

# 1           **Optimization of rearing *Transeius montdorensis* under laboratory conditions**

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## 5   **Abstract**

6   The global application of *Transeius montdorensis* (Acari: Phytoseiidae) as a biological control agent across  
7   various protected crops has proven effective against a range of insect pests like thrips and whiteflies, as well as  
8   pest mites like broad mites and russet mites. Optimization of rearing *T. montdorensis* under laboratory  
9   conditions is crucial for further studies of this species to improve their application in Integrated Pest  
10   Management (IPM) programs. Here, we evaluated the development and reproduction of *T. montdorensis* when  
11   fed on four different diets, including cattail pollen (*Typha latifolia*), living dried fruit mites (*Carpoglyphus*  
12   *lactis*), frozen *C. lactis* eggs, and a mixed diet of frozen *C. lactis* eggs and *T. latifolia* pollen. Females  
13   consuming the mixed diet exhibited superior total fecundity and daily oviposition rate, along with the highest  
14   intrinsic rate of increase ( $r_m$ ) and net productive rate ( $R_0$ ) among all diets tested. The immature period was  
15   significantly longer for mites on a diet of living *C. lactis* compared to those on other diets. Importantly, utilizing  
16   frozen *C. lactis* eggs and *T. latifolia* pollen mitigates the risk of infestation and contamination from the living  
17   dried fruit mites, which is important for laboratory and field settings when releasing the predator colonies. Our  
18   findings not only present an optimized rearing method for predatory mites under laboratory conditions but also  
19   suggest potential broader applications for enhancing the effectiveness and sustainability of biological control  
20   strategies across various agroecosystems and reducing dependency on chemical pesticides.

21   Keywords: Phytoseiidae, *Transeius montdorensis*, life table, frozen *Carpoglyphus lactis* eggs, optimization

## 22   **Introduction**

23   Within the group of phytophagous predatory arthropods that feed on both plants and animals, predacious mites  
24   of the Phytoseiidae family play a vital role in controlling key agricultural pests, including thrips, whiteflies, and  
25   phytophagous mites, which negatively affect economically important crops (Bazgir et al., 2019; Chant, 1985;  
26   Samaras et al., 2019). Demite et al. (2014) reported that the Phytoseiidae family comprises approximately 2,709  
27   formally recognized species classified into 91 distinct genera. Based on their feeding habits, biological traits,  
28   and morphological features, those predatory mites were categorized into four primary types (with several sub-

29 types): specialized mite predators of Tetranychus species, selective predators of tetranychid mites, generalist  
30 predators, and polliniphagous generalist predators (JA McMurtry & Croft, 1997). This family stands out as the  
31 initial and primary group of predatory mites that colonize foliage environments in various ecosystems  
32 (McMurtry, 2010). Their increasing incorporation into Integrated Pest Management (IPM) programs across  
33 different cultivated crops highlights their crucial role in reducing reliance on chemical insecticides, offering a  
34 sustainable approach to managing agricultural insect pests (Javier et al., 2010; Van Lenteren, 2012).

35 *Transeius montdorensis* (formerly known as *Amblyseius montdorensis*/*Typhlodromips montdorensis*/  
36 *Typhlodromus montdorensis*) (Acari: Phytoseiidae), a polyphagous predatory mite, was originally found in New  
37 Caledonia and was first described by Schicha (Schicha, 1979; Steiner et al., 2003). According to their food  
38 preference, *T. montdorensis* was categorized into type III, generalist predators (JA McMurtry & Croft, 1997).  
39 This predator targets a variety of pests, including *Frankliniella occidentalis* (Labbé et al., 2019; Rahman et al.,  
40 2011), whiteflies (Cuthbertson, 2014; Sun et al., 2022), spider mites (Schicha, 1979), and broad mites (Steiner &  
41 Goodwin, 2002). Though originating from the subtropical areas of Australia, *T. montdorensis* thrives in  
42 temperatures between 20 °C and 30 °C (Cox et al., 2006) and has adapted to colder environments, with a  
43 developmental threshold between 10.3 °C and 10.7 °C observed in the UK (Hatherly et al., 2004). Due to their  
44 wide range of prey, high adaptation in different environments as well as the potential to sustain their population  
45 at relatively high levels even at low prey or in the absence of prey (JA McMurtry & Croft, 1997; McMurtry et  
46 al., 2013), *T. montdorensis* has been positioned as a commercial beneficial agent for pest control in global  
47 agricultural settings, being available in Australian and European markets since 2003 (Van Lenteren, 2012).  
48 Furthermore, Steiner et al. (2003) and Buitenhuis et al. (2010) indicated that *T. montdorensis* outperforms  
49 *Neoseiulus cucumeris* in intrinsic rate of increase, daily oviposition rate, and thrips larvae consumption. Another  
50 study also reported that *T. montdorensis* is more effective than *Amblydromalus limonicus* in controlling two  
51 whitefly species, *Bemisia tabaci* and *Trialeurodes vaporariorum*, especially under cold temperatures (Richter,  
52 2016). On the contrary, Mouratidis et al. (2023) concluded that *A. limonicus* and *Amblyseius swirskii* performed  
53 better in controlling *Scirtothrips dorsalis* on strawberries than *T. montdorensis* and *N. cucumeris*. In Australia, *T.*  
54 *montdorensis* has been successful in managing *F. occidentalis* across various crops (Manners et al., 2013;  
55 Steiner & Goodwin, 2002), showing a higher intrinsic rate of increase ( $r_m$ ) than *N. cucumeris* (Steiner et al.,  
56 2003) and superior in daily *F. occidentalis* consumption than *N. cucumeris* on strawberry (Rahman et al., 2012).  
57 Type III predatory mites can develop and reproduce on diverse diets, including natural prey like phytophagous  
58 mites, thrips, and whiteflies, as well as alternative food sources like pollen, honeydew, and factitious prey like

59 storage mites (Jaques et al., 2015; Nguyen et al., 2013). Although pollen quality differs among plant species and  
60 environmental conditions (Lundgren, 2009), it is favored due to the high quality and abundant of pollen grains,  
61 cost-effectiveness, ease of collection, and its suitability in enhancing the development and reproduction of  
62 phytoseiid mites (Irina Goleva & Claus PW Zebitz, 2013; Gravandian et al., 2022; Samaras et al., 2015;  
63 Vangansbeke et al., 2014). Schreiber (2018) noted that pollen serves as both an alternative and supplementary  
64 food source for feeding predatory mites. Interestingly, several predacious mites are capable of growing and  
65 reproducing solely on pollen (Ranabhat et al., 2014; Steiner et al., 2003). Among a number of plant pollen  
66 species, cattail pollen (*Typha latifolia*) is a commercial product and a promising alternative food source for  
67 mass-rearing predatory mites (Gravandian et al., 2022).

68 Due to the high costs associated with maintaining and rearing generalist predators using natural prey, storage  
69 mites have been applied as a cost-effective and less laborious candidate for rearing various predatory mites (JA  
70 McMurtry & Croft, 1997; Pirayeshfar et al., 2020). These factitious hosts are employed not only to feed  
71 predatory mites under laboratory conditions or in mass-rearing programs but also serve as an additional food  
72 source to sustain populations of predatory mites in the crops when their preferred prey is at low density or  
73 absent (Barbosa & de Moraes, 2015; Massaro et al., 2021). Moreover, prey mites do not support the  
74 development and reproduction of thrips (Pirayeshfar et al., 2020). Among various storage mites, *Carpoglyphus*  
75 *lactis* has been extensively researched and utilized for rearing different generalist predators and has shown  
76 promising results (Nguyen, 2015; Wang et al., 2024). However, the required space and labor make them costly  
77 (Gravandian et al., 2022). Additionally, their potential to cause infestations and health issues, such as  
78 gastrointestinal acariasis, allergic dermatitis, and respiratory allergies in humans, poses significant risks in food  
79 production (Hubert et al., 2018; Jan Hubert, 2011; F. Pirayeshfar et al., 2021b).

80 Alongside the mentioned diets, including plant food and living storage mites, frozen stages of factitious hosts  
81 were recognized as a potential diet for feeding several species of predatory mites such as *A. swirskii* (Pirayeshfar  
82 et al., 2020) and *Blattisocius mali* (F. Pirayeshfar et al., 2021a). Moreover, a combination of factitious host and  
83 pollen has been applied to investigate the developmental and reproductive responses of predacious mites,  
84 including *Amblydromalus lomoniscus* (Samaras et al., 2019) and *B. mali* (Pirayeshfar et al., 2022). Similarly,  
85 another research demonstrated that a mixture of almond pollen and the flour moth *E. kuehniella* eggs  
86 significantly enhanced the oviposition of *N. cucumeris* (Etienne et al., 2021). However, no studies have  
87 examined the impact of frozen factitious food alone or mixed with pollen on *T. montdorensis*. Therefore, this  
88 study assessed life table parameters using four diets, including cattail pollen (*T. latifolia*), living dried fruit mite

89 (*C. lactis*), frozen *C. lactis* eggs, and a mixture of frozen *C. lactis* eggs and *T. latifolia* pollen to evaluate their  
90 nutritional value and suitability for rearing *T. montdorensis* under laboratory conditions. Overall, the application  
91 of frozen *C. lactis* eggs and *T. latifolia* pollen significantly reduced the immature development time and  
92 enhanced the reproductive capacity of *T. montdorensis*, compared to using living *C. lactis* as food for this  
93 predator. More importantly, this method can be applied to multiply several other Phytoseiidae species, thereby  
94 improving the effectiveness of biological control strategies across various crops.

## 95 **Materials and methods**

### 96 **Stock colony of *T. montdorensis* and culture methods**

97 The laboratory cultures of the predatory mites *T. montdorensis* were initially supplied by Bugs for Bugs  
98 (Queensland 4350, Australia) with food sources including mixed stages of living *C. lactis* and vermiculite as a  
99 shelter for the mites. Subsequently, this colony was maintained in a climatic set to  $25 \pm 1$  °C,  $70 \pm 5$  % RH, and  
100 a 16:8 h (L:D) photoperiod. The mites were maintained on white tiles (200 x 100 x 7 mm) (Johnson Tiles,  
101 Victoria 3153, Australia) that were placed on top of wet, thick foam pads (200 x 80 x 100 mm) (QEP company,  
102 Victoria 3175, Australia) inside a plastic tray (40 x 32 x 17 cm) filled with water. To prevent the mites from  
103 escaping and to ensure optimal humidity for predator development, the edges of the tiles were surrounded by  
104 tissue papers soaked in water. A curved piece of black filter paper (Westlab Pty Ltd, Victoria 3355, Australia)  
105 was placed in the centre of the tile to serve as shelter for the mites (Fig. 1a). Mixed stages of living *C. lactis*  
106 were added as food when needed.

### 107 **Diet preparation**

#### 108 *Pollen*

109 The fresh cattail pollen (*T. latifolia*) was collected in the cattail field in Menangle, New South Wales 2568,  
110 Australia, in December 2022 by tapping flowers on a tray and then transferred into a 50 ml tube (Thermo Fisher  
111 Scientific Australia Pty Ltd, Victoria 3179, Australia) before freezing at -20 °C. Prior to use in the experiments,  
112 the pollen was thawed and then stored at a temperature of 4 °C for up to one week.

#### 113 *Living C. lactis*

114 A colony of *C. lactis* was also supplied by the Bugs for Bugs. The storage mites were reared in Petri dishes (90  
115 x 15 mm). The prey mites were fed exclusively on instant dry yeast (Lowan Australia Ltd, NSW 2761,  
116 Australia) to collect frozen eggs of *C. lactis* by sieving through a fine net mesh. The Petri dishes, containing

117 living *C. lactis* and its foods, were sealed with cling wrap (MPM Marketing Services, QLD 4008, Australia) and  
118 then placed on tiles (200 x 100 x 7 mm) (Johnson Tiles, Victoria 3153, Australia). These tiles were positioned on  
119 thick sponge pads inside a plastic box (40 x 32 x 17 cm) filled with water. A sharp needle was used to puncture  
120 tiny holes in the cling wrap to ensure ventilation within the Petri dishes while preventing prey escape. The  
121 stocks were kept at  $25 \pm 2$  °C,  $85 \pm 5$  % RH, and in complete darkness (Bakr et al., 2021) by covering the lids  
122 and all sides of the rearing containers with thick black plastic bags. The mixed life stages of *C. lactis* served as  
123 the food source for one colony of *T. montdorensis* and were used in experiments.

#### 124 *Frozen C. lactis* eggs

125 To collect the frozen eggs of *C. lactis*, entire Petri dishes containing all life stages of the prey mites, as well as  
126 their food diets and feces, were frozen at -20 °C for at least 48 hours to ensure complete mortality. Subsequently,  
127 the contents were sieved through a fine mesh net (100µm) (Pathtech, Victoria 3072, Australia) to collect only *C.*  
128 *lactis* eggs. These eggs were then stored at - 20 °C for no longer than 4 weeks to ensure their quality  
129 (Pirayeshfar et al., 2020).

### 130 **Experimental design and statistical analyses**

#### 131 *Stock colony of predatory mites T. montdorensis*

132 There were four stocks of *T. montdorensis*, each fed on one of four different food diets: cattail pollen (*T.*  
133 *latifolia*), mixed stages of living *C. lactis*, frozen *C. lactis* eggs, and a combination of *T. latifolia* pollen and  
134 frozen *C. lactis* eggs in a 1:1 ratio (mixed diet). The rearing setup was similar to the main stock, except the  
135 white tiles were replaced by blue ones (97 x 97 mm). This alteration aimed to enable the easy detection and  
136 differentiation of male and female predators. Food was replenished every two days, with the removal of mouldy  
137 residues as necessary to prevent adverse effects on mite population development. Each population was reared  
138 for at least 2 generations before collecting fresh eggs for experimental setups.

#### 139 *Rearing microcosms*

140 Small Petri dishes (35 x 12 mm) were used to examine the development and reproduction of individual *T.*  
141 *montdorensis*. To improve their development's visibility, each Petri dish's exterior was coated with black acrylic  
142 paint (Shamrock company, VIC 3130, Australia). Each dish featured a small hole at the bottom, into which a  
143 piece of cotton thread was inserted. A total of eight small Petri dishes were arranged within a single takeaway  
144 box equipped with a lid, which had eight corresponding holes, allowing the cotton threads to extend to the  
145 bottom of the box filled with tap water. This setup provided a water source to support the development of the

146 predators. The Petri dishes were covered with cling wrap to prevent the mites from escaping while still allowing  
147 easy observation of their development and reproduction (Fig. 1b).

#### 148 *Experimental setup*

149 To examine the development and reproduction of individual *T. montdorensis*, freshly laid eggs were collected  
150 within 8 hours and transferred each one into a separate small Petri dish for individual observation (n=36 for *T.*  
151 *latifolia* pollen and living *C. lactis* diets and n=32 for frozen *C. lactis* eggs and mixed diets). Upon egg hatching,  
152 the corresponding diets of *T. latifolia* pollen, living *C. lactis*, frozen *C. lactis* eggs, and mixed diet were  
153 introduced in the rearing microcosms. All food diets were replenished every two days except for the second diet,  
154 where living *C. lactis* in mixed stages were added as necessary. Observations were conducted every 12 hours  
155 until the mites reached the adult stage to collect data on the duration of each developmental stage, including egg  
156 and immature stages (the period after the egg hatched until the mite reached adulthood), as well as their survival  
157 rate. Developmental stages were determined based on the presence of cast skin in the rearing microcosms  
158 (Steiner et al., 2003). After emergence, each female was paired with a male fed on the same diet. Males that died  
159 during the experiment were replaced by other males that had been reared on the same diet. Subsequently, daily  
160 observations were made to assess oviposition periods, female longevity, and fecundity, while the preoviposition  
161 period was examined every 12 hours. To obtain the data on offspring's sex ratio and survival proportion, eggs  
162 laid were collected daily, transferred to new rearing microcosms, and provided with the same diets as their  
163 parents until they reached adulthood. Mites that died as a result of non-natural causes, including accidental  
164 fatalities while attempting to recover the cling wrap on the rearing microcosm or escape from the rearing arena,  
165 were excluded from data analysis. The experiments were conducted in a growth chamber set to a constant  
166 environment of  $25 \pm 1$  °C,  $70 \pm 5$  % RH, and a 16:8 hours (L:D) photoperiod.

#### 167 *Statistical analysis*

168 Statistical analysis was conducted using R software version 4.4.0 to evaluate the impact of diet on various  
169 aspects of the predatory mite's biology, including the development time of egg and immature stages,  
170 preoviposition and oviposition duration, daily and total eggs laid, proportion of female offspring and female  
171 longevity. Initially, the Shapiro-Wilk test was performed to assess data normality. For non-normally distributed  
172 data, means were calculated using the "dplyr" package and compared using the "dunn.test" package. Dunn's test  
173 with Bonferroni correction was used in conjunction with the Kruskal-Wallis test to calculate the chi-square ( $\chi^2$ ),  
174 degrees of freedom (df), and P-value. To analyse the proportion of female offspring across four treatments and

175 to compare their means, a Generalized Linear Model (GLM) was employed. This was followed by post-hoc  
176 analysis using Dunn's test with Bonferroni correction to determine statistical significance and assign  
177 significance labels. Pairwise comparisons were made to identify differences between treatments, with a  
178 conventional alpha level of 0.05 used to assess significance. Therefore, P-values of 0.05 or lower were  
179 considered significant.

#### 180 *Life table parameters*

181 The life table parameters, including the net productive rate ( $R_0$ ), generation time ( $T_c$ ), and intrinsic rate of  
182 increase ( $r_m$ ), were calculated employing the Jackknife procedure as described by Maia et al. (2000).

## 183 **Results**

### 184 **Developmental time**

185 The survival proportion of individuals at the immature stages across all four treatments was 100%. For females,  
186 the total immature developmental time was shortest when fed on the mixed diet ( $5.30 \pm 0.14$  days) and longest  
187 for those reared on living *C. lactis* ( $6.05 \pm 0.21$  days). Notably, the developmental time of female deutonymphs  
188 fed on the mixed diet ( $1.15 \pm 0.05$  days) was significantly shorter compared to those reared on *T. latifolia* pollen  
189 ( $1.43 \pm 0.08$  days), living *C. lactis* ( $1.79 \pm 0.15$  days), and frozen *C. lactis* eggs ( $1.55 \pm 0.08$  days). There were  
190 no significant differences in the developmental time of egg, larval, and protonymph stages for females across  
191 the various diets. For males, the shortest total developmental time was observed with frozen *C. lactis* eggs ( $5.00$   
192  $\pm 0.17$  days), which was significantly different from that recorded with cattail pollen ( $5.54 \pm 0.14$  days) and  
193 living *C. lactis* ( $5.97 \pm 0.09$  days), but not significantly different from the mixed diet ( $5.38 \pm 0.20$  days).  
194 Notably, the developmental time of the eggs that hatched into males, laid by females fed on frozen *C. lactis* eggs  
195 ( $1.65 \pm 0.19$  days) was significantly shorter than that of eggs from females reared on *T. latifolia* pollen ( $2.19 \pm$   
196  $0.17$  days) and living *C. lactis* ( $2.18 \pm 0.07$  days). Regarding the larval stage, the developmental time of males  
197 fed on *T. latifolia* pollen ( $0.62 \pm 0.06$  days) was significantly shorter than those fed on the mixed diet ( $0.71 \pm$   
198  $0.07$  days), living *C. lactis* ( $0.88 \pm 0.05$  days), and frozen *C. lactis* eggs ( $0.96 \pm 0.09$  days). However, the  
199 developmental time of the protonymph stage did not differ among the four diets. Meanwhile, male  
200 deutonymphs developed significantly faster when fed on frozen *C. lactis* eggs ( $1.15 \pm 0.07$  days) compared to  
201 those fed on *T. latifolia* pollen ( $1.38 \pm 0.08$ ), the mixture ( $1.38 \pm 0.09$  days), and living *C. lactis* ( $1.53 \pm 0.08$   
202 days).

203

204

## 205 **Reproduction**

206 Diet significantly influenced the reproductive parameters of the predatory mite *T. montdorensis*, including  
207 oviposition period, proportion of female offspring, daily oviposition rate, fecundity, and survival proportion of  
208 offspring (Table 2). Preoviposition was shortest in females fed on the mixed diet ( $2.08 \pm 0.11$  days), followed by  
209 frozen *C. lactis* eggs ( $2.28 \pm 0.12$  days), *T. latifolia* ( $2.61 \pm 0.12$  days), and living *C. lactis* ( $2.63 \pm 0.17$  days). In  
210 contrast, no significant differences were observed in the oviposition period and female longevity across the four  
211 diets, with duration ranging from  $21.4 \pm 1.12$  days to  $23.7 \pm 1.62$  days for the oviposition period and from  $26.8$   
212  $\pm 1.22$  days to  $30.9 \pm 1.83$  days for female longevity. Fecundity was highest in females reared on the mixed diet  
213 ( $43.1 \pm 2.07$  eggs), followed by those on frozen *C. lactis* eggs ( $33.2 \pm 2.10$  eggs) and on *T. latifolia* pollen ( $29.6$   
214  $\pm 1.55$  eggs) while the lowest fecundity was observed in females fed on living *C. lactis* ( $27.3 \pm 1.38$  eggs). The  
215 daily oviposition rate followed a similar trend, with the highest rate recorded in the mixed diet ( $1.59 \pm 0.07$   
216 eggs/female/day) and the lowest one observed in living *C. lactis* ( $1.00 \pm 0.06$  eggs/female/day). Peak oviposition  
217 occurred on days 6, 8, 10, and 12 after emergence for diets of frozen *C. lactis* eggs, the mixed diet, *T. latifolia*  
218 pollen, and living *C. lactis*, respectively (Fig. 2). The proportion of female progeny was significantly higher in  
219 offspring reared on diets of *T. latifolia* pollen and the mixed diet ( $0.65 \pm 0.03$  %) compared to those on frozen *C.*  
220 *lactis* eggs ( $0.59 \pm 0.04$  %) and living *C. lactis* diets ( $0.46 \pm 0.05$  %).

## 221 **Life table parameters**

222 The net reproductive rate ( $R_0$ ) of *T. montdorensis* females that fed on the mixed diet ( $26.50 \pm 0.13$   
223 females/female) was significantly higher than that of mites fed on *T. latifolia* pollen ( $17.50 \pm 0.07$   
224 females/female) and living *C. lactis* ( $10.85 \pm 0.10$  females/female) (Table 2). The generation time of females fed  
225 on living *C. lactis* ( $12.79 \pm 0.05$  days) was significantly longer than those reared on the mixed diet ( $12.03 \pm 0.02$   
226 days), *T. latifolia* pollen ( $11.96 \pm 0.02$  days). Meanwhile, females *T. montdorensis* that consumed frozen *C.*  
227 *lactis* eggs had the shortest generation period ( $11.47 \pm 0.04$  days). The intrinsic rate of increase ( $r_m$ ) for the  
228 females reared on the mixed diet ( $0.41 \pm 0.02$  female/female/day) was higher than those reared on frozen *C.*  
229 *lactis* eggs ( $0.38 \pm 0.02$  female/female/day), and those fed on *T. latifolia* pollen ( $0.36 \pm 0.01$  female/female/day),  
230 and significantly higher compared to those fed on living *C. lactis* ( $0.26 \pm 0.01$  female/female/day).

## 231 **Discussion**



232 The primary objective of our research was to optimize the rearing method for the predatory mite *Transeius*  
233 *montdorensis* under controlled laboratory conditions. Our findings demonstrated a significant enhancement in  
234 development and reproduction for *T. montdorensis* females when fed on a combination of *T. latifolia* pollen and  
235 frozen *C. lactis* eggs. Particularly, the mixed diet reduced the total immature developmental time to  $5.30 \pm 0.14$   
236 days, notably shorter than that of females reared on other diets, especially living *C. lactis*, where the total  
237 development time of females was longest, at  $6.05 \pm 0.21$  days. On the contrary, another study reported an  
238 immature development time of  $7.5 \pm 0.16$  days for *T. montdorensis* fed on a combination of *Tetranychus urticae*  
239 and cattail pollen at 25 °C (Hatherly et al., 2004). Our study further highlights the value of the mixed diet by  
240 demonstrating superior life table parameters. Specifically, the highest value of both intrinsic rates of increase  
241 ( $r_m$ ) and net reproductive rate ( $R_0$ ) in *T. montdorensis* females reared on the combination of frozen *C. lactis* eggs  
242 and *T. latifolia* pollen. Females fed exclusively on cattail pollen exhibited a lower intrinsic rate of increase ( $r_m =$   
243 0.36) than those fed on the mixed diet ( $r_m = 0.41$ ). However, it was higher than reported by Steiner et al. (2003)  
244 under the same laboratory conditions (25 °C, 70 % RH, and a 16:8 h (L:D) photoperiod) ( $r_m = 0.32$ ). This  
245 indicated that the mixed diet is preferable for *T. montdorensis*, as superior life table parameters result from  
246 dietary preference in arthropods, which subsequently leads to a high population build-up (Grenier & Clercq,  
247 2003). Furthermore, the total number of eggs laid per female reared on the mixed diet was highest, averaging  
248  $43.1 \pm 2.07$  eggs per female, and almost double compared to that by females fed on living *C. lactis*, averaging  
249  $27.3 \pm 1.38$  eggs per female.

250 Pollen has been recognized as a promising alternative food source during periods of prey scarcity (He et al.,  
251 2022) and as a dietary supplement for large-scale rearing practices (Eini et al., 2022; I. Goleva & C. P. W.  
252 Zebitz, 2013; Yazdanpanah et al., 2021a). Despite recommendation by Hulshof and Vanninen (2002) to reduce  
253 pollen availability to manage *F. occidentalis*, subsequent research has shown that pollen positively affected the  
254 increase of predatory mite populations such as *A. limonicus* (Lee & Zhang, 2018; Samaras et al., 2019), *A.*  
255 *swirskii* (Barzka et al., 2023), and *N. cucumeris* (Han et al., 2024). Some species, for example, *Amblyseius*  
256 *cucumeris*, *Typhlodromus pyri*, and *Amblyseius andersoni* have been found to achieve comparable or even  
257 superior reproductive outcomes when fed on pollen rather than on their natural prey (Duso & Camporese, 1991;  
258 Marisa & Sauro, 1990). In a separate study, the supplementary of corn pollen to feed *Euseius scutalis* and *A.*  
259 *swirskii* resulted in a tenfold and twofold increase in their populations, respectively (Adar et al., 2014).  
260 Additionally, Gravandian et al. (2022) underscored the utility of *T. latifolia* pollen in sustaining immature  
261 development and fecundity of *N. cucumeris* females over 25 consecutive generations. However, pollen lacks  $\beta$ -

262 carotene (Hatherly et al., 2004), which is rich in yeast, the primary food of *C. lactis*. Additional studies have  
263 also indicated the advantages of incorporating pollen into the diets of predatory mites under laboratory  
264 conditions (Eini et al., 2022; Irina Goleva & Claus P. Zebitz, 2013; Yazdanpanah et al., 2021b). Therefore, a  
265 combination of pollen and a protein-rich diet is necessary to improve the development and reproduction of  
266 predatory mites. This aligns with the results of this study, where life table parameter values, including  $R_0$ ,  $r_m$ ,  
267 and fecundity, for *T. montdorensis* females fed on the mixed diet were significantly higher than those fed on  
268 pollen alone. These results support the hypothesis that a mixed diet can enhance growth rates and reproductive  
269 outcomes, suggesting a synergistic effect from combining pollen with frozen prey, potentially due to improved  
270 nutritional balance.

271 The utilization of the storage mite *C. lactis* as an alternative diet offers several advantages over natural prey  
272 species. Studies have shown that *C. lactis* can be easily reared on simple media like yeast and sugar (Barbosa &  
273 de Moraes, 2015; San et al., 2020). Additionally, the high reproduction and rapid population growth of *C. lactis*  
274 ensures a consistent supply of food resources for the large-scale production of predatory mites. Consequently, *C.*  
275 *lactis* has been employed as a promising alternative food resource for rearing various predatory mites, including  
276 *T. montdorensis* and *A. swirskii* (Pirayeshfar et al., 2020; Zhang & Zhang, 2021). However, this study's  
277 outcomes showed the lowest effectiveness of living *C. lactis* in both the development and reproduction of the  
278 predatory mites. *T. montdorensis* females fed on living *C. lactis* had the longest immature duration but the  
279 lowest net reproductive rate ( $R_0$ ), intrinsic rate of increase ( $r_m$ ), and total number of eggs laid. Moreover, using  
280 *C. lactis* as factitious prey is not without any drawbacks. Occasional observations during the experiments  
281 revealed that mature *C. lactis* occasionally preyed on the eggs and larvae of *T. montdorensis*, which likely  
282 contributed to the reduced fecundity observed in the predatory mites fed exclusively on this diet. Jan Hubert  
283 (2011) highlighted that the risk of *C. lactis* contamination has increased in the Czech Republic because *C. lactis*  
284 can migrate between packages in supermarkets. Additionally, contact with storage mites or even agricultural  
285 products that are infested with the mites can cause itching and redness or trigger storage-mite allergies in  
286 humans. The application of frozen *C. lactis* eggs can prevent the contamination risks associated with living *C.*  
287 *lactis*, thereby reducing the environmental impact when releasing *T. montdorensis* and frozen *C. lactis* eggs into  
288 greenhouse or field, rather than a combination involving living *C. lactis*.

289 To our knowledge, this study is the first to investigate the application of frozen *C. lactis* eggs in rearing the  
290 predatory mite *T. montdorensis*. Prior research on the efficacy of frozen eggs and the early stages of storage  
291 mites in supporting the development and fecundity of predators is limited. Contrary to our findings, such studies

292 generally indicated that the performance of these alternative diets did not surpass those of living prey. Li and  
293 Zhang (2016) reported an extended juvenile development time for *N. cucumeris* when consuming frozen eggs of  
294 *T. urticae* as opposed to fresh eggs. In addition, research conducted by Xu et al. (2023) on the influence of  
295 frozen and fresh *T. urticae* eggs on the immature development, consumption rates, and oviposition rates of  
296 *Phytoseiulus persimilis* under laboratory conditions suggested that the nutritional quality of frozen eggs might  
297 be inadequate, leading to reduced oviposition rates and increased consumption rates when females are fed  
298 frozen prey mite eggs. Similarly, a study examining the impact of frozen eggs of storage mites *Tyrophgus*  
299 *putrescentiae* on the development and reproduction of *A. swirkii* illustrated that frozen *T. putrescentiae* eggs  
300 with cattail pollen did not significantly enhance the daily oviposition rate of this predator. The contrasting  
301 results regarding the effectiveness of frozen storage mite eggs for the development and reproduction of their  
302 predators between our study and others may be due to differences in prey and predator species, experimental  
303 setups, and methodology of each research (Pirayeshfar et al., 2020). Fatemeh Pirayeshfar et al. (2021) also  
304 reported that females of *B. mali* fed on one-day frozen eggs of storage mite *T. putrescentiae* exhibited higher life  
305 table parameters ( $r_m$ ,  $R_0$ , and  $\lambda$ ) than those fed on eggs frozen for 90 days. It is, therefore, crucial to evaluate the  
306 developmental and reproductive performance of *T. montdorensis* females reared on frozen *C. lactis* eggs stored  
307 for different durations. Assessing the optimal ratio of frozen *C. lactis* eggs and *T. latifolia* pollen is also  
308 essential. Following these trials, further experiments should be scheduled to evaluate the feasibility of applying  
309 this mixed diet in mass-rearing.

310 Our results highlight the superiority of a combination of frozen *C. lactis* eggs and *T. latifolia* pollen in rearing *T.*  
311 *montdorensis* under laboratory conditions. This approach may not only support an effective mass-rearing  
312 program but also mitigate concerns associated with contamination and infestation by living *C. lactis*, as reported  
313 in previous studies (Çobanoğlu, 2009; Hubert et al., 2015; Jan Hubert, 2011). However, the duration for which  
314 prey mites are frozen could influence the reproduction outcomes of predatory mites. Moreover, extending this  
315 study to other predatory mites that consume *C. lactis*, such as *A. swirskii* (Asgari et al., 2020; Nguyen et al.,  
316 2013; San et al., 2020), *Phytoseiulus persimilis* (Tabic et al., 2022), *N. cucumeris* (Ji et al., 2015), and  
317 *Neoseiulus californicus* (Sweelam & Nasreldin, 2023; Wang et al., 2024) could provide broader implications for  
318 biological control strategies across different agricultural contexts.

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325

326

### 327 **Author contributions**

328 Conceptualization: Hung Nguyen, Maciej Maselko, Bishwo Mainali, Binh Nguyen; Methodology: Hung  
329 Nguyen, Maciej Maselko, Bishwo Mainali, Binh Nguyen; Formal analysis and investigation: Hung Nguyen;  
330 Writing-original draft preparation: Hung Nguyen; Writing-review and editing: Maciej Maselko, Hung Nguyen,  
331 Binh Nguyen, Bishwo Mainali; Funding acquisition: Maciej Maselko; Resources: Department of Agriculture,  
332 Fisheries and Forestry; Supervision: Maciej Maselko

### 333 **Declarations**

334 **Conflict of interest:** The authors have no financial or proprietary interests in any material discussed in this  
335 article.

336 **Informed consent:** The study does not contain any person's data. Hence, informed consent is not applicable.

337 **Ethics:** Biosafety approval was granted by the Macquarie University Institutional Biosafety Committee.

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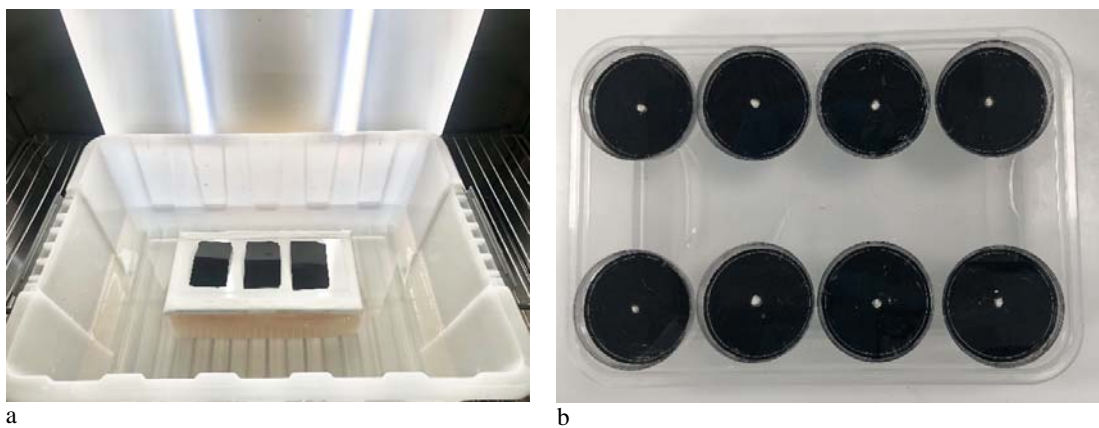
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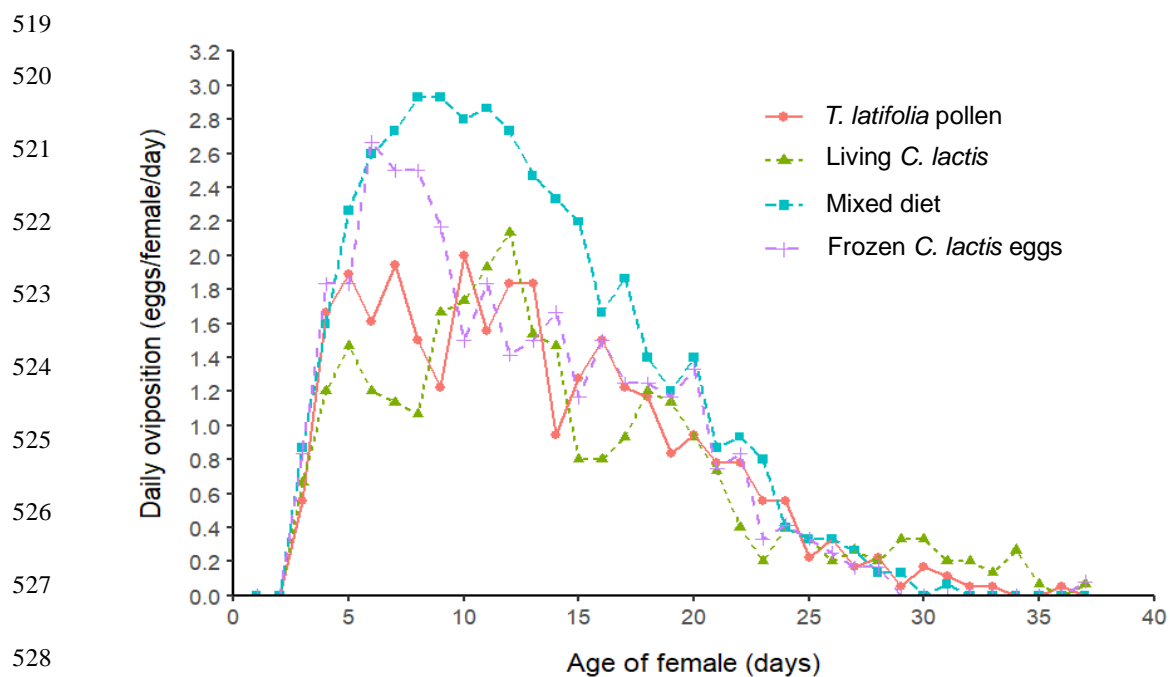
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518

**Fig. 1** Rearing arenas of *Transeius montdorensis*. a. Stock colony, b. Rearing microcosms



529

**Fig. 2** Daily oviposition rate of *Transeius montdorensis* fed on four diets at 25 °C

530 **Table 1** Developmental time (days) of the egg and immature stages of *Transeius montdorensis*  
 531 fed on four diets at 25 °C

Diet	n	Developmental stage				
		Egg	Larva	Protonymph	Deutonymph	Total immature
<b>Females</b>						
<i>T. latifolia</i> Pollen	23	1.78 ± 0.10a	0.83 ± 0.06a	1.54 ± 0.07a	1.43 ± 0.08a	5.59 ± 0.12ab
Living <i>C. lactis</i>	19	2.00 ± 0.08a	0.87 ± 0.05a	1.39 ± 0.08a	1.79 ± 0.15a	6.05 ± 0.21a
Mixed diet	20	1.72 ± 0.15a	0.83 ± 0.05a	1.60 ± 0.08a	1.15 ± 0.05b	5.30 ± 0.14b
Frozen <i>C. lactis</i> eggs	19	1.63 ± 0.13a	0.84 ± 0.05a	1.34 ± 0.08a	1.55 ± 0.08a	5.37 ± 0.14ab
$\chi^2$		4.28	0.50	6.71	20.87	9.99
df		3	3	3	3	3
<i>P</i>		0.233	0.919	0.082	<0.001	0.019
<b>Males</b>						
<i>T. latifolia</i> Pollen	13	2.19 ± 0.17a	0.62 ± 0.06c	1.35 ± 0.10a	1.38 ± 0.08ab	5.54 ± 0.14b
Living <i>C. lactis</i>	17	2.18 ± 0.07a	0.88 ± 0.05ab	1.38 ± 0.08a	1.53 ± 0.08a	5.97 ± 0.09a
Mixed diet	12	1.96 ± 0.23ab	0.71 ± 0.07bc	1.33 ± 0.07a	1.38 ± 0.09ab	5.38 ± 0.20bc
Frozen <i>C. lactis</i> eggs	13	1.65 ± 0.19b	0.96 ± 0.09a	1.23 ± 0.07a	1.15 ± 0.07b	5.00 ± 0.17c
$\chi^2$		6.99	12.42	1.67	10.10	20.04
df		3	3	3	3	3
<i>P</i>		0.722	0.006	0.643	0.018	<0.001

532 Means ± SE within a column and sex followed by the same letter are not significantly different.

533 *n* number of individuals reaching the adult stage.

534

**Table 2** Reproduction and life table parameters of *Transeius montdorensis* fed on four diets at 25 °C

Parameter	<i>T. latifolia</i> pollen (n = 18)	Living <i>C. lactis</i> (n = 15)	Mixed diet (n = 15)	Frozen <i>C. lactis</i> eggs (n = 12)
Preoviposition period (days)	2.61 ± 0.12a (n=23)	2.63 ± 0.17a (n=19)	2.08 ± 0.11b (n=19)	2.28 ± 0.12ab (n=18)
Oviposition period (days)	21.4 ± 1.12a	23.7 ± 1.62a	22.0 ± 0.92a	21.8 ± 1.50a
Female longevity (days)	26.8 ± 1.22a	30.9 ± 1.83a	30.00 ± 1.09a	30.1 ± 1.22a
Female proportion of the progeny (%)	0.65 ± 0.03a	0.46 ± 0.05b	0.65 ± 0.03a	0.59 ± 0.04ab
Oviposition rate (eggs/female/day)	1.25 ± 0.05b	1.00 ± 0.06c	1.59 ± 0.07a	1.24 ± 0.09b
Total number of eggs laid (eggs/female)	29.6 ± 1.55b	27.3 ± 1.38c	43.1 ± 2.07a	33.2 ± 2.10b
Offspring survival proportion (%)	0.94 ± 0.01a	0.87 ± 0.01b	0.94 ± 0.01a	0.94 ± 0.01a
Net reproductive rate $R_0$ (females/female)	17.50 ± 0.07b	10.85 ± 0.10c	26.50 ± 0.13a	18.41 ± 0.17ab
Generation time $T_c$ (days)	11.96 ± 0.02b	12.79 ± 0.05a	12.03 ± 0.02b	11.47 ± 0.04c
Intrinsic rate of increase $r_m$ (females/female/day)	0.36 ± 0.01a	0.26 ± 0.01b	0.41 ± 0.02a	0.38 ± 0.02a

535 Means ± SE within a row followed by the same letter are not significantly different.

536 *n* number of reproducing females observed