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Article

## Relative fitness of avermectin-resistant strain of *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae)

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### Abstract

The predacious mite *Neoseiulus cucumeris* (Oudemans) is an effective natural enemy of pest insects and mites. To identify the relative fitness of the avermectin-resistant strain of *N. cucumeris*, the life history parameters of avermectin resistant (R) and susceptible (S) strains of *N. cucumeris* were observed under experimental conditions ( $25 \pm 1^\circ\text{C}$ ,  $90 \pm 5\%$  RH and L: D = 14:10 h) feeding upon *Tetranychus truncatus* (Ehara). Fertility, net reproductive rates ( $R_0$ /female), intrinsic rates of increase ( $r$ /day) and development durations of the two strains were compared. The abamectin resistant strain of *N. cucumeris* had significantly shorter developmental duration and longevity than the sensitive stain. However, the mean fecundity of the resistant strain was significantly higher than that of the susceptible strain. The net reproductive rate ( $R_0=30.3833$  offspring), the intrinsic rate of increase ( $r=0.2231\text{ d}^{-1}$ ) and the finite rate of increase ( $\lambda=1.2499\text{ d}^{-1}$ ) of the resistant strain were only slightly higher than those of the susceptible strain ( $R_0=29.5333$  offspring,  $r=0.2130\text{ d}^{-1}$ ,  $\lambda=1.2373\text{ d}^{-1}$ ); the differences were not significant. However, the mean generation time ( $T=15.1768\text{ d}$ ) of the resistant strain was significantly shorter than that of the susceptible strain ( $T=16.0314\text{ d}$ ).

**Key words:** *Neoseiulus cucumeris*; avermectin; insecticide resistance; relative fitness

### Introduction

Relative fitness is an important index to evaluate biological changes in populations of pesticide resistant strains and is used to measure the adaptability of insects to pesticides. Relative fitness is calculated as the net reproductive rate ( $R_0$ ) of resistant populations divided by the net reproductive rate of susceptible populations (Liu & Lu 2016). Compared to the sensitive strain, pesticide resistant strains of arthropods usually have lower fertility and longer development periods (Georghiou 1972; Janmaat and Meyers 2011). There is much research concerning the relative fitness of important agricultural pests and the health of pesticide resistant populations (Meng *et al.* 2007; Feng *et al.* 2009; Chen *et al.* 2011a; Abbas *et al.* 2012, 2014; Mansoor *et al.* 2013; Zhou *et al.* 2015).

The predacious mite *Neoseiulus cucumeris* (Oudemans) is an effective natural enemy of pest insects and mites (Gillespie, 1988; Zhang *et al.* 2006; Zhang, 2006; McMurtry *et al.* 2013). However, this predatory mite is very susceptible to insecticides (Chen *et al.* 2004, 2005, 2006, 2007a, 2007b, 2010; Zhang *et al.* 2011). This shortcoming has severely restricted its scope of use and effectiveness in pest control. We had bred an avermectin-resistant strain of *N. cucumeris*; the  $\text{LC}_{50}$  of susceptible strain was only 2.5270 mg/L, whereas that of the resistant strain was increased to 163.2930 mg/L.

The resistant multiple of resistant strain was 64.62-fold compared to susceptible strain. Routine use concentration of avermectin was 3.60–18.00 mg/L. The LC<sub>50</sub> of chlorpyrifos to susceptible strains and resistant strains were 7.4116 mg/L and 205.6392 mg/L respectively, and the resistance multiple was 26.75-fold. The LC<sub>50</sub> of imidacloprid to susceptible strains and resistant strains were 132.5151 mg/L and 2497.8167 mg/L respectively, and the resistance multiple reached to 17.85-fold (Chen *et al.* 2011b, 2013). To date, we have not reported on the relative fitness of the resistant strain of *N. cucumeris*. Thus, we compare the life history parameters between the resistant strain and the susceptible strain, to identify the relative fitness of the avermectin-resistant strain of *N. cucumeris*. Predatory mites with pesticide resistance will be employed to improve the biological control performance and the coordination of chemical control with biological control.

## Materials and Methods

### Mites

The susceptible (S) strain of *N. cucumeris* was purchased from BCP (BCP Certis<sup>®</sup>, UK) and was reared in the laboratory for at least ten years with *Aleuroglyphus ovatus* Troupeau supplied as prey at 25 ± 1 °C, 90 ± 5% RH and a 14:10 h (L:D) photoperiod at the Institute of Plant Protection, Fujian Academy of Agriculture Sciences, China. The avermectin-resistant (R) strain of *N. cucumeris* was established after 60 generations selection of the susceptible strain (S) by using avermectin. By then, the LC<sub>50</sub> (median lethal concentration) of resistant strain was 64.62-fold compared to susceptible strain (Chen *et al.* 2011b). *Tetranychus truncatus* Ehara was collected from a tomato garden in Fuzhou, Fujian Province, China, and was reared on bean plants (*Phaseolus vulgaris* L.) in the laboratory for at least two years.

### Experimental cage

Each experimental cage was constructed from an acrylic plate (30.0×25.0×1.5 mm) with a hole (6.0 mm in diameter) in the center, a transparent glass plate (30.0×30.0×1.0 mm) and a sheet of black nylon screen mesh (800-mesh). The nylon screen mesh was melted and bonded onto the hole of the acrylic plate with heat-sealing. The acrylic plate and the transparent glass were clamped together with two clips on either side of the acrylic plate with a rearing space for the predatory mites (Toyoshima & Amano 1998; Ji *et al.* 2015a; Ji *et al.* 2015b).

### Developmental duration

A single egg (approx. 8 h old) of each strain of *N. cucumeris* was introduced into an experimental cage together with *T. truncatus*, and each strain had 60 replicates. The experiments were conducted in a rearing chamber under a 14:10 h (L: D) photoperiod at 25±1°C and 90±5% RH. The development of the mites was observed twice daily, at 8 a.m. and 5 p.m., and the stage of development was recorded at each observation. More fresh *T. truncatus* were added if the food in cages became scarce during the observation period.

### Life table study

A newly emerged female of each strain from the development experiment (several females reared in parallel to the developmental duration experiment) was mated with an unmated male (3 days old) for one day in the experimental cage. The male was removed the following day. Five days later, the female was again allowed to mate with another unmated male (3 days old) for one day (Ji *et al.* 2015b). The predators were provided sufficient amounts of *T. truncatus* eggs daily. The oviposition and survival of the predatory females were observed daily until death. The sex ratio of

the offspring was determined by rearing all eggs deposited by the predator females to adulthood with *T. truncatus* as prey. The experiments were maintained in rearing chambers under a 14:10 h (L: D) photoperiod at  $25 \pm 1^\circ\text{C}$  and  $90 \pm 5\%$  RH. The experiments were replicated 35 times for the susceptible strain and 32 times for the resistant strain. A two-sample t-test were used to examine the means ( $\pm$ SE) of two strains are significantly different from one another ( $p=0.05$ ).

#### Life table data analysis

The raw life history data for survival, longevity and female daily fecundity of *N. cucumeris* individuals were analyzed using the TWOSEX-MSChart (Chi 2015) program, based on the age-stage, two-sex life table theory and methods described by Chi and Liu (1985) and Chi (1988). The TWOSEX-MSChart program is available at <http://140.120.197.173/ecology/>. The survival rate ( $s_{xj}$ ) ( $x$  = age,  $j$  = stage), which is the probability that a newly laid egg will survive to age  $x$  and stage  $j$ , and fecundity  $f_{xj}$ , which is the number of hatched eggs produced by a female adult at age  $x$  were calculated. According to Chi and Liu (1985), the specific survival rate ( $l_x$ ) is then calculated as:

$$l_x = \sum_{j=1}^m s_{xj} \quad (1)$$

where  $m$  is the number of stages. To take individuals of different stages at age  $x$  into account, the age-specific fecundity ( $m_x$ ) is calculated as:

$$m_x = \frac{\sum_{j=1}^m s_{xj} f_{xj}}{\sum_{j=1}^m s_{xj}} \quad (2)$$

The total number of offspring that an individual can produce during its lifetime, i.e., the net reproductive rate ( $R_0$ ), is calculated as:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (3)$$

The intrinsic rate of increase ( $r$ ) using the Lotka–Euler equation with age indexed from zero (Goodman 1982) is calculated as:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (4)$$

The mean generation time ( $T$ ) represents the period that a population requires to increase to  $R_0$ -fold of its size as time approaches infinity and the population growth rate settles down to the intrinsic rate and finite rate. Mean generation time is calculated as:

$$T = \frac{\ln R_0}{r} \quad (5)$$

The standard errors of developmental time, longevity, fecundity, and population parameters were calculated by using the bootstrap method with 200,000 bootstrap replicates (Efron and Tibshirani 1993, Huang and Chi 2012). Differences between treatments were compared using the paired bootstrap test (Efron and Tibshirani 1993, Smucker *et al.* 2007, Polat Akköprü *et al.* 2015).

## Results

The developmental times of the egg, larval and protonymph stage of the resistant strain of *N. cucumeris* were significantly shorter than that of the susceptible strain (Table 1). However, there were no significant differences in the developmental times of deutonymph stage and adult stage between two strains (Table 1). The total developmental time for the preadult stages was 5.79 d in resistant strain, which was significantly shorter than 6.72 d in susceptible strain.

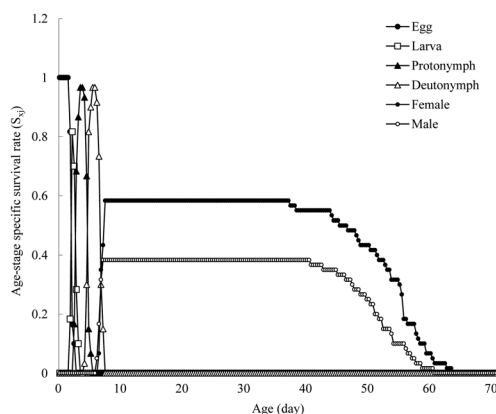
**TABLE 1.** Developmental time, APOP, TPOP, longevity, survival, and fecundity (mean± SE) of resistant (R) and susceptible strains (S) of *Neoseiulus cucumeris* with prey *Tetranychus truncatus*.

Parameter	Stage	R strain		S strain		p
		n	Mean±SE	n	Mean±SE	
Developmental time(d)	Egg	57	1.37±0.02a	59	2.03±0.04b	0.0000
	Larva	55	0.61±0.02a	58	0.71±0.02b	0.0000
	Protonymph	55	1.78±0.03a	58	1.89±0.02b	0.0027
	Deutonymph	55	2.04±0.03a	58	2.10±0.03a	0.2185
	Preadult	55	5.79±0.04a	58	6.72±0.05b	0.0000
	Adult	55	44.52±0.70a	58	45.86±0.77a	0.1972
APOP (d)	Female	32	1.82±0.05a	35	1.91±0.04a	0.1371
TPOP (d)	Female	32	7.74±0.05a	35	8.76±0.07b	0.0000
Total longevity (d)	Whole life span	60	46.26±1.86a	60	50.91±1.38b	0.0453
Female total longevity (d)	Whole life span	32	51.78±0.85a	35	53.30±1.05a	0.2623
Male total longevity (d)	Whole life span	23	48.28±1.09a	23	51.51±1.06b	0.0342
Mean longevity (d)	Whole life span	60	46.26±1.86a	60	50.91±1.38b	0.0453
Survival	Preadult	60	0.92±0.02a	60	0.97±0.04a	0.2474
Fecundity (F; eggs per female)	Female	32	55.38±0.63a	35	52.09±0.67b	0.0004

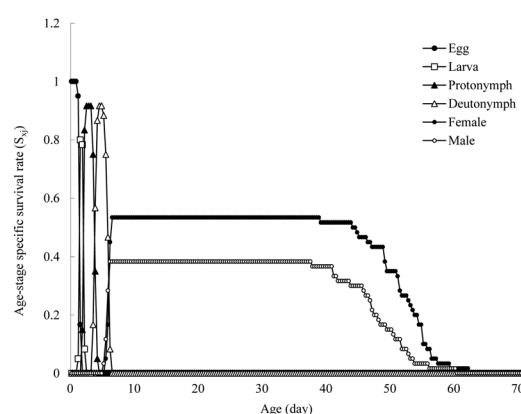
Means in the same row followed by different letters are significantly different ( $P < 0.05$ ) by using the paired bootstrap test.

There was no significant difference in adult pre-oviposition period (APOP) between resistant and susceptible strains. However, there was significant difference in the total pre-oviposition period between two strains. There were also significant differences in mean longevity and male total longevity between resistant and susceptible strains. However, the female total longevities and preadult survival were not significantly different between resistant and susceptible strains (Table 1). The fecundity of the resistant strain was significantly higher than that of the susceptible strain (Table 1).

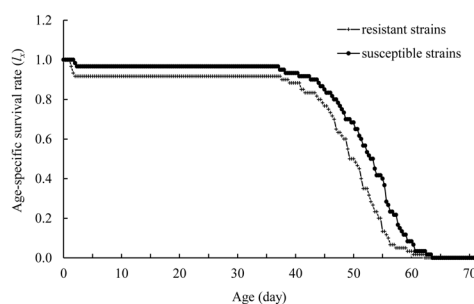
The detailed age-stage-specific survival rates ( $s_{xj}$ ) of the resistant and susceptible strains of *N. cucumeris* are plotted in Figs. 1 and 2. The parameter  $s_{xj}$  represents the probability that an egg of *N. cucumeris* will survive to age  $x$  and stage  $j$ . Overlapping among stages were observed. The probability that an individual surviving to the egg and larva stage of resistant strains were lower than susceptible strains, which is also consistent with the lower total longevities (51.78 d for female and 48.28 d for male) in resistant strains shown in Table 1.



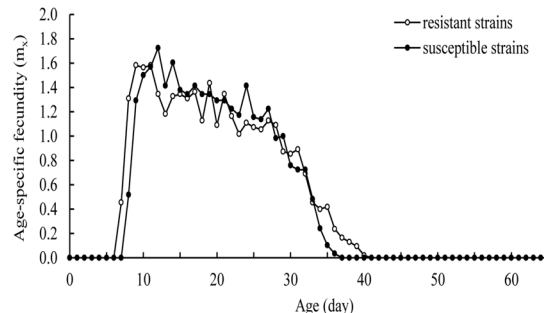
**FIGURE. 1** Age-stage specific survival rate ( $s_{xj}$ ) of susceptible strains of *N. cucumeris*.



**FIGURE. 2** Age-stage specific survival rate ( $s_{xj}$ ) of resistant strains of *N. cucumeris*.



**FIGURE. 3** Age-specific survival rate ( $l_x$ ) of resistant and susceptible strains of *N. cucumeris*.



**FIGURE. 4** Age-specific fecundity ( $m_x$ ) of resistant and susceptible strains of *N. cucumeris*.

When the survival rates  $s_{xj}$  of different stages are pooled, the age-specific survival rate ( $l_x$ ) produced a simplified overview of the survival history of the entire population (Fig. 3). The survival rates of the female of resistant and susceptible strains of *N. cucumeris* were similar.

In resistant strains, the first reproduction occurred at age 7 d and reached its peak between 8~28 d; however, in susceptible strains, reproduction began on age 8 d and reached peak fecundity at age 9~27 d. The detailed age-stage-specific fecundity ( $m_{xj}$ ) of the resistant and susceptible strains of *N. cucumeris* is plotted in Fig. 4; they were similar.

**TABLE 2.** Life history parameters of resistant (R) and susceptible (S) strains of *Neoseiulus cucumeris* with prey *Tetranychus truncates*.

Parameter	R strain	S strain	p
Net reproductive rate, $R_0$ (offspring)	30.3833±3.3344a	29.5333±3.5833a	0.8596
Mean generation time, $T$ (d)	15.1768±0.1942a	16.0314±0.1589b	0.0012
Intrinsic rate of increase, $r$ (d <sup>-1</sup> )	0.2231±0.0104a	0.2130±0.0085a	0.4563
Finite rate of increase, $\lambda$ (d <sup>-1</sup> )	1.2499±0.0130a	1.2373±0.0105a	0.4561

Means in the same row followed by different letters are significantly different ( $P < 0.05$ ) using the paired bootstrap test.

The life history parameters of the resistant and susceptible strains of *N. cucumeris* are shown in Table 2. The net reproductive rate ( $R_0=30.3833$  offspring), the intrinsic rate of increase ( $r=0.2231\text{ d}^{-1}$ ) and the finite rate of increase ( $\lambda=1.2499\text{ d}^{-1}$ ) of the resistant strain were only slightly higher than those of the susceptible strain ( $R_0=29.5333$  offspring,  $r=0.2130\text{ d}^{-1}$ ,  $\lambda=1.2373\text{ d}^{-1}$ ); the differences were not significant. However, the mean generation time ( $T=15.1768\text{ d}$ ) of the resistant strain was significantly shorter than that of the susceptible strain ( $T=16.0314\text{ d}$ ).

## Discussion

Fitness is a biological response to the external environment conditions. Pest insects evolve resistance in order to survive pesticides. As opposed to the rest of the population, the mites that have developed resistance pass along the genes that improve the survival of their offspring. In most cases, increasing the survival rate will decrease the fitness of the resistance stain. They will be less competitive; they will show longer developmental duration, higher mortality rate, lower fertility and lower overwinter survival (Li 2000; Meng 1998). In general, arthropods that have developed resistance to insecticides will have reduced fitness (Denholm 1992; Carriere 2003). Moreover, when reared at the same temperature, the abamectin resistance strain of *T. cinnabarinus* had longer developmental duration and lower fertility than the susceptible stain (He *et al.* 2008). However, fitness cost can't always be observed among all the resistant strains (Wu 1994). When compared to the susceptible strain, the intrinsic rate of increase ( $r_m$ ), and net reproduction rate ( $R_0$ ) of the propargite-resistant strain of *T. cinnabarinus*, were 0.24 and 25.34 for susceptible strains, and 0.26 and 28.99 for resistant strains, respectively (Luo *et al.* 2015). Furthermore, the relative fitness of resistance strains was 1.14, which showed an advantage over the susceptible strain (Luo *et al.* 2015).

This study demonstrated the advantages of using the age-stage, two-sex life table theory in describing demography (Chi 1988, Yu *et al.* 2005). Our results indicate that, when reared at the same temperature, the abamectin resistant strain of *N. cucumeris* had significantly shorter developmental duration and higher fertility than the susceptible strain, contrary to the pattern in abamectin resistant strain of *T. cinnabarinus* (He *et al.* 2008). However, the longevity of the resistant strain was significantly shorter than that of the susceptible strain. The mean generation time ( $T$ ) of the resistant strain of *N. cucumeris* was significantly shorter than that of the susceptible strain. However, other parameters (the net reproductive rate, the intrinsic rate of increase and the finite rate of increase) were not significantly different between the two strains.

Since the traditional female age-specific cannot describe the stage differentiation and ignores the male population, the practical applications of traditional female age-specific life tables in population ecology and pest management are limited. The problems associated with the traditional female age-specific life table are discussed in detail in Huang and Chi (2011).

*N. cucumeris* is one of the main products of natural enemies among international biocontrol companies and is mainly used to control the thrips, mites and other pests (Gillespie, 1988; Zhang *et al.* 2006). Now, *N. cucumeris* has been widely used in China to control spider mites and other pests on bamboo, citrus, tea, apple, cotton, strawberry, loquat, and peach crops with remarkable results (Zhang *et al.* 2006; Zhang, 2006).

We have compared the predation rates between resistant strains and susceptible strains and also measured potency detoxification enzyme activity to reveal the biochemical mechanism of *N. cucumeris* resistance to abamectin. The results will be presented in another article. The resistant strain, which was mass-reared in the laboratory and applied in the field, can reduce the conflict between biological control and chemical control. Predatory mites will be employed for rational and

efficient use to improve the biological control performance, and the coordination of chemical control with biological control in integrated pest management programs.

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