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ORIGINAL CONTRIBUTION

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Abundance of natural enemies of *Nezara viridula* (Hemiptera: Pentatomidae) on three cultivars of sweet alyssum

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Abstract

The southern green stink bug, *Nezara viridula* is one of the most serious pests of tomatoes in Florida. During 2014 and 2015 tomato cropping seasons, three cultivars of sweet alyssum, *Lobularia maritima* (Brassicales: Brassicaceae) were evaluated in north Florida to determine their effectiveness in conserving the natural enemies of the southern green stink bug in open field conditions. The experimental refuge crops were cultivated on two outer rows and one center row of the tomato crop. Each section of the rows of trap crops and refuge crops was 20 × 3 ft. (6 × 0.91 m) in size. Pest insects and their natural enemies were collected weekly and identified. On refuge crops, big-eyed bug, *Geocoris punctipes* (Hemiptera: Geocoridae); minute pirate bug, *Orius insidiosus* (Hemiptera: Anthocoridae), ladybird beetles (Coleoptera: Coccinelidae), assassin bugs (Hemiptera: Reduviidae), damsel bugs (Hemiptera: Nabidae), hover flies (Diptera: Syrphidae) and spiders (Arachnida) were collected. All cultivars of *L. maritima* attracted the natural enemies of the southern green stink bug; however 'carpet of snow' was the most effective cultivar followed by 'tall white' in attracting *G. punctipes* in the tomato crop. *N. viridula* population first peaked in July and declined in week 11 and then had a sharp increase in weeks 12 and 13 when *G. punctipes* population was comparatively high. Data on the availability of suitable refuge crops which provide nectar and shelter to natural enemies are useful for integrated pest management of the southern green stinkbug, because these plants provide necessary resources to conserve beneficial species.

KEYWORDS
conservation, natural enemies, refuge crops, southern green stink bug

1 | INTRODUCTION

Florida ranks first in production of fresh market tomatoes. In 2016, tomatoes were planted in 30,000 acres with a sale value of more than $3.82 billion in the Sunshine State (USDA, National Agricultural Statistics Service, 2017). Florida serves as the major tomato supplier throughout the United States during the late fall, winter and early spring months (Freeman, Dittmar, & Vallad, 2015). Production cost for tomato in Florida often exceeds $16,000 per acre in large part due to the high cost of pest management. Exotic insect pests are considered serious threat to sustainable tomato production and food security in Florida. The known common insect pests on tomatoes in Florida include: Southern Green Stink Bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae); Brown Stink Bug, *Euschistus servus* (Say) (Hemiptera: Pentatomidae); Silverleaf Whitefly, *Bemisia argentifolii* (Bellows & Perring) (Hemiptera: Aleyrodidae); Green Peach Aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), Tomato Fruitworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), Tomato Hornworm, *Manduca quinquemaculata* (Haworth) (Lepidoptera: Sphingidae) and Western Flower Thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The greatest challenge to tomato producers in Florida is the management of serious insect pests especially *Nezara viridula*

(Linnaeus) (Hemiptera: Pentatomidae), a highly polyphagous feeder which attacks many important food crops (Squitier, 2013). In tomato crops, *N. viridula* populations reach their peak in late summer (Kamminga, Koppel, Herbert, & Kuhar, 2012) and the economic injury potential of *N. viridula* feeding varies to other host crops.

Nezara viridula has piercing-sucking mouthparts with which they puncture plant tissues and suck the sap. As a result, plant shoots may wither or, in severe cases, may eventually die (Squitier, 2013). Feeding damage on young tomatoes induces early maturity and reduces fruit size and weight (Lye, Story, & Wright, 1988). Direct feeding is the main cause of yield loss. Adult injects saliva into plant parts via stylet penetration damaging plant tissue, causing blemishes, discoloration and malformation; while sucking removes plant nutrient resources resulting in retarded growth (Plantwise, 2015). The pest reduces the quality and quantity of tomatoes, making them unmarketable; thus affecting grower's profitability and productivity.

Nezara viridula is tolerant to many insecticides and thus it is very difficult to control. The possible buildup of toxic residues from some pesticides in the environment and the development of pesticide resistance leads to loss of pesticides effectiveness (Lewis, Van Lenteren, Phatak, & Tumlinson, 1997). The establishment of habitats that are favourable to beneficial fauna within the agroecosystem can boost the survival, reproduction, dispersal and ultimately the regulation of pest populations by natural enemies (Flint & Gouveia, 2001; Landis, Menalled, Lee, Carmona, & Perez-Valdez, 2000). Developing improved methods to establish beneficial habitat and conserve natural enemies can improve ecosystem sustainability and help growers produce quality marketable produce.

Currently, limited options are available to vegetable growers in Florida to combat *N. viridula*. In addition, lack of awareness of beneficial species and reliance on conventional pesticides are some of the major contributing factors to pest problems, including pesticide resistance, pest resurgence, residue and secondary pest outbreaks. (NRDC, 2013). A substantial amount of research has been done on the use refuge crops to conserve natural enemies of certain serious pests in vegetable and other crops production, as well as research that has examined the relationship between refuge crops and main crops (Eyre & Leifert, 2011; Lundgren, Wyckhuys, & Desneux, 2009; Reeves, Greene, Reay-Jones, Toews, & Gerard, 2010). The refuge crops are planted within and adjacent to high cash crops; refuge crops serve to attract, maintain or increase predator and parasitoid populations by providing them with food source and more suitable habitat for the population to grow and feed on the pests attacking the main crop (Rechcigl & Rechcigl, 2000).

Conservation of natural enemies in agricultural systems is important for biological control of pests. Beneficial species such as tachinid fly, *Trichopoda pennipes* Fabricius (Diptera: Tachinidae), Big-eyed Bug, *Geocoris punctipes* Fallen (Hemiptera: Geocoridae); and Minute Pirate Bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae) are usually present in agroecosystems and feeds on eggs of *N. viridula*, therefore, improvement in habitat for these beneficial species will enhance pest management in tomatoes. In addition, numerous natural enemies including parasitoids, predators and entomopathogens (Todd, 1989) feeds on *N. viridula*. Among these natural enemies are the hymenopteran scelionid parasitoid *Trissolcus basalis* (Wollaston) which oviposits in the eggs of *N. viridula* and a tachinid parasitoid *Trichopoda pennipes* (F.) feeds on the late-instar nymphs and adults (Panizzi, McPherson, James, Javahery, & McPherson, 2000; Todd, 1989). Several African and Asian egg parasitoids in the genera *Trissolcus*, *Telenomus* and *Cryon* were recorded feeding on *N. viridula* (Jones, 1988). For example, the scelionid parasitized 45% of *N. viridula* egg masses in earlier studies in Florida during 1974-1975 (Buschman & Whitcomb, 1980). Many generalist predators, including spiders, ants, frogs, crickets, beetles and predatory bugs prey on *N. viridula* (Stam, Newsom, & Lambremont, 1987; Van Den Berg, Bagus, Hassan, Muhammed, & Zega, 1995). In this study, we determined the abundance of natural enemies of *N. viridula* on three cultivars of sweet alyssum (refuge crops) with a view to protect tomatoes in north Florida.

2 | MATERIALS AND METHODS

2.1 | Study site and crops

The study was conducted at the Vegetable IPM Demonstration Site, located at the Center for Viticulture and Small Fruit Research (CVSFR), Florida Agricultural and Mechanical University, in Leon County, Florida (at approximately 30°28′39″ N, 84°10′16″ W). The size of the plot was 0.137 acre (0.055 ha). West of the study site were cultivated muscadine grapes cultivar: majesty, carlos), and several small fruit trees (peach (cultivar: gulfcrest); pear (cultivar: baldwin); apple; fig (cultivar: brown turkey); Citrus (cultivar: satsuma). On the East, persimmon (cultivar: fuyu), and oriental chestnuts (Chinese) were grown, while on the north side citrus (cultivar: satsuma) was grown. The land to the south side was not cultivated. The soil was primarily sandy loam, fairly well drained with a good infiltration rate and a pH of 6.5. The study was carried out from May to July in 2014 and 2015.

2.2 | Experiment layout

Refuge crops and main crops were sown in seedling trays and transplanted to the experimental plots in 60 ft. (18.2 m) rows (Table 1). Proper fertilization and irrigation were provided to promote suitable plant establishment. Drip irrigation was provided, one hour in the morning and one hour at the night.

The experimental refuge crops were cultivated on two outer rows and one center row of the major crop (tomato) plot (Figure 1). Each treatment area consisted of three cultivars of sweet alyssum (*Lobularia maritima*): royal carpet, carpet of snow and tall white. The control was a similar plot of six rows of tomatoes grown under the same agronomic practices in a plot about 70 ft. (21 m) north of the experimental plot. Each section of the rows of trap crops and refuge crops was 20 × 3 ft. (6 × 0.91 m) in size (Figure 1). The row to row and plant to plant distance was kept at 36 and 18 ft, respectively.

TABLE 1 Tomato and refuge crops monitored from May to July 2014 and 2015

Common name	Family	Scientific name	Collection method/tool
Tomato var			
Marglobe	Solanaceae	*Solanum lycopersicum*	Handpicked/Insect net
Sweet alyssum var			
Carpet of snow	Brassicaceae	*Lobularia maritima*	Vacuum aspirator[a]
Royal carpet			
Tall white			

[a]Hand-held insect vacuum aspirator (Craftsman): 19.2 volt.

Treatments	Replicates		
	←20 ft→	←20 ft→	←20 ft→
Refuge crop	TC1	TC2	TC3
Main crop		Tomato	
Main crop		Tomato	
Main crop		Tomato	
Refuge crop	TC3	TC1	TC2
Main crop		Tomato	
Main crop		Tomato	
Main crop		Tomato	
Refuge crop	TC2	TC3	TC1

↕ 63.75 ft

FIGURE 1 Field layout (TC_1: Carpet of snow; TC_2: Royal carpet and TC_3: Tall white)

2.3 | Insect collection

In summer 2014 and 2015, tomato and refuge crop (sweet alyssum) were cultivated to determine the presence or absence of insect pests and natural enemies. Every week insects (pest and their natural enemies) were collected. Adult and late instar nymphs of *N. viridula* were hand-picked and also collected with an insect collection net. The natural enemies were collected using a battery operated vacuum aspirator. Unidentified insects and parasitized (eggs, nymphs and adults) were and brought back to the laboratory and partially reared in the incubator set at 75 ± 3°F; 65% ± 5% RH and 14:10 hr photophase. Partial rearing was done to determine any eggs, nymphs or adults were parasitized. Upon emergence, parasitoids were identified using diagnostic characters under the microscope and/or hand lens (20×).

2.4 | Abundance of beneficial species

Sampling was performed weekly from May to July in 2014 and 2015 to know the number of natural enemies on the three refuge crops (carpet of snow, royal carpet and tall white). Each treatment block was vacuum-sampled for 20-s on the sampling date. The entire procedure was repeated in the summer of 2015. Sampling continued through until all tomatoes were harvested. Insects were sampled in the morning time (9:00–11:00 a.m.) Since most insects were less mobile under cooler temperatures. Stink bugs were sampled with sweep nets (three sweeps per treatment). We used the sampling procedure of Todd and Herzog (1980) with some modifications. Natural enemies were sampled using a vacuum sampler (30-s per treatment) in an area of about 30 ft^2. Some qualitative data from general inspections were also recorded. The insects collected via sweep net were recorded on a data sheet and were placed in plastic vials and ziplock bags (quart, BioQuip) and their respective sample numbers were recorded. Insects were identified using microscopes, hand lens and taxonomic keys. Insects collected from trap crops, refuge crops and tomatoes were identified, tabulated and grouped into families, genera and species. *N. viridula* adults collected in the field were brought in the laboratory and partially reared to determine parasitism rates of *T. pennipes* in the field conditions. In case of egg parasitoid, *T. basalis*, number of egg batches of *N. viridula* were collected and partially reared to determine the parasitism rates.

2.5 | Statistical analyses and evaluation

All data were checked for normality and homoscedasticity and were $\log_{10}(x + 1)$ transformed when necessary. Data on number of insect pests for each trap crop were analysed to determine if treatment effects were statistically significant. Data were subjected to Two-Way Analysis of Variance (ANOVA) and treatment means were separated using Tukey's HSD (honestly significant difference) Test. Alpha level of 0.05 was used to determine statistical significance for all major variables (SAS Institute, 2013).

3 | RESULTS

3.1 | Major insect pests and their natural enemies in tomatoes and refuge crops

Insect pests found in this study were identified to genus and species levels. Four major species of natural enemies (two parasitoids and two predators) were identified. In addition, predators belonging to order: Diptera, Araneae, Coleoptera and Hemiptera were recorded (Table 2). Three major insect pests, *Nezara viridula*;

TABLE 2 List of hemipteran pests and natural enemies collected from cultivated crop (tomato) and refuge crops during summer of 2014 and 2015

Orders/status	Species/status	Tomato (*Solanum lycopersicum*)	Sweet alyssum (*Lobularia maritima)* Carpet of snow	Royal carpet	Tall white
Insect pests					
Hemiptera	*Nezara viridula*	+	–	–	–
Hemiptera	*Euschistus servus*	+	–	–	–
Hemiptera	*Leptoglossuss phyllopus*	+	–	–	–
Natural enemies					
Diptera	*Trichopoda pennipes*	+	–	–	–
Hymenoptera	*Trissolcus basalis*	+	–	–	–
Hemiptera	*Geocoris punctipes*	+	+	+	+
Hemiptera	*Orius insidiosus*	+	+	+	+

Note. Species present (+) & Species absent (–).

Euschistus servus and *Leptoglossuss phyllopus* were collected and a parasitoid, *Trichopoda pennipes* was associated with all these pests. The parasitism rate on these three species ranged from 18%–35% in north Florida conditions. The parasitoid preferred adults and later instar nymphs for oviposition. *T. basalis* was the only egg parasitoid recorded on *N. viridula* in this study. The highest egg parasitism rates were recorded in May (27%) and June (33%). For the refuge crops, two predators *Geocoris punctipes* and *Orius insidiosus* were collected. Other predators including ladybird beetles (Coccinellidae), assassin bugs (Reduviidae), damsel bugs (Nabidae), hover flies (Syrphidae) and spiders were found in small numbers. These insects were not identified at species level. However, the pest most frequently recovered throughout the season were the *N. viridula*; *E. servus* and *L. phyllopus* and among the natural enemies *G. punctipes* was the dominant predator species found throughout the season.

3.2 | Abundance of beneficial species

The big eyed bug, *Geocoris punctipes* was the most dominant natural enemy species found on all three cultivars of sweet alyssum (*Lobularia maritima*). Other natural enemies included the minute pirate bug (*Orius insidiosus*), and predators, such as hover flies, spiders, ladybird beetles, assassin bugs and damsel bugs (Table 2). The higher number (143 adults) *G. punctipes* were recorded on the carpet of snow variety (F = 1.38; df =1, 24; p = 0.2520) followed by tall white (131) (F = 0.01; df = 1, 24; p = 0.9125) and royal carpet (31) (F = 0.77; df = 1, 24; p = 0.3886) (Figure 2). *Goecoris punctipes* was recorded on all three cultivars of *L. maritima*, starting on the first week of sampling (Figure 3) during the cropping seasons of 2014 and 2015. All three cultivars attracted the predators. However, the carpet of snow attracted the most adults of *G. punctipes* in May, June and July. The predator numbers were significantly higher in May and June and then declined at the end of season in the July. The royal carpet attracted the least number of predator during the 2 years of study (Figures 3 and 4). In the control treatment, very few number (0–5 adults/month) of predators were recorded in both years.

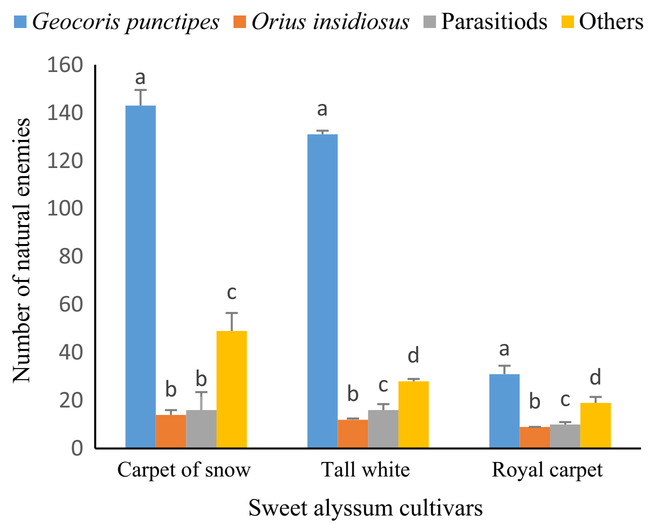

FIGURE 2 Cumulative number of natural enemies captured (Mean ± *SE*) on three refuge crops for two cropping season (2014 and 2015)

3.3 | Effect of *Geocoris punctipes* on *Nezara viridula* population

In 2014, when the *Geocoris punctipes* population was high the number of *N. viridula* was low in the week 4–9 (Figure 5). *Nezara viridula* population first peaked in week 10 (July) declined in week 11 and then had a sharp increase in week 12 and 13 when *Geocoris punctipes* population was comparatively high. There was a sharp decline in week 14. *Geocoris punctipes* highest peak was in week 7. Increased *N. viridula* numbers from week 9–13 were found when precipitation rates and *G. punctipes* numbers declined. Unknown factors may have caused decline in *N. viridula* numbers (Figure 5). Similarly in 2015, in weeks 1–7, *G. punctipes* population was the highest and *N. viridula* was low except for week 4.

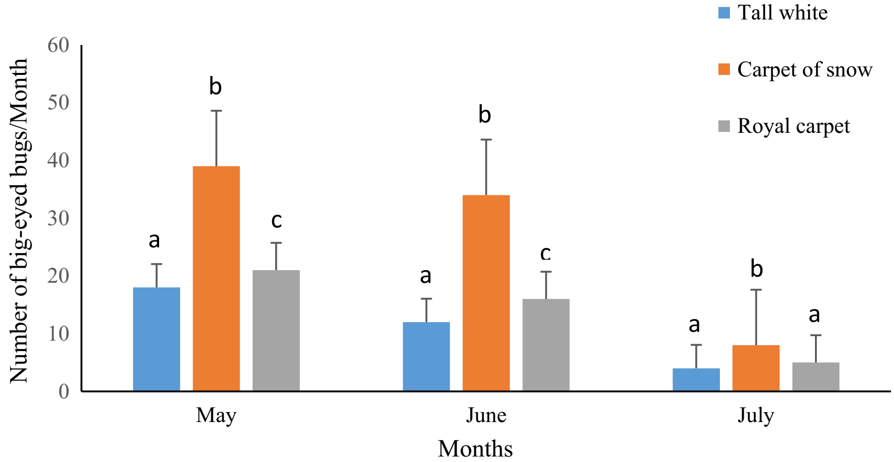

FIGURE 3 Natural enemies captured (Mean ± SE) on three refuge crops during the 2014 (May-July) cropping season

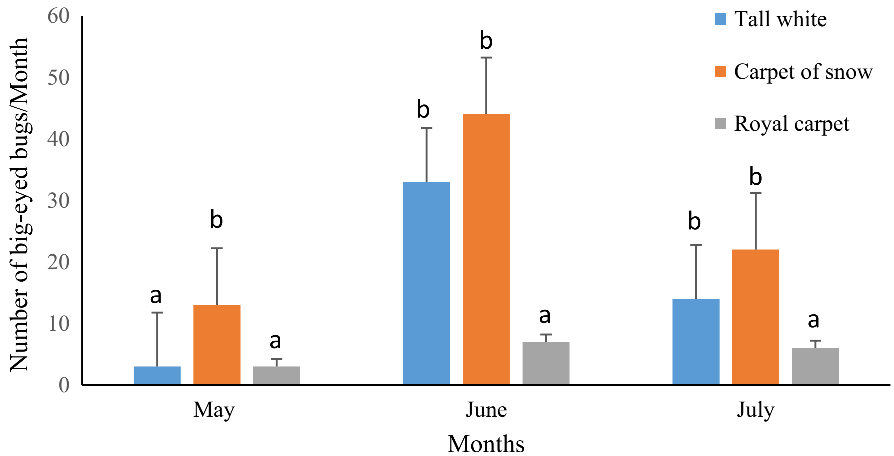

FIGURE 4 Natural enemies captured (Mean ± *SE*) on three refuge crops during the 2015 (May-July) cropping season

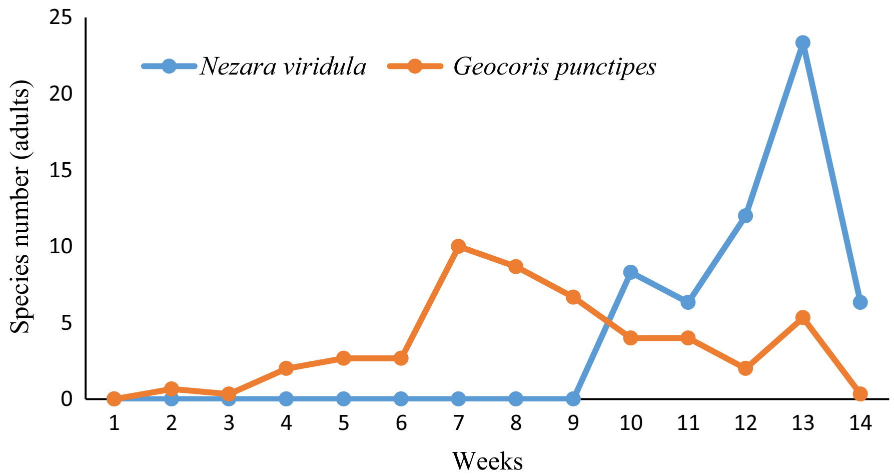

FIGURE 5 Relationship between *Geocoris punctipes* and *N. viridula* in summer 2014 (May-August) treatments

4 | DISCUSSION

The carpet of snow, tall white and royal carpet all started blooming at the same time. All cultivars of *L. maritima* attracted the natural enemies of the southern green stink bug; however, carpet of snow was the most effective followed by tall white in attracting *G. punctipes* to the tomato crop during both years. Natural enemies showed preference for the flowers that had white blooms; carpet of snow and tall white. Flower colour may influence beneficial species choice (Colley & Luna, 2000; Smith & Capinera, 2011). Cowgill (1989) surveyed wild plants on farmlands and noted that white and yellow flowers were particularly attractive to natural enemies. Our results are consistent with previous reports on attractiveness of insects to flower colors.

In an earlier study, *N. viridula* population were managed by *T. basalis* in Hawaii (Jones, 1995) and Australia (Clarke & Walter, 1993). It is probably the best known *Trissolcus* species, due to its widespread introduction against *N. viridula* and other pentatomid crop pests (Plantwise, 2015). In a study conducted by Corrêa-Ferreira and Moscardi (1995), inundative releases of *T. basalis* (15,000/ha) were made in soybean trap crops, and the population density of *N. viridula* consequently decreased by 58% in the main crop. The effectiveness of both introduced and native natural enemies can be enhanced by modifying the environment to favor predators and parasitoids. The big-eyed bugs, ladybird beetles, syrphid flies and lacewings are examples of predators that can be conserved around the tomato field. Similarly, the association of egg and nymphal parasitoids of the southern stinkbugs can be enhanced using conservation biological control in tomatoes. Conservation often involves establishing plants that provide alternate food sources, such as nectar and pollen for beneficial insects, or selective use of insecticides so as not to injure the beneficial species. Many beneficial insects feed on the pollen of plants, such as sweet alyssum, cilantro, fennel and buckwheat. It may be possible to increase the numbers of beneficial insects by including such plants in a farm.

Indeed, tomato IPM is a highly complex practice in Florida and it depends on numerous factors, especially the conservation of natural enemies to control *N. viridula*. In general, the refuge crops prevent pesticide resistance development and sustain the cropping systems in the long run. In addition, information on the availability of refuge crops which provide nectar and shelter to natural enemies are very useful. Further studies are necessary to determine the comparative effects of sweet alyssum to potential natural enemies of other pests of tomato like western flower thirps, whiteflies and leafminers. We recommend tomato growers to use either carpet of snow or tall white because both cultivars attracted similar natural enemies. An IPM strategy which consist of three components that include physical control (trap and refuge crops), biological control (predators and parasitoids) and possibility of using biobased selective chemicals could provide better pest management option against *N. viridula*. This will certainly increase the tomato grower's crop productivity and profitability.

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AUTHOR CONTRIBUTION

All authors contributed to the design of the research. The experiments and analyses were carried out by TLG, MH and JCL, supervised by LHBK. The writing up of the manuscript was led by MH with contributions from all authors.

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