Suitable Food for the Mass Rearing of *Wollastoniella rotunda* (Heteroptera: Anthocoridae), a Predator of *Thrips palmi* (Thysanoptera: Thripidae)

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Abstract

Wollastoniella rotunda Yasunaga et Miyamoto is an effective predator of *Thrips palmi* Karny and a potential biological control agent for this pest of Japanese greenhouse vegetables. To determine alternative sources of prey for the mass rearing of *W. rotunda* used for *T. palmi* augmentative biocontrol, we examined the life history parameters of this predator as reared on two different prey species: *Tyrophagus putrescentiae* (Schrank) and *Ephestia kuehniella* Zeller eggs. When the predator nymphs were reared on *T. putrescentiae* and *E. kuehniella* at 25°C, their developmental times were 21.0 and 16.7 days, respectively, and their survival rates on *T. putrescentiae* and *E. kuehniella* at 25°C were 43% and 91%, respectively. The longevity of the predator females was 13.1 days on *T. putrescentiae* and 19.2 days on *E. kuehniella*, and their total fecundity was 15.0 eggs on *T. putrescentiae* and 56.5 eggs on *E. kuehniella*. The intrinsic rate of natural increase per day of *W. rotunda* on *E. kuehniella* (r_m = 0.087) was greater than that on *T. putrescentiae* (r_m = 0.031). We thus concluded that *E. kuehniella* is more suitable than *T. putrescentiae* as food for the mass rearing of *W. rotunda*.

Introduction

Thrips palmi Karny native to the Malaysian-Indonesian region[25] is a worldwide serious pest that attacks various vegetables including eggplant in both greenhouses and open fields. *Orius* species are effective biological control agents against the pest in greenhouses[6,10]. However, the use of most *Orius* species is seasonally limited as the reproductive diapause of these predators is induced by short-day conditions[2,7,8,16,18,23]. In Japan, some strains of *Orius strigicollis* (Poppius) with a low incidence of reproductive diapause have been used over the past ten years for the biological control of *T. palmi* on eggplant

grown in greenhouses. However, the use of *O. strigicollis* as a control agent against thrips is difficult because the reproduction of this predator is reduced under the low temperature and short-day conditions in winter, even when released in heated greenhouses[19]. Therefore, non-diapausing natural enemies from tropical or sub-tropical regions should be used to extend the biological control of *T. palmi* to greenhouses in Japan during winter. The possibility of such use had already been suggested[4,11,14] when the use of *O. strigicollis* as a biological control agent against *T. palmi* in Japanese greenhouses was initiated.

In different parts of Thailand (Chiang Mai, Suphan Buri, Nakhon Pathom, and Bangkok), *Wollastoniella rotunda* Yasunaga et Miyamoto (initially recorded as *Bilia*

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sp.[3], but later described as a new species of *Wollastoniella*[27]) was discovered and evaluated as an effective predator of *T. palmi* on eggplant[3]. *W. rotunda* diapause was not induced under relatively low temperature and short-day conditions[15]. Moreover, the developmental threshold in immature stages of *W. rotunda* (at 11.5°C) was below the average winter temperature (approximately 13°C) in Japanese greenhouses that grow eggplant[17]. These life history traits of *W. rotunda* are suitable as those of a biological control agent against *T. palmi* on vegetables in Japanese greenhouses during winter. In fact, the results from cage trials of *W. rotunda* showed that this predator successfully developed, reproduced, and suppressed *T. palmi* populations on eggplant in winter greenhouses[15]. The release of *W. rotunda* against *T. palmi* populations on eggplant in greenhouses during two winters proved successful in controlling pest populations[22]. *W. rotunda* is thus a promising predator for augmentative biological control agents against *T. palmi* on greenhouse vegetables during winter in Japan. However, no method of mass rearing for this predator has been explored.

In the present study, we chose *Tyrophagus putrescentiae* (Schrank)[5,9,12,13] and the eggs of *Ephestia kuehniella* Zeller[13,23,24,26] as possible foods for rearing *W. rotunda*, as both species have been used as food for rearing other *Orius* species preying on thrips. We evaluated the suitability of these foods for the mass rearing of *W. rotunda* as based on the intrinsic rates of natural increase per day.

Materials and methods

1. Laboratory culture of *W. rotunda* and *T. putrescentiae*

W. rotunda: Adults and nymphs collected from eggplants infested with *T. palmi* in Prachin Buri, Kamphaengsaen, and Suphan Buri, Thailand, in 1995 were used to initiate a laboratory colony for our study and other studies[15,17,20,21]. The *W. rotunda* colony was reared through several generations on potted eggplants infested with *T. palmi* in a thermostatic incubator at 25°C ± 1°C and 16L8D without controlled RH. *W. rotunda* adults obtained from this laboratory culture were used in the present study.

T. putrescentiae: A stock culture of *T. putrescentiae* was obtained from Hyogo Prefectural Agricultural Institute. The stock culture was maintained using an artificial diet prepared by mixing 50 g of wheat bran, 50 g of dried bear yeast, 100 g of rice hulls, and 50 ml of water[12]. The culture was kept in a thermostatic incubator at 25°C ± 1°C, 75% ± 10% RH, and 16L8D. A fine brush was used to collect *T. putrescentiae* kept in rearing containers prior

to use.

2. *E. kuehniella* egg

Mr. Wakisaka (Otsuka Chemical Co. Ltd.) provided the eggs on embryos killed by ultraviolet rays. The eggs were sealed in glass vials and preserved in a freezer at -30°C. Frozen eggs were taken out from the freezer and then defrosted in a refrigerator at ca. 5°C prior to use.

3. Development and survival of *W. rotunda* immatures

A young stem section of kidney bean with fresh eggs (within 24 hours) deposited by *W. rotunda* females collected from the laboratory colony was kept in a glass vial in a thermostatic incubator at 25°C ± 1°C, 75% ± 10% RH, and 16L8D. Egg hatching was monitored under a binocular microscope every 24 hours until 16 days after oviposition, and egg duration was also evaluated. This procedure and rearing conditions (temperature and photoperiod) were the same as those employed by Shima and Hirose (2002)[17].

First, stadium nymphs of *W. rotunda* within 24 hours after hatching were placed individually on a reversed eggplant leaf section (*Solanum melongena* L., cv. 'Senryo', 2 cm × 2 cm) on absorbent cotton soaked with water in a petri dish (Fig. 1 A) in a thermostatic incubator at 25°C ± 1°C, 75% ± 10% RH, and 16L8D. Approximately 100 live *T. putrescentiae* or approximately 100 *E. kuehniella* eggs were placed on the eggplant leaf section every day. Surplus amounts of prey were given to the nymphs on the eggplant leaf section. A bent filter paper section (Whatman No. 2, 0.5 cm × 0.5 cm) was placed on the eggplant section as a shelter for *W. rotunda*. To prevent nymphs from drowning in dewdrops forming on the surface of the eggplant leaf section, the petri dish lid was pierced with nine air holes (each 3 mm in diameter) and covered with filter paper. The leaf section was renewed every five to seven days. The developmental stage and the number of surviving *W. rotunda* were examined under a binocular microscope every 24 hours. The presence of nymph exuvia was used as an indicator of molting. Shima and Hirose (2002)[17] reared nymphs of *W. rotunda* in glass vials, but other procedures were similar to those used in this experiment. Rearing conditions (temperature and photoperiod) also were the same as those utilized by Shima and Hirose (2002)[17].

4. Female longevity and fecundity of *W. rotunda* adults

Pairs of *W. rotunda* adults (within 24 hours old) were collected from the laboratory colony, confined in a glass vial (3.5 cm in diameter × 7.5 cm in height) with a young

stem section (3 cm in length) of kidney bean (*Phaseolus vulgaris* L., cv. 'Uzuramame') (Fig. 1 B). The pairs were divided into two groups: one was fed approximately 100 live *T. putrescentiae* per pair; the other was fed approximately 100 *E. kuehniella* eggs per pair. Both kinds of prey were replenished in the same amounts every 24 hours. Males that died before female oviposition were replaced with healthy males. Kidney bean stem sections were renewed every 24 hours, and then the number of eggs laid in the stem was counted under a binocular microscope. Adult female mortality was also recorded every 24 hours. Experiments were conducted at 25°C ± 1°C, 75% ± 10% RH, and 16L8D. This procedure was almost the same as that employed by Uefune et al. (2008)[20], and the rearing conditions (temperature and photoperiod) of this experiment were the same as those utilized by Uefune et al. (2008)[20].

Based on the results obtained from the experiments above, the mean generation times (*T*), net reproductive rates (*R*$_0$), and intrinsic rates of natural increase (*r*$_m$) of *W. rotunda* reared on two prey species were calculated by using the Birch's method[1]. However, the female sex ratio was assumed to be 0.5.

5. Statistical analysis

The nymph developmental time, longevity of adult females, preoviposition period, total eggs laid per female, and number of eggs laid per female per day of *W. rotunda* were analyzed by conducting a *t*-test. Survival rates throughout nymphal duration and the ovipositing female rates were analyzed using Fisher's exact probability test and chi-squire test, respectively.

Results and discussion

The duration and hatchability of *W. rotunda* eggs laid in young kidney bean stem sections were 7.9 ± 0.1 days (n = 66) and 98.5% (n = 67), respectively. This egg duration was similar to the result of eggplant leaf sections (8.2 days)[17], The hatchability in young kidney bean stem sections, however, was higher than that laid in eggplant leaf sections (80.7%)[17]. The difference in hatchability between these two studies was probably due to the difference between oviposition substrates, resulting in the young stem sections of kidney bean maintaining vigor longer than the eggplant leaf sections.

The total developmental time of *W. rotunda* nymphs reared on *E. kuehniella* eggs (16.7 days) was significantly shorter than that of *W. rotunda* nymphs reared on *T. putrescentiae* (21.0 days) (*t*-test, *p* <0.05) (Table 1). The survival rates throughout the nymphal period were 43% on *T. putrescentiae* and 91% on *E. kuehniella* eggs, with the former being significantly lower than the latter (χ^2-test, *p* <0.05) (Table 2). The developmental time of *W. rotunda* nymphs reared on *E. kuehniella* eggs was almost the same as for those reared on *T. palmi* (16.8 days: average of females and males) at 25°C[17]. The survival rate of nymphs reared on *E. kuehniella* eggs (91%) in our study was slightly higher than that of those reared on *T. palmi* at 25°C (83.3%)[17].

The longevity of *W. rotunda* females reared on *E. kuehniella* eggs (19.2 days) was significantly greater than that of those reared on *T. putrescentiae* (13.1 days) (*t*-test, *p* <0.05) (Table 3). The maximum longevity of *W. rotunda* adult females reared on *E. kuehniella* eggs (33 days) was greater than that of those reared on *T. putrescentiae*
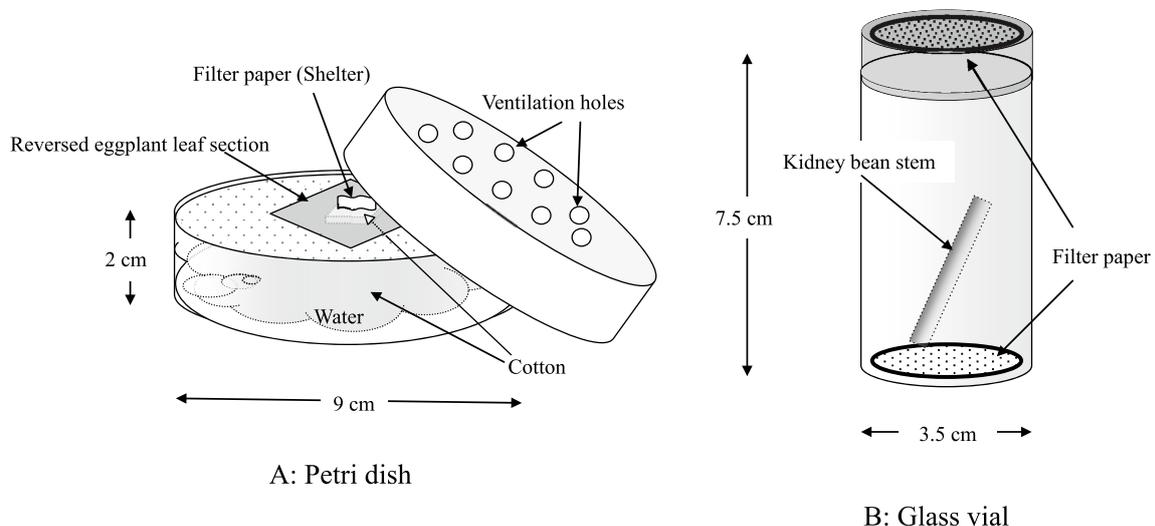
Fig. 1. Rearing containers for adults (A) and nymphs (B) of *W. rotunda*

(22 days) (Fig. 2). The longevity when reared on *E. kuehniella* eggs in our study was greater than that when reared on *T. palmi* (12.3 days) and *T. kanzawai* (11.1 days)[20], indicating that *E. kuehniella* eggs are more suitable than *T. palmi* as a natural diet of the predator species.

The percentage of ovipositing females was 90% on *T. putrescentiae* and 100% on *E. kuehniella* eggs, and the preoviposition periods were 4.3 days on *T. putrescentiae* and 3.8 days on *E. kuehniella* eggs (Table 3). The differences in these measures between prey species were not significant (for the percent of ovipositing females, Fisher's exact probability test, *p* >0.05; for the preoviposition period, *t*-test, *p* >0.05.). However, the total fecundity and daily oviposition rates per female reared on *E. kuehniella* eggs (56.5 eggs and 4.3 eggs) were significantly greater than those per female reared on *T. putrescentiae* (15.0

eggs and 1.8 eggs) (*t*-test, *p* <0.05) (Table 3). Oviposition of *W. rotunda* continued until 33 days after emergence when reared on *E. kuehniella* eggs, but only 18 days when reared on *T. putrescentiae* (Fig. 3). The total fecundity was also greater in our study for feeding on *E. kuehniella* than for feeding on *T. palmi* (40.3) and *T. kanzawai* (34.6)[20].

The mean generation time (*T*) of *W. rotunda* reared on *E. kuehniella* eggs (38.7 days) was almost the same as that for those reared on *T. putrescentiae* (37.7 days) (Table 4). The net reproductive rate (R_0), r_m value per day, and reproductive rate per month of *W. rotunda* reared on *E. kuehniella* eggs (25.9, 0.087, and 13.6) were greater than those of *W. rotunda* reared on *T. putrescentiae* (3.15, 0.031 and 2.49) (Table 4). Judging from a comparison of these two intrinsic rates of natural increase per day, *E.*

Table 1. Developmental times in nymphal stages of *W. rotunda* reared on *T. putrescentiae* and *E. kuehniella* eggs[1,2]

Prey	Developmental time (Mean ± S.E.) (day)					
	1st stage nymph	2nd stage nymph	3rd stage nymph	4th stage nymph	5th stage nymph	Nymph to adult
T. putrescentiae	3.8 ± 0.2a (18)	3.4 ± 0.2a (18)	3.4 ± 0.4a (13)	4.6 ± 0.5a (13)	6.4 ± 0.7a (9)	21.0 ± 1.1a (9)
E. kuehniella eggs	3.2 ± 0.2a (22)	2.8 ± 0.2a (20)	2.5 ± 0.1b (20)	3.0 ± 0.2b (20)	5.2 ± 0.1a (20)	16.7 ± 0.3b (20)

[1] The number of samples tested for each prey is denoted in parentheses.
[2] Means followed by the same letter in the same column are not significantly different at *p*= 0.05 (*t*- test).

Table 2. Survival rates in nymphal stages of *W. rotunda* reared on *T. putrescentiae* and *E. kuehniella* eggs

Prey	Survival rate (%)[1,2]					
	1st stage nymph	2nd stage nymph	3rd stage nymph	4th stage nymph	5th stage nymph	Nymph to adult[3]
T. putrescentiae	86 (21)	100 (18)	72 (18)	100 (13)	69 (13)	43 (21)
E. kuehniella eggs	100 (22)	91 (22)	100 (20)	100 (20)	100 (20)	91 (22)

[1] The number of samples tested for each prey is denoted in parentheses.
[2] The number of individuals that survived during the stage(s).
[3] The survival rates throughout nymphal duration between both prey are significantly different at *p*=0.05 (χ^2-test).

Table 3. Adult longevity, total fecundity, and other related measures of *W. rotunda* reared on *T. putrescentiae* and *E. kuehniella* eggs

Prey	Longevity of females (day)[1,2]	% of ovipositing females[1,3]	Preoviposition period (days)[1,2]	Total eggs laid per female[1,2]	No. of eggs laid per female per day[1,2]
T. putrescentiae	13.1 ± 1.0a (21)	90 (21)	4.3 ± 0.3a (19)[4]	15.0 ± 2.0a (19)	1.8 ± 0.2a (19)[4]
E. kuehniella eggs	19.2 ± 1.4b (21)	100 (21)	3.8 ± 0.2a (20)[4]	56.5 ± 6.2b (21)	4.3 ± 0.6b (20)[4]

[1] The number of samples tested for each prey is denoted in parentheses.
[2] Mean ± S.E. Means followed by the same letter in the same column are not significantly different at *p* = 0.05 (*t*- test).
[3] The percentages of ovipositing females are not significantly different at *p* = 0.05 (Fisher's exact probability test).
[4] The data on one female was excluded, as the female began oviposition after the dead male was replaced by a healthy male.

kuehniella eggs are a more suitable diet for *W. rotunda* than *T. putrescentiae*. And as previously mentioned, both the longevity and fecundity of *W. rotunda* females reared on *E. kuehniella* eggs in our study are greater than those of *W. rotunda* females reared on *T. palmi* and *T. kanzawai*[20], suggesting that *E. kuehniella* eggs are a suitable diet for the mass rearing of *W. rotunda*.

Our study provides information that may prove useful for the mass rearing of *W. rotunda* in augmentative biological control programs against *T. palmi* in winter greenhouses. Our study also suggests that a young kidney bean stem is better than an eggplant leaf as an oviposition substrate of *W. rotunda*, because the kidney bean stem enables higher hatchability than the eggplant leaf[17]. As shown by our study and that conducted by Uefune et al. (2008a)[21], both the hatchability and development of *W. rotunda* were apparently affected by plant species. For the mass rearing of *W. rotunda*, future studies should examine the effect of plant species provided with this predator.

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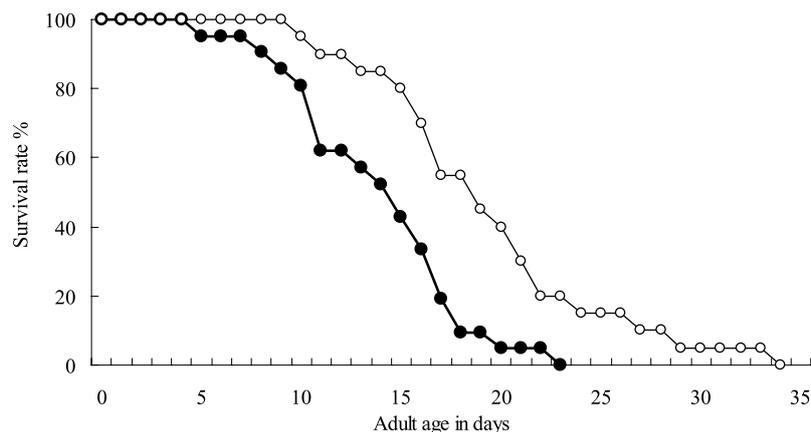
Fig. 2. Survivorship curves of adult females of *W. rotunda* reared on two different prey species, *T. putrescentiae* and *E. kuehniella* (eggs)
—●— : *T. putrescentiae*, —○— : *E. kuehniella*.
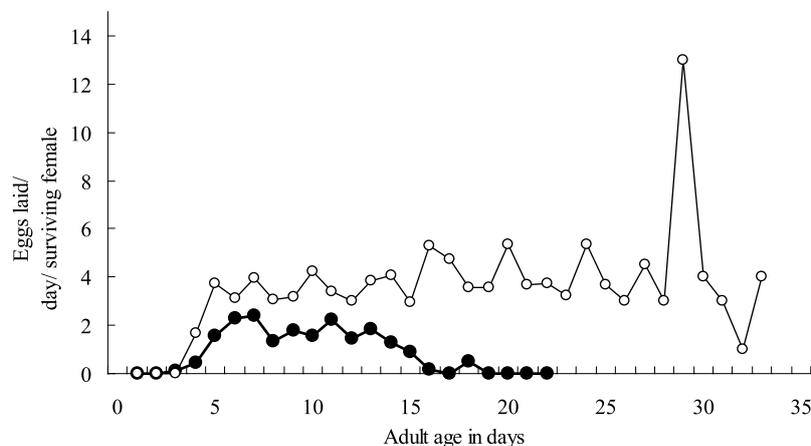
Fig. 3. Adult oviposition curves of *W. rotunda* reared on two different prey species, *T. putrescentiae* and *E. kuehniella* (eggs)
—●— : *T. putrescentiae*, —○— : *E. kuehniella*.

Table 4. Population growth parameters for *W. rotunda* reared on *T. putrescentiae* and *E. kuehniella* eggs

Prey	Mean generation time (T) (days)	Net reproductive rate (R_0)	Intrinsic rate of natural increase/day (r_m/day)	Reproductive rate per month
T. putrescentiae	37.7	3.15	0.031	2.49
E. kuehniella eggs	38.7	25.9	0.087	13.6

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