Bulletin of Entomological Research (2019) **109**, 419–425 doi:10.1017/S0007485318000299

Chinese chives and garlic in intercropping in strawberry high tunnels for *Neopamera bilobata* Say (Hemiptera: Rhyparochromidae) control

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Abstract

Strawberry is affected by several pests and diseases. *Neopamera bilobata* is an emerging pest that has been reported by several strawberry growers, usually associated with catfacing symptoms in fruits. We evaluated intercropping garlic or Chinese chives on *N. bilobata* populations on strawberry crops grown in high tunnels in two experiments. In the first experiment, we evaluated *N. bilobata* populations on strawberry intercropping with garlic plants (three densities: 8, 16, 24 GP – garlic plant per plot) on the bags by taking 12 samples from December 2015 to April 2017. *N. bilobata* populations on strawberry were also assessed when Chinese chives were grown under the suspended wooden structures in which strawberry plants are grown ('undercropping') (14 samples), in two high tunnels, from November 2016 to March 2017. The number of nymphs and adults on 14 randomly selected fruits per plot were assessed. During the garlic intercropping experiment, the treatments of three densities of garlic reduced *N. bilobata* populations; however, the 24 GP treatment caused a greater reduction than the 8 GP treatment. Garlic densities reduced *N. bilobata* populations by 35, 50, and 64% for the 8, 16, and 24 GP treatments, respectively. Chinese chives cultivated under the structures reduced *N. bilobata* populations by 47%. The results suggest that intercropping garlic or undercropping Chinese chives are suitable tools to be tested in integrated pest management in strawberry crops.

Keywords: Companion planting, *Allium sativum*, *Allium tuberosum*, *Fragaria* × *ananassa*, seed bugs, suspended system

(Accepted 2 April 2018; First published online 8 May 2018)

Introduction

Strawberry is affected by several pests and diseases, which can reduce yields and fruit quality. In Brazil, the most important strawberry pests are aphids (*Chaetosiphon fragaefolii* Cockerell and *Aphis forbesi* Weed), mites (*Tetranychus urticae* Koch and *Phytonemus pallidus* Banks), and thrips *Frankliniella occidentalis* (Pergande) (Bernardi *et al.*, 2015). Most recently, three species with high potential to become important pests have been reported as causing injuries and damage to strawberries fruits or plants: *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Deprá *et al.*, 2014), *Duponchelia fovealis* (Zeller) (Lepidoptera: Crambidae) (Zawadneak *et al.*, 2016), and *Neopamera bilobata* Say (Hemiptera: Rhyparochromidae) (Kuhn *et al.*, 2014).

N. bilobata feeds on strawberry achenes and causes imperfections in fruits ('catfacing') (Bernardi *et al.*, 2015). Although these insects feed on strawberry leaves, they complete their

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development only when feeding on green or mature strawberry fruits (Kuhn *et al*., 2014). The life of cycle of this species is completed within 42 days (egg-adult), and females lay up to 320 eggs (Bernardi *et al*., 2015). A higher incidence of *N. bilobata* in strawberry fields was observed in Mexico, at temperatures above 20°C and low precipitation (Gallardos-Granados *et al*., 2016).

N. bilobata has been reported in several strawberry fields in Paraná State, Brazil. High *N. bilobata* populations are mostly seen during the warm periods between November and January. Based on *N. bilobata* thermal requirements studies and historical mean temperatures, it is estimated that until five generations of this insect per year may be achieved in Parana State (Kuhn *et al*., 2018). In Minas Gerais State, Brazil, a study shows that *N. bilobata* was the most abundant arthropod species associated with strawberry (De Melo, 2017). Transitioning strawberry production from open fields to high tunnels and the popularity of day-neutral cultivars could have contributed to a continuous presence of suitable host (fruits) for *N. bilobata* resulting in increased pest populations. In addition, to date, no insecticide has been registered for *N. bilobata* control.

Vegetation management could add an important tool to Integrated Pest Management programs (Stenberg, 2017). Intercropping is defined as growing two or more plant species simultaneously (Vandermeer, 1989). Amaryllidaceae plants, such as garlic or onion, have been introduced in intercropping, and pest population or damage reduced in several crops (Noman *et al*., 2013; Tiroesele & Matshela, 2015; Hata *et al*., 2016). Chinese chives have also been proposed as an intercrop plant candidate for disease management: *Pseudomonas solanacearum* control on tomato (Yu, 1999) and *Fusarium oxysporum* f. sp. cubense control on banana (Zhang *et al*., 2013).

Besides pest and disease reductions, intercropping with Amaryllidaceae plants has also been associated with higher Land Equivalent Ratio (LER) indexes. LER index above one means a higher efficiency of soil use by intercropping, compared with monocrops. Garlic-pea (Anjum *et al.*, 2015), garlic-eggplant (Islam *et al.*, 2016), onion-strawberry (Karlidag & Yildirim, 2009) intercropping LER indices of 1.29, 1.97, 1.90, respectively, indicating that such use of intercropping leads to more effective utilization of soils.

Intercropping garlic between strawberry plants reduces *T. urticae* populations in the field and greenhouse (Hata *et al*., 2016). We tested the influence of this approach on *N. bilobata* populations. Then, we evaluated intercropping garlic or Chinese chives on *N. bilobata* populations on strawberry crops grown in high tunnels, in two experiments.

Materials and methods

Experiments were conducted in two high tunnels located in Marialva County, Paraná State, Brazil, described as: area one: 23° 29′ 09.15″ S, 51° 45′ 57.45″ W, 568 m a.s.l. and area two: 23° 28′ 13.59″ S, 51° 47′ 50.54″ W, 614 m a.s.l. The climate is classified as Cfa (humid sub-tropical) according to Köppen, with hot humid summers.

Strawberry plants in both areas were planted in March 2015 in bags (1.2 m length and 0.3 m width). Seven strawberry plants were grown in double rows (0.30 m between plants and 0.25 between rows) in each bag, which contained organic compost plus carbonized rice husks. The plants were drastically pruned in March 2016 and February 2017, at the outset of new crop cycles. The bags were suspended by a wooden structure (1.0 m above the ground). Experimental plots consisted of two bags (14 strawberry plants per plot) placed side by side, for the two experiments.

Management of *T. urticae* was undertaken with predatory mites *Neoseiulus californicus* McGregor with field releases on 6 and 8 July 2016, for areas one and two, respectively.

Garlic intercropping

This experiment was conducted during three crop cycles (2015/16, 2016 and 2016/17) in area one, only. Garlic plants (0.30 m between plants and 0.25 between rows) were intercropped between strawberries (cv. Monterey) with planting taking place in September 2015, March 2016, and November 2016 for the first, second and third cycles, respectively.

The treatments were monocropping (strawberry alone) and 8 GP (garlic plants per plot), 16 GP or 24 GP grown between strawberry plant rows (fig. 1). Each plot received different densities of garlic intercropping. A border area of 2.4 m was used between treatments.

In preliminary tests, we first observed whether *N. bilobata* was present walking on strawberry fruits or hiding inside the crown. Then, the number of nymphs and adults of *N. bilobata* on 14 randomly selected fruits per plot was assessed. For more accurate sampling, a certain distance was maintained for the insect observations so as not to disturb them. For the first cycle, assessments were conducted on 14, 21, and 28 December 2015, and on 4 January 2016. For the second cycle, assessments were conducted on 5 and 26 July, and on 13 and 20 September 2016. For the third cycle, assessments were conducted on 8 and 20 March, and on 4 and 11 April 2017. For each cycle, means were calculated using all the assessments, and the data from all assessments (cycles one, two, and three) were summarized.

Chinese chives 'undercropping'

The assessments were conducted in area one and two. The treatments were a single row of Chinese chives (0.50 m within the plants) cultivated under the suspended strawberry (cv. Albion) structures vs control – natural infestation of weeds under the bags (fig. 2).In each area, the high tunnel area was divided into two 100 m^2 blocks one with the treatment and other as the control. Chinese chives were planted in March 2016 and were used because farmers previously observed that this plant develops well even in the shaded environment. The weed community grown under the structures included *Commelina benghalensis* L., Commelinaceae (35%), *Digitaria sanguinalis* L. Scop., Poaceae (25%), *Ipomoea* spp., Convolvulaceae (15%), *Parthenium hysterophorus* L., Asteraceae (15%), *Mentha piperita* L., Lamiaceae (5%), and other species + bare soil (5%) in the control treatment; and Chinese chives (60%), *Paspalum notatum* Flüggé, Poaceae (20%), and other species + bare soil (20%) in the 'undercropping' treatment.

The number of nymphs and adults of *N. bilobata* on 14 randomly selected fruits per plot was assessed. For more accurate sampling, a certain distance was maintained for the insects' observations so as not to disturb them. Assessments were conducted on 21 November and 13 and 27 December 2016, and on 15 January, 23 February, and 8 and 20 March 2017. The mean number of *N. bilobata* in each area was calculated, and the data from the two areas were summarized.

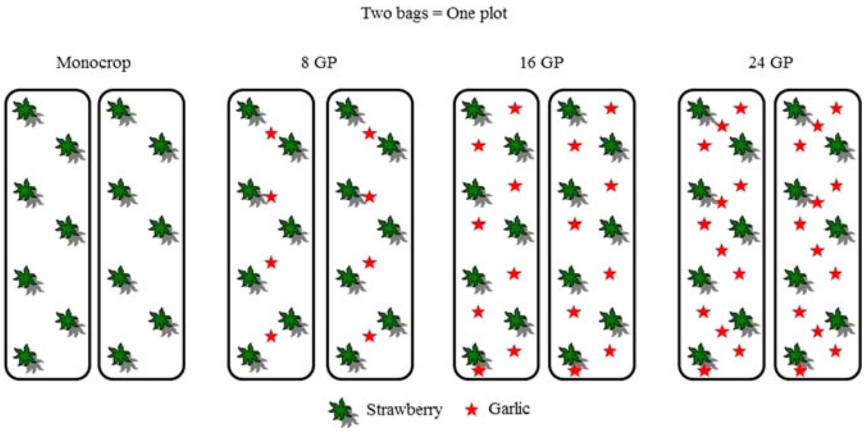

Fig. 1. Garlic-strawberry intercropping design of strawberry grown in slabs, under high tunnels on suspended system. GP = Garlic plant per plot.

Experimental design and statistical analysis

For garlic intercropping, the treatments were replicated five times in a randomized complete block design. For Chinese chives undercropping, the treatments were replicated eight times in a completely randomized design.

To verify the assumptions for the analysis of variance, tests of the variance homogeneity (Hartley's Fmax test) and normality (Shapiro–Wilk test) were performed. Thereafter, analysis of variance was performed, and means were compared using Tukey's test ($\alpha = 0.05$); if the tests of the assumptions failed, the data were analyzed with non-parametric tests: the Friedman test for the garlic intercropping experiment and the Kruskal–Wallis test using the Student-Newman–Keuls (SNK) method to compare means for the Chinese chives experiment. The BioEstat 5.0 (Ayres, 2007) and SASM-Agri (Canteri *et al*., 2001) software packages were used.

Results

Garlic intercropping

Neopamera bilobata populations were significantly reduced by garlic intercropping on 14, 21, and 28 December 2015, and 4 January 2016, for the first experiment; on 5 July and 20 September 2016, for the second experiment; and on 20 March 2017. On the other hand, on 4 April, the 8 GP treatment presented higher *N. bilobata* populations than the monocrop treatment in the third experiment (table 1).

Fig. 2. Strawberry grown in slabs, under high tunnels on suspended system. (a) Control, with natural infestation of weeds; (b) Chinese chives undercropping.

https://doi.org/10.1017/S0007485318000299 Published online by Cambridge University Press

Table 1. Mean (± SD) numbers of *Neopamera bilobata* adults and nymphs on strawberry fruits (Monterey cv.) in monocrop or intercropped with different densities of garlic plants per plot during three crop cycles. Marialva, Paraná, Brazil, from December 2015 to April 2017.

	First cycle				
	14 December	21 December	28 December	4 January	Mean
Monocrop	6.00 ± 1.73a	8.00 ± 4.01a	6.75 ± 0.96a	6.75 ± 0.96a	6.88 ± 0.21a
8 GP	4.33 ± 1.53ab	3.50 ± 1.30b	4.50 ± 1.00ab	5.75 ± 1.71ab	4.52 ± 0.30b
16 GP	1.67 ± 1.16b	7.00 ± 4.00ab	4.00 ± 1.16ab	3.25 ± 1.26b	4.13 ± 0.37b
24 GP	2.00 ± 1.73b	3.75 ± 2.76b	2.75 ± 1.26b	3.75 ± 0.50b	3.06 ± 0.20b
C.V. (%)	34.67	32.31	25.66	23.44	17.14
F-value	8.53	6.42	8.38	8.36	16.33
	Second cycle				
	5 July	26 July	13 September	20 September	Mean
Monocrop	2.50 ± 1.91a	0.25 ± 0.50a	1.25 ± 0.50a	1.75 ± 1.26a	1.44 ± 0.09a
8 GP	0.75 ± 0.96ab	0.25 ± 0.50a	0.50 ± 0.58a	0.75 ± 0.76ab	0.56 ± 0.07b
16 GP	1.00 ± 0.82ab	0.00 ± 0.00a	0.50 ± 0.58a	0.50 ± 0.58ab	0.50 ± 0.06b
24 GP	0.50 ± 0.58b	0.00 ± 0.00a	0.50 ± 0.58a	0.00 ± 0.00b	0.25 ± 0.00b
C.V. (%)	69.11	230.94	60.61	99.38	34.28
F-value	5.29	3.00	4.20	2.10	19.32
	Third cycle				
	8 March	20 March	5 April	12 April	Mean
Monocrop	1.50 ± 2.38a	1.25 ± 0.50a	0.13 ± 0.25b	2.38 ± 1.25a	1.38 ± 0.40a
8 GP	1.25 ± 0.96a	1.50 ± 1.29a	1.33 ± 0.47a	0.75 ± 0.96ab	1.02 ± 0.49ab
16 GP	0.25 ± 0.50a	0.00 ± 0.00a	0.00 ± 0.00b	0.50 ± 0.58ab	0.38 ± 0.32b
24 GP	0.25 ± 0.50a	0.00 ± 0.00a	0.50 ± 0.58ab	0.00 ± 0.00b	0.25 ± 0.20b
C.V. (%)	145.41	97.72	81.56	84.40	49.77
F or *Fr** value	1.24	5.68	9.07	8.18*	8.06

Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test ($P < 0.05$) or the Friedman test (*) ($P < 0.05$).

It was evident that *N. bilobata* numbers progressively decreased with increasing garlic plant density. During the first cycle, a significant reduction was observed in just one assessment (56% on 21 December) (of four assessments conducted) when 8 GP (garlic plants per plot) were intercropped, two assessments for 16 GP (72 and 52% on 14 December and 4 January, respectively), and all assessments for 24 GP (reductions of 67, 52, 59, and 44% on 14, 21, and 28 December and 4 January, respectively). In the second cycle, the 24 GP intercropping treatment reduced *N. bilobata* adults and nymphs by 80 and 100% (5 July and 20 September, respectively) compared with the strawberry monocrop in two assessments (total of four assessments). In the third cycle, on 5 April, the *N. bilobata* population was significantly higher in the 24 GP treatment than in the monocrop or 16 GP treatments. On 11 April, the 24 GP treatment reduced the *N. bilobata* population by 100% compared with the monocrop treatment. In the first and second cycles, the means of all assessments showed significantly lower *N. bilobata* populations in treatments with garlic plants (8, 16, and 32 GP) than in the strawberry monocrop. Reductions were 34, 40, and 56 and 61, 65, and 83% for the 8, 16, and 24 GP treatments in the first and second cycle, respectively. In the third cycle, only the 16 and 24 GP treatments decreased the populations of these insects, with significant reductions of 73 and 82%, respectively.

Analysis of the three cycles confirms a reduction in the *N. bilobata* populations compared with the strawberry monocrop for the three garlic plant densities used (fig. 3). The 32 GP treatment showed a significantly greater reduction than the 8 GP treatment. Intermediate results were observed in the treatment with 16 GP. The observed reductions compared with the monocrop treatment were 35, 50, and 64% for 8, 16, and 24 GP, respectively.

Chinese chives undercropping

Reductions of *N. bilobata* populations also occurred when Chinese chives were cultivated under the suspended

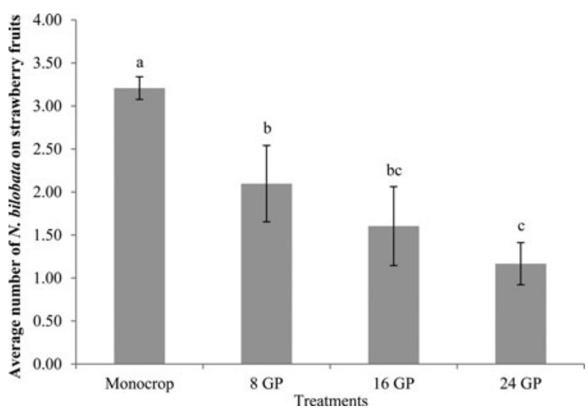

Fig. 3. Mean number (±SD) of *Neopamera bilobata* adults and nymphs on strawberry fruits (Monterey cv) in monocrop or intercropped with different densities of garlic plants per plot (GP; 8, 16, and 24 GP), with overall data from three crop cycles. Marialva, Paraná, Brazil, from December 2015 to April 2017.

https://doi.org/10.1017/S0007485318000299 Published online by Cambridge University Press

Table 2. Mean (± SD) numbers of *Neopamera bilobata* adults and nymphs on strawberry fruits (Albion cv.) in control or undercropped with Chinese chives, 2016/17. Marialva, Paraná, Brazil, from November, 2016 to March, 2017.

Treatments	21 November	13 December	27 December	15 January	23 February	8 March	20 March	Mean
Area one								
Control	4.63 ± 3.07a	1.87 ± 0.99a	1.71 ± 0.95a	3.75 ± 2.82a	0.13 ± 0.35a	1.25 ± 0.89a	1.71 ± 0.57a	2.16 ± 0.65a
Undercrop	1.71 ± 1.70b	0.38 ± 0.52b	0.38 ± 0.51b	2.25 ± 1.98a	0.25 ± 0.46a	1.13 ± 0.99a	0.57 ± 0.61b	1.07 ± 0.41b
C.V. (%)	93.42	70.24	74.95	81.16	219.67	79.17	84.32	31.50
F or *H** value	6.77	8.93*	11.92	1.52	0.37	0.07	4.92	18.30
Area two								
Control	0.38 ± 0.74a	1.50 ± 1.07a	0.38 ± 0.74a	2.67 ± 1.21a	3.57 ± 1.51a	2.14 ± 0.69a	0.67 ± 0.82a	1.52 ± 0.35a
Undercrop	0.63 ± 0.92a	0.50 ± 0.53b	0.38 ± 0.52a	1.00 ± 0.89b	1.63 ± 1.41b	0.86 ± 0.69b	0.00 ± 0.00a	0.86 ± 0.54b
C.V. (%)	166.90	67.51	170.90	58.07	57.51	55.51	173.21	43.76
F or *H** value	0.36	5.05*	0.00	7.35	6.64	6.67	4.00	6.03

Means ± SD within a column followed by the same letter are not significantly different based on Tukey's test ($P < 0.05$) or the Kruskal-Wallis and SNK test (*) ($P < 0.05$)

strawberry plants in the two high tunnels. In both high tunnels, statistically significant reductions were observed in four assessments (total of seven assessments) with decreases of 63, 78, and 67% being observed on 21 November, 13 and 27 December, and 20 March, respectively, in area one and of 67, 63, 54, and 60% on 13 December, 15 January, 23 February, and 8 March, respectively, in area two (table 2). The means of all assessments within an area confirm the reductions observed on the assessment dates: 51 and 43% for area one and two, respectively.

Analysis of the data from the two high tunnels shows a significant reduction of 47% with Chinese chives intercropping (fig. 4).

Discussion

In general, intercropping reduced *N. bilobata* populations when using garlic plants between strawberry rows or Chinese chives under the strawberry plants cultivated in suspended bags (tables 1 and 2, figs 3 and 4). Significant differences in *N. bilobata* populations were observed mostly because insect populations increased in the strawberry monocrop treatment. We did not find any previous study on the use of intercropping against *N. bilobata* in the literature. Several studies show a reduction in populations of other pests with intercropping. *T. urticae* populations were lower in strawberry intercropped with garlic than in monocropped strawberry (Hata *et al.*, 2016). *Tetranychus evansi* Baker and Pritchard were also reduced by intercropping garlic in a tomato crop (Mtambo & Zeledon, 2000). Garlic or onion intercropping with mustard (*Brassica rapa* L.) reduced populations of *Lipaphis erysimi* Kalt. (Homoptera: Aphididae) (Sarker *et al.*, 2007; Noman *et al.*, 2013).

The deleterious effects previously found against insects and mites (Sarker *et al.*, 2007; Mtambo & Zeledon, 2010; Noman *et al.*, 2013; Hata *et al.*, 2016) have been attributed to volatile organic compounds (VOCs), such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) released by garlic leaves or dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) released by the leaves of Chinese chives (Yabuki *et al.*, 2010). In a bioassay with 16 essential oils from Chinese herbs, two major compounds of garlic essential oil (DADS and DATS)

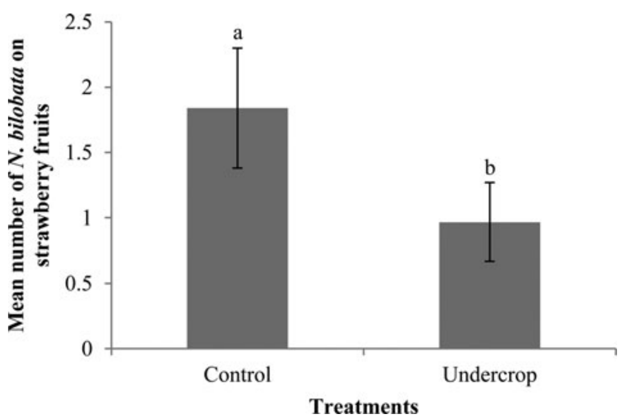

Fig. 4. Mean number (±SD) of *Neopamera bilobata* adults and nymphs on strawberry fruits (Albion cv) in control or undercropped with Chinese chives, with overall data from two high tunnels. Marialva, Paraná, Brazil, 2017.

https://doi.org/10.1017/S0007485318000299 Published online by Cambridge University Press

presented the strongest fumigant effect against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), with LC50 values of 0.12 and 0.08 (μg/l air) for DADS and DATS, respectively (Liu *et al*., 2014). DADS disrupts digestive enzymes (α-amylase, alkaline phosphatase, alanine amino-transferase, lactate dehydrogenase, and proteases), which reduces arthropod biological activities and eventually their populations (Shahriari & Sahebzadeh, 2017). In another study, a semiochemical dispenser filled with DADS or garlic intercropping reduced populations of *Sitobion avenae* Fabricius (Hemiptera: Aphididae) and attracted more natural enemies of aphids than control plots in wheat fields (Zhou *et al*., 2013).

When *T. urticae* was reduced by intercropping garlic in strawberry fields (Hata *et al*., 2016), associational resistance was suggested as the underlying mechanism, and the same may be occurring for *N. bilobata*. The mixture of Chinese chives or garlic VOCs may be passively absorbed for later re-release (Choh *et al*., 2004; Himanen *et al*., 2010), or the induction of resistance mediated by volatile compounds may have been achieved through associational resistance (Tscharntke *et al*., 2001). It is also possible that the pattern of VOCs released by strawberry was changed by Chinese chives or garlic volatiles, which consequently affected *N. bilobata* acceptance or orientation towards fruits and plants. Previously, the volatile profile of potato plants was shown to be altered by onion VOCs: a higher terpenoid concentration was found in the headspace of onion-exposed potato plants, which reduced aphid populations in an intercropped field and caused deterrent effects in a laboratory bioassay (Ninkovic *et al*., 2013). These two terpenoids were not previously identified among the onion VOCs bouquet, and the authors suppose that a yet unknown mechanism that differs from the passive absorption/re-release concept guides potato response to onion VOCs.

The results presented in our study indicate that intercropping garlic plants in strawberry crops and the cultivation of Chinese chives under suspended bags are options to be included in *N. bilobata* integrated management. This is true for both conventional and organic farmers because no established biological or even chemical control options for *N. bilobata* have been officially recorded in Brazil. Intercropping garlic also reduces populations of twospotted spider mite (TSSM) (Hata *et al*., 2016), one of the most important pest species in strawberry, and further investigations must be conducted to assess the effects of introducing Chinese chives into strawberry crops on this key pest and other pests (i.e., aphids, thrips) and diseases. The simultaneous effects of the two strategies could also be investigated with the aim to obtain additive or even synergic effects. Introducing aromatic Amaryllidaceae plants substantially changes the odor headspace of the strawberry crop, which could also affect the performance of predatory mites. Currently, augmentative biological control using these mites is a common practice in high tunnel-grown strawberry. This question also must be answered in future studies.

Growing Chinese chives under the suspended structure that supports the bags for strawberry cultivation is a simple practice that also does not interfere with crop management, e.g. fertilization. This is possible because the plant develops well even under some level of shading. The critical need for more efficient land use makes 'undercropping' Chinese chives and intercropping garlic profitable approaches for pest management and may increase growers' incomes with the commercialization of these aromatic plants.

In summary, *N. bilobata*, an emerging pest species in strawberry crops, was reduced by intercropping garlic plants between strawberry plants or introducing Chinese chives under the suspended bags containing strawberry plants.

Acknowledgements

The authors would like to thank Adilson Gallo and Tamura's Family, for the research areas, and the research funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, for the scholarships granted.

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