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Risk assessment of non-target effects caused by releasing two exotic phytoseiid mites in Japan: can an indigenous phytoseiid mite become IG prey?

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Abstract Two exotic phytoseiid mites, *Neoseiulus cucumeris* and *Amblyseius swirskii*, are commercially available in Japan for the control of thrips and other pest insects. As part of a risk assessment of the non-target effects of releasing these two species, we investigated intraguild predation (IGP) between these exotic phytoseiid mites and an indigenous phytoseiid mite *Gynaeseius liturivorus*, which is promising as an indigenous natural enemy for the control of thrips in Japan. To understand IGP relations between the exotic and indigenous phytoseiid mites after use of the exotic mites for biological control, we investigated IGP between them in the absence of their shared prey. When an IG prey was offered to an IG predator, both exotic and indigenous females consumed the IG prey at all immature stages (egg, larva, protonymph, deutonymph), especially at its larval stages. The propensity for IGP in a no-choice test was measured by the survival time of IG prey corrected using the survival time of thrips offered to the IG predator. There was no significant difference in the propensity for IGP between *N. cucumeris* and *G. liturivorus*, but the propensity was significantly higher in *A. swirskii* than *G. liturivorus*. The propensity for IGP in a choice test was measured by the prey choice of the IG predator when a conspecific and a heterospecific larva were offered simultaneously as IG prey. Both exotic females consumed the heterospecific larva only. The indigenous female preferentially consumed the heterospecific larva when the heterospecific larva was *N. cucumeris*, but consumed the conspecific larva when the heterospecific larva was *A. swirskii*. We concluded that further investigation would be necessary for the exotic mites' risk assessment, since the propensity for IGP of the two exotic females was similar to or higher than that of the indigenous female in both the no-choice and choice tests.

Keywords Intraguild predation · Intra-specific predation · Risk assessment · *Gynaeseius liturivorus* · *Neoseiulus cucumeris* · *Amblyseius swirskii*

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Introduction

Use of natural enemies instead of chemical pesticides is increasingly common worldwide, but unwelcome non-target effects caused by the release of exotic natural enemies are of growing concern (e.g., van Lenteren et al. 2006; Babendreier et al. 2006). In Japan, most commercially available biological control agents are exotic, and the quantity and variety have been increasing year by year. However, very few risk assessments of these commercially available exotics have been carried out in Japan (Mochizuki and Mitsunaga 2004; Naka et al. 2005, 2006; Mochizuki et al. 2006). Phytoseiid mites are a very common choice as introduced biological control agents, in a number of countries. Eight species of exotic phytoseiid mites, one of which is the same species as the indigenous type but originates from an exotic population, have been introduced to control crop pests in Japan, and four of these species are commercially available at present (Mochizuki 2010; registration of biological control agents in Japan, December 31, 2009, supplied by the Japan Plant Protection Association). *Neoseiulus cucumeris* (Oudemans) and *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) are exotic phytoseiid mites commercially provided to control thrips, whiteflies and other pest insects. These two species feed on mites, scale insects, honeydew and pollen, and show a broader host range than the remaining two commercially introduced species, *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor), which are available to control pest spider mites (for review, see McMurtry and Croft 1997). A biological control agent with a broad host range is often advantageous, since the predator population can survive on alternative food such as pollen when its normal prey population is low (e.g., Sabelis and van Rijn 1997; van Veen et al. 2006). On the other hand, a broad host range can cause undesirable side effects, such as exclusion of innocuous native insects by predation (Strong and Pemberton 2000). Therefore, it is necessary to assess the risk of non-target effects caused by releasing these two exotic species with a broad host range.

When assessing the risk of exotic species, the choice of non-target species is important (Kuhlmann et al. 2006). Three categories have been proposed for the selection of test species: (1) ecological similarities, (2) phylogenetic/taxonomic affinities, and (3) safeguard considerations (Kuhlmann et al. 2006). The two exotic mites in question are often released on greenhouse vegetable crops in Japan (e.g., green pepper, cucumber and eggplant), but several indigenous phytoseiid mites have also been found on these greenhouse vegetables (Komi et al. 2008a). Among these indigenous mites, *Gynaeseius liturivorus* (Ehara) actively preys on thrips, and is promising as an indigenous natural enemy for their control in Japan (Komi et al. 2008b; Mochizuki 2009). It is expected that *G. liturivorus* is appropriate as a non-target species of category 1, in the risk assessment of the two exotic species concerned. Competition for resources is usually regarded as having a potential impact on non-targets in category 1. However, intraguild predation (IGP), a mixture of competition and predation, is often observed in phytoseiid mites. Since IGP is thought to be an important factor in the displacement of species (e.g., Holt and Polis 1997), it is necessary to understand their IGP relations.

In this study, we investigated IGP between two exotic phytoseiid mites, *N. cucumeris* and *A. swirskii*, and an indigenous phytoseiid mite, *G. liturivorus*. We focused on their IGP relations in the absence of shared prey, since it is necessary to understand the relations after use of these exotic mites for pest control, as part of an initial risk assessment. After they had eaten up their prey, they would be forced to prey on each other to survive. We first investigated whether or not the two exotic mites would prey on the indigenous mite, and vice versa, by offering an immature mite (IG prey) to a starved female (IG predator).

Next, we measured the propensity for IGP in a no-choice test using the survival time of IG prey. Since native predation speed and/or tolerance to starvation varies among species, the survival time of IG prey was corrected using the survival time of one target pest insect, the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The propensity for IGP was compared between the exotic and indigenous mites. We also measured the respective mites' propensity for IGP in a choice test. Intraspecific predation (ISP or cannibalism) has been reported in many phytoseiid mites (Schausberger 2003). They may adopt ISP instead of IGP when there is no food except for each other, and ISP would partly reduce the effect of IGP on population dominance between predators that feed on the same prey (van Rijn et al. 2005). On the other hand, superior ability to discriminate between con- and heterospecies, and preferential consumption of the latter, has been reported in generalist phytoseiid mites, including *N. cucumeris* (Schausberger and Croft 1999, 2000). We investigated the respective mites' predation preference by offering a conspecific and a heterospecific larva simultaneously to a starved female.

Materials and methods

Mites and thrips

Neoseiulus cucumeris was obtained from a commercial producer (Arysta LifeScience, Japan), and reared on mold mites, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) on wheat bran and yeast in petri dishes, surrounded by water. *A. swirskii* was obtained from a commercial producer (Koppert Biological Systems, The Netherlands), and reared on sugar mites, *Carpoglyphus lactis* (Linnaeus) (Acari: Carpoglyphidae) on low-refined sugar and yeast in petri dishes, surrounded by water. The prey mites used for the rearing of these phytoseiid mites were obtained from the contents of the bottle containing the phytoseiid mites. *F. occidentalis* had been collected from kidney beans in Itoman (Okinawa Prefecture) in March 1996, and reared on detached kidney bean leaves resting on wetted cotton in a plastic case. *G. liturivorus* had been collected from Akitsu, Hiroshima Prefecture in 2005, and reared in artificial arenas consisting of a 24-well tissue culture plate and woolen yam surrounded by wetted tissue paper (Kishimoto 2005). The first-instar larvae of western flower thrips and detached kidney bean leaves were supplied to the arenas a few times per week as prey. These cultures were maintained in the laboratory at 18–25°C, 50–70% RH and photoperiod of LD 15:9 h, and kept under conditions of 25 ± 2°C, 50–70% RH and LD 15:9 h photoperiod from 1 month before the start of the following experiments.

Experiment 1: Propensity for IGP in a no-choice test

An immature indigenous mite was offered to a starved female of exotic mites, and an immature exotic mite was offered to a starved female of indigenous mites. As a control, an immature western flower thrips, *F. occidentalis* was offered to a starved female of exotic or indigenous mites. The cage used for the experiment consisted of an acrylic glass cell 15 mm in diameter and 5 mm high, with a kidney bean leaf at the bottom and a fine mesh screen on each side, closed at the top with a cover glass. Female mites were randomly selected from cultures and placed into the cages. After being starved for 24 h, female mites who had oviposited during starvation were placed singly in the new cage, and heterospecific mites or western flower thrips were offered as prey. The following were used as heterospecific mite samples: eggs oviposited within 3 h, larvae less than 3 h after hatching, and protonymphs and deutonymphs less than 3 h after molting. Eggs were collected by rearing females on first-instar larvae of western flower thrips in the cage. Larvae were collected by hatching the eggs. Protonymphs and deutonymphs were collected by rearing the larvae on first-instar larvae of western flower thrips in the cage. The following samples of western flower thrips were used: first-instar larvae less than 3 h after hatching (early first-instar larva), first-instar larvae of 24–27 h after hatching (late first-instar larva), second-instar larvae less than 3 h after first molting (early second-instar larva), and second-instar larvae of 24–27 h after first molting (late second-instar larva). Eggs of western flower thrips were first collected by allowing females to oviposit into water in a petri dish covered with stretched Sealonfilm® membrane (Fuji Photo Film Co., Japan), which was laid in the plastic case for rearing western flower thrips (Murai and Ishii 1982; Murai and Loomans 2001). And then, larvae of each stage were collected by rearing the eggs on detached kidney bean leaves resting on wetted cotton in a plastic case. Survival of the prey was checked 1, 2 and 3 h after prey introduction, and observation ended when the prey was consumed. Each procedure was replicated 30 times. It was confirmed that the prey did not develop to the following stage, and died within 3 h in this cage, when they were introduced alone ($n = 30$ in each prey stage). These experiments were conducted under controlled conditions of 25 ± 2°C, 50–70% RH, and LD 15:9 h photoperiod.

Experiment 2: Propensity for IGP in a choice test

A conspecific larva and a heterospecific larva (two larvae in total) were offered simultaneously as prey to a starved adult female of the exotic and indigenous mites, respectively. We used samples from the larval stage as prey in this experiment, since this life stage of these mites is vulnerable to IGP, as shown in the previous experiment. The cage used in this experiment was the same as that used in the former experiment. Adult females were randomly selected from cultures and placed into the cages. After being starved for 24 h, each female who oviposited eggs during starvation was placed singly into the cage. Conspecific and heterospecific larvae less than 3 h old were collected by hatching the eggs collected by rearing females on first-instar larvae of western flower thrips, and offered as prey. Since there are reports that phytoseiid mites discriminate between kin and non-kin, and prey on the latter preferentially (Faraji et al. 2000; Schausberger and Croft 2001; Schausberger 2007), this study ensured that the conspecific larvae used in its experiments were not the progeny of the predator, by dividing the rearing unit. Survival of the prey was checked 1, 2 and 3 h after prey introduction, and observation ended when the predator had consumed at least one of the two larvae. When both larvae had disappeared at the same observation, this data was not used in the analysis. The survivor was reared on early first-instar thrips until it became an adult, and the species was then identified. These experiments were conducted under controlled conditions of 25 ± 2°C, 50–70% RH, and photoperiod of LD 15:9 h.

Statistics

A Cox proportional hazards regression model with Efron approximation was used in the comparison of predation speed on thrips among these three phytoseiid mites, and in the comparison of the propensity for IGP in the no-choice test between exotic and indigenous mites in experiment 1. For the comparison of predation speed on thrips, a model was constructed using the data set of survival time of prey thrips offered to females of the three phytoseiid mite species. For the comparison of the propensity for IGP, two models were constructed using the data set of survival time of prey mites when immature *G. liturivorus* and *N. cucumeris* were offered to *N. cucumeris* and *G. liturivorus* females and the data set of survival time of prey mites when immature *G. liturivorus* and *A. swirskii* were offered to *A. swirskii* and *G. liturivorus* females. The explanatory variables in these three models included the predatory mite species type and the prey life-stage. In addition to these explanatory variables, an offset variable was incorporated into the two models for the comparison of the propensity for IGP, to correct the difference in predation speed among predatory mite species. Log-transformed hazard ratios, obtained by the model involving the offering prey thrips to the predatory mites, were employed as an offset variable.

An exact binomial test was used in experiment 2 to compare the proportion of females choosing the conspecific or heterospecific larva to the expected proportion of 0.5. All data analysis was performed using the freeware statistical package *R*, ver. 2.10.0 (available at http://www.r-project.org).

Results

Experiment 1: Propensity for IGP in a no-choice test

Predatory mite species type had a significant effect on the survival time of prey thrips (Table 1). The mortality rate of prey thrips offered to *N. cucumeris* and *A. swirskii* females was significantly lower than the mortality rate of prey thrips offered to the *G. liturivorus* female (Table 1; Fig. 1). This indicates that there was a difference either in the native predation speed or the effect of 24 h starvation among the predatory mite species. To incorporate this difference in the comparison of the propensity for IGP, coefficients of the model in Table 1 (log-transformed values of hazard ratios) were used as an offset variance in the prey mite model. Prey life-stage had a significant effect on the survival time of prey thrips, and the mortality rate of early first-instar larva was highest among the four life-stages of the prey thrips (Table 1; Fig. 1).

An increase in mortality rate was observed in *Gynaeseius liturivorus* at all immature stages, when it was offered to *Neoseiulus cucumeris* and *A. swirskii* females (Fig. 2a, b). A similar phenomenon was observed in the reverse combination, where the prey was *Neoseiulus cucumeris* and *Amblyseius swirskii*, and the predator was *G. liturivorus*

Table 1 Cox proportional hazards regression model involving the offering prey thrips, *Frankliniella occidentalis* to the predatory mites

Variable	Coefficient	SE	*z*	*P*
Predatory mite species type				
Gynaeseius liturivorus (indigenous)	0	–	–	–
Neoseiulus cucumeris (exotic)	−0.800	0.156	−5.137	<0.001
Amblyseius swirskii (exotic)	−1.192	0.168	−7.111	<0.001
Prey life-stage				
Early first-instar larva	0	–	–	–
Late first-instar larva	−0.825	0.169	−4.892	<0.001
Early second-instar larva	−0.718	0.169	−4.254	<0.001
Late second-instar larva	−2.288	0.235	−9.742	<0.001

Full model: likelihood ratio test $\chi^2_5 = 167.8$, $P < 0.001$, $n = 360$

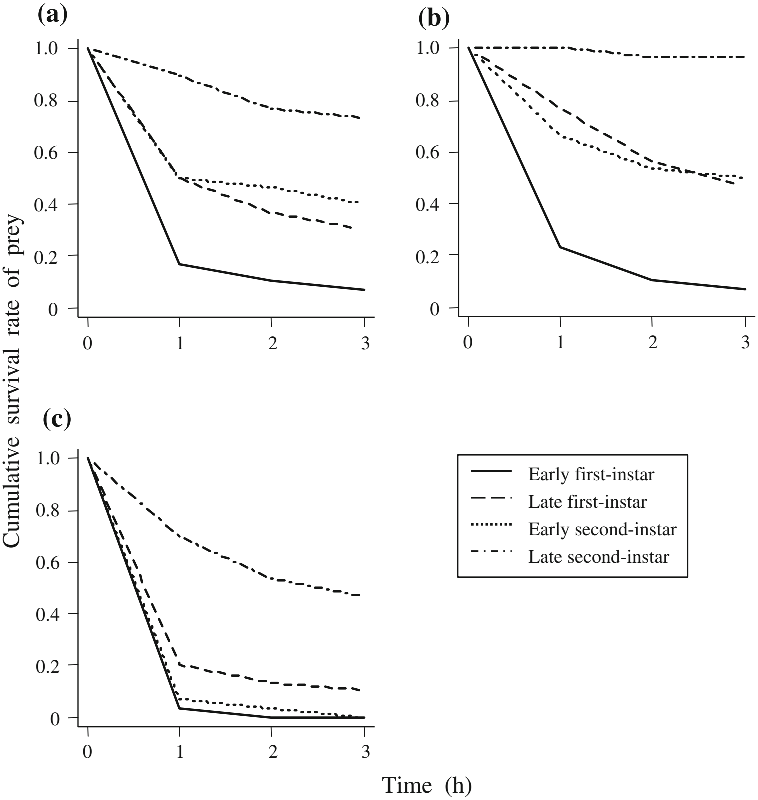

Fig. 1 The cumulative survival rates of prey thrips (*Frankliniella occidentalis*) after 3 h, when one immature thrips was offered to **a** one *Neoseiulus cucumeris* female (exotic), **b** one *Amblyseius swirskii* female (exotic), and **c** one *Gynaeseius liturivorus* female (indigenous). Each procedure was replicated 30 times

(Fig. 2c, d). These results indicate that these exotic and indigenous mites prey on each other when there is no other food available.

The effect of predatory mite species type on the survival time of the prey mite was not significant in the model involving the offering of prey mites (immature *G. liturivorus* and *N. cucumeris*) to *N. cucumeris* and *G. liturivorus* females (Table 2). On the other hand, this effect was significant in the model involving the offering of prey mites (immature *G. liturivorus* and *A. swirskii*) to *A. swirskii* and *G. liturivorus* females: the propensity for IGP was significantly higher in *A. swirskii* than *G. liturivorus* (Table 3). The effect of prey life-stage on the survival time of the prey mite was significant in both models, and the mortality rate of the larval stage was highest among the four life stages of prey mite (Tables 2, 3; Fig. 2).

Experiment 2: Propensity for IGP in a choice test

When a conspecific and a heterospecific larva were offered simultaneously, *N. cucumeris* and *A. swirskii* females preyed on heterospecifics only (Fig. 3a, b). On the other hand, *G. liturivorus* consumed the heterospecific larvae rather than the conspecific larvae, when

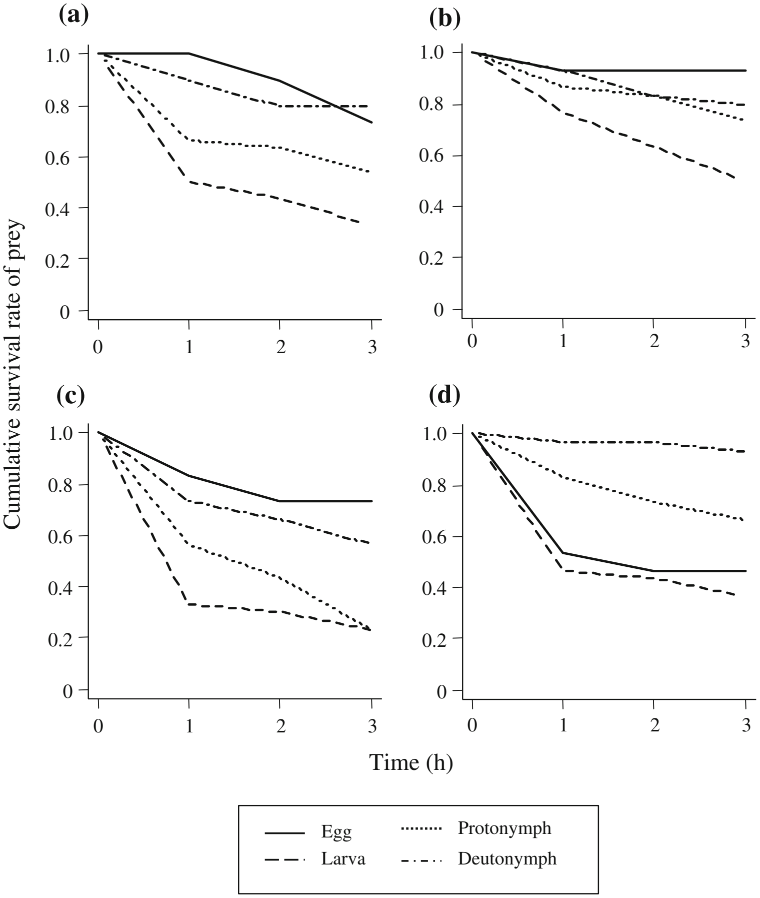

Fig. 2 The cumulative survival rates of prey mites after 3 h, when one immature mite was offered to one predatory female mite. The combinations of prey and predator are **a** prey: *Gynaeseius liturivorus* (indigenous), predator: *Neoseiulus cucumeris* (exotic); **b** prey: *G. liturivorus* (indigenous), predator: *Amblyseius swirskii* (exotic); **c** prey: *N. cucumeris* (exotic), predator: *G. liturivorus* (indigenous); **d** prey: *A. swirskii* (exotic), predator: *G. liturivorus* (indigenous). Each procedure was replicated 30 times

the heterospecific larva was *N. cucumeris* (Fig. 3c); and consumed the conspecific larvae rather than the heterospecific larvae, when the heterospecific larva was *A. swirskii* (Fig. 3d). These proportions were significantly different from the 0.5 expected (Fig. 3).

Discussion

IGP in the absence of shared prey was investigated in two exotic phytoseiid mites, *N. cucumeris* and *A. swirskii*, and an indigenous phytoseiid mite, *G. liturivorus*, as an initial risk assessment of non-target effects caused by releasing these exotic mites. Both exotic females consumed the indigenous mite at all immature stages (egg, larva, protonymph, deutonymph), especially at its larval stages (Fig. 2a, b). Mite in the egg stage is immobile and at the youngest of the immature stages of the prey mite, but both exotic

Table 2 Cox proportional hazards regression model involving the offering of prey mites (immature *G. liturivorus* and *N. cucumeris*) to *N. cucumeris* and *G. liturivorus* females

Variable	Coefficient	SE	z	P
Predatory mite species type				
Gynaeseius liturivorus (indigenous)	0	–	–	–
Neoseiulus cucumeris (exotic)	0.288	0.190	1.517	0.129
Prey life-stage				
Egg	0	–	–	–
Larva	1.515	0.294	5.147	<0.001
Protonymph	1.142	0.300	3.810	<0.001
Deutonymph	0.249	0.339	0.735	0.46

Full model: likelihood ratio test $\chi^2_4 = 44.11$, $P < 0.001$, $n = 240$

Table 3 Cox proportional hazards regression model for prey mite involving the offering of prey mites (immature *G. liturivorus* and *A. swirskii*) to *A. swirskii* and *G. liturivorus* females

Variable	Coefficient	SE	z	P
Predatory mite species type				
Gynaeseius liturivorus (indigenous)	0	–	–	–
Amblyseius swirskii (exotic)	0.564	0.232	2.425	<0.05
Prey life-stage				
Egg	0	–	–	–
Larva	0.763	0.292	2.615	<0.01
Protonymph	−0.132	0.334	−0.397	0.69
Deutonymph	−1.050	0.426	−2.466	<0.05

Full model: likelihood ratio test $\chi^2_4 = 35.67$, $P < 0.001$, $n = 240$

females preyed less on the indigenous egg (Fig. 2a, b), in spite of showing an interest in it (frequently touching it, in our observation, and seemingly attempting to pierce it). The lower level of predation on the indigenous egg might be explained by the difficulty in piercing the phytoseiid egg, as described previously in several phytoseiid mite studies (e.g., Schausberger 1997). On the other hand, the indigenous female consumed the exotic mites at all immature stages, especially at their larval stages (Fig. 2c, d), and consumed the egg of *A. swirskii* as well as its larva, unlike the two exotic females (Fig. 2). It appears that the *A. swirskii* egg is different from the other two species' eggs, although we could see no superficial differences. There was a slight difference in vulnerable life stage among these phytoseiid mites, but it was clearly revealed that both the exotic and indigenous phytoseiid mites prey on each other if there is no other food available.

There was no significant difference between *N. cucumeris* and *G. liturivorus* in the propensity for IGP in the no-choice test (Table 2). In addition, the propensity for IGP in the no-choice test was significantly higher in *A. swirskii* than *G. liturivorus* (Table 3). It thus appears that IGP relations between these exotic and indigenous mites cannot be disregarded in risk assessment. However, in this study, *G. liturivorus* was reared on thrips, whereas *N. cucumeris* and *A. swirskii* were reared on mold mites and sugar mites, respectively. There are reports that the experience of consuming a given prey affects the response to the prey (Castagnoli and Simoni 1999; Castagnoli et al. 2001; Rahmani et al.

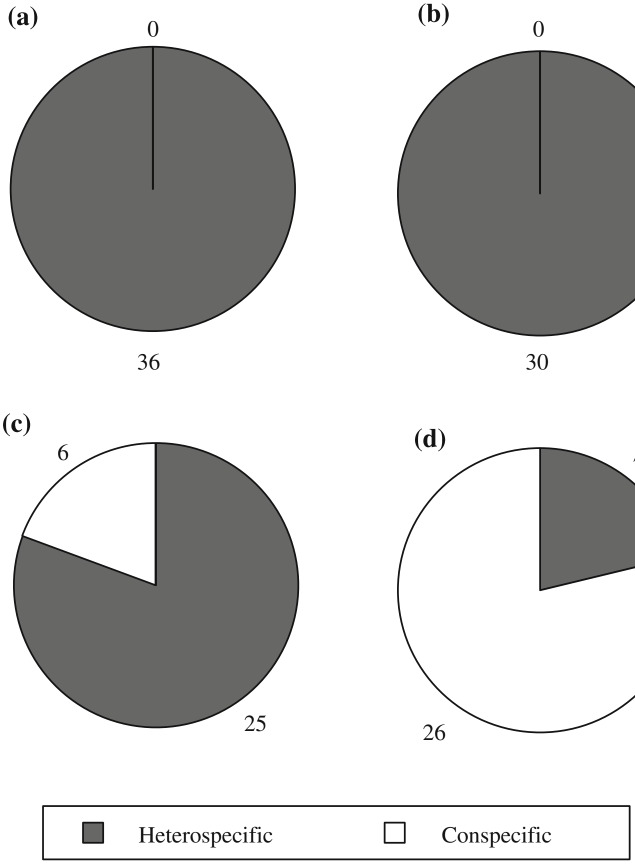

Fig. 3 Prey choice by exotic and indigenous predatory mites, when one pair of con- and heterospecific larva was offered to one starved female for 3 h. The combinations of prey and predator are **a** prey: *Neoseiulus cucumeris* (exotic) and *Gynaeseius liturivorus* (indigenous), predator: *N. cucumeris* (exotic); **b** prey: *Amblyseius swirskii* (exotic) and *G. liturivorus* (indigenous), predator: *A. swirskii* (exotic); **c** prey: *G. liturivorus* (indigenous) and *N. cucumeris* (exotic), predator: *G. liturivorus* (indigenous); **d** prey: *G. liturivorus* (indigenous) and *A. swirskii* (exotic), predator: *G. liturivorus* (indigenous). *P* values derived from the exact binomial tests, comparing the observed frequency with the expected frequency of 0.5, were **a** $n = 36$, $P < 0.001$; **b** $n = 30$, $P < 0.001$; **c** $n = 31$, $P < 0.001$; **d** $n = 33$, $P < 0.01$

2009), and thus the higher mortality rates of prey thrips offered to *G. liturivorus* might be caused by the difference in their experience of prey (Table 1; Fig. 1). If so, the correction using the survival time of thrips, in comparing the propensity for IGP in the no-choice test, might have been too severe for *G. liturivorus*.

However, the necessity of further investigation for the risk assessment was also suggested by the propensity for IGP in the choice test. When a conspecific and a heterospecific larva were offered simultaneously, *N. cucumeris* and *A. swirskii* females preferentially consumed the heterospecific larva (Fig. 3a, b). Their ability to discriminate between conspecific and heterospecific larvae was extremely high, since all of the females consumed the heterospecific larva only, in both species (Fig. 3a, b). On the other hand, *G. liturivorus* females consumed the conspecific rather than heterospecific larva when the heterospecific larva was *A. swirskii* (Fig. 3d). There is no denying that *A. swirskii* larvae might have preyed on *G. liturivorus* larvae, since *A. swirskii* larvae facultatively feed (Wimmer et al. 2008). However, it is more likely that *G. liturivorus* females consumed the conspecific larvae, since the predation ability would be much higher in the starved female of *G. liturivorus* than in *A. swirskii* larvae. When the heterospecific larva was *N. cucumeris*, *G. liturivorus* females consumed the heterospecific larva rather than the conspecific larva, but the accuracy of prey choice was incomplete and lower than those of the exotic mites (Fig. 3c). ISP would partly reduce the effect of IGP on population dominance (van Rijn et al. 2005), thus the results of the choice test suggest the risk that *G. liturivorus* might be replaced by these exotic mites.

These results suggest that there would be a risk of non-target effects in releasing of these exotic mites. However, in this study we focused only on their IGP relations involving ISP in the absence of shared prey. A more precise estimation of their risk would involve the simultaneous consideration of a number of factors (Holt and Polis 1997; van Rijn et al. 2005). The data in this study would be useful for establishing the parameters of a simulation model that would allow us to consider several factors together. As a next step, it is necessary to investigate the parameters of other important factors that would affect population dominance between exotic and indigenous mites, such as the nutritional value of IG prey, the predation rate, and their IGP relations in the presence of shared prey.

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