lactis* (along with its diet medium) and inspected every 2 weeks. All cultures were kept in an incubator at 25 °C, 70% RH, and a 16 h light: 8 h dark photoperiod. According to previous studies, *A. swirskii* developed from eggs to adults in approximately 7.2 days and laid 16.1 eggs at 25 °C and 73% RH, when provided with *C. lactis* (P. P. San, M. Tuda and M. Takagi, unpublished data). *C. lactis* developed to adults in 10.1 days and laid 37.3 eggs at 25 °C and 74% RH on a 1:1 mixed diet of white sugar and brewer's yeast (Okamoto 1984).

Experimental rearing of *A. swirskii* and *C. lactis*

A variety of different initial predator:prey ratios was used to determine the most suitable medium for *A. swirskii* reproduction. Mites were reared in ventilated plastic containers (8 × 3 × 5.5 cm), covered with 47-micron gauze to ensure gas and heat exchange and to prevent the mites' escape. Treatment diets were brown sugar (S), baker's yeast *Saccharomyces cerevisiae* (Y) (Fig. 1a), and the mixture of brown sugar + baker's yeast diets (SY) (S: Y = 4:6) (Fig. 1b). The baker's yeast was in the form of coarse, oblong granules. A preliminary experiment using a mixed diet of brown sugar + brewer's yeast showed a limited increase in both predator and prey densities than the diet of brown sugar + baker's yeast. In the absence of predatory mites, *C. lactis* populations increased at higher rates on baker's yeast than brewer's yeast. This is because the brewer's yeast was a powdery medium that acted as an adhesive on the legs of *C. lactis*, inhibiting their movement. Therefore, the baker's yeast was used as the diet media instead of brewer's yeast.

Each experimental rearing unit consisted of 2 g of one of the 3 diet treatments as well as one of 3 initial predator:prey ratios: 1:7 (14 *A. swirskii* and 98 *C. lactis*), 1:12 (14 *A. swirskii* and 168 *C. lactis*), and 1:20 (14 *A. swirskii* and 280 *C. lactis*). Mixed developmental stages of prey were used in all experiments, and the sex ratio of *A. swirskii* was 5:2 (females: males). To set up the experiment, *C. lactis* were first transferred from the stock culture to a rearing unit using a wet camel hairbrush (to prevent injury) and allowed to settle for one day (to become accustomed to the change). Then, 14 adult individuals (10 females and 4 males) of *A. swirskii* were transferred from the stock culture to each rearing unit. Sewing threads were placed in the rearing units to serve as ovipositional sites for *A. swirskii*. Both *A. swirskii* and *C. lactis* were observed using a binocular microscope. Each treatment (diet medium × initial predator:prey ratio) was replicated 5 times. The experiment was carried out under the same controlled environmental conditions as the stock culture. After 1 month (30 days), the final predator and prey population densities were estimated by taking five 0.1 g samples from each rearing unit and

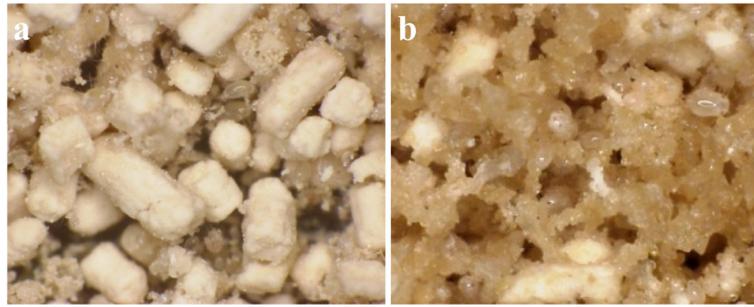


Fig. 1 Rearing medium for the predatory mite, *Amblyseius swirskii*, and food for its prey, dried fruit mite, *Carpoglyphus lactis*. **a** Baker's yeast (and *C. lactis*) and **b** Brown sugar and baker's yeast mixture (and *C. lactis*)

multiplying the average number of mites to express the population densities per 2 g of diet medium.

Statistical analysis

First, the effects of diet, initial predator:prey ratio, and their interaction on the final size of the predator and prey populations (after 1 month of rearing) were analyzed by generalized linear models with a Poisson distribution and a log-link function (Nelder and Wedderburn 1972). Overdispersion of data was corrected. If an effect was significant, a post hoc contrast test was performed. Second, the effects of diet, initial predator:prey ratio, and their interaction on the final predator:(prey + 0.5) ratio was tested with a logistic regression model. JMP 13.2.1 (SAS Institute Inc.) was used for all statistical analyses at the significance level $P = 0.05$ (Goodman 1993).

Results and discussion

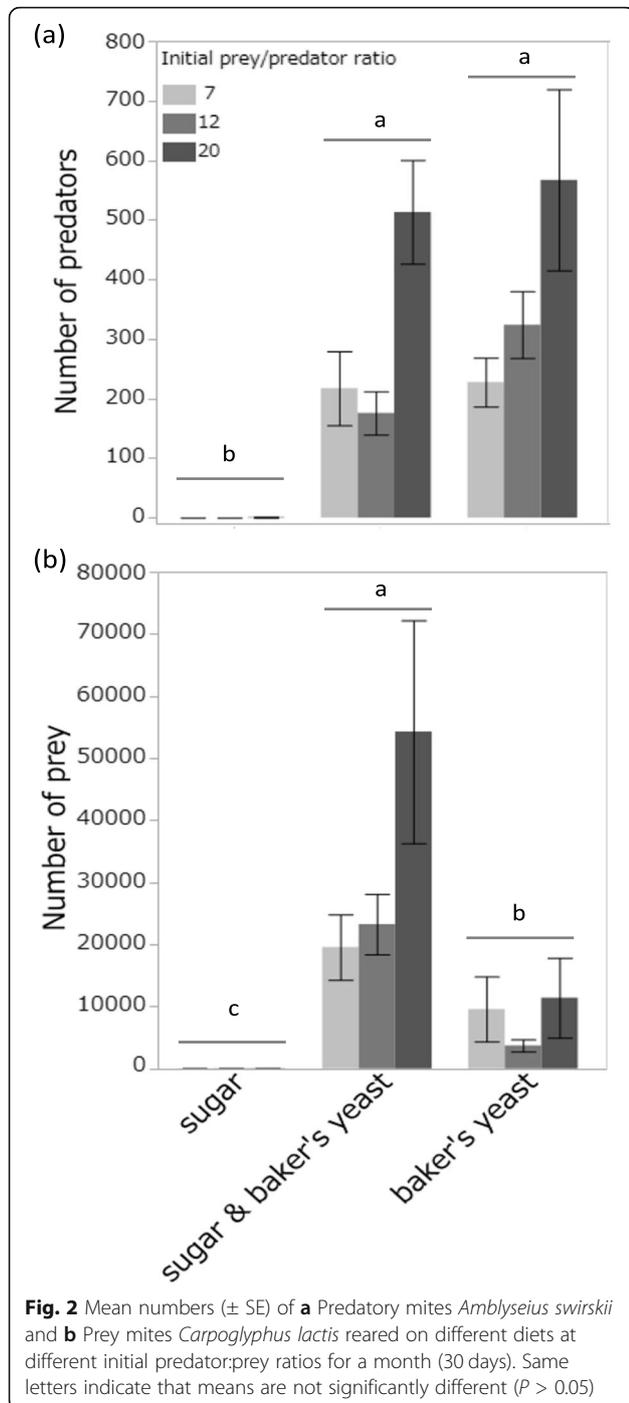
The diets supplied for *C. lactis* significantly affected the final population densities of *A. swirskii* [likelihood ratio (LR) $\chi^2_2 = 130.51$, $P < 0.0001$] and *C. lactis* (LR $\chi^2_2 = 65.37$, $P < 0.0001$) (Fig. 2). Final densities of *A. swirskii* were significantly higher when prey was supplied by the SY and Y diets, and prey density was significantly higher on the SY diet than on the S and Y diets alone.

In contrast, the initial predator:prey ratio did not affect the final population densities of *A. swirskii* (LR $\chi^2_1 = 0.34$, $P = 0.56$) or *C. lactis* (LR $\chi^2_1 = 0.02$, $P = 0.90$). There was also no significant interaction effect of diet and initial predator:prey ratio on the final population densities of *A. swirskii* (LR $\chi^2_2 = 0.22$, $P = 0.89$) or *C. lactis* (LR $\chi^2_2 = 1.09$, $P = 0.58$). The final predator:prey ratio varied among the different diets (LR $\chi^2_2 = 6030.68$, $P = 0.0001$) but was highest on the Y diet (Fig. 3). The final predator:prey ratio increased with the initial ratio (LR $\chi^2_1 = 6.06$, $P = 0.01$). There was a significant interaction between diet and initial predator:prey ratio (LR $\chi^2_2 = 131.38$, $P = 0.0001$).

This experiment showed that higher numbers of *A. swirskii* were produced when prey was provided by the

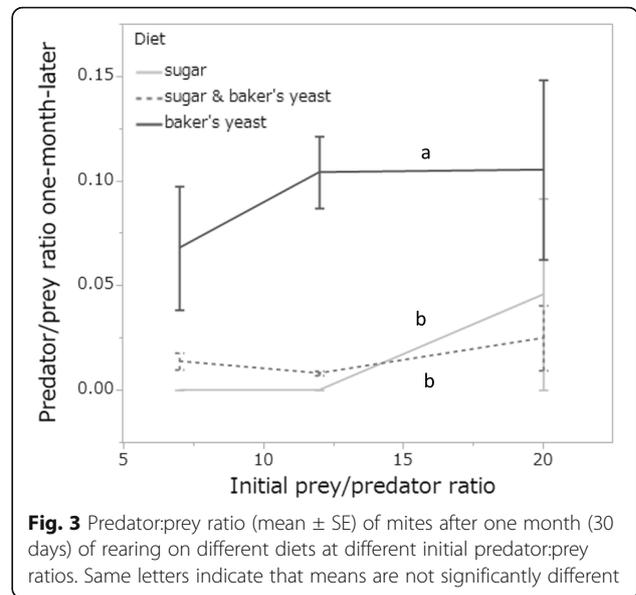
SY and Y diets. However, the highest prey densities were observed on the SY diet, so the final predator:prey ratio was highest on the Y diet. Therefore, the Y diet was the most suitable medium for high rates of *A. swirskii* population growth. Because moisture increased in the SY diet after 2 weeks, this may have disturbed the foraging and reproduction of *A. swirskii* (even though *C. lactis* was still able to reproduce in these conditions). High prey densities might interfere with *A. swirskii* on the SY diet (Chant 1961); additionally, a saturating type II functional response as well as mutual interference by *A. swirskii* itself (Fathipour et al. 2020) may limit oviposition rates and fecundity (Nakamichi et al. 2020). In this study, *A. swirskii* populations (both males and females) increased 40.5 times after 30 days without additional prey at an initial predator:prey ratio of 1:20, on the baker's yeast diet. In a previous study, with the same initial number of females and predator:prey ratio, the female-only population increased 21.5 times after 24 + 4 days when provided with an additional supply of prey (Ji et al. 2016). While it is difficult to compare these 2 studies because of differences in methodology and unreported sex ratios, this combination of dietary resources and prey appears to support sufficiently high rates of reproduction and survival for the predatory mite *A. swirskii*.

C. lactis exhibited a higher population growth on the S diet than on a diet of white sugar because of the higher water and nitrogen contents of the S diet (Iimuro 1956). The relatively low densities of both mites on the S diet during this study may be due to the nutrient content of the S diet, which was too poor for *C. lactis* (so the predator could not find enough prey), or because the S medium was too sticky for both mites to successfully forage and reproduce. While *C. lactis* can be reared on the Y diet alone (baker's yeast, Chmielewski 1971), an SY mixture was optimal (4:6 ratio of white sugar and brewer's yeast, Matsumoto 1964 and Okamoto 1984). Baker's yeast is available in several different forms; coarse, oblong granules of dry yeast was used in this study. This form served as a porous medium and



allowed free movement of both mites and provided sufficient nutrition for *C. lactis*. Therefore, the Y diet (baker's yeast) was a suitable diet for *C. lactis* when rearing *A. swirskii*.

Rearing programs that involve factitious hosts can reduce the complexity and cost of producing biological control agents. Even though the predatory mite *A. swirskii* can reproduce on pollen, the collection of pollen is a



laborious process and *A. swirskii* reared on cattail pollen do not reproduce as well as those provided with *C. lactis* (Nguyen et al. 2013). Moreover, when the rearing hosts themselves are crop pests, there can be a risk of spreading them along with the control agent when they are used in the mass culture of natural enemies. For instance, this became a problem in Spain when rearing *Eretmocerus mundus* (Mercet) on the Q strain of the whitefly *Bemisia tabaci* (Gennadius) (Van Driesche et al. 2008). However, the dried fruit mite *C. lactis*, which was used as an alternative prey in this study, did not damage crops.

Conclusion

The baker's yeast is a suitable (but not optimal) diet for the prey mite *C. lactis* and an optimal medium for the predatory mite *A. swirskii*. An additional benefit is that baker's yeast is a relatively low-cost material. This study highlighted the high population increase of the predatory mite *A. swirskii* when provided with the prey mite *C. lactis* in a medium of baker's yeast alone. This efficient rearing method can be applied safely and effectively as a short-term method for maintaining populations of *A. swirskii*.

Abbreviations

S: Brown sugar; Y: Baker's yeast; SY: Brown sugar and baker's yeast mixture; RH: Relative humidity; LR: Likelihood ratio; χ^2 : Chi-square

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Authors' contributions

PPS conducted the experiment and wrote the original manuscript. M Tuda analyzed the data, prepared the figures, and revised the final version of the manuscript. M Tagaki and KN supervised the experiment. All authors have approved the final version of the manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants or vertebrates performed by any of the authors.

Consent for publication

Consent for publication was obtained from all individual participants included in the study.

Competing interests

All authors declared that they have no conflict of interest.

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