

# Challenges for Sustainable Pest Management Utilizing Indigenous Natural Enemies in Chrysanthemum Fields in Okinawa

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## Abstract

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*Thrips nigropilosus* is the most economically important pest of chrysanthemum. To manage this species, there is a need for integrated pest management (IPM), including the use of natural enemies on chrysanthemums, as an environmentally friendly and sustainable method of pest control. We conducted the following investigations to develop a technique for utilizing indigenous natural enemies of this pest in chrysanthemum: (1) exploration of natural enemies of *T. nigropilosus* in chrysanthemum fields; (2) evaluation of the predation ability of a potential natural enemy, *Campylomma livida*, against *T. nigropilosus*; and (3) field trials to assess the effectiveness of augmentative release methods for *C. livida*. In terms of the identified natural enemies, *Campylomma* spp., *Geocoris ochropterus*, *Orius* spp., *Haplothrips* spp., and *Ceranisus* sp. were found in chrysanthemum fields, with *C. livida* being the most abundant. In the laboratory, we observed that an adult *C. livida* could feed on at least 9.0 adults and 9.9 larvae of *T. nigropilosus* in 24 h. Therefore, a field trial was conducted using *C. livida* to examine its effects on lowering the density of *T. nigropilosus* and reducing the damage it causes. Consequently, the number of plants damaged by thrips decreased to one-fifth in the biological control plot (*C. livida* released), compared with the findings in the conventional plot where pesticides were sprayed regularly or to the control plot where no pesticides were sprayed. Damage to chrysanthemum was also reduced to less than one-third in the biological control plot, providing evidence for *C. livida*'s effectiveness for managing thrips in chrysanthemum fields.

**Key words:** *Thrips nigropilosus*, Ryukyu, Biological control, *Campylomma livida*.

## INTRODUCTION

The regular application of synthetic pesticides has significant impacts on ecosystems (Goulson 2013; Chagnon *et al.* 2015) and facilitates the development of pesticide resistance in pest populations. For example, *Frankliniella occidentalis* and *Tetranychus urticae*, which are important

pests of chrysanthemum (de Jager *et al.* 1995; Ganaha-Kikumura *et al.* 2012; OPPPC 2018), have developed a high degree of resistance to various pesticides (Osakabe *et al.* 2009; Ohno *et al.* 2010), resulting in control measures against these pests becoming inadequate. The extensive use of ineffective pesticides is also laborious and expensive for growers and is particularly

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undesirable in Japan, where the population engaged in agriculture is aging.

In Japan, 59 billion yen worth of *Chrysanthemum morifolium* Ramat (Asteraceae) was produced in 2023, with Okinawa accounting for 10% of that (MAFF 2024). Chrysanthemum cultivation in Okinawa Prefecture is conducted almost yearly and December and March are the main shipping periods to meet high demand, as chrysanthemum can be produced in Okinawa at a lower cost than in other Japanese regions by taking advantage of the mild winter climate and growing in open fields. The most important pests of chrysanthemum in Okinawa are thrips, spider mites, aphids, bugs, and lepidopteran larvae, with *Thrips nigropilosus* (Thysanoptera: Thripidae) being the most important (Ganaha-Kikumura *et al.* 2012). *T. nigropilosus* is widely distributed all over the world (Mound 2010) and is known to attack a variety of crops (Sakimura 1939; Bullock 1965; Stannard 1968; Mound *et al.* 1976; Umeya & Okada 2003).

However, *T. nigropilosus* has been regarded as a minor pest since the widespread use of pesticides reduced its pest status (Walker & Michaux 1989), and most reports on this species as a pest were published more than 40 years ago. Moreover, Ganaha-Kikumura *et al.* (2014) demonstrated that organophosphates, carbamate, synthetic pyrethroids, neonicotinoids, and other pesticides were highly effective against Okinawan populations of *T. nigropilosus*. However, in farmers' fields, the control of thrips has not been as effective as expected based on pesticide efficacy. Kijima *et al.* (2014) suggested that the cultivation of chrysanthemums in densely planted arrangements in Okinawa, where 18,000–22,000 plants are planted per 1,000 m<sup>2</sup>, prevents the sprayed chemical solution from reaching the microhabitat of the pest, such as the underside of leaves of plants grown inside the ridges. There is thus a need to establish an integrated pest management (IPM) technique, regardless of pesticide resistance.

Various control methods that can be

incorporated into IPM approaches against thrips have been evaluated for their effectiveness. Recently, it was reported that red light irradiation of plants changes the behavior of *Thrips palmi*, with fewer individuals being attracted to plants irradiated with red light than to plants subjected to normal light illumination (Murata *et al.* 2018). In addition, Ganaha-Kikumura & Zakimi (2018) reported that irradiation with red LED lights was significantly effective at lowering the density of *T. nigropilosus*, but was insufficient for the reduction of damage to chrysanthemum leaves. Because *T. nigropilosus* has been documented to attack a variety of plants, the population may be suppressed by controlling weeds around chrysanthemum fields that act as hosts of it. However, a survey of various weed species in areas in Okinawa where horticultural crops are cultivated showed that *T. palmi* was the dominant species, while *T. nigropilosus* was only found on a few Asteraceous weeds in chrysanthemum fields (Ganaha-Kikumura *et al.* 2022). In addition, netting houses, which have been primarily used for growing chrysanthemum to mitigate typhoon damage since 2000, have been shown not a function to reduce the number of thrips intruding into them (Ganaha-Kikumura *et al.* 2012). It thus seems to be more important to control the population density of *T. nigropilosus* in chrysanthemum fields, rather than that of individuals invading such netting houses.

In this study, we aimed to develop a technique for utilizing indigenous natural enemies of the thrips, which has rarely been attempted, for the establishment of an effective IPM system in chrysanthemum production. We conducted the following investigations: (1) exploration of potential natural enemies in open fields of chrysanthemum; (2) evaluation of the predation ability of one of the promising natural enemies, *Campylomma livida* (Hemiptera: Miridae), against *T. nigropilosus*; and (3) field trials to assess the effectiveness of augmentative release methods for *C. livida*.

## MATERIALS AND METHODS

### Search for indigenous natural enemies in chrysanthemum

We set the survey period in chrysanthemum fields in Okinawa Island as May–June in 2023 and 2024 for two reasons: (1) In Okinawa, blooming chrysanthemum flowers are present in the fields only during April and June since flowering is completely controlled by using electric night lights. (2) The fields that have not been sprayed with pesticides for a period of time are limited in those seasons. In total, we investigated 27 fields in the northern, central, and southern parts of Okinawa Island.

To search for natural enemies of chrysanthemum pests, 10 fully opened chrysanthemum flowers per field were collected in 70% ethanol. In some fields ( $n = 15$ ) where the chrysanthemum leaves were vibrant, we collected phytophagous thrips and potential candidates of natural enemies against the thrips by beating the plants by hand ten times at each of the three locations in the field. The insects that fell onto a tray (20 cm × 60 cm, covered with Tyvek® 700AG sheet; DuPont™, Wilmington, DE, USA) were immediately stored in 70% ethanol. Potential candidates, such as *Campylomma* spp., Geocorid species (Hemiptera: Geocoridae), and *Orius* spp. (Hemiptera: Anthocoridae), were identified based on Rural Culture Association Japan (2016), as well as the reports of Yasunaga *et al.* (2001, 2015), Oida (2009), and Ishikawa *et al.* (2012). For phlaeothripid thrips, specimens were macerated with 10% KOH and mounted in Hoyer's medium or Canada balsam after dehydration in ethanol and clearing in clove oil. Species identification was performed based on the report of Okajima (2006). If a female of either of the two morphologically closely related species, *Haplothrips brevitubus* and *Haplothrips chinensis*, was found, we treated it as *H. chinensis* because *H. brevitubus* has never been reported in Okinawa (Okajima 2006; Ganaha-

Kikumura *et al.* 2022). For thripid thrips, the specimens were prepared in the same way as for the phlaeothripid ones. Species identification was based on the reports published by Wilson (1975), Palmer (1992), Mound & Kibby (1998), and Masumoto & Okajima (2004). Some specimens were identified based on the work of Chiwaki *et al.* (1994) and Ganaha-Kikumura & Kawamura (2020) without specimen preparation because they had characteristic morphologies, such as for *Frankliniella cephalica*, *Microcephalothrips abdominalis*, and *T. nigropilosus*.

### Evaluation of the predatory ability of the indigenous natural enemy, *C. livida*, against the chrysanthemum thrips

In accordance with the procedure described by Nakao (1993), a disk of chrysanthemum leaves (20 mm in diameter) was placed on the bottom of a cylindrical plastic container (inner diameter 18 mm, height 49 mm) and fixed with Parafilm®. Ten adults or second-instar larvae of *T. nigropilosus* were placed in the container, and the top of the container was sealed with Parafilm® soon after the release of a single adult *C. livida* that had been deprived of food for 24 h. The containers were placed in a 14L : 10D thermostatic chamber at 25°C, and the number of dead thrips was counted 24 h after releasing the predacious bug. As a control, containers without the bug were also established. In the experiments on adults, 5 replicates and 3 control replicates were performed. Meanwhile, in the experiments on larvae, 16 replicates and 3 control replicates were performed.

### Trial of *C. livida* release in chrysanthemum fields

In September 2023, chrysanthemums (variety: “Kinshu”) were planted in nine netting houses (2.5 m × 6 m) at Okinawa Agricultural Research Center, Itoman City. These nine houses were divided into three treatment plots as follows: (1) a predatory bug, *C. livida*, was introduced to

manage pests (hereafter, biological control plot); (2) chemical pesticides were applied regularly (hereafter, conventional plot); and (3) neither chemical pesticides nor natural enemies were applied (hereafter, control plot). In the biological control plot, one adult of *C. livida* per m<sup>2</sup> was released twice at a 2-wk interval. The release density was based on that of *Orius strigicollis*, a species that is commercially available in Japan as a biological control agent against thrips and that shares several biological characteristics with *C. livida*. Two holy basil plants were planted per netting house as insectary plants for the bugs. Each house was randomly assigned within an 800 m<sup>2</sup> field, ensuring a minimum distance of 3.0 m between them. To prevent indigenous natural enemies from intruding into the houses, broad-spectrum pesticides were sprayed around the houses at least once a week. In addition, to minimize potential spatial bias, the three treatments were systematically allocated across the houses. The insect pests and their natural enemies were surveyed at 5–12 d intervals, each covering 30 plants (90 leaves) per house. Chrysanthemums were harvested on December 14. The cut flowers were trimmed to a length of 70 cm and the leaves on the lower 20 cm of the plant were removed to meet standards for shipping. Among the remaining leaves, the number of damaged leaves was counted. For the plants that exhibited core stoppage symptoms, as caused by *Taylorilygus apicalis* (Hemiptera: Miridae), the number of sites of damage was also counted.

### Statistical analysis

To evaluate the difference in the abundance of thrips among the three treatments (biological control plot, conventional plot, and control plot), we conducted statistical analysis using two hurdle models since the dataset comprised a substantial number of zeros. For both models, a binomial distribution with a logit link was used to model the absence or presence of thrips, after which Poisson and negative binomial distributions with a log link function were used for the count for each model. As a result of comparing the fitting of the two hurdle models

by performing a likelihood ratio test, we chose the model with a negative binomial distribution. The dispersion parameter [Log( $\theta$ )] was also significant (estimate = -1.19,  $P = 0.00020$ ), confirming the appropriateness of the model. A Kruskal-Wallis test was conducted to examine the significance of differences in spider mite and scale insect occurrences across the three treatments. Because almost all of the data were 0 in some treatments, we assessed normality using the Shapiro-Wilk test. As the data deviated from normality, we used the non-parametric Kruskal-Wallis test, followed by pairwise Wilcoxon rank-sum test with Bonferroni correction applied to adjust for multiple comparisons.

The effect of treatment on the number of damaged leaves caused by thrips per plant was analyzed using a generalized linear mixed model (GLMM). This model included treatment as a fixed effect and the netting house as a random effect. The total number of leaves per plant was included as an offset to standardize the response variable to a per-plant scale. The analysis was performed using the “glmmTMB” package in R, assuming a Poisson distribution with a log link function.

The effect of treatment on the number of sites damaged by *T. apicalis* per plant was analyzed using GLMM assuming a negative binomial distribution with a logit link function.

To test the overall significance of the treatment effect, a type II Wald chi-square test was conducted using the “ANOVA” function in the “car” package. Pairwise comparisons between treatments were performed using Tukey’s method for multiple comparisons with the “emmeans” package.

### Comparison of net production value

To examine the management implications of reducing the number of pesticide applications through the release of *C. livida*, the net production value was calculated by subtracting the cost of materials and labor for chemical application from the production value. The unit price of the pesticides was calculated from the retail price as of December 2024, and the time required for

pesticide/natural enemy application based on the amount of pesticide, the number of units shipped per unit area, and the unit sales price were calculated based on the regional crop-specific management indicators (OPG 2018). Since *C. livida* is not available on the market in Japan, the unit sales price of *O. strigicollis*, which is already commercially available as a natural enemy formulation, was used as a reference to calculate the unit sales price. The time required to apply *C. livida* was set to 15 min 1,000 m<sup>-2</sup>; this setting was based on the standard release density of *O. strigicollis* in Japan, with which the release density of *C. livida* was aligned in the present study. The presence or absence and extent of thrips damage have a major impact on the unit price of chrysanthemums. However, there are no fixed standards for the difference in price due to insect damage because the unit price is greatly influenced by market trends and the balance of supply and demand at any given time. Therefore, in this study, the unit prices of high- and medium-grade products were calculated as 25 yen and 12 yen, respectively, by considering the plants with 0–19% and ≥ 20% damaged leaves as those of high and medium grade, respectively.

## RESULTS

### Search for indigenous natural enemies in chrysanthemum

Table 1 shows the results of our survey of chrysanthemum flowers. The candidate natural enemies found were *Campylomma* spp., *Orius* spp., *Haplothrips* spp., and parasitoid wasps including the genus *Ceranisus* sp. (Hymenoptera: Eulophidae) with herbivorous thrips. Except parasitoids, *Campylomma* spp. were the most abundant in terms of both the number of locations and the number of individuals found and they included two species, *C. livida* and *C. lividicornis*. We could not identify *Orius* spp. at the species level due to a lack of adult specimens. We collected three individuals of phlaeothripid thrips, two *Haplothrips chinensis*, and one *Haplothrips gowdeyi*. We collected 897 thripid

thrips in the 27 fields. A total of 347 individuals were identified as *F. cephalica*, *Frankliniella intonsa*, *M. abdominalis*, *Thrips hawaiiensis*, *T. nigropilosus*, *T. palmi*, and *T. tabaci*. Among them, *F. intonsa* and *M. abdominalis* were the most abundant species, while *T. nigropilosus* was relatively uncommon (7.5% of the total). The remaining 549 individuals were not identified due to their immaturity, while one specimen was in a poor condition.

Table 2 shows the results of the survey based on beating the plants in the chrysanthemum fields 10 times. We performed investigations in 15 fields in various localities on Okinawa Island and identified two candidate natural enemies: *Campylomma* spp. and *G. ochropterus*. *Campylomma* spp. were the more common of these and were found in all surveyed areas. Twelve individuals were identified as *C. livida*, whereas *C. lividicornis* was not found in this survey. Larvae of this genus were often collected sympatrically with its adults. We collected 337 thripid thrips from the 15 fields. A total of 89 individuals were identified as *F. cephalica*, *F. intonsa*, *M. abdominalis*, *T. hawaiiensis*, *T. nigropilosus*, and *T. palmi*. Compared with the flower survey results, the proportion of *T. nigropilosus* was higher, accounting for 34.7% of the total.

### Evaluation of the predatory ability of the indigenous natural enemy, *C. livida*, against chrysanthemum thrips

Despite the limited sample size, the outcome was unambiguous- both adult and larval thrips offered were almost entirely preyed upon. The adults of *C. livida* preyed on an average of 9.0 adult thrips (range 7–10 individuals) and an average of 9.9 larval thrips (range 9–10) per 24 h. No thrips died in either the adult or the larval controls.

### Trial of *C. livida* release in the field

Figure 1 shows the numbers of *T. nigropilosus* and *C. livida* per shoot. *C. livida* was established on all three biological control plots throughout

**Table 1.** Species composition of thripid thrips and candidate natural enemies against thrips collected from chrysanthemum flowers on Okinawa Island in 2023–2024.

Area	NS <sup>z</sup>	Number of fields where natural enemies were found (number of individuals)														
		Number of thripid thrips					Number of fields where natural enemies were found (number of individuals)									
		<i>Frankliniella intonsa</i>					<i>Campylomma</i> spp. <sup>x</sup>					<i>Haplothrips</i> spp.				
		Total					Total					Total				
		<i>Microcephalothrips abdominalis</i>					Immature stages					<i>Orius</i> spp.				
		<i>Thrips nigropilosus</i>					Others <sup>y</sup>					Geocorid species				
		<i>Thrips palmi</i>					Immature stages					Immature stages				
		5					<i>C. livida</i>					<i>H. chinensis</i> <sup>w</sup>				
		11					<i>C. lividicornis</i>					<i>H. gowdeyi</i>				
		22					Total					Total				
		38					Total					Total				
		10					Total					Total				
		26					Total					Total				
		120					Total					Total				
		154					Total					Total				
		46					Total					Total				
		73					Total					Total				
		35					Total					Total				
		897					Total					Total				
		206					Total					Total				
		417					Total					Total				
		274					Total					Total				
		897					Total					Total				
Okinawa Island		8					Total					Total				
Northern region		11					Total					Total				
Central region		8					Total					Total				
Southern region		27					Total					Total				
Total		897					Total					Total				

<sup>z</sup> NS: Number of fields surveyed. All fields surveyed in the same year were at least 200 m apart.

<sup>y</sup> The category "Others" includes the following species: *Frankliniella cephalica*, *Thrips hawaiiensis*, *Thrips tabaci*, and an unidentified individual.

<sup>x</sup> Species identification was performed using only adult males.

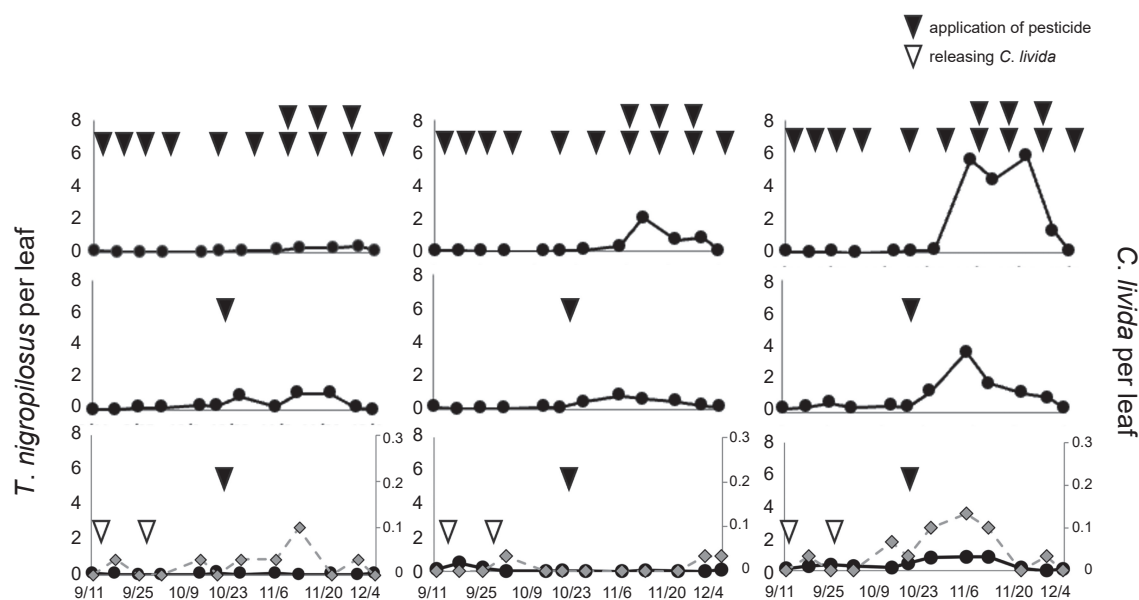
<sup>w</sup> Data include male specimens only.

<sup>v</sup> Potential thrips-parasitizing wasps including the genus *Ceramistis*.

**Table 2.** Species composition of candidate natural enemies against thrips collected by beating chrysanthemum plants on Okinawa Island during 2023–2024.

Area	NS <sup>z</sup>	Number of thripid thrips										Number of fields where natural enemies were found (number of individuals)								
		<i>Frankliniella intonsa</i>	<i>Microcephalothrips abdominalis</i>	<i>Thrips nigropilosus</i>	<i>Thrips palmi</i>	Others <sup>y</sup>	Immature stages	Total	<i>C. livida</i>	<i>C. lividicornis</i>	Immature stages	<i>Geocoris ochropterus</i> <sup>w</sup>	<i>Orius</i> spp.	Total	<i>H. chinensis</i>	<i>H. gowdeyi</i>	Immature stages	Parasitoid wasps <sup>v</sup>		
Okinawa Island																				
Northern region	2	3	0	0	3	0	0	0	0	0	0	2 (4)	0	1 (2)	0	0	0	0	0	0
Central region	8	154	17	35	9	0	5	88	5 (17)	5 (6)	0	5 (17)	0	1 (2)	1 (2)	0	0	0	0	0
Southern region	5	180	4	0	14	1	1	160	4 (12)	4 (6)	0	4 (12)	0	2 (3)	1 (1)	0	0	0	0	0
Total	15	337	21	35	26	1	6	248	11 (33)	9 (12)	0	11 (33)	0	4 (7)	2 (3)	0	0	0	0	0

<sup>z</sup> NS: Number of fields surveyed. Almost all fields surveyed were included among the fields in Table 1.<sup>y</sup> The category "Others" includes *Frankliniella cephalica* and *Thrips hawaiiensis*.<sup>x</sup> Species identification was performed using only adult males.<sup>w</sup> The central region contained a larval stage.<sup>v</sup> Potential thrips-parasitizing wasps.



**Fig. 1.** The numbers of individuals of *T. nigropilosus* (solid black line) and *C. livida* (dashed grey line) per chrysanthemum leaf.

the study period. The abundance in the biological control plots was significantly lower than that in the conventional plots [exp(estimate) = 0.20, 95% CI: 0.08–0.47,  $z = -3.65$ ,  $P < 0.05$ ]. Despite the regular application of pesticides in the conventional plots (Table 3), there was no significant difference in the abundance of thrips between the conventional and control plots in the counting model [exp(estimate) = 0.58, 95% CI: 0.25–1.34,  $z = -1.28$ ,  $P > 0.05$ ]. The zero hurdle component (binomial with a logit link) revealed that the control plots was associated with a significant increase in the probability of non-zero observation [exp(estimate) = 10.00, 95% CI: 1.18–84.8,  $z = 2.11$ ,  $P < 0.05$ ], whereas the biological control plots had no significant effect on this parameter [exp(estimate) = 1.18, 95% CI: 0.38–3.70,  $z = 0.29$ ,  $P > 0.05$ ], when compared with the conventional plots.

The GLMM indicated that the treatment significantly affected the number of leaves damaged by thrips per plant (type II Wald chi-square test,  $\chi^2 = 643.02$ ,  $P < 0.001$ ). Pairwise comparisons adjusted by Tukey's method revealed that the number of damaged leaves in the biological control plots was significantly

lower than in the conventional [GLMM Poisson, exp(estimate) = 0.271, 95% CI: 0.24–0.30,  $z = -23.69$ ,  $P < 0.0001$ ] and control plots [GLMM Poisson, exp(estimate) = 0.267, 95% CI: 0.24–0.30,  $z = -24.28$ ,  $P < 0.0001$ ]. There was no significant difference in this variable between the conventional and control plots [GLMM Poisson, exp(estimate) = 0.984, 95% CI: 0.91–1.07,  $z = -0.47$ ,  $P > 0.05$ ].

We observed some *T. apicalis* on the terminal buds of chrysanthemums after November. In the survey of the quality of products conducted after the harvest, no significant differences were identified among the three treatments in the number of leaves damaged by *T. apicalis* per plant (type II Wald chi-square test,  $\chi^2 = 2.463$ ,  $P > 0.05$ ).

Shapiro-Wilk tests indicated that the data within each treatment deviated significantly from normality (all  $P < 0.05$ ). Therefore, we employed the non-parametric Kruskal-Wallis test. The rate of occurrence of *Tetranychus urticae* (green form) differed significantly among the three treatments, as indicated by the Kruskal-Wallis test ( $\chi^2 = 64.15$ ,  $df = 2$ ,  $P < 0.001$ ). The number of spider mites was significantly higher in the conventional plots

**Table 3.** History of chrysanthemum and pesticide use in the 2023 *C. livida* release trial.

Date	Crop management	Active ingredient			IRAC code <sup>y</sup>
		Biological control plot	Control plot	Conventional plot <sup>z</sup>	
4 Sep	Transplanting				
12 Sep		<i>C. livida</i> [1.0 individuals m <sup>-2</sup> ]		Imidacloprid 20% SC	4A
15 Sep	Pinching				
20 Sep				Abamectin 1.8% EC	6
26 Sep		<i>C. livida</i> [1.0 individuals m <sup>-2</sup> ]		Chlorfenapyr 10% SC	13
28 Sep	Pruning				
2 Oct				Prothiofos 45% EC	1B
		<i>Bacillus thuringiensis</i> and the insecticidal proteins it produces			11A
20 Oct				Tebufenpyrad 10% EW	21A
31 Oct				Milbemectin 1.0% EC	6
1 Nov	Turning off supplemental lights				
9 Nov				Cyenoxyrafen 30% SC Tolfenpyrad 15% EC	25A 21A
16 Nov				Spinosad 25% WG Acequinocyl 15% SC	5 20B
27 Nov				Sulfoxaflor 9.5% SC Bifenthrin 7.2% SC	4C 3A
1 Dec				Abamectin 1.8% EC	6
14 Dec	Harvesting				
Total number of pesticides		1	1	13	

<sup>z</sup> SC: suspension concentrate; EC: emulsifiable concentrate; EW: emulsion, oil in water; WG: water dispersible granules. See Appendix 2 for the cost per unit, dilution ratio, and applied amount of each pesticide.

<sup>y</sup> IRAC: Insecticide Resistance Action Committee.

than in the biological control and control plots ( $P < 0.001$ , Wilcoxon rank-sum test). No significant difference in this variable was observed between the biological control and control plots ( $P > 0.05$ ). The occurrence of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), which is not considered to be economically important, also differed significantly among the three treatments, as shown by the Kruskal-Wallis test ( $\chi^2 = 22.33$ ,  $df = 2$ ,  $P < 0.001$ ). The number of scale insects was significantly lower in the conventional plots than in the biological control and control plots ( $P < 0.001$ , Wilcoxon rank-sum test). However, there was no significant difference in this variable between the biological control and control plots ( $P > 0.05$ ).

## Comparison of net production value

The net production value of each plot is shown in Table 4. As supplemental data, the breakdown of production value by quality, pesticide costs per unit area, and labor costs for pesticide application are provided in Appendices 1, 2, and 3, respectively. The net production value of the conventional plot was 434,430/1,000 m<sup>2</sup>, while the control plot was expected to increase revenue by 72,809 yen and the biological control plot by 245,598 yen, compared with the level for the conventional plot.

## DISCUSSION

In this study, we explored potential natural

**Table 4.** Comparison of the net production value per 1,000 m<sup>2</sup> between treatments.

Treatment	Production value (JPY) <sup>z</sup>	Pesticide/biological control agent costs (JPY) <sup>y</sup>	Labor costs for pesticide application (JPY) <sup>x</sup>	Net production value (JPY)
Biological control plot	784,029 (627,223–940,835)	104,004	3,500	680,028 (523,221–836,834)
Control plot	512,160 (409,728–614,592)	4,921	2,625	507,239 (404,807–609,671)
Conventional plot	512,160 (409,728–614,592)	77,730	39,375	434,430 (331,998–536,862)

<sup>z</sup> The production value was estimated based on the unit prices and the quantities. Based on the proportion of damaged leaves in the harvest survey, the quality of chrysanthemums was divided into high grade and medium grade, with unit prices incorporating a  $\pm$  20% variation. The quantities were calculated based on the composition of each grade. See Appendix 1 for details.

<sup>y</sup> The costs consist of the unit price, dilution ratio of each pesticide, and amount used per unit area. See Appendix 2 for details.

<sup>x</sup> The hourly rate was set at 1,750 JPN based on the guidelines of OPG (2018). See Appendix 3 for details.

enemies in chrysanthemum fields. As a result, we identified *Campylomma* spp., *G. ochropterus*, *Orius* spp., and *Haplothrips* spp. in chrysanthemum fields on Okinawa Island. Among these, *C. livida*, which has been considered an effective natural enemy of thrips and whitefly (Wang 1994; Qin *et al.* 2004; Kijima *et al.* 2013; Nakaishi 2013), was the most frequent and abundant (Tables 1 and 2).

*C. livida* is known to prey on up to 482.5 second-instar larvae of *T. palmi* per day (Nakaishi 2013). Since no previous findings of whether it preys on *T. nigropilosus* could be identified, we fed adult and larval thrips in the laboratory to determine whether the *C. livida* adults preyed on them. The results showed that the adult *C. livida* could feed on an average of 9.0 adults or 9.9 second-instar larvae out of 10 *T. nigropilosus* individuals provided within 24 h, suggesting that it can be considered an effective natural enemy of *T. nigropilosus*. Although further study is needed to determine the exact level of predation, it could be close to the amount of predation by *T. palmi* because the body sizes of the thrips and *T. nigropilosus* are comparable.

In the field trials, *C. livida* significantly reduced the number of thrips-damaged plants compared with that in the conventional treatment. The high proportion of zeros likely reflects the patchy distribution of thrips and the effectiveness of treatments in eliminating the pest from many sampling points. Furthermore, the damage to chrysanthemum products was

significantly reduced in the biological control plots (Fig. 2). Although the present field study was conducted only single season, these results strongly suggest that the biological control plots are the most effective option for reducing thrips populations and the damage they cause to chrysanthemum products. In conventional chrysanthemum cultivation in Okinawa, pesticides are regularly applied throughout the cultivation period (Fig. 1). Therefore, our surveys for potential natural enemies were limited to the period of May–June, which corresponds to the time when residues have remained in the field for a relatively long time after harvest. However, *C. livida* was established in our field trial from September to December (Fig. 1). As Yasunaga *et al.* (2015) mentioned, this predacious bug is multivoltine, indicating that it has the potential for year-round use for controlling thrips in chrysanthemum. Although there was a concern about it potentially damaging chrysanthemum as *C. livida* has been reported to damage crops (Luo *et al.* 1996), no such damage was observed in this study. These results provide evidence for the effectiveness of *C. livida* for controlling thrips in chrysanthemum.

The findings of this study also suggested that the use of *C. livida* would be associated with a significant increase in profitability, and labor costs would be significantly lower for maintaining the biological control plots than for the conventional plots (Table 4). However,



**Fig. 2.** Examples of leaf damage caused by *T. nigropilosus*. (A) Undamaged leaf. (B) Slightly damaged leaf: visible but minor damage. (C) Heavily damaged leaf: significant damage affecting a large portion of the leaf surface. Leaves classified as “damaged” include both slightly and heavily damaged leaves.

there is a need for more detailed confirmation of the actual profitability relative to the net production value because, despite the fact that unit prices and quality evaluation criteria are significantly influenced by fluctuations in market conditions, we adopted predetermined values for both high- and medium-grade products in our calculation. Moreover, the cost of *C. livida* was calculated based on the price of *O. strigicollis*, assuming that it would also become available on the market. However, since it is the most common indigenous natural enemy found in chrysanthemum, it may be possible to apply it at a lower cost by using insectary plants to attract or retain it.

There was no significant difference between the conventional and control plots in terms of the number of leaves damaged by thrips per plant. The factors contributing to this are unclear, but it is possible that the dense planting of chrysanthemums prevented the chemical solution from reaching the thrips’ habitat, as shown by Kijima *et al.* (2014). Our results may indicate that the chemical was not sufficiently effective due to it being applied unevenly. More research is needed to determine whether other factors may have been involved in the lack of a significant difference mentioned above. To prevent the movement of *C. livida* between treatment plots, a variety of pesticides were applied around the netted enclosures at least once a week. However, because the dispersal capacity of *C. livida* is not clearly understood, it remains

uncertain whether inter-plot movement was fully prevented. If *C. livida* had moved between plots, such movement would likely have reduced the differences between the natural enemy release plots and the others. Nevertheless, the clear differences detected in this study strongly suggest the effectiveness of releasing *C. livida* as a biological control agent.

Biological control in chrysanthemum cultivation may involve both positive and negative side effects on pests other than thrips. Spider mites, which are known to be the second most important chrysanthemum pest after *T. nigropilosus* (Ganaha-Kikumura *et al.* 2012), were significantly less abundant in the biological and control plots than in the conventional plots. Meanwhile, no significant difference in this regard was observed between the biological and control plots. It was not possible to determine why there was a low level of spider mite infestation in this trial, but the presence of natural enemies of spider mites may have had an effect, as predacious gall midges and predatory mites have been observed on chrysanthemum in other surveys. In other words, as clarified by Bommarco *et al.* (2011) in a study on cabbage, pesticide application may eliminate the natural enemies of spider mites, resulting in a surge in their population. Future research should be performed to clarify this. However, reducing the frequency of pesticide application caused a significantly higher occurrence of a new pest, *P. solenopsis*. Multiple infestations of this scale insect could

cause the wilting of new shoots. Besides, this species is known to be polyphagous (Tanaka & Uesato 2012) and can utilize a variety of weeds, so we should aware of it when reducing pesticide application. There is also an important pest of chrysanthemum, *T. apicalis* (Yasuda 1993). As it belongs to the same family as *C. livida*, it is anticipated that the use of pesticides to control *T. apicalis* may negatively affect *C. livida*. From this point of view, it is thus crucial to develop IPM techniques that do not rely on pesticides.

In this experiment, the density of the release of *C. livida* was set based on that of *O. strigicollis*, and releases were carried out twice- immediately after planting and two weeks later- primarily for convenience. During the trial, the natural enemy was frequently associated with newly emerging shoots (personal observation). This indicates that optimizing the release timing with shoot development may improve establishment. Mass-rearing of this natural enemy is technically feasible, and the availability of commercialized indigenous predatory bugs suggests that its commercialization is also achievable.

It should be noted that using a single natural enemy may induce an enemy-risk effect in pest populations (Culshaw-Maurer *et al.* 2020) and increase the risk of control failure over time due to intraguild predation (Rosenheim 2005). To mitigate or avoid such risks, the development of techniques that enhance the diversity of natural enemies around the fields- such as placing flowering chrysanthemums near field margins- and the use of natural enemies with different ecological niches and functional traits (e.g. activity periods or seasonal occurrences) could contribute to stabilizing pest control. The genus *Ceranisus*, parasitoid wasps identified in this study, known to parasitize multiple thrips species, could be considered as one of the potential candidates.

For large-scale field trials, additional studies will be required, including the assessment of the effects of major insecticides and fungicides, elucidation of developmental traits, determination of optimal release timing and density, evaluation of the necessity of banker plants for conservation

of natural enemies and selection of appropriate plant species, as well as the development of countermeasures against emerging pests including scale insects.

Once these aspects are clarified, growers should recognize that the effectiveness of natural enemy releases in chrysanthemum production can vary greatly depending on environmental conditions and crop management practices, which are performed based on their judgment, including the types and timing of pesticide applications.

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**Appendix 1.** Breakdown of production value by quality.

Treatment	Quality grade <sup>z</sup>	Unit price (JPY/unit) <sup>y</sup>	Quantity <sup>x</sup>	Production value (JPY) <sup>w</sup>
Biological control plot	High grade	25 (20–30)	20,913	522,825 (418,260–627,390)
	Medium grade	12 (9.6–14.4)	21,767	261,204 (208,963–313,445)
	Subtotal			784,029 (627,223–940,835)
Control plot	High Grade	25 (20–30)	0	0
	Medium Grade	12 (9.6–14.4)	42,680	512,160 (409,728–614,592)
	Subtotal			512,160 (409,728–614,592)
Conventional plot	High Grade	25 (20–30)	0	0
	Medium Grade	12 (9.6–14.4)	42,680	512,160 (409,728–614,592)
	Subtotal			512,160 (409,728–614,592)

<sup>z</sup> A damaged leaf rate of < 20% was considered to represent high grade, while ≥ 20% was considered to represent medium grade. See Fig. 2 for the definition of damaged leaves.

<sup>y</sup> Unit prices for high grade and medium grade were taken with reference to guidelines from OPG (2018). Values in parentheses indicate the range based on a ± 20% variation in unit price.

<sup>x</sup> The total number of chrysanthemums per 1,000 m<sup>2</sup> was set at 42,680 for all treatments. Quantity by grade was calculated according to the percentage of each grade in our harvesting survey.

<sup>w</sup> Values in parentheses indicate the range based on a ± 20% variation.

**Appendix 2.** Pesticide costs per 1,000 m<sup>2</sup> in Okinawa, Japan.

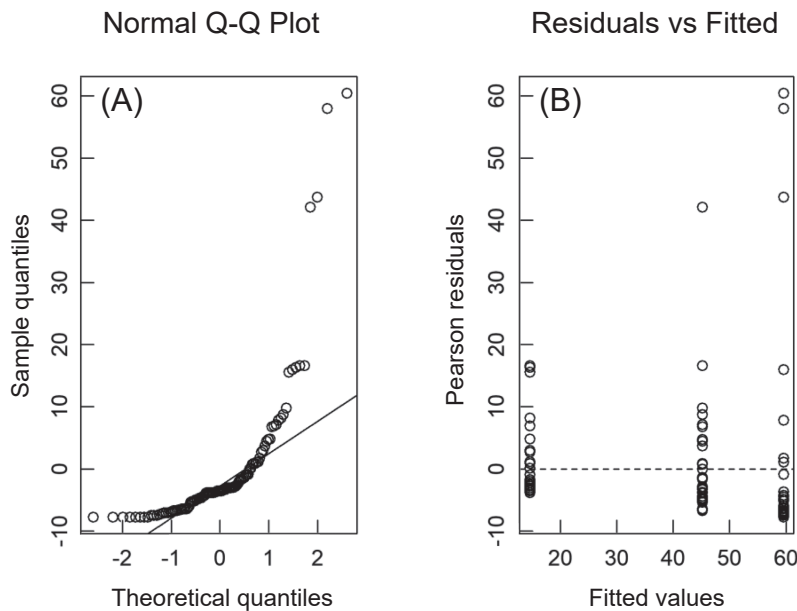
Treatment	Pesticide/biological control agent	Cost per unit (JPY)	Dilution ratio	Applied amount (l)	Quantity used	Unit	Cost per unit area (JPY)
Biological control plot	<i>C. livida</i>	49.1 <sup>y</sup>	-	-	2,000	individual	98,208
	<i>BT</i> <sup>z</sup>	11.5	1,000	200	200	g	2,296
	Subtotal						104,004
Control plot	<i>BT</i>	11.5	1,000	200	200	g	2,296
	Subtotal						2,296
Conventional plot	Imidacloprid	21.0	2,000	150	75	mL	1,574
	Abamectin	9.9	500	450	900	mL	8,952
	Chlorfenapyr	27.4	2,000	150	75	mL	2,058
	Prothiofos	10.0	1,000	200	200	mL	2,004
	Tebufenpyrad	11.1	1,000	200	200	mL	2,224
	Milbemectin	10.2	1,500	200	133	mL	1,362
	Cyenoxyrafen	22.6	2,000	300	150	mL	3,395
	Tolfenpyrad	13.1	1,000	300	300	g	3,928
	Spinosad	62.9	5,000	300	60	g	3,775
	Acetaminocyl	11.2	1,000	300	300	mL	3,374
	Sulfoxaflor	11.7	1,000	300	300	mL	3,517
	Bifenthrin	29.2	4,000	300	75	mL	2,192
	Subtotal						38,355

<sup>z</sup> *Bacillus thuringiensis* and the insecticidal proteins it produces.

<sup>y</sup> The cost per unit was calculated based on the retail price of *Orius strigicollis* because *C. livida* is not commercially available in Japan.

**Appendix 3.** Labor costs for pesticide application in Okinawa, Japan.

Treatment	Task	Labor period (h)	Hourly rate (JPY h <sup>-1</sup> )	Total cost (JPY)
Biological control plot	<i>C. livida</i>	0.50	1,750	875
	<i>BT</i>	1.50	1,750	2,625
	Subtotal	2.00		3,500
Control plot	<i>BT</i>	1.50	1,750	2,625
	Subtotal	1.50		2,625
Conventional plot	Imidacloprid	1.25	1,750	2,188
	Abamectin	3.25	1,750	5,688
	Chlorfenapyr	1.50	1,750	2,625
	Prothiofos	1.50	1,750	2,625
	Tebufenpyrad	1.50	1,750	2,625
	Milbemectin	1.50	1,750	2,625
	Cyenoxyrafen	2.00	1,750	3,500
	Tolfenpyrad	2.00	1,750	3,500
	Spinosad	2.00	1,750	3,500
	Acequinocyl	2.00	1,750	3,500
	Sulfoxaflor	2.00	1,750	3,500
	Bifenthrin	2.00	1,750	3,500
	Subtotal	22.50		39,375



**Supplementary Fig. 1.** Residual diagnostic plots for the count component of the hurdle model. (A) Quantile-quantile (Q-Q) plot of Pearson residuals shows no major deviation from normality. (B) The residuals versus fitted values plot indicates homoscedasticity and no apparent pattern, suggesting an adequate model fit.

# 利用本土天敵進行沖繩菊花田害蟲永續管理的挑戰

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## 摘要

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菊褐斑薊馬 (*Thrips nigropilosus*) 是沖繩所產菊花最重要的經濟害蟲。為了管理該物種，需進行害蟲整合管理，包括在菊花上使用天敵，以作為友善且永續的害蟲防治方法。為開發以本土天敵防治此菊花害蟲的技術，遂進行下列研究：(1) 在菊花田中調查菊褐斑薊馬的天敵；(2) 評估潛在天敵中華微刺盲椿象 (*Campylomma livida*) 對菊褐斑薊馬的捕食能力；(3) 以田間試驗評估增強釋放 *C. livida* 的有效性。已鑑定天敵包含 *Campylomma* spp.、*Geocoris ochropterus*、*Orius* spp.、*Haplothrips* spp. 以及 *Ceranisis* sp.，其中以 *C. livida* 的數量最多。在實驗室中，每隻 *C. livida* 成蟲於 24 小時內，可取食菊褐斑薊馬之成蟲與若蟲，分別為 9 隻與 9.9 隻。因此，我們利用中華微刺盲椿象進行實地試驗，以檢查其降低菊褐斑薊馬密度與減少損害的效果，並與定期噴灑農藥的傳統樣區或未噴灑農藥的對照樣區進行比較，結果顯示在生物防治樣區 (釋放了 *C. livida*) 所受薊馬危害的植物數量減少 1/5，菊花受損程度亦降低至 1/3 以下，顯示中華微刺盲椿象可在菊花田有效防治菊褐斑薊馬。

**關鍵詞：**菊褐斑薊馬、琉球、生物防治、中華微刺盲椿象。

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