



# Performance of four species of phytoseiid mites on artificial and natural diets



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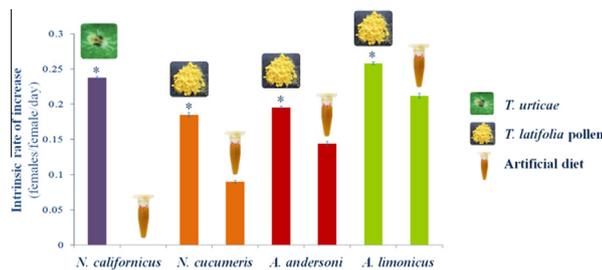
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## HIGHLIGHTS

- Population growth of four phytoseiids was studied on natural and artificial diets.
- A liquid artificial diet was enriched with extract of *Artemia franciscana* cysts.
- Growth rates were lower on artificial diet than on natural food, but survival was similar.
- The more generalist predators performed better on the artificial diet.
- The artificial diet has potential for the mass rearing or for use in the crop.

## GRAPHICAL ABSTRACT



\* Asterisks indicate significant differences among diets within a species ( $P < 0.05$ )

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## ABSTRACT

The phytoseiid mites *Neoseiulus californicus* (McGregor), *Neoseiulus cucumeris* (Oudemans), *Amblyseius andersoni* Chant, and *Amblydromalus limonicus* Garman & McGregor (Acari: Phytoseiidae) are being used on a commercial scale as biological control agents of spider mites, thrips and whiteflies in protected crops. The present laboratory study was conducted to determine the life table parameters of these predatory mites when presented with a liquid artificial diet (consisting of honey, sucrose, tryptone, yeast extract and egg yolk) enriched with extract of dry decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae), as compared to feeding on two-spotted spider mites *Tetranychus urticae* Koch (*N. californicus*) or *Typha latifolia* L. pollen (*N. cucumeris*, *A. andersoni* and *A. limonicus*). Diet had no influence on the immature survival rate, ranging from 92% to 98% for all species. Female developmental times were significantly shorter for predators offered spider mites or pollen than for those fed the artificial diet, except in *A. limonicus*. The fecundity of *N. californicus*, *N. cucumeris* and *A. limonicus* females given spider mites or pollen was significantly higher than that of females presented with the artificial diet, whereas no differences among diets were observed in *A. andersoni*. When *N. californicus* females were fed on the artificial diet, none of their offspring succeeded in reaching adulthood. Our findings indicate the potential of this artificial diet for use as a supplemental food source to maintain populations in the crop after release or for use in the mass production, especially for the more generalist predatory mites *A. andersoni* and *A. limonicus*.

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## 1. Introduction

Phytoseiid predatory mites are important biocontrol agents of tetranychid mites and small, soft-bodied insects like thrips and whiteflies (Chant, 1985). In augmentation biological control, large numbers of predaceous mites are released in the field (Collier and Van Steenwyk, 2004; Stinner, 1977). Hence, a cost-effective method for their mass-rearing is an essential prerequisite (van Lenteren, 2003). Rearing phytoseiid mites on plant materials infested with natural prey has several disadvantages, such as large space requirements, inconsistent yields of predators, harvesting difficulties and variable results with different species (McMurtry and Scriven, 1965). Rearing procedures based on factitious prey like storage mites (Bolckmans and van Houten, 2006; Zhang, 2003) also involve space and labor to maintain large parallel cultures of the factitious prey. Further, there may be health risks for workers in production facilities or releases in the crop caused by allergens associated with the factitious mite prey (Bolckmans and van Houten, 2006; Fernandez-Caldas et al., 2007). The availability of an adequate artificial diet could eliminate many of the above-mentioned problems associated with the mass production of predatory mites (Kennett and Hamai, 1980). In addition, these artificial diets may be useful as food supplements to support predator populations after release in the crop (Wade et al., 2008).

Several artificial diets have been developed for phytoseiid mites, but the results were mostly inferior to those on natural or factitious prey. McMurtry and Scriven (1966) reported longer developmental times and lower oviposition rates when four phytoseiids (*Amblydromalus limonicus* Garman and McGregor, *Amblyseius hibisci* Chant), *Typhlodromus occidentalis* Nesbitt and *Typhlodromus rickeri* Chant (Acari: Phytoseiidae) were fed on various artificial diets compared with mite prey and pollen as food sources. Shehata and Weismann (1972) tested three artificial diets for the specialist spider mite predator *Phytoseiulus persimilis* Athias-Henriot. Their results indicated that the larvae could develop to adults but the females failed to produce viable eggs. Kennett and Hamai (1980) investigated oviposition rate and developmental capacity of 9 predaceous mites (*A. hibisci*, *A. limonicus*, *Amblyseius largoensis* (Muma), *Metaseiulus pomoides* Schuster & Pritchard, *T. occidentalis*, *Typhloseiopsis arboreus* (Chant), *Typhloseiopsis pyri* Scheuten, *P. persimilis*, and *Iphiseius degenerans* (Berlese)) fed on artificial and natural diets. The authors reported that complete development and oviposition occurred for seven out of nine species when fed on an artificial diet consisting of bee honey, sugar, yeast flakes, yeast hydrolysate, enzymatic casein hydrolysate and fresh egg yolk. Oviposition rates of all species fed on the artificial diet were lower than those on a natural diet. Ochieng et al. (1987) reported that *Amblyseius teke* Pritchard and Baker could complete more than 25 generations when reared on a liquid diet composed of bee honey, milk powder, egg yolk and Wesson's salt. Abou-Awad et al. (1992) noted that the predaceous mites *Amblyseius gossipi* El-Badry and *Amblyseius swirskii* Athias-Henriot developed and reproduced successfully on artificial diets composed of yeast, milk, cysteine, proline, arginine, sucrose, glucose, streptomycin sulfate and sorbic acid. However, fecundity of both species fed on the artificial diet was lower than on natural prey, although the eggs showed no abnormalities. Shih et al. (1993) conducted experiments to investigate the responses of *Euseius ovalis* (Evans) to natural food resources and two artificial diets. Immature development was successful in the first generation but offspring was not able to complete its life cycle when maintained on the artificial diets. The females of *E. ovalis* fed on artificial diets showed a shorter oviposition period, lower daily and total reproductive rates, and shorter longevity than those fed on natural diets. Ogawa and Osakabe (2008) investigated the development and survival of *Neoseiulus californicus* (McGregor) on an artificial diet. The phytoseiid successfully developed on the artificial diet, but only few eggs were deposited.

In our previous studies we found that artificial diets enriched with an extract of dry decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae) or pupal hemolymph of the Chinese oak silkworm (*Antheraea pernyi* (Guérin-Méneville)) supported development and reproduction of the generalist predatory mite *A. swirskii*. The females fed on these two artificial diets displayed higher intrinsic rates of increase than those fed on several natural prey and performed as well as those reared on the factitious prey *Carpoglyphus lactis* L. (Acari: Carpocephidae), which is routinely used in the mass rearing of this phytoseiid (Nguyen et al., 2014a, 2013). The objectives of the present study were to assess the suitability of the artificial diet enriched with *A. franciscana* as an alternative food for several other economically important predatory mites by performing full life table studies under controlled laboratory conditions. The phytoseiids selected for testing belong to different types based on their level of food specialization (McMurtry et al., 2013): *N. californicus* is a selective predator of tetranychid mites (type II), whereas *A. andersoni* Chant, *N. cucumeris* (Oudemans), and *A. limonicus* are more generalist predators (type III).

## 2. Materials and methods

### 2.1. Stock colonies of predatory mites

Laboratory cultures of *N. cucumeris* and *A. limonicus* were initiated with specimens supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands) and *A. andersoni* was supplied by Biobest N.V. (Westerlo, Belgium). The mites were reared on green plastic arenas (10 × 10 × 0.3 cm) (Multicel, SEDPA, France), placed on a wet sponge in a plastic tray containing water (Nguyen et al., 2013). The edges of the arenas were covered with tissue paper immersed in the water to provide moisture and deter the mites from escaping. Every two days the mites were fed with fresh cattail pollen (*Typha latifolia* L.), which was also supplied by Koppert B.V. and stored at −18 °C. For the experiments, pollen was thawed and kept in a refrigerator at 5 °C for max. 1 week. A small piece of sewing thread was placed on the arenas to serve as an oviposition substrate. Every two days the eggs were collected and transferred to new arenas.

A culture of *N. californicus* was initiated with mites acquired from Koppert B.V. and was reared on kidney bean leaves heavily infested with two-spotted spider mites (*Tetranychus urticae* Koch). The leaves were placed upside down on a layer of water-saturated cotton in a glass petri dish (ϕ 133 mm), with an extra cotton layer on the leaf edges to provide free water and prevent the mites from escaping.

Predatory mites were cultured in a growth chamber set at 25 ± 1 °C, 70 ± 5% RH and a 16:8 h (L:D) photoperiod.

### 2.2. Preparation of artificial diet

Artificial diets were prepared according to Nguyen et al. (2014a): 80% basic artificial diet supplemented with 20% (w/w) extract of dry decapsulated *A. franciscana* cysts, which were provided by the *Artemia* Reference Center of Ghent University (Ghent, Belgium) and originated from the Great Salt Lake (Utah, USA). The basic artificial diet was composed of 5% honey (Meli N.V., Veurne, Belgium), 5% sucrose (MP Biomedicals LLC, Illkirch, France), 5% tryptone (a pancreatic digest of casein; Fluka Analytical, Sigma-Aldrich Co., St. Louis, USA), 5% yeast extract (Duchefa, Haarlem, The Netherlands), 10% fresh hen's egg yolk, and 70% distilled water, and was prepared according to Nguyen et al. (2013). The diet was dispensed into 2 ml Eppendorf tubes and stored at −18 °C.

### 2.3. Experimental setup

Eight hours before start of the experiments, new pieces of sewing thread were placed in the stock colony of *N. cucumeris*, *A. limonicus* and *A. andersoni* or fifty females of *N. californicus* were transferred to new bean leaves infested with mixed life stages of *T. urticae*. Deposited eggs (less than 8 h old) were transferred individually to the rearing microcosms that were modified from Munger cells as described by Ogawa and Osakabe (2008), Nguyen et al. (2013). Development and reproduction of the tested phyto-seiids on the artificial diet was compared with that on a natural food source (spider mites for *N. californicus* or cattail pollen for *N. cucumeris*, *A. andersoni* and *A. limonicus*). All food sources were offered ad libitum from the larval stage of the predator on and were refreshed every 2 days. For the artificial diet, approximately 2  $\mu$ l of diet was absorbed on a small piece of filter paper (2  $\times$  2 mm) placed on the bottom board of the rearing microcosms. To obtain data on the duration of each developmental stage of the predatory mites and on mortality and escape rates, observations were made every 24 h until all individuals had reached adulthood. The developmental stage of each individual was determined based on the presence of exuviae in the Munger cells. After completing immature development, each female was paired with a male that was reared on the same diet as the female. Males that died during the experiment were replaced with males that had been fed on the same diet. Adults were observed daily to determine the preoviposition and oviposition period, longevity and fecundity. Eggs from females of the same age were transferred to new Munger cells and fed on the same diet as their parents in order to determine the sex ratio of the offspring; all eggs laid were collected throughout the lifetime of all tested females. Mites that escaped or died due to unnatural causes were excluded from data analysis. Females that died before laying eggs were excluded from calculation of reproductive parameters. The experiments were done in a growth chamber at 23  $\pm$  1  $^{\circ}$ C, 65  $\pm$  5% RH and a 16:8 h (L:D) photoperiod.

### 2.4. Life table parameters

The intrinsic rate of increase ( $r_m$ ) was calculated according to the formula of Birch (1948):

$$\sum l_x m_x e^{-r_m x} = 1$$

**Table 1**

Results of a logistic regression and a two-way ANOVA indicating the effect of diet (artificial diet versus *Typha latifolia* or *Tetranychus urticae*) and phytoseiid species (*Neoseiulus californicus*, *Neoseiulus cucumeris*, *Amblyseius andersoni*, *Amblydromalus limonicus*) on immature survival and developmental time, reproduction parameters, sex ratio and life table parameters.

Parameter	Diet			Species			Diet $\times$ species			Error term df
	F	df	P	F	df	P	F	df	P	
Immature survival <sup>a</sup>	0.11	1	0.739	3.09	3	0.378	1.25	3	0.74	–
Female development time <sup>b</sup>	248.85	1	<0.001	86.19	3	<0.001	41.34	3	<0.001	227
Male development time <sup>b</sup>	123.29	1	<0.001	42.48	3	<0.001	4.85	3	0.003	193
Preoviposition period <sup>b</sup>	66.80	1	<0.001	25.66	3	<0.001	23.93	3	<0.001	204
Oviposition period <sup>b</sup>	24.13	1	<0.001	113.41	3	<0.001	22.49	3	<0.001	204
Female longevity <sup>b</sup>	7.66	1	0.006	96.34	3	<0.001	7.81	3	<0.001	204
Oviposition rate <sup>b</sup>	502.47	1	<0.001	164.92	3	<0.001	34.45	3	<0.001	204
Total number of eggs <sup>b</sup>	116.51	1	<0.001	15.32	3	<0.001	14.59	3	<0.001	204
Female proportion of the progeny <sup>a</sup>	0.44	1	0.505	17.47	3	<0.001	9.76	2	0.008	–
$R_0$ <sup>b</sup>	47.80	1	<0.001	19.16	3	<0.001	11.38	2	<0.001	195
$T$ <sup>b</sup>	410.70	1	<0.001	405.66	3	<0.001	153.48	2	<0.001	195
$r_m$ <sup>b</sup>	860.90	1	<0.001	444.86	3	<0.001	56.88	2	<0.001	195

Life table parameters ( $r_m$ ,  $R_0$  and  $T$ ) and female proportion of the progeny were not calculated for *N. californicus* fed on artificial diet.

<sup>a</sup> Probit.

<sup>b</sup> Two-way ANOVA.

where  $x$  equals the female age from the time of hatching (days),  $l_x$  is the age specific survival of the females at age  $x$  and  $m_x$  is the number of daughters produced per female at age  $x$ . The latter parameter is obtained by multiplying the mean number of eggs laid per female by the proportion of female offspring produced at age  $x$ . The Jackknife procedure was used according to Meyer et al. (1986), Hulting et al. (1990) to calculate the standard error of  $r_m$ . Other parameters calculated according to Maia et al. (2000) were the generation time  $T$ , i.e. mean time span between the birth of individuals of a generation and that of the next generation (days),

$$T = \frac{\sum x l_x m_x}{\sum l_x m_x}$$

and the net reproductive rate,  $R_0$ , i.e. the mean number of female offspring produced per female (females/female)

$$R_0 = \sum l_x m_x$$

### 2.5. Statistical analysis

Two-way analysis of variance (ANOVA) (IBM, SPSS Statistics 20) was conducted to evaluate the effects of diet and species on the duration of the immature stages, preoviposition and oviposition period, daily and total oviposition, adult longevity and life table parameters. When an interaction was detected between the main factors, means were compared among species and a pairwise multiple comparison procedure was used (Kutner et al., 2005). When a Kolmogorov–Smirnov test indicated that data were normally distributed, the pairwise comparisons among diets in each species were analyzed using Student  $t$ -tests. When data were not normally distributed, a nonparametric Mann–Whitney  $U$  test was used. Generalized linear model with a probit (log odds) link function and a binomial error distribution was used to compare immature survival rates and progeny sex ratios. Each test consisted of a regression coefficient that was calculated and tested for being significantly different from zero, for which  $P$ -values are presented (McCullagh and Nelder, 1989). In all tests,  $P$ -values smaller than or equal to 0.05 were considered significant.

## 3. Results

Two-way ANOVA indicated no interaction between diet and species for immature survival (Table 1), whereas interactions were

**Table 2**  
Immature survival and developmental time of different phytoseiid mites fed on artificial diet versus natural food sources.

Species	Diet	Proportion surviving	Developmental duration (days) <sup>a</sup>			
			n	Females	N	Males
<i>N. californicus</i>	<i>T. urticae</i>	0.92 ± 0.04 a	32	5.09 ± 0.05 a	24	5.38 ± 0.18 a
	Artificial	0.96 ± 0.03 a	20	7.90 ± 0.31 b	28	6.46 ± 0.15 b
	$\chi^2/U$	0.818		4.500		81.500
	$df/Z$	1		-6.546		-4.979
	P	0.366		<0.001		<0.001
<i>N. cucumeris</i>	<i>T. latifolia</i>	0.98 ± 0.02 a	33	6.67 ± 0.09 a	22	6.45 ± 0.11 a
	Artificial	0.98 ± 0.02 a	29	9.00 ± 0.21 b	24	8.25 ± 0.11 b
	$\chi^2/U$	0.001		0.000		5.000
	$df/Z$	1		-6.988		-5.927
	P	0.979		<0.001		<0.001
<i>A. andersoni</i>	<i>T. latifolia</i>	0.96 ± 0.03 a	27	5.74 ± 0.10 a	26	5.81 ± 0.12 a
	Artificial	0.98 ± 0.02 a	34	6.79 ± 0.12 b	20	6.55 ± 0.14 b
	$\chi^2/U$	0.338		118.000		113.500
	$df/Z$	1		-5.387		-3.523
	P	0.561		<0.001		<0.001
<i>A. limonicus</i>	<i>T. latifolia</i>	0.98 ± 0.02 a	30	5.77 ± 0.11 a	30	5.50 ± 0.17 a
	Artificial	0.97 ± 0.02 a	30	5.77 ± 0.09 a	27	6.41 ± 0.12 b
	$\chi^2/U$	0.371		443.000		150.500
	$df/Z$	1		-0.127		-4.304
	P	0.542		0.899		<0.001

n and N: number of tested female and male individuals, respectively.

<sup>a</sup> Means ± SE; means within a column and a species followed by the same letter are not significantly different ( $P > 0.05$ ), according to Probit (Wald Chi-square) test (Immature survival), or Mann–Whitney  $U$  test (Female and male developmental time).  $\chi^2$ -,  $df$ - and  $P$ -values refer to Probit (Wald Chi-square) test,  $U$ -,  $Z$ -, and  $P$ -values refer to Mann–Whitney  $U$  test.

**Table 3**  
Reproductive parameters of several phytoseiid mites fed on artificial diet versus natural food sources.

Species	Diets	n	Preoviposition period (days) <sup>a</sup>	Oviposition period (days) <sup>a</sup>	Female longevity (days) <sup>a</sup>	Oviposition rate (eggs/female/day) <sup>a</sup>	Total number of eggs (eggs/female) <sup>a</sup>	Female proportion of the progeny <sup>a</sup>
<i>N. californicus</i>	<i>T. urticae</i>	32	2.22 ± 0.10 a	19.44 ± 0.70 a	34.25 ± 2.40 a	2.76 ± 0.07 a	53.34 ± 2.05 a	0.63 ± 0.02
	Artificial	10	14.30 ± 4.84 b	15.50 ± 3.40 a	38.00 ± 6.10 a	0.85 ± 0.09 b	12.90 ± 3.41 b	–
	$U/t \chi^2$		1.000	1.135	-0.688	15.035	9.763	
	$Z/df$		-5.482	9.772	40	40	40	
	P		<0.001	0.283	0.495	<0.001	<0.001	
<i>N. cucumeris</i>	<i>T. latifolia</i>	33	2.52 ± 0.11 a	30.97 ± 1.43 a	52.64 ± 3.37 a	1.51 ± 0.06 a	45.45 ± 2.26 a	0.73 ± 0.02 a
	Artificial	27	4.00 ± 0.16 b	50.52 ± 3.27 b	71.56 ± 4.22 b	0.52 ± 0.02 b	25.78 ± 1.68 b	0.65 ± 0.02 b
	$U/t \chi^2$		88.500	-5.475	-3.550	14.989	7.003	9.226
	$Z/df$		-5.531	35.747	58	38.894	56.053	1
	P		<0.001	<0.001	0.001	<0.001	<0.001	0.002
<i>A. andersoni</i>	<i>T. latifolia</i>	27	2.26 ± 0.11 a	33.07 ± 2.15 a	55.70 ± 3.30 a	1.49 ± 0.07 a	48.56 ± 3.44 a	0.70 ± 0.01 a
	Artificial	32	4.34 ± 0.18 b	50.91 ± 2.02 b	68.16 ± 2.62 b	0.92 ± 0.03 b	45.97 ± 2.10 a	0.71 ± 0.01 a
	$U/t \chi^2$		37.000	-6.034	-2.993	7.736	0.642	0.308
	$Z/df$		-6.189	57	57	40.001	43.942	1
	P		<0.001	<0.001	0.004	<0.001	0.524	0.579
<i>A. limonicus</i>	<i>T. latifolia</i>	30	1.73 ± 0.08 a	16.40 ± 0.99 a	22.37 ± 1.83 a	2.57 ± 0.06 a	42.20 ± 2.81 a	0.65 ± 0.02 a
	Artificial	21	1.90 ± 0.07 a	11.38 ± 0.84 b	13.10 ± 0.86 b	1.90 ± 0.08 b	22.05 ± 2.40 b	0.69 ± 0.03 a
	$U/t \chi^2$		261.000	3.853	4.583	6.458	5.447	0.971
	$Z/df$		-1.503	48.985	40.254	49	48.964	1
	P		0.133	<0.001	<0.001	<0.001	<0.001	0.324

n: number of tested females.

<sup>a</sup> Means ± SE; means within a column and a species followed by the same letter are not significantly different ( $P > 0.05$ ) according to Mann–Whitney  $U$  test (preoviposition period), Student's  $t$ -test (oviposition period, female longevity, oviposition rate, total number of eggs) or Probit (Wald Chi-square) test (female proportion of progeny).  $U$ -,  $Z$ -, and  $P$ -values refer to Mann–Whitney  $U$  test;  $t$ -,  $df$ - and  $P$ -values refer to Student's  $t$ -test;  $\chi^2$ -,  $df$ - and  $P$ -values refer to Probit (Wald Chi-square) test.

found to be significant for all other parameters. Diet had no influence on the immature survival rate of all tested species (Table 2). Female developmental times were significantly shorter on natural diets (*T. urticae* or *T. latifolia*) than on the artificial diet, except for *A. limonicus*. In all species, males developed faster on natural diets than on the artificial diet.

Preoviposition periods of *A. limonicus* females fed on *T. latifolia* and the artificial diet were similar, whereas females of the other species fed on their respective natural diets had significantly

shorter preoviposition periods than those fed on the artificial diet (Table 3). Oviposition period of *N. californicus* did not differ among diets, whereas in *A. limonicus* females reared on artificial diet had a shorter oviposition period than those reared on cattail pollen. In contrast, *N. cucumeris* and *A. andersoni* fed on the artificial diet had longer oviposition periods than those reared on cattail pollen. Whereas no difference in female longevity was observed among diets for *N. californicus*, females reared on artificial diet lived shorter in *A. limonicus* and *A. andersoni* and longer in *N. cucumeris*, than their

**Table 4**  
Life table parameters of different phytoseiid mites fed on artificial diet versus natural food sources.

Species	Diets	n	Net reproductive rate ( $R_0$ , females per female) <sup>a</sup>	Generation time (T, days) <sup>a</sup>	Intrinsic rate of increase ( $r_m$ , females/female/day) <sup>a</sup>
<i>N. californicus</i>	<i>T. urticae</i>	32	29.45 ± 1.18	14.21 ± 0.17	0.238 ± 0.002
	Artificial	–	–	–	–
<i>N. cucumeris</i>	<i>T. latifolia</i>	33	32.97 ± 1.74 a	18.95 ± 0.27 a	0.185 ± 0.003 a
	Artificial	27	17.16 ± 1.04 b	31.74 ± 0.71 b	0.090 ± 0.002 b
<i>t</i>			7.804	–16.850	24.357
<i>df</i>			51.076	33.386	58
<i>P</i>			<0.001	<0.001	<0.001
<i>A. andersoni</i>	<i>T. latifolia</i>	27	33.74 ± 2.62 a	18.03 ± 0.34 a	0.195 ± 0.002 a
	Artificial	32	33.16 ± 1.82 a	24.29 ± 0.37 b	0.144 ± 0.003 b
<i>t</i>			0.185	–12.429	13.653
<i>df</i>			47.776	57	57
<i>P</i>			0.854	<0.001	<0.001
<i>A. limonicus</i>	<i>T. latifolia</i>	30	26.96 ± 1.67 a	12.79 ± 0.19 a	0.258 ± 0.002 a
	Artificial	21	13.83 ± 1.21 b	12.39 ± 0.27 a	0.212 ± 0.003 b
<i>t</i>			6.385	1.248	12.533
<i>df</i>			48.169	49	49
<i>P</i>			<0.001	0.218	<0.001

n: number of tested females.

<sup>a</sup> Means ± SE; means within a column and a species followed by the same letter are not significantly different ( $P > 0.05$ ) according to Student's *t*-test.

counterparts fed on pollen. The total number of eggs deposited by *N. californicus*, *N. cucumeris* and *A. limonicus* females reared on natural diets was significantly higher than that of females maintained on the artificial diet but fecundity of *A. andersoni* was not affected by diet. Whereas the proportions of female offspring in *A. andersoni* and *A. limonicus* were similar on artificial and natural diets, in *N. cucumeris* a lower percentage of female offspring was observed on the artificial diet than on cattail pollen. Because the offspring of *N. californicus* fed on the artificial diet did not develop successfully to adulthood, the female proportion of the progeny and life table parameters were not calculated for this treatment (see Table 4).

Net reproductive rate ( $R_0$ ) of *N. cucumeris* and *A. limonicus* females offered pollen was significantly higher than that of females given the artificial diet, whereas in *A. andersoni* this parameter did not differ among diets. Females reared on pollen had a shorter generation time than those on artificial diet for *N. cucumeris* and *A. andersoni*, whereas there were no differences for *A. limonicus*. For all species, a higher intrinsic rate of increase ( $r_m$ ) was calculated when the mites were maintained on their respective natural foods as compared to the artificial diet (Table 4).

#### 4. Discussion

The developmental and reproductive performances of the four predatory mites studied were differentially affected by diet. Where differences were observed, the mites developed faster and produced more eggs on their natural diets (spider mites for *N. californicus* or cattail pollen for *N. cucumeris*, *A. andersoni* and *A. limonicus*) than on the artificial diet. Overall, the percentage decrease in developmental rate, oviposition rate and intrinsic rate of increase of the predatory mites fed on artificial diet as compared with natural diet was greatest in *N. californicus*, intermediate in *N. cucumeris* and smallest in *A. andersoni* and *A. limonicus*. This order reflects the level of polyphagy of the different species. *N. californicus* is classified as a selective predator of tetranychid mites (McMurtry and Croft, 1997), which may explain its relatively poor performance on the artificial diet. The more polyphagous species *A. andersoni* and *A. limonicus*, which readily feed and reproduce also on pollens (Duso and Camporese, 1991; van Houten et al., 1995), were also able to develop and reproduce well on the artificial diet.

Our findings are consistent with those reported by McMurtry and Scriven (1966) who tested several artificial diets for four phytoseiid mites. In the latter study, the studied type II phytoseiids of the genus *Typhlodromus* (*T. occidentalis* and *T. rickeri*) did not perform well on the tested artificial diets, whereas better results were obtained for two *Amblyseius* species (*A. limonicus* and *A. hibisci*) characterized by a more generalist feeding habit.

The artificial diet in the present study supported development of *N. californicus* well, with 96% of the mites reaching adulthood. Immature development of females fed on the artificial diet was longer than that on *T. urticae* but was similar to that reported by Rencken and Pringle (1998), Kim et al. (2009) when the predatory mite was reared at 25 °C with mixed stages of *T. urticae*. *N. californicus* fed on the artificial diet at 23 °C in our study had similar developmental rates to those of the predator reared at 25 °C on an artificial diet formulated by Ogawa and Osakabe (2008). However, whereas only few eggs were produced on the diet used by Ogawa and Osakabe (2008), *N. californicus* females in the present study produced 0.85 eggs/day on a similar diet supplemented with *A. franciscana*. Nonetheless, egg production on the artificial diet was substantially lower than on the natural prey, *T. urticae*, and offspring could not develop successfully to adulthood.

To our knowledge, this is the first time that an artificial diet was tested for *N. cucumeris*. The predatory mite developed successfully on the artificial diet, with an immature survival of 98% and produced viable eggs, the offspring of which succeeded in reaching adulthood (>75% survival). A positive intrinsic rate of increase of 0.09 females/female/day<sup>-1</sup> was calculated on the artificial diet, showing that *N. cucumeris* is still able to maintain its population when exclusively fed on the artificial diet. Thus, whereas the diet is not suitable for the mass production of this phytoseiid, it still may have some potential to be used as a food supplement to sustain populations of *N. cucumeris* in the crop (Nguyen et al., 2014b). Our results also show the suitability of *T. latifolia* pollen as a supplemental food for *N. cucumeris*. The intrinsic rate of increase of *N. cucumeris* fed on *T. latifolia* pollen (0.185 females/female/day) is higher than the values reported for the phytoseiid when fed on pollen of apple, birch, Christmas cactus, horse-chestnut, maize, or tulip (0.149, 0.127, 0.155, 0.180, 0.101 or 0.167 females/female/day, respectively) at 25 °C (Ranabhat et al., 2014), or on castor pollen and *T. urticae* (0.179 and 0.147 females/female/day,

respectively) at 25 °C (van Rijn and Tanigoshi, 1999). The  $r_m$  value of *N. cucumeris* on *T. latifolia* pollen calculated in our study was slightly lower than the value of 0.208 reported by van Rijn and Tanigoshi (1999) when the predator was offered pollen of broad bean (*Vicia faba* L.), but this difference may in part be attributed to the higher experimental temperature (25 °C) in the latter study.

*Amblyseius andersoni* females reared on the artificial diet produced less eggs per day than those given *T. latifolia* pollen. However, the artificial diet prolonged the oviposition period, so that an equal amount of eggs was produced on both diets. The life time fecundity of *A. andersoni* females reared on *T. latifolia* and the artificial diet (48.56 and 45.97 eggs/female, respectively) in this study is substantially higher than the total fecundities reported by Lorenzon et al. (2012) for females of the species fed on *T. latifolia* pollen, *Eotetranychus carpini* (Oudemans), *Panonychus ulmi* (Koch), *Colomerus vitis* (Pagenstecher), or the mycelium of grape downy mildew *Plasmopara viticola* (Berk. & Curt.) Berl. (25.7, 24.2, 22.2, 25.6, and 9.3 eggs/female, respectively).

The artificial diet fully supported the development and reproduction of *A. limonicus*. Developmental duration of females was similar on *T. latifolia* pollen and the artificial diet (5.8 days). Development was shorter than that observed on dry decapsulated cysts of *A. franciscana* and *E. kuehniella* eggs at the same temperature (6.58 and 6.85 days, respectively) (Vangansbeke et al., 2014). The oviposition rate of *A. limonicus* on the present artificial diet (1.90 eggs/female/day) was higher than that on the artificial diet designed by Kennett and Hamai (1980) (1.22 eggs/female/day) and on the diets (sucrose, molasses, yeast + sucrose and yeast + molasses) used by McMurtry and Scriven (1966) (0.11, 0.25, 1.13 and 1.18 egg/female/day, respectively).

The use of food supplements, consisting of alternative or artificial foods, can increase the abundance and impact of arthropod natural enemies in crops where target prey or plant foods like pollen and nectar are absent or only present at low densities (Wade et al., 2008). While various supplemental foods appear to have potential for enhancing establishment of insect natural enemies, only few have been reported to support beneficial Acarina (Wade et al., 2008). For generalist predatory mites, pollen has been reported to be an adequate supplemental food source to enhance the biological control of thrips and whiteflies on cucumber (Nomikou et al., 2010; van Rijn et al., 2002). *Typha angustifolia* L. pollen (Nutrimite™, Biobest NV) is commercially available to support pollen-feeding phytoseiids in the crop. Drawbacks associated with the application of pollen are the relatively high costs (Messelink et al., 2014) and the potential beneficial effects on pollen-feeding pests, such as *Frankliniella occidentalis* Pergande (Ramakers, 1995). Certain animal foods also have potential to support populations of phytoseiid mites, such as sterilized eggs of *Ephestia kuehniella* Zeller and decapsulated cysts of *A. franciscana* (Vangansbeke et al., 2014). Finding inexpensive alternative food sources is one of the major opportunities for enhancing biological control in protected crops (Messelink et al., 2014). Several artificial diets have been developed with the aim to reduce the production cost of phytoseiid mites or provide supplemental foods for supporting their populations after release in the crop. For example, a simple mixture of yeast, sugar and protein applied on chrysanthemum increased population levels of *A. swirskii* (Messelink et al., 2009). In our previous studies, artificial diets enriched with *A. franciscana* or *A. peryi* were found to support the development and production of this economically important species (Nguyen et al., 2014a, 2013). The findings of the present study suggest that the same diet may also be useful to support populations of other commercially available phytoseiids, either in the laboratory or in the field. Future studies should focus on the optimization of artificial diets for phytoseiids in terms of their nutritional value and formulation. In addition, when evaluating an artificial diet for field

support, researchers may not only limit their focus on promoting the predator, but also diminishing potential beneficial effects for omnivorous pests. More work is also needed to optimize the application of artificial diets as food sprays in the crop. Possible limitations of the use of artificial diets as food sprays include desiccation, and chemical or microbial degradation of the foods, and crop pollution or damage.

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