Greenhouse Evaluation of Neonate and Adult Applications of Coleomegilla maculata (Coleoptera: Coccinellidae) to Control Twospotted Spider Mite Infestations

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Greenhouse evaluation of neonate and adult applications of *Coleomegilla maculata* (Coleoptera: Coccinellidae) to control twospotted spider mite infestations

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Abstract

The lady beetle *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is a euryphagous predator that is known to consume the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Spider mites are frequently problematic in greenhouse operations and often cause damage to the ornamental and bedding plant *Impatiens walleriana* Hook f. (Ericales: Balsaminaceae). Increasing demand for alternatives to pesticides combined with resistance to registered pest control products necessitates the evaluation of viable alternatives. In this study, naïve impatiens were intentionally infested with twospotted spider mites. When damage was visible on experimental plants, they were treated with lady beetles in containment cages. One treatment was adult *C. maculata* and another treatment was neonate *C. maculata* using recently developed methods for rearing and harvesting eggs. Levels of spider mite infestation were assessed after treatments, and images of the treated and untreated plants were collected to assess the effectiveness of *C. maculata* in a greenhouse environment. Plants with *C. maculata* treatments were essentially free of spider mites. There is potential to use mass-reared *C. maculata* eggs as treatment for spider mites in contained crops or indoor decorative plants.

Key Words: lady beetle; *Tetranychus urticae*; impatiens; ornamental plant; generalist predator

Resumen

La maraquita *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) es un depredador eurífago conocida de consumir la arañita de dos manchas, *Tetranychus urticae* Koch (Acari: Tetranychidae). Las arañitas son frecuentemente problemáticas en las operaciones de invernaderos, y con frecuencia causan daño a la planta ornamental y de cobertura, *Impatiens walleriana* Hook f. (Ericales: Balsaminaceae). La creciente demanda de alternativas a los plaguicidas, combinadas con la resistencia a los productos registrados para el control de plagas requiere la evaluación de alternativas viables. En este estudio, impatiens ingenuos fueron infestadas intencionalmente con arañitsa de dos manchas. Cuando hubo daño visible en las plantas experimentales, ellas fueron tratadas con las mariquitas en jaulas de contención. Uno de loso tratamientos fue con adultos de *C. maculata* y el otro tratamiento fue con neonatos de *C. maculata* utilizando métodos recientemente desarrollados para la cría y la recolección de huevos. El nivel de infestación de las arañitas fue evaluado después de los tratamientos, y se recolectaron imágenes de las plantas tratadas y no tratadas para evaluar la eficacia de *C. maculata* en un ambiente de invernadero. Las plantas tratadas con *C. maculata* fueron esencialmente libre de la las arañitas rojas. Existe la potencial de utilizar huevos de *C. maculata* criados en masa como un tratamiento para las arañitas rojas en los cultivos contenidos o plantas decorativas de interior.

Palabras Clave: mariquita; *Tetranychus urticae*; impatiens; alegría de la casa; miramelindo; plantas ornamentales; depredador generalista

Lady beetles are iconic in any discussion of biological control. Unlike many species in the family Coccinellidae, the lady beetle *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) is a generalist predator, described as "perhaps the most euryphagous coccinellid known" (Hodek 1973). Recent research on mass rearing and predatory efficiency of *C. maculata* has demonstrated that the species has good probability for success as a commercially raised augmentative biological control agent (Lucas et al. 2004; Allen & Riddick 2012). Commercial production of *C. maculata* is possible, but mass rearing suppliers are scarce (Hunter 1997). Several species in the family Coccinellidae are commercially available for garden and commercial applications. Those recommended for control of mites are from the genus *Stethorus* Weise, and are specialized mite predators. While these species may be effective in some biological control applications (reviewed in Biddinger et al. [2009]), additional effective agents may be needed depending on the plants and pests to be controlled. Success in augmentative biological control applications must be based on an expectation that the agent will effectively suppress target organisms that are damaging to commercially relevant plants.

The twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a serious pest of many plants, both agricultural and horticultural. Because spider mites have a high reproductive rate and disperse rapidly, they are particularly problematic in greenhouse-grown plants (van de Vrie 1985). Populations of spider mites can rapidly es-

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tablish and reach critical damaging levels in contained crop situations, and early detection of infestation can be difficult (Alatawi et al. 2005).

Impatiens *Impatiens walleriana* Hook f. (Ericales: Balsaminaceae) is a widely used landscape and ornamental plant. Impatiens can be purchased at most US nurseries for use as ground cover or bedding plants and are often sold in hanging baskets for decoration. Sales of impatiens in the US for commercial landscaping and ornamental use ranged from $12–20 million between 2002 and 2011 (NASS 2012). Impatiens are known to be susceptible to spider mite infestations and have been utilized for study of population estimation (Alatawi et al. 2005, 2007) and biological control efficacy (Holt et al. 2007).

This study constitutes the first greenhouse evaluation of neonate and adult stages of *C. maculata* against spider mites. The goal of the study was to determine whether this lady beetle could serve as an effective biological control agent in a realistic setting. The experiment was regionally relevant, because the plant used for the study is of commercial relevance and the experiment was performed in a greenhouse similar to commercial greenhouses in the local area. Commercial greenhouse operators suffer losses from spider mite infestations, and there is a broad regional need for an effective control. A supplementary file for this article is online in Florida Entomologist 98(2) (June 2015) at http://purl.fcla.edu/fcla/entomologist/browse, and is referred to herein as Suppl. File.

Materials and Methods

PRELIMINARY STUDY

In an initial study interrupted by the US Government shutdown in the fall of 2013, all treatment tents were set up, infested, and treated with the same numbers of *C. maculata* insects, adults and neonates, in a similar fashion to the study described below in detail. During the furlough, no observations were collected and plants were untended for 17 d.

PLANTS

In the initial (preliminary) study, impatiens plants were purchased from a Mississippi nursery (Flowers Green Growcery, Greenville, Mississippi, USA). For the second (definitive) study, plants were grown from seed (Impatiens Sunny Lady White Hybrid, Park Seed, Greenwood, South Carolina, USA) in a controlled environment room in the National Biological Control Laboratory, located in Stoneville, Mississippi, USA. Seeds were planted in peat seed flats as per instructions on the seed packet. Starting the plants from seed was required to match the availability of greenhouse space. As described on the seed packet, after germination, seedlings were allowed to grow 2 true leaves, and then were transplanted to seventy-two 10 cm (4 inch) diameter pots, with 3 to 5 seedlings per pot (2 or 3 peat cylinders). After a month of growth, potted plants were mature, with several adult leaves per plant. At this point the plants were similar in maturity to those that would be available in a commercial nursery, were judged ready for the experiment, and were distributed to tents. Plants were distributed to tents in order of relative size to provide roughly the same quantity and quality of plants in each tent. Each tent contained 4 pots.

INSECTS

Colonies of *C. maculata* were raised as described in Allen & Riddick (2012). Insects were maintained in 16:8 h L:D lighting conditions, at 23–26 °C temperature in a Percival E30B plant growth chamber (Percival Scientific, Perry, Iowa, USA). Both immatures and adults were fed a diet including bee pollen, brewer's yeast, Daphnia, and insect eggs ad libitum during rearing. Mature adult specimens were released into a BugDorm-2120 insect tent (Megaview Science Co., Ltd, Taiwan) and observed as they formed mating pairs. Mating pairs were isolated in 10 × 35 mm Petri dishes (Falcon) for use in the adult treatments. Egg masses were collected from 4 oviposition cages daily as described (Allen & Riddick 2012) and held in 10 × 100 mm Petri dishes for use as neonate treatment. Egg masses (12) that hatched on the day of treatment application were used; neonates were transferred with a sable paint brush to sixteen 10 × 35 mm Petri dishes containing a 2 × 2 cm piece of damp Kimwipe (Kimberly-Clark, Roswell, Georgia, USA) and powdered crushed bee pollen/brewer's yeast/Daphnia (roughly 7 mg) to a total of 5 neonates per dish. Neonates were chosen from largest to smallest, and distributed to dishes sequentially until the hatched mass was exhausted; thence to the next hatched egg mass. This order was followed to randomize the size and genetic makeup of the insects used for the neonate treatments. A total of 6 egg masses were utilized, with between 10 and 20 neonates per mass. Study treatments of adults (3 pairs per tent) or neonates (5 individuals per plant pot, totaling 20 individuals per tent) were added to spider mite–infested plants 5 d after infestation.

Replicated treatment groups were BugDorm-2120 insect cages (BioQuip Products, Rancho Dominguez, California, USA), which will be described here as "tents." The tents served as enclosures and exclosures, maintaining lady beetle treatments inside with a designated number of potted plants, and excluding migration of insects between treatments. Tents were arranged in an 8 × 8 m square greenhouse room on 3 aluminum wire and pipe tables in a modified Latin square and numbered sequentially from the northeastern corner of the greenhouse. Because the spider mites were known to be able to pass through the screen of the tents and infest neighboring plants, negative controls (tents not infested with spider mites) were placed adjacent to treated tents in all cases, and never adjacent to positive control (infested with spider mites but with no lady beetle treatment) tents. Each tent was considered a replicate (4 replicates per treatment). A diagram of the experimental layout is shown in Fig. 1. Four naïve pots of plants were enclosed in each tent, while plants not used in the experiment were kept unenclosed by insect tents at the west side of the greenhouse as sentinels to indicate spider mite escape or infestation of the greenhouse by other pests. Greenhouse conditions varied dramatically with daylight and ambient temperatures, and frequently reaching high temperature and relative humidity exceeding 40 °C and 90% (Fig. 2).

MITE INFESTATION

Lima bean leaves severely infested with twospotted spider mites were distributed among the treatment tents (Fig. 1, Suppl. File). Three leaflets of roughly similar size were placed on tissues in the center of the tent with the 4 plants spaced toward the corners of the tent, to allow mites to distribute to the living impatiens plants from the bean leaflets. Signs of infestation (leaf stippling) were visually detected after 5 d.

DATA COLLECTION AND ANALYSIS

Observations regarding plant status, infestation status, and recordings of conditions in the greenhouse were recorded daily with the exception of some weekend days and holidays. A data logger set to collect temperature, relative humidity, and illumination (Onset Computer Corporation, Bourne, Massachusetts, USA) was kept in the experimental greenhouse room during the experiment. Samples of plant leaves were collected 20 d after treatments were applied, and on that day plants were "pinched back" to promote new growth after sample collection

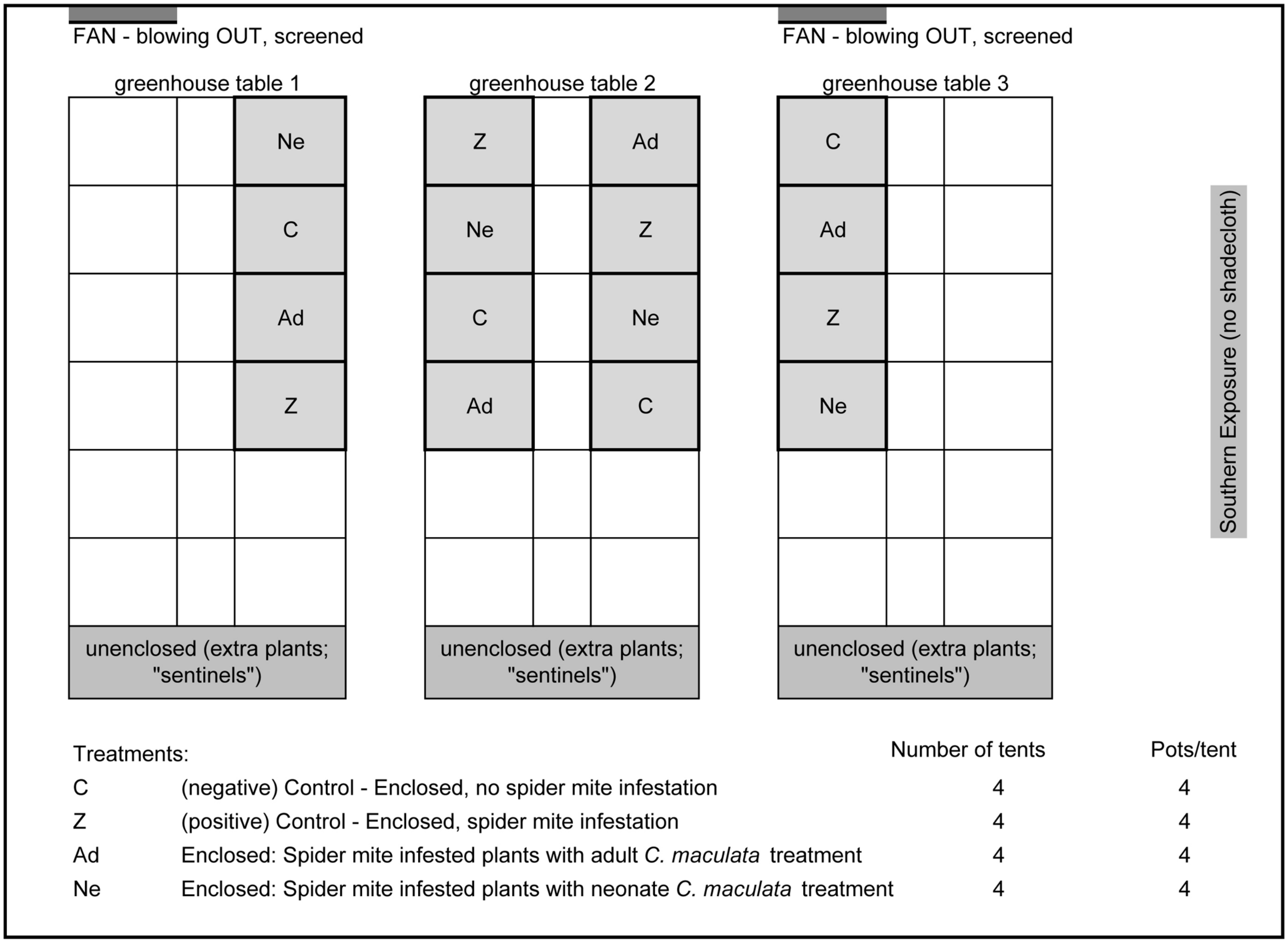

Fig. 1. Diagram of greenhouse layout. Experimental design was Latin square, non-random by design (see text for explanation). Uninfested (C, negative control) cages (tents) were placed between treated cages to minimize contamination from infested positive control cages.

(Park Seed 2012). Leaf collections were wrapped gently in Kimwipes and stored in individual sealed plastic bags in an ultralow temperature freezer for later examination. Observations of plants in tents were continued after sample collection for another 18 d. Leaf surface area was estimated by placing leaves on a grid of 2 × 2 mm squares, photographing, and counting squares covered by the leaf. Leaves were examined using a stereomicroscope (Nikon SMZ 1500, Nikon Instruments Inc., Melville, New York, USA) and numbers of spider mite eggs, juveniles (having 6 legs), and adults were recorded for each leaf. Spider mites, when present, were found on the abaxial side of the leaf, thus only this side was used in counting for infestation calculations. Three leaves were counted from each tent, and the mean number of spider mites of each stage per square cm of leaf abaxial surface was calculated using the total abaxial leaf surface area. Each tent was considered a replicate of a treatment. The calculated levels of infestation by eggs, juveniles, or adults per square cm of leaf (3 leaf subsamples per tent) were analyzed using SigmaPlot (SSI 2012) 2-way repeated measures ANOVA. Because the raw data failed to pass Shapiro-Wilk or Kolmogorov-Smirnov tests for normality, the data were transformed by $\sqrt[2]{x + 0.05}$. All pairwise multiple comparison procedures using the Holm-Sidak method were used to determine significance at $P < 0.05$.

Results

Several important observations were obtained from the failed preliminary experiment, most importantly that the treatment of spider mite–infested impatiens plants with *C. maculata* convincingly benefitted the plants. Following the 17 d of neglect, plants in the first experiment were tended and observed, and those plants that had been treated with either adult or neonate *C. maculata* recovered and grew new leaves and those without treatment did not (Fig. 3). Without a complete data set, however, the experiment was otherwise a loss. The spider mite infestations were observed to spread outside of the containment tents, infesting negative control plants in tents and sentinel plants at the ends of the greenhouse tables. Infestation was characterized by stippled leaves and presence of webbing and mites. Once the mites and webbing were visible to the unaided eye, the infested leaves were beyond recovery. These leaves dried, yellowed, and dropped off the plants. Plants thus defoliated were unable to recover and grow new leaves (Fig. 3). Plants placed at the southern side of the greenhouse also appeared more stressed from heat and dried more quickly than those further from the southern windows.

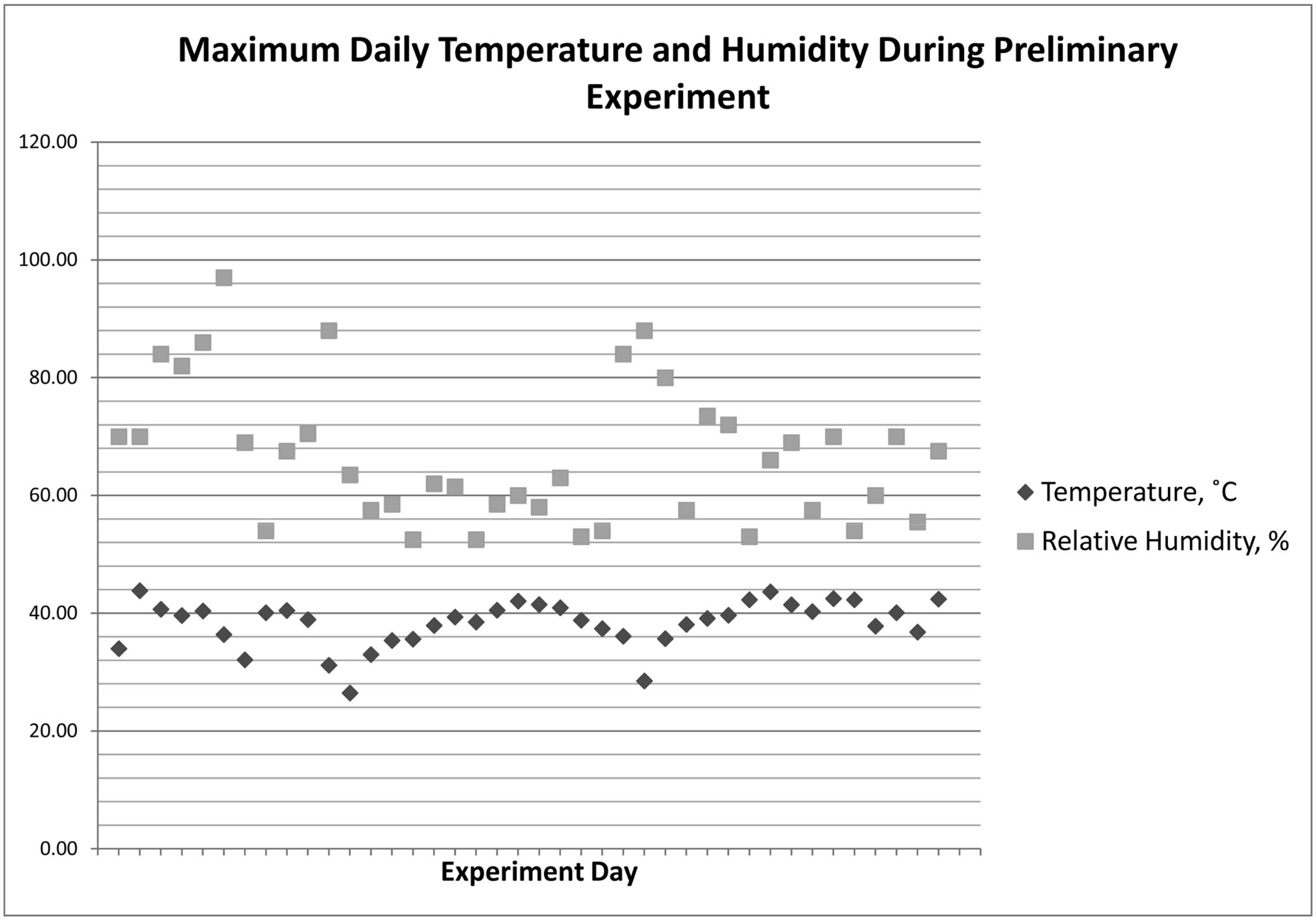

Fig. 2. Temperature fluctuations (only daily high temperatures are charted) during the course of the experiment.

The second, definitive study demonstrated that spider mite–infested impatiens plants could be recovered to a condition with little to no spider mite infestation using either adult or neonate applications of *C. maculata*. As shown in Fig. 4, all stages of spider mites were greatly reduced to near absence from leaves after treatment. Treatments were applied 5 d after spider mite introduction when impatiens plants began exhibiting evidence of infestation. Black particles of waste material from the insides of the mites were noted on leaves collected from tents treated with *C. maculata*. Larval puparia were also noted as evidence that mites had been consumed by lady beetles in the tents treated with neonates. Microscopic examination of the leaves collected from all tents treated with *C. maculata* only identified one adult mite on a leaf from a neonate-treated tent and one juvenile mite on a leaf collected from an adult-treated tent. The raw infestation data did not pass the Shapiro-Wilk test for normality and thus were square root transformed. The transformed data were analyzed by 2-way repeated measures ANOVA. Egg infestation levels were significant between treatments ($F = 11.552$, $P = 0.002$, df = 3), juvenile mite infestation levels were significant ($F = 5.237$, $P = 0.033$, df = 3), and adult mite infestation levels were significant ($F = 40.576$, $P < 0.001$, df = 3). All treated groups were significantly different ($P < 0.05$) from the infested control group (Holm-Sidak method). These results were substantiated by ongoing observation and image collection, documenting plant characteristics (Suppl. File, time course images). Plants treated with either adult or neonate *C. maculata* were leafy and flowered whereas the untreated infested plants withered and died. Another observation worth noting is that few insects were observed during the experiments. Thus the eventual fate of the control agents was not determined. Other than the small amount of diet provided for the neonate larvae when they were added to tents, no supplementary food was added to tents. Thus the insects may have fed to prey exhaustion and perished. Spider mites are not an ideal exclusive diet for the omnivorous *C. maculata* (Riddick et al. 2014).

Discussion

The popularity of impatiens as an ornamental plant and the problems from spider mite infestations are well documented, as are efforts to implement biological control in greenhouses (Holt et al. 2007; Opit et al. 2009). The leaves of the impatiens variety grown for this research project were glabrous on all leaf and stem surfaces. As the plants were raised in a controlled environmental room in the National Biological Control Laboratory, the risk of contamination from non-study organisms was low.

Commercially available biological control agents for spider mites are primarily predacious mites (Holt et al. 2007) or coccinellids in the group Stethorini (Biddinger et al. 2009). The results presented here

Fig. 3. Representative samples from initial experiment interrupted and left untended for 17 d. Plants treated with lady beetles recovered from extreme stress; infested and untreated plants could not grow new leaves (no recovery).

support consideration of *C. maculata* as an additional biological control agent. One drawback of adult beetles used for biological control is that they may fly away from the application site (Obrycki & Kring 1998). They are, in a manner consistent with predator biological imperatives, repelled from one another, and suited to disperse to seek prey. Chemical cues left by predators are repellent to both prey and predator (Ninkovic et al. 2013). Although the results here show effective spider mite control by adult and neonate beetles, the adults used in the project would undoubtedly have flown away had they not been contained by the tents. *Coleomegilla maculata* is not the most dispersive of lady beetles but is certainly capable of flight and dispersal (Hodek 1973). The most important result is that neonate insects deposited on plants in greenhouse conditions are suitable control agents. The implication is that mass-reared *C. maculata,* maintained to produce large quantities of high-quality eggs for application to greenhouse crops, could be a realistic addition to an Integrated Pest Management (IPM) strategy. Combining predators that are complementary in a biological control setting, called beneficial pairing, has been advocated by research on hemipteran predators (Northfield et al. 2014). Coccinellids and syrphid flies have also been demonstrated as complementary predators in field ecosystems (Grez et al. 2014). So, although understanding the specifics of the pest components in an agricultural setting is important in choosing control methods, the idea of a one-to-one control to pest strategy should not be considered the only option. In fact, generalist and omnivorous predators may even be effective biological control IPM components if some intraguild predation is known or expected in the system (Messelink & Janssen 2014).

Additional questions remain concerning the applicability of *C. maculata* as a viable IPM component and a commercially viable biological control agent. The plant used for this project was glabrous, and interactions between plants, pests, and predators can vary depending on the types of plant surfaces, particularly on plants with glandular trichomes (Riddick & Simmons 2014a,b). Further research may reveal additives such as pollen, known to be an important component of *C. maculata* diet (Lundgren & Wiedenmann 2004), or artificial diets that enhance performance in an IPM strategy. These results show that *C. maculata*

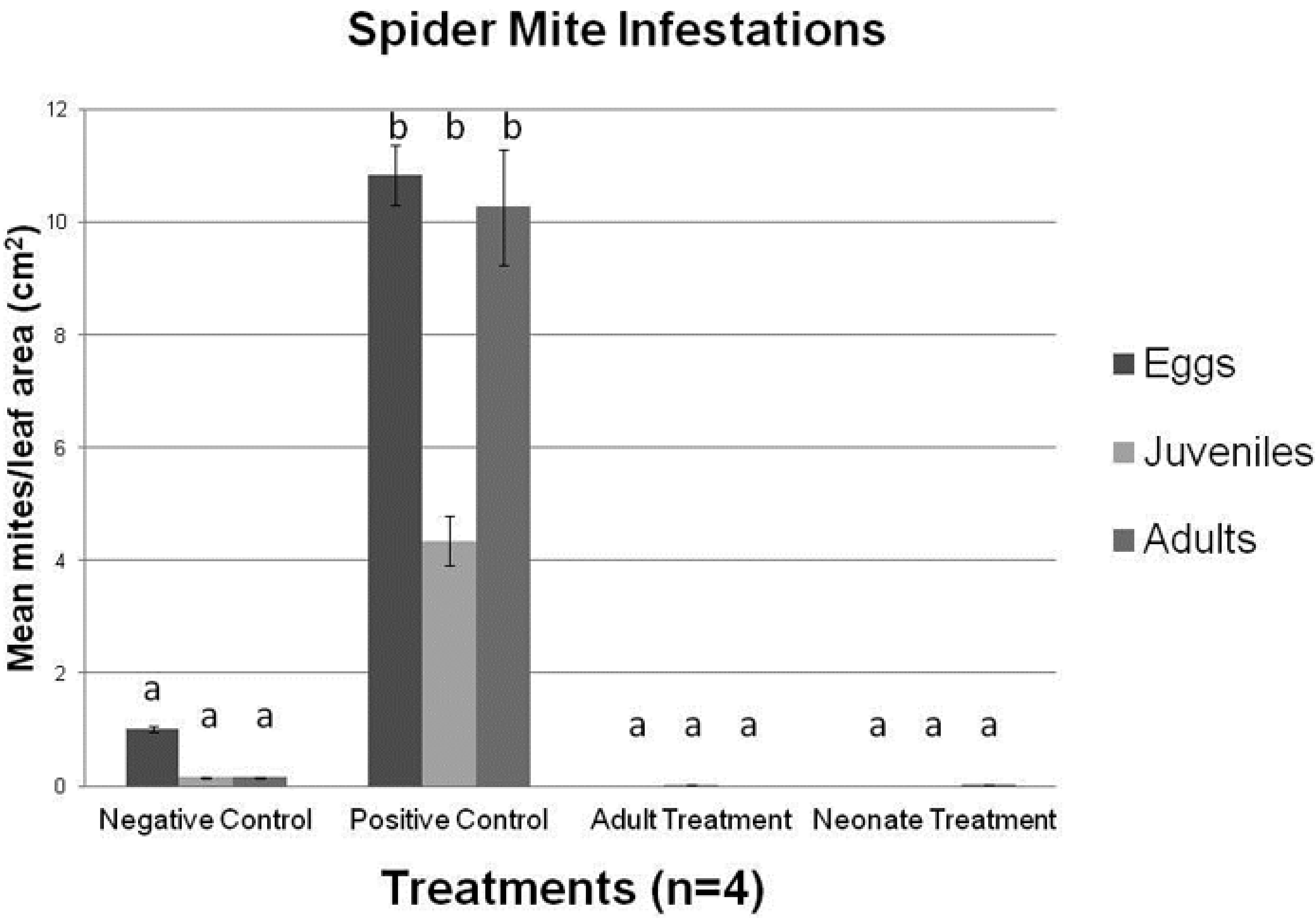

Fig. 4. Spider mite infestation levels in each treatment 20 d after treatment application. Data represent the non-transformed mean number of mites, at specified developmental stage, per square cm of leaf surface (abaxial) ± SEM (n = 4). Different letters above bars indicate significant difference (P < 0.05, Holm-Sidak multiple comparisons).

can be an effective control of spider mites on impatiens; with some further work, *C. maculata* could become a helpful component of pest management in crops that are sensitive to chemical pesticides or that require pesticide-free product.

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