Pollen Alone or a Mixture of Pollen Types? Assessing Their Suitability for Mass Rearing of *Neoseiulus cucumeris* (Acari: Phytoseiidae) Over 20 Generations

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Abstract

The generalist predatory mite, *Neoseiulus cucumeris* (Oudemans) is known as one of the most effective natural enemies on many pests. This economically important biocontrol agent was reared for 20 generations on date palm and castor bean pollen as well as a mixture of pollen types, including date palm, castor bean, and almond. The performance of this predator was evaluated by comparing its life table parameters after different generations (G1–G20) fed on each diet in a laboratory at 25 ± 1°C, 60 ± 5% RH, and a photoperiod of 16:8 (L: D) h. The development time of the predator reared on all tested diets had no significant difference in G20. The intrinsic rate of increase (*r*) of *N. cucumeris* by feeding on the mixed pollen (0.197 day⁻¹) was significantly higher than that on castor bean, or date palm pollen (0.146 or 0.152 day⁻¹ in G1, respectively). Our results indicated that the predator’s performance was not affected by long-term feeding on the pollen diets, as well as there was no considerable difference between pollen alone and pollen mixture diets. Furthermore, mites reared on pollen diets had higher quality than those reared on natural prey, *Tetranychus urticae* Koch.

Key words: Phytoseiidae, alternative food, life table, main prey, mass rearing

Biological control has long been known as a highly efficient and environmentally safe pest management procedure, and predatory mites as biocontrol agents are increasingly used in pest control programs across the globe (van Lenteren 2012). The phytoseiid predatory mites can control tiny insect and mite pests, which have been classified as specialists and generalists. The generalist predatory mites can feed on different food and diets, including small arthropods, nematodes, fungi, and pollen (McMurtry et al. 2013).

*Neoseiulus cucumeris* (Oudemans) is a generalist phytoseid predator known because of its success in controlling a wide range of pests in greenhouses (Zhang et al. 2011, Delisle et al. 2015). Feeding and reproducing on a pollen diet is a considerable characteristic of this predatory mite that facilitates the cost-effective production of large numbers of this predator so-called mass rearing. Since the performance of a predator is conditioned by long-term mass rearing (Nemati and Riahi 2020), the potential of *N. cucumeris* has already been evaluated on *Tetranychus urticae* Koch (Acari: Tetranychidae) as main prey and *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) as factitious prey for 30 generations (Yazdanpanah et al. 2022).

It is generally accepted that both inbreeding-mediated reduction in fitness and loss of genetic variation induced during long-term rearing can lead to poor quality natural enemies, inappropriate control results, and unpredictable consequence of augmentative biological control (van Lenteren 2003, Consoli et al. 2010). Therefore, the quality of mass-reared organisms should be evaluated during mass rearing to monitor their quality to be sure they are still in a condition to be reared and appropriately control the target pest after release (van Lenteren 1991, Bellutti 2011, Sørensen et al. 2012).

Although several pollen grains such as castor bean and date palm are suitable alternative diets for *N. cucumeris* (Yazdanpanah et al. 2021a), they have not been tested as a long time food source. Therefore, in the current study, we investigated the multigenerational impacts of date palm pollen, castor bean pollen, and a mixture of two mentioned pollen as well as almond pollen on *N. cucumeris* over 20 consecutive generations. Furthermore, the data were compared with the previously obtained data on a long time rearing of the predator on the main prey, *T. urticae* (Yazdanpanah et al. 2022).

Materials and Methods

**Pollen Collection**

Pollen of castor bean (*Ricinus communis* L.) was collected from the plants grown at the campus of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Pollen of date palm (*Phoenix*...
The values of all life table parameters, including the net reproductive rate ($R_0$), gross reproductive rate ($GRR$), finite rate of increase ($\lambda$), intrinsic rate of increase ($r$), and mean generation time ($T$), as well as age-stage-specific survival rate ($s_{x}$), age-specific survival rate ($l_x$) (the probability that a newborn will survive to age $x$, calculated by pooling all of the surviving individuals of different stages), age-stage-specific fecundity ($f^x_0$), and age-specific fecundity ($m_x$) were calculated according to the age-stage, two-sex life table procedure (Chi and Liu 1985, Chi 1988) using the TWWSEX-MSChart software (Chi 2021) at different generations on different diets. The variances and standard errors of all life table parameters were estimated using the bootstrap procedure with 100,000 samples. Multiple comparisons among different generations of each diet and the corresponding generation among different diets were carried out using the paired bootstrap test (Reddy and Chi 2015, Bahari et al. 2018).

Results

Effects of Pollen Diets on Life Table Parameters of *N. cucumeris*

The deutonymphal durations did not differ among generations on all diets except for castor bean pollen. Overall, no significant difference in the total pre-adult duration was observed between G1 and G20, whereas the shortest developmental time was recorded in G10 when pollens were offered separately. Furthermore, when the mixed pollen was a diet, the developmental time was longest in G20, whereas there was no significant difference between G1 and G10 (Table 1). Adult pre-oviposition period (APOP) of females on castor bean and mixed diet did not differ among three generations, whereas it was longest in G20 on date palm pollen. When the date palm pollen was the food source, the total pre-oviposition period (TOPP) was longest in G20 and shortest in G10, while on the castor bean pollen, this parameter was not affected by the generation. The oviposition period and fecundity of *N. cucumeris* did not differ among generations on all diets tested, except for date palm pollen. The mentioned parameters in G10 were lower than those in G1 and G20 on date palm pollen. Male longevity had no significant difference among tested generations on all diets except for castor bean pollen which males in G10 had shorter longevity than G10 and G20. Female longevity and the total life span (from birth to death) of *N. cucumeris* were not significantly different among different generations. When date palm pollen was offered, the female longevity and total life span in G20 was longer than G10, while on the mixture of pollen grains, the mentioned parameters were not different between G10 and G20. Whereas the immature survival of *N. cucumeris* did not differ among three generations on all diets; survival in all tested generations was 100% when the mixed diet was offered (Table 1).

Age-stage survival rate ($s_{x}$) of *N. cucumeris* fed in the first, 10th, and 20th generation on different diets are shown in Fig. 1. The adult stage began around the age of 6, 5, and 6 d in G1, G10, and G20, respectively, when the diet was date palm pollen. Furthermore, females of the first generation and males of the 20th generation lived more (Fig. 1). The adult stage began around the age of 5, 4, and 6 d on castor bean pollen in G1, G10, and G20, respectively. In addition, females in G1 and G20 lived more, whereas males of G10 had the shortest lifetime (Fig. 1). The beginning of the adult stage on the mixed diet was around the age of 4, 6, and 6 d in G1, G10, and G20, respectively, and females in G10 lived more (Fig. 1). Based on the fecundity curves, the peak of fecundity when date palm pollen was offered was observed in G10 (2.32 eggs/female) at the age of 10 d, followed by G20 at the age of 13 d (2.17 eggs/female) (Fig. 2). It was highest in G10 (1.77 eggs) at the age of 30 d, followed by G20 (1.72 eggs) at the age of 20 d when castor bean was offered, while in G1 (2.85 eggs) at the age of 18 d on the mixed diet (Fig. 2).
Table 1. Long-term effects of feeding on date palm pollen, castor bean pollen, and mixture of date palm, castor bean and almond pollen types on duration of different life stages (days), fecundity (eggs/female), and immature survival (%) (mean ± SE) of *Neoseiulus cucumeris* compared with a natural prey, *Tetranychus urticae*.

| Parameter                        | Date palm | Castor bean | Mixed pollen | *Tetranychus urticae* |
|----------------------------------|-----------|-------------|--------------|-----------------------|
|                                  | G1        | G10         | G20          | G1        | G10         | G20          | G1        | G10         | G20          |                             |
| Egg                              | 2.39 ± 0.121<sup>a</sup> | 1.52 ± 0.149<sup>b</sup> | 2.190 ± 0.145<sup>b</sup> | 2.12 ± 0.124<sup>a</sup> | 1.48 ± 0.161<sup>a</sup> | 1.74 ± 0.146<sup>a</sup> | 1.64 ± 0.217<sup>a</sup> | 2.05 ± 0.158<sup>a</sup> | 2.45 ± 0.107<sup>a</sup> | 2.42 ± 0.116<sup>a</sup> |
| Larva                            | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> | 1 ± 0.000<sup>a</sup> |
| Protonymph                       | 1.88 ± 0.057<sup>c</sup> | 1.96 ± 0.042<sup>b</sup> | 2.23 ± 0.093<sup>a</sup> | 2.37 ± 0.076<sup>a</sup> | 1.91 ± 0.064<sup>a</sup> | 2.16 ± 0.083<sup>a</sup> | 2.00 ± 0.000<sup>a</sup> | 1.91 ± 0.092<sup>a</sup> | 2.13 ± 0.073<sup>a</sup> | 1.7 ± 0.103<sup>a</sup> |
| Deutonymph                       | 1.94 ± 0.087<sup>c</sup> | 1.82 ± 0.104<sup>a</sup> | 2.05 ± 0.082<sup>a</sup> | 1.65 ± 0.038<sup>a</sup> | 1.91 ± 0.089<sup>a</sup> | 1.89 ± 0.103<sup>a</sup> | 1.86 ± 0.094<sup>b</sup> | 2.00 ± 0.067<sup>a</sup> | 1.82 ± 0.083<sup>a</sup> | 2.75 ± 0.157<sup>c</sup> |
| Pre-adult                        | 7.19 ± 0.121<sup>a</sup> | 6.27 ± 0.159<sup>b</sup> | 7.47 ± 0.160<sup>a</sup> | 7.08 ± 0.134<sup>a</sup> | 6.23 ± 0.192<sup>a</sup> | 6.79 ± 0.159<sup>a</sup> | 6.50 ± 0.242<sup>a</sup> | 6.95 ± 0.158<sup>a</sup> | 7.41 ± 0.105<sup>a</sup> | 7.25 ± 0.157<sup>c</sup> |
| APOP                             | 2.466 ± 0.162<sup>a</sup> | 2.369 ± 0.111<sup>ab</sup> | 2.94 ± 0.177<sup>a</sup> | 2.68 ± 0.200<sup>a</sup> | 2.79 ± 0.176<sup>a</sup> | 2.36 ± 0.165<sup>a</sup> | 2.22 ± 0.215<sup>a</sup> | 2.36 ± 0.194<sup>ab</sup> | 2.67 ± 0.278<sup>a</sup> | 3.4 ± 0.424<sup>a</sup> |
| TPOP                             | 9.73 ± 0.244<sup>a</sup> | 8.63 ± 0.238<sup>b</sup> | 10.53 ± 0.190<sup>a</sup> | 9.82 ± 0.296<sup>a</sup> | 9.05 ± 0.338<sup>a</sup> | 9.07 ± 0.279<sup>c</sup> | 8.89 ± 0.340<sup>b</sup> | 9.50 ± 0.335<sup>a</sup> | 10.00 ± 0.241<sup>a</sup> | 10.8 ± 0.506<sup>b</sup> |
| Oviposition days                  | 20.67 ± 1.790<sup>c</sup> | 15.79 ± 1.067<sup>c</sup> | 21.88 ± 0.946<sup>a</sup> | 27.92 ± 1.823<sup>a</sup> | 25.53 ± 2.173<sup>a</sup> | 23.64 ± 1.657<sup>a</sup> | 23.55 ± 3.125<sup>a</sup> | 27.57 ± 0.993<sup>a</sup> | 23.84 ± 2.382<sup>a</sup> | 15.2 ± 2.390<sup>c</sup> |
| Male longevity                   | 45.41 ± 3.391<sup>b</sup> | 44.46 ± 9.952<sup>a</sup> | 52.34 ± 8.586<sup>a</sup> | 64.94 ± 5.733<sup>a</sup> | 20.17 ± 5.586<sup>c</sup> | 70.18 ± 14.557<sup>a</sup> | 50.83 ± 12.967<sup>a</sup> | 55.15 ± 8.159<sup>a</sup> | 51.42 ± 5.655<sup>a</sup> | 22.23 ± 4.95<sup>c</sup> |
| Female longevity                 | 48.4 ± 5.861<sup>a</sup> | 39.10 ± 3.649<sup>a</sup> | 54.64 ± 3.041<sup>a</sup> | 56.69 ± 4.974<sup>a</sup> | 59.96 ± 4.547<sup>a</sup> | 63.37 ± 6.229<sup>a</sup> | 48.86 ± 7.634<sup>a</sup> | 72.46 ± 6.976<sup>a</sup> | 54.01 ± 6.835<sup>a</sup> | 28.70 ± 3.396<sup>a</sup> |
| Total life span                  | 54.0 ± 3.213<sup>c</sup> | 46.36 ± 3.230<sup>c</sup> | 61.80 ± 2.728<sup>a</sup> | 67.48 ± 3.802<sup>a</sup> | 60.89 ± 4.921<sup>a</sup> | 71.76 ± 5.540<sup>a</sup> | 56.08 ± 6.439<sup>a</sup> | 73.64 ± 5.594<sup>a</sup> | 60.24 ± 4.404<sup>a</sup> | 25.22 ± 2.485<sup>a</sup> |
| Fecundity                        | 32.88 ± 2.411<sup>a</sup> | 25.37 ± 1.831<sup>c</sup> | 36.40 ± 1.690<sup>a</sup> | 38.20 ± 2.910<sup>a</sup> | 43.32 ± 3.766<sup>a</sup> | 40.35 ± 4.114<sup>a</sup> | 46.54 ± 6.538<sup>a</sup> | 55.79 ± 1.856<sup>a</sup> | 47.58 ± 7.474<sup>a</sup> | 38 ± 0.76 ± 2.897<sup>c</sup> |
| Immature survival (%)            | 97 ± 0.030<sup>a</sup> | 96 ± 0.042<sup>a</sup> | 95 ± 0.044<sup>a</sup> | 93 ± 0.039<sup>a</sup> | 95 ± 0.042<sup>a</sup> | 100 ± 0.000<sup>a</sup> | 100 ± 0.000<sup>a</sup> | 100 ± 0.000<sup>a</sup> | 100 ± 0.000<sup>a</sup> | 83 ± 0.076<sup>a</sup> |

G, generation; APOP, adult pre-ovipositional period (from adult emergence to first oviposition); TPOP, total pre-ovipositional period (from egg to the first oviposition). The means followed by different lowercase letters in the same row are comparisons among different generations of each diet. The means followed by different capital letters within the same row are comparisons between the corresponding generations of four diets (*P* < 0.05, paired-bootstrap).

* For more details on comparisons among tested generations when *T. urticae* was the diet, please see Yazdanpanah et al. (2022).
Fig. 1. Age-stage survival rate ($s_{x}$) of sequential generations (G) of Neoseiulus cucumeris reared on date palm pollen, castor bean pollen, and mixed pollen diet.

Fig. 2. Age-specific survivorship ($l_{x}$), age-specific fecundity ($m_{x}$), and age-stage-specific fecundity ($f_{x,j}$) of sequential generations (G) of Neoseiulus cucumeris reared on date palm pollen, castor bean pollen, and mixed pollen diet.
The highest gross reproductive rate (GRR) and net reproductive rate ($R_0$) when the food was date palm pollen were observed in G20; however, there was no significant difference between G20 and G10 (Table 2). In addition, the highest values of the intrinsic and finite rate of increase ($r$ and $\lambda$, respectively) were observed in G10, whereas there was no significant difference in the mentioned parameters between G1 and G20 (Table 2). When castor bean was the diet, $R_0$ and $r$ in G10 and G20 were significantly higher than G1. The GRR, $R_0$, and $r$ parameters were not influenced by the generation when the mixed diet was offered. Neoseiulus cucumeris individuals reared on the mixed diet for one generation had the shortest mean generation time. In contrast, they had no significant difference with those fed on the mentioned diet for 10 generations (Table 2).

Comparing Life Table Parameters of N. cucumeris Reared on Pollen Diets and T. urticae

Although the pre-adult duration of N. cucumeris was shortest in G1 when the mixed diet was offered, developmental time was significantly shorter in G10 when date palm pollen and castor bean pollen were offered compared with both the mixed pollen and the main prey (Table 1). TPOP was shortest on a mixed diet in G1, while it was the fastest in G20 on T. urticae and castor bean pollen. The predator had the shortest oviposition days and lowest fecundity by feeding on the prey in all generations tested; however, no significant difference was observed between predators reared on date palm pollen and the prey in G1 (Table 1). In addition, in all generations tested, the shortest total life span was recorded when the predator individuals fed on T. urticae. In G1, immature survival rate differed significantly among different treatments, while in other generations, there was no significant difference among food diets (Table 1).

Females reared on the prey had the highest GRR in G1; however, it did not differ from those fed on the mixed diet, but the mentioned treatment showed the lowest value in the other generations (Table 2). In G1, $R_0$ did not differ among treatments, while it was highest on the mixed diet and castor bean pollen in G20. In G1, the females reared on the mixed diet showed the highest growth rate, similar to those raised on the prey. No differences were observed among different pollen diets in terms of the intrinsic and finite rate of increase in G10 and G20. In G1, the females supplied with castor bean pollen had the longest mean generation time than others. In contrast, feeding on castor bean pollen and the mixed diet resulted in the longest $T$ in G10, while it was longest on the mixed diet and date palm pollen in G20 (Table 2).

Discussion

Since pollen from different plant species may differ in their nutritional value and affect the predatory mites’ performance (McMurtry and Scriven 1966), the current study investigated the potential of three types of pollen diets for N. cucumeris during 20 generations. Additionally, the results of long-term rearing on these alternative diets were compared to the effects of long-term rearing of the predator on T. urticae as a main prey.

The long-term effects of feeding on pollen grains on a predator’s performance must be evaluated if such a diet is used for mass rearing (Belluti 2011, Sørensen et al. 2012). The results of the present study revealed that N. cucumeris could be reared for 20 generations on castor bean, date palm pollen, and the mixture of pollen types. Similarly, this predator was able to develop and reproduce on almond pollen for 50 generations (Yazdanpanah et al. 2021b) and
cattail pollen for 25 generations (Gravandian et al. 2022). Successful long-term rearing on pollen grains for other phytoseiid mites such as Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) (Khanamani et al. 2017) and Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) (Nemati and Riahi 2020) have been reported beyond 20 and 6 generations, respectively when fed on almond pollen.

Our results showed that the performance of N. cucumeris had no reduction during long-term feeding on date palm pollen. In addition, the highest value of $r$ in G10 showed the most adaptation of this value ($0.208$ day$^{-1}$) was close to the value obtained on tomato rust mite Acerion lycopersici (Massee) (Acari: Eriophyidae) ($0.211$ day$^{-1}$) (Al-Azzazy and Alhewairini 2018). Date palm pollen has been recommended as a favorable diet for mass rearing of other phytoseiid mites such as A. swirskii (Riahi et al. 2017), and Typhlodromus bagdasarjani Wainstein & Arutunjan (Acari: Phytoseiidae) (Riahi et al. 2016).

Although some researchers indicated low quality or even toxicity of castor bean pollen for phytoseiid species (Ranabhat et al. 2014, Khanamani et al. 2016), we found that not only this pollen is not toxic for this predator, but also it could support its long-term rearing at least for 20 generations. Actually, the nutritional quality of pollen which can be influenced by environmental conditions, method of pollen preparation (Bogdanov 2006, Dabija 2010), plant genotype (Linskens and Pfahler 1977), and plant age (Brodbeck et al. 2001) may be a reason for different performance of predators on them. The high value of $R_p$ and $r$ in G10 and G20 indicated that the castor bean pollen could be a suitable diet for the long-term rearing of this predator. Similarly, the suitability of this pollen for N. cucumeris (van Rijn and Tanigoshi 1999, Yazdanpanah et al. 2021a), Amblyseius zaberi Yousef and El-Borolossy (Acari: Phytoseiidae), and Euseius yousefi Zaher & El-Borolossy (Acari: Phytoseiidae) (Momen 2004) has been recorded previously. In addition, the value of the intrinsic rate of increase on castor bean in G1 ($0.146$ day$^{-1}$) was close to the value of this parameter by feeding on apple pollen ($0.149$ day$^{-1}$), Christmas cactus ($0.156$ day$^{-1}$) (Ranabhat et al. 2014), and T. urticae ($0.131$ day$^{-1}$) (Al-Azzazy et al. 2018).

Some important parameters such as total life span, oviposition days, fecundity, $R_o$, and $r$ had no significant difference among all tested generations by feeding on the mixed pollen, which means that this diet is a suitable food for long-term rearing of N. cucumeris. The estimated values of $r$ in the current study at the first generation were higher than the value of $r$ when the predator fed on cattail pollen ($0.120$ day$^{-1}$) (Gravandian et al. 2022) or fed on almond pollen ($0.128$ day$^{-1}$) (Yazdanpanah et al. 2021b) in G1.

Based on Yazdanpanah et al. (2022), long-term feeding on T. urticae caused no decline in N. cucumeris performance, and its predation potential remained high; therefore, T. urticae can be one of its main prey species. Comparison of the life table parameters between the reared predators on the pollen diets and natural prey (T. urticae) during 20 generations revealed that although pre-adult duration, especially incubation and larval periods were affected by maternal diets, developmental time in G20 had no significant difference in all pollen diets versus prey (T. urticae) diet. Consequently, long-term rearing and adaptation to the diet have a similar effect on the pre-adult period in all diets tested, including alternative or main food.

The values of $R_p$, $r$, and $\lambda$ had no significant difference among all tested diets at the first generation, but the values of the fecundity, oviposition days, and total life span were the lowest in all tested generations by feeding on T. urticae, while all mentioned parameters were the highest when the predator fed on castor bean or mixed pollen diets (especially in the later generations). This may be due to prey movement and the time spent subduing a prey individual (Hassell 1978, Yazdanpanah et al. 2022). Actually, pollen grains as immotile food sources are more available than prey, conserving energy for the rest of the predator’s life. Although N. cucumeris was successfully reared on T. urticae for up to 30 generations (Yazdanpanah et al. 2022), rearing the phytoseiid mites on natural prey gives rise to high labor costs.

In conclusion, mass rearing of N. cucumeris on castor bean, date palm, and mixed pollen (castor bean + date palm + almond) did not decrease its performance during 20 generations of rearing. Furthermore, mites reared on pollen diets had higher quality than those reared on natural prey (T. urticae); therefore, it can be concluded that castor bean or date palm alone, and mixed pollen are good candidates for the mass rearing of N. cucumeris for use in augmentative biological control programs.

Author Contributions
S.Y.: conceptualization; investigation; data curation; formal analysis; methodology; writing original draft. Y.E.: project administration; funding acquisition; supervision; methodology; validation; review and editing. E.R.: methodology; validation; review and editing.

M.P.Z.: validation; methodology; review and editing.

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