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Cage evaluation of augmentative biological control of *Thrips palmi* with *Wollastoniella rotunda* (Heteroptera: Anthocoridae) in winter greenhouses

Yoshitaka Nakashima [a, d], Masayoshi Uefune [b], Eiko Tagashira [b], Seiko Maeda [b], Katsuya Shima [b], Kazuya Nagai [c], Yoshimi Hirose [b, e] and Masami Takagi [b*]

[a] Obihiro University of Agriculture and Veterinary Medicine, Obihiro, 080-8555, Japan

[b] Faculty of Agriculture, Kyushu University, Fukuoka, 812-8581, Japan

[c] Okayama Prefectural Agricultural Experiment Station, San'yo-cho, Okayama 709-0801, Japan

[d] Current address: Rothamsted Research, Harpenden, Herts. AL5 2JQ UK

[e] Current address: 349 Asano, Munakata 811-3415, Japan

* To whom correspondence should be addressed at Faculty of Agriculture, Kyushu University, Fukuoka, 812-8581, Japan. Fax: +81(0)92 642 3040.

E-mail: mtakagi@grt.kyushu-u.ac.jp

Abstract

A cage trials of an anthocorid predator, *Wollastoniella rotunda* Yasunaga et Miyamoto, as a biological control agent of *Thrips palmi* Karny were conducted in Fukuoka, Japan (33˚35´N, 130˚23´E), under winter greenhouse production conditions. Females of *W. rotunda* were released on caged eggplants, placed in two greenhouses on 27 October. Development, population growth and effectiveness of *W. rotunda* were observed until early March. Results from the cage trials showed that *W. rotunda* successfully developed, reproduced and suppressed *T. palmi* populations under conditions of winter greenhouses. During the experiment, one full generation and a second generation of adult predators occurred. The *T. palmi* population exposed to predators remained at low density throughout the trial period, yet increased dramatically on eggplants without *W. rotunda*. The maximum difference between predator treatments and controls was approximately 10- fold at the end of January. *Wollastoniella rotunda* is potentially an effective control agent for *T. palmi* on eggplant even during the winter in temperate regions.

Key Words: reproductive diapause; photoperiod; development; winter; *Wollastoniella rotunda*; *Orius*; biological control; *Thrips palmi*; *Solanum melongena*; *Dicyphus tamaninii*; *Frankliniella occidentalis*; *Piocoris varius.*

Introduction

Thrips palmi Karny, which was accidentally introduced into Japan, is the major pest of vegetable crops, including eggplants, water melons and sweet peppers, grown both in greenhouses and in open fields (Kawai, 2001). It is believed that *T. palmi* cannot overwinter in the field in the area of Japan north of Kyushu, but it can survive under the conditions in greenhouses (Ikeda, 1983; Makino and Horikiri, 1983; Tsumuki *et al*., 1987). After overwintering in greenhouses, the thrips disperse into open fields late in the growing season of greenhouse crops (Makino and Horikiri, 1983; Hirose, unpublished data).

Several species of *Orius*(Hemiptera: Anthocoridae) are effective biological control agents of thrips in greenhouses (Jacobson, 1993; van de Veire and Degheele, 1993; Kawai, 1995). Their efficiency, however, is seasonally-limited because these predators enter reproductive diapause under short-day conditions (van den Meiracker, 1994; Kohno, 1997; Shimizu and Kawasaki, 2002). Ito and Nakata (1998) demonstrated that adult females of *Orius sauteri* (Poppius) and *O. minutus* (L.) did not enter reproductive diapause, even under short-day conditions (11 hrs photoperiod), if they were reared under a long daylength (16 hrs photoperiod) during their nymphal stage. They proposed the use of these non-diapausing adults for controlling thrips in winter greenhouses, although effective use cannot be expected over the complete winter period.

To extend the seasonal limit of thrips biological control in greenhouses, the use of non-diapausing predator populations from lower latitudes has been suggested.

The anthocorid predator, *Wollastonniela rotunda* Yasunaga et Miyamoto (= *Bilia* sp. in Hirose *et al*., 1993), was first described from Thailand (Yasunaga and Miyamoto, 1993) as an effective predator of *T. palmi* (Hirose *et al*., 1993). According to laboratory trials, reproductive diapause of *W. rotunda* is not induced under short-day conditions (Shima, 1997) and developmental thresholds of immature stages are below the average winter temperature of eggplant greenhouses (Shima and Hirose, 2002). However, what is not known is if this tropical predator can successfully develop, reproduce and control *T. palmi* under winter greenhouse conditions in Japan. Therefore, the objective of this study is to determine the effectiveness of *W. rotunda* for biological control of *T. palmi* on eggplant grown under winter production conditions.

Materials and methods

Insects

A colony of *W. rotunda* was established using adults and nymphs collected from eggplant gardens in Kamphaengsaen and Nakhon Pathom, Thailand in February and October 1995, and February 1996. The colony was maintained using methods developed by Shimizu and Kawasaki (2001) except that an eggplant leaf was provided as an oviposition substrate in place of a branch of the pickle, *Othonna capensis* L. H. Bailey. Plastic boxes (15 x 10 x 5 cm), with a 2 cm mesh-covered hole on one side, were used as rearing units. A mesh sheet (15 x 10 cm) and a piece of moist cotton wool were put in each box as a shelter for bugs and moisture regulator, respectively. Eggs of *Ephestia kuehniella* Zeller, killed by ultraviolet irradiation, were provided as a food source for the predators. A new eggplant leaf and *Ephestia* eggs were added every two or three days, and these eggplant leaves, moist cotton, mesh sheet and *Ephestia* eggs were renewed once a week. *Wollastoniella rotunda* was reared at the quarantine facility of the Institute of Biological Control at Kyushu University. The cage experiment was conducted under permission of the ministry of agriculture, forestry and fisheries of Japan.

Wollastoniella rotunda adults within 24 h after emergence were removed from the laboratory colony and reared individually in glass vials (2.5 cm diameter, 7.0 cm high) containing sufficient eggs of *E. kuehniella* for survival and reproduction, an eggplant leaflet (1.5 x 1.5 cm) and filter paper (1.5 x 1.5 cm). The prey eggs, leaflet and filter paper were changed daily. 24h after female emergence, an adult male was placed in a vial with an unmated female for one day, with prey. After the male was removed, each female was maintained in the glass vials, as described. Four or five-day-old mated females were used in the cage experiment.

A *T. palmi* colony was established from insects collected from an eggplant field in San'yo-cho, Okayama Prefecture, in summer 1993. The *T. palmi* were reared on kidney bean plants. Adult thrips collected from the colony were used to initiate the cage experiment. All the colonies of *W. rotunda* and *T. palmi* were kept at 25 ± 1˚C with a 16L: 8D photoperiod.

Cage experiment

The experiment was conducted in greenhouses at Kyushu University in Fukuoka city (33˚35´N, 130˚23´E), Fukuoka Prefecture, Japan from autumn 1999 to spring 2000. Fourteen eggplants (cv. chikuyo), *Solanum melongena* L., were grown individually in plant pots measuring 30 cm in height and 24 cm in diameter at the bottom and 30 cm in diameter at the top. On each eggplant that started fruiting and was at the 70- to 100-leaf stage, 120 adults of *T. palmi* were released on 6 October 1999. Seven cages measuring 1.2 m high by 1.0 m wide by 1.0 m long and covered with fine-mesh polyester organdy were positioned in each of two greenhouses (5.8 m x 3.7 m). A side of each cage has two zip fasteners that can be opened to allow entry into the cages. The thrips-infested eggplants were individually placed into each cage on 24 October. The thrips were allowed to acclimate for a 72-h period before to the introduction of predators. Five adult females of *W. rotunda* were released on each eggplant in the "predator" greenhouse on 27 October. No *W. rotunda* were released in the "without predator" greenhouse. At the initiation of the experiment (just before releasing *W. rotunda*), plants with and without predator had 1.74 ± 0.34 and 1.79 ± 0.44 (mean ± SE) thrips (adults and larvae) per leaf, respectively on 27 Octorber (Mann-Whitney *U* test, $P > 0.05$).

Population sampling from each cage took place weekly from 27 October 1999 to 1st of March 2000. Cages were zipped closed while insects were counted to prevent insects from escaping. All leaves, buds (both leaf and flower buds), stems and flowers of each eggplant were checked, and the number of *T. palmi* larvae and adults and *W. rotunda* were counted. For the predator, the 1st to 5th nymphal instars and adults were recorded.

The two greenhouses, which have the same structure, were spaced 1 m apart. The light conditions of greenhouses were similar as there are no shading of sunlight around the greenhouses. The soil in plant pots and watering frequencies were same among pots. Temperatures in each greenhouse were recorded hourly with digital thermometers, allowing the calculation of daily minimum and maximum temperatures in each greenhouse. During the experimental period, the average, and average minimum and average maximum temperatures were 17.8˚C ± 0.4, 14.0˚C ± 0.1 and 26.0˚C ± 0.4 (mean ± SE) for the predator treatment greenhouses, respectively, and 17.9˚C ± 0.1, 14.6˚C ± 0.1 and 26.2˚C ± 0.5 (mean ± SE) for the greenhouse without any predators, respectively. Thus, potential greenhouse effects on plants and insects were controlled for as much as was possible.

Analysis

A repeated measures ANOVA was conducted to detect significant differences in thrips and predator densities between treatments. In the analysis, the number of insects was log-transformed. The analysis was carried out in JMP® ver 4.0 (SAS Institute Inc., 2000).

RESULTS

The percentage of a four-month average of *W. rotunda* found on each plant structure was 83.5% (leaf), 15.4% (bud), 0% (flower) and 1.1% (stem) for adults, and 88.1% (leaf), 6.1% (bud), 0.1% (flower) and 3.7% (stem) for nymphs. The percentage of *T. palmi* found on each plant structure was 97.5% (leaf), 2.0% (bud), 0.04% (flower) and 0.07% (stem) for adults, and 98.7% (leaf), 0.07% (bud), 0% (flower) and 0.02% (stem) for larvae. Almost all *T. palmi* were found on leaves, while the majority of *W. rotunda* were found on the leaves and buds. Thus, the density per leaf and bud for *W. rotunda,* and density per leaf for *T. palmi* were used for analysis.

Released adults of *W. rotunda* successfully established and reproduced on all eggplants. The total density per leaf and bud for all stages gradually increased through November and remained at 0.1 until mid January, before increasing dramatically (Fig. 1). This population increase, from mid January, was composed mainly of nymphs (Fig. 2). *Wollastniella rotunda* density peaked on 23 February, when 10 times more predators were present than at the initiation of the experiment (0.04/leaf) (Fig. 1).

Two peaks of first-instar nymphs of *W. rotunda* appeared during the survey period (10 November and 9 February) (Fig.2). First-instar nymphs of the first generation were found one week after adult females were released. These nymphs developed through November and December, and the number of first generation adults started to increase in late December. No first-instar nymphs were found at the end of December and beginning of January, but the number dramatically increased during January. These nymphs of the second generation continued to develop until the end of the experiment.

The release of *W. rotunda* significantly lowered the density of *T. palmi*, and trends in population increase were found to be significantly different between treatments (Table 1: Fig. 3). For the control treatment, *T. palmi* density increased until the beginning of February and then declined as plant quality deteriorated. In contrast, *T. palmi* density remained at a much lower level in the predator release treatment (Table 1: Fig. 3). The maximum difference between the predator release and control treatments was approximately 10-fold at the end of January. Average densities of *T. palmi* larvae, adults and total number of larvae and adults (mean ± SE) throughout the experiment were 2.95 ± 0.92, 0.58 ± 0.15 and 3.53 ± 1.07 for the predator release treatment, and 10.08 ± 1.67, 1.91 ± 0.30 and 11.89 ± 2.03 for control, respectively.

Discussion

Wollastoniella rotunda successfully developed, reproduced and suppressed the population levels of *T. palmi* on eggplants under winter greenhouse production conditions. At the end of the experiment, one full generation and a second generation of adults had occurred.

First instar nymphs were found one week after the initial release of females. In spite of the fact that the first generation nymphs and adults were exposed to short-day conditions and relatively low temperatures (around 10h photoperiod and 17˚C), these individuals developed and reproduced. *Wollastoniella rotunda* does not oviposit without mating and has a preoviposition period following mating (Uefune, personal observation), like *Orius* species (Honda *et al*., 1998). Thus, most of December may be required for mating and egg maturation (Fig. 2). It is probable that these females continued to oviposit until the end of experiment, because first-instar nymphs were abundant over this period and would have continued to appear if the experiment continued. These observations would indicate that *W. rotunda* can reproduce independently of photoperiod even in relatively low temperature conditions.

Wollastoniella rotunda suppressed the thrips population to low levels throughout the survey period (Fig. 3). Economic thresholds for *T. palmi* on eggplant, which Matsuzaki & Ichikawa (1985) calculated based on the percentage of fruit scarred, was 0.49 thrips larvae and adults per leaf. Both maximum and average densities of *T. palmi* were higher than this threshold density on most caged plants with *W. rotunda*. Initial predator-prey ratios may be a critical factor determining the effectiveness of biological control agents in augmentative biological control programs (Castañé *et al.*, 1996). Thus, for effective biological control, release ratios should be evaluated for the system in the future.

The use of a non-diapausing predator species has required the introduction of non-indigenous, sub-tropical or tropical natural enemies to replace domestic natural enemies which are in active in winter. Hirose *et al.* (1999a) classified the search for non-diapausing natural enemies into two approaches: (1) seeking tropical or subtropical natural enemy species different from the domestic natural enemy species; and (2) seeking non-diapausing geographic races of the natural enemy species in subtropical or tropical regions if their ranges extend to these regions. We have successfully adopted the first approach, although several authors have also proposed the second approach. For example, Hirose *et al* (1999b) recorded that some natural enemies of *T. palmi* from the subtropical Ryukyu Islands of Japan, suggesting the possible use of these natural enemies, such as *Piocoris varius* (Uhler) (Hemiptera: Lygaeidae) and *O. strigicollis*, in winter greenhouses. Furthermore, importing a non-diapausing natural enemy species, which is commercially available, is also a useful approach. However, as any approaches above may have potential risks for native ecosystems, the ability of exotic species or strains of predator to overwinter should be carefully tested before the introduction (Shimizu and Kawasaki, 2001), even though non-diapausing species are unlikely to overwinter in temperate regions.

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REFERENCES

Castañé, C., Alomar, O., and Ridavets, J., 1996. Management of western flower thrips on cucumber with *Dicyphus tamaninii* (Heteroptera; Miridae). *Bio.l Contr*. **7**, 114-120.

Gillespie, D. R., Quiring, D. M. J., 1993. Extending seasonal limits on biological control. *IOBC/WPRS Bulletin* **16**, 43-45.

Hirose, Y., Kajita, H., Takagi, M., Okajima, S., Napompeth, B., Buranapanichpan, S., 1993. Natural enemies of Thrips palmi and their effectiveness in the native habitat, Thailand. *Biol. Contr.* **3**, 1-5.

Hirose Y., Nakashima Y., Shima K., Takagi M., Nagai K., Kajita H., and Urano S., 1999a. Surveying for non-diapausing predatory bugs for biological control of thrips pests in greenhouses during winter. *IOBC/WPRS Bulletin,* **22**, 105-108.

Hirose, Y., Nakashima, Y., Takagi, M., Nagai, K., Shima, K., Yasuda, K., Kohno, K., 1999b. Survey of indigenous natural enemies of the adventive pest *Thrips palmi* (Thysanoptera: Thripidae) on the Ryukyu Islands, Japan. *Appl. Entomol. Zool.* **34**, 489-496.

Honda, J. Y., Nakashima, Y., and Hirose, Y., 1998. Development, reproduction and longevity of *Orius minutus* and *Orius sauteri* (Heteroptera: Anthocoridae) when reared on *Ephestia kuehniella* eggs. *Appl. Entomol. Zool.* **33**, 449-453.

Ikeda, F., 1983. Ecology and control of *Thrips palmi* Karny on the muskmelon in green houses in Shizuoka Prefecture. *Shokubutsu boeki* **37**, 291-293 (in Japanese).

Ito, K., and Nakata, T., 1998. Effect of photoperiod on reproductive diapause in the predatory bugs, *Orius sauteri* (Poppius) and *O. minutus* (Linnaeus) (Heteropera: Anthocoridae). *Appl. Entomol. Zool*. **33**, 115-120.

Jacobson, R., 1993. Control of *Frankliniella occidentalis* with *Orius majusculus*: Experiences during the first full season of commercial use in the U. K. *SROP/WPRS Bull*. **16**, 81-84.

Kawai, A., 1995. Control of *Thrips palmi* Karny (Thysanoptera: Thripidae) by *Orius* spp. (Heteroptera: Anthocoridae) on greenhouse eggplant. *Appl. Entomol. Zool*. **30**, 1-7.

Kawai. A., 2001, Population management of *Thrips palmi* Karny. *Jpn. J. Appl. Entoml*. **45**, 39-59 (in Japanese with English summary).

Kohno, K. 1997. Photoperiodic effect on incidence of reproductive diapause in *Orius sauteri* and *O. minutus* (Heteroptera: Anthocoridae). *Appl. Entomol. Zool*. **32**, 644-648.

Makino, S., and Horikiri, M., 1983. Ecology and control of *Thrips palmi* Karny on the cucumber in Kagoshima Prefecture. *Shokubutsu boeki* **37**, 287-290 (in Japanese).

Matsuzaki, T., and Ichikawa, K., 1985. Injury on eggplant by *Thrips palmi* Karny in greenhouse. *Bull. Kochi Inst. Agr. & Forest. Sci.* **17**, 25-31 (in Japanese).

SAS, 2000. JMP® ver 4.0. SAS Institure Inc., Cary, North Carolina.

Shima, K., 1997. An ecological study on the predator, *Wollastoniella rotunda* (Heteroptera: Anthocoridae), with special reference to its effectiveness as a possible control agent of Thrips palmi (Tysanoptera: Thripidae) in greenhouse in Japan during the winter. M. S. thesis, Kyushu University.

Shima, K., and Hirose, Y., 2002. Effect of temperature on development and survival of *Wollastoniella rotunda* (Heteroptera: Anthocoridae), a predator of *Thrips palmi* (Thysanoptera: Thripidae). *Appl. Entomol. Zool.* **37**, 465-468.

Shimizu, T., and Kawasaki, K., 2001. Geographic variability in diapause response of Japanese *Orius* species. *Entomol. Exp. Appl.* **98**, 303-316.

Tsumuki, H., Nagai,K., and Kanehashi, K., 1987. Cold Hardiness of *Thrips palmi* Karny. 1. Survival period of winter and summer populations at low temperatures. *Jpn. J. Appl. Entomol. Zool.* **31**, 328-332 (in Japanese with English summary).

van de Veire, M., and Degheele, D., 1993. Control of western flower thrips, *Frankliniella occidentalis* with the predator *Orius insidiosus* on sweet peppers. *SROP/WPRS Bull*. 16,185-188.

van den Meiracker, R. A. F. 1994. Induction and termination of diapause in *Orius* predatory bugs. *Entomol. Exp. Appl*. 73, 127-137.

Yasunaga, T., and Miyamoto, S., 1993. Three anthocorid species (Heteroptera: Anthocoridae), predators of *Thrips palmi* (Thysanoptera) in eggplant gardens of Thailand. *Appl. Entomol. Zool*. **28**, 227-232.

Figure legends

Fig. 1. Changes in mean densities (N=7) of *W. rotunda* (all stages) in caged eggplants. Vertical lines indicate ±1 SEM.

Fig. 2. Changes in the age structure of *W. rotunda* population in caged eggplants (mean densities of N=7).

Fig. 3. Changes in mean densities of *T. palmi* (all stages) in caged eggplants with and without predators (N=7). Vertical lines indicate ±1 SEM.

Table 1. Repeated measures ANOVA for effect of predator release and time on number of *Thrips palmi* per leaf

Factor	df	*F*	*P*
Predator release	1, 12	17.64	0.0012
Time	18, 216	17.06	< 0.0001
Predator release x Time	18, 216	9.74	< 0.0001

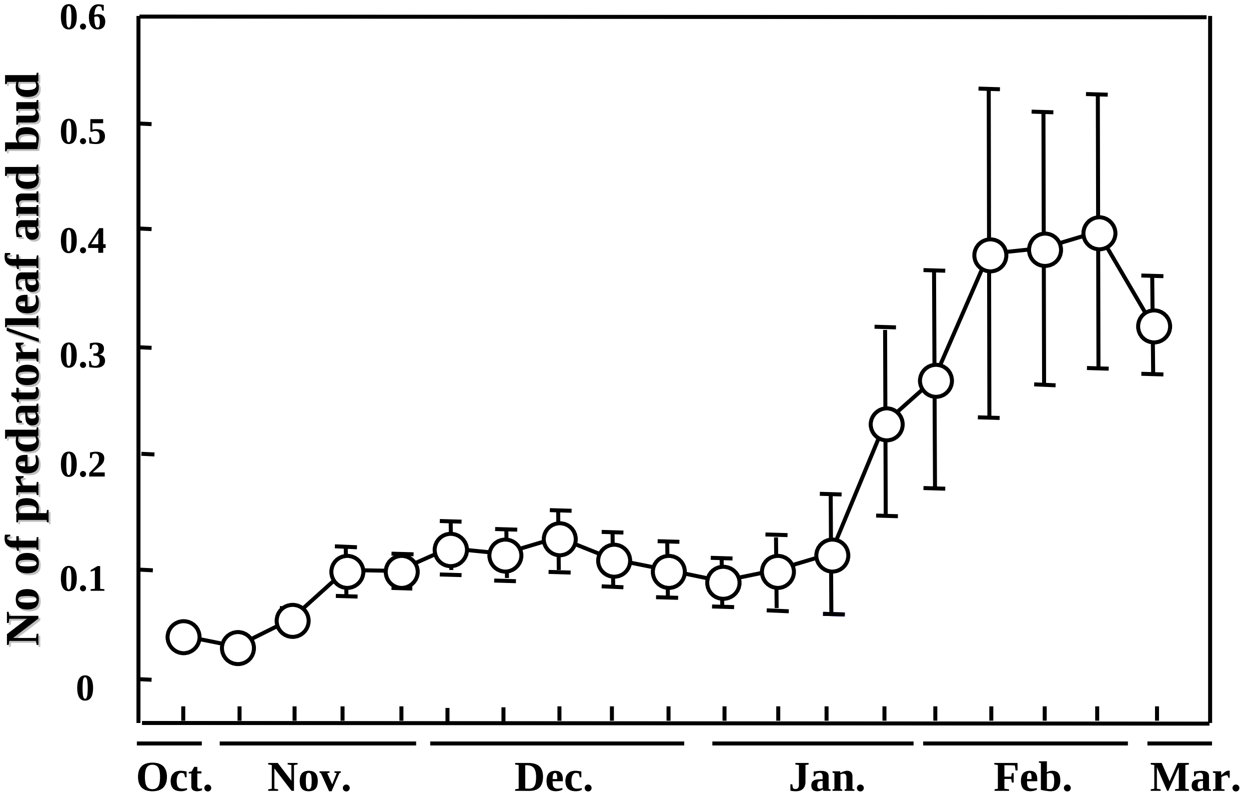

Fig. 1. Nakashima *et al.*

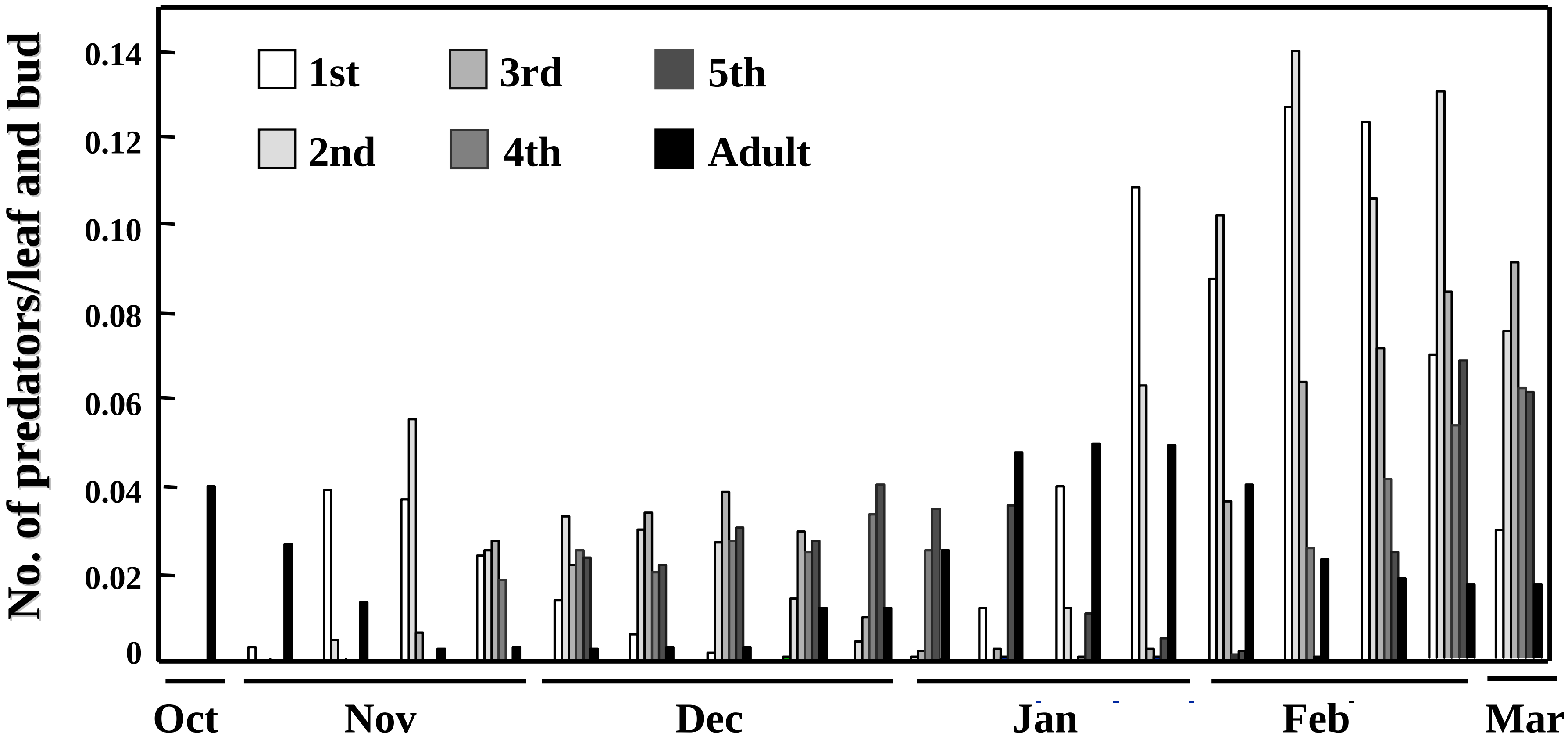

Fig. 2. Nakashima *et al.*

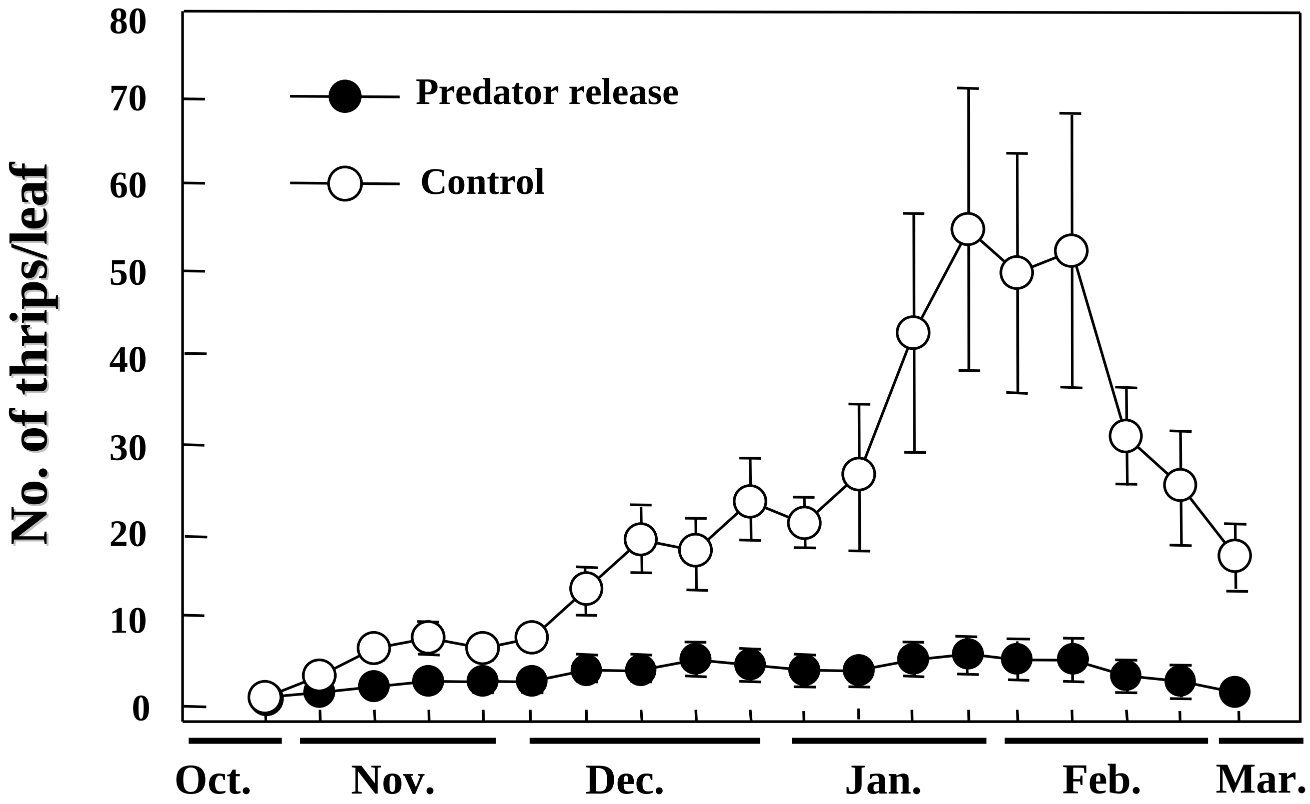

Fig. 3. Nakashima *et al.*