Comparative studies of predation among feral, commercially-purchased, and laboratory-reared predators

James Hagler

Received: 15 November 2007 / Accepted: 19 May 2008 / Published online: 31 May 2008 International Organization for Biological Control (IOBC) 2008

Abstract The predatory activities of commerciallypurchased adult Hippodamia convergens Guérin-Méneville and two laboratory-reared strains of adult Geocoris punctipes (Say) were compared with their feral counterparts. In single prey choice feeding tests, commercially-purchased and feral H. convergens were provided copious amounts of silverleaf whitefly, Bemisia argentifolii Bellows & Perring adults or eggs of pink bollworm, Pectinophora gossypiella (Saunders). Commercially-purchased H. convergens devoured more pink bollworm eggs and at a faster rate than the feral H. convergens. In contrast, feral H. convergens consumed more adult whiteflies and at a faster rate than commercial H. convergens. In multiple feeding choice tests, two distinctly different laboratory-reared strains and feral G. punctipes were provided a cotton leaf disk containing copious amounts of silverleaf whitefly eggs, nymphs, and adults. Virtually no predation was observed on whitefly eggs, but both laboratory strains of G. punctipes fed on more whitefly nymphs and adults

Handling editor: Patrick De Clercq.

J. Hagler (\boxtimes) Arid-Land Agricultural Research Center, USDA-ARS, Maricopa, AZ, USA e-mail: james.hagler@ars.usda.gov

than the feral G. punctipes. Moreover, both of the laboratory strains had a significantly faster feeding rate on adult whiteflies and spent significantly less time feeding on plant tissue than the feral strain. These results suggest that the augmentative biological control candidates retained their ability to prey on these selected prey items, even after being displaced from their overwintering site (H. convergens) or being reared in captivity (G. punctipes) for over 40 generations.

Keywords Predation *Hippodamia convergens* Geocoris punctipes · Bemisia argentifolii · Pectinophora gossypiella \cdot Quality control \cdot Augmentative/inundative biological control Feeding behavior

Introduction

The use of broad spectrum insecticides continues to be the most common pest control tactic used throughout the world. However, with increasing concerns about groundwater contamination, pesticide residues in foods, insecticide resistance, secondary pest outbreaks, and the mounting cost of pesticides, growers are seeking more environmentally safe and cost-effective methods for controlling insect pests. One such method may include using natural enemies of insect pests that are released as part of an inundative (augmentative) biological control program. There are dozens of

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commercial producers worldwide that sell predators and parasitoids for inundative biological control (van Lenteren [2003\)](#page-10-0). Unfortunately, the quality of most commercially available natural enemies has not been thoroughly evaluated. This is especially true for predaceous biological control agents. Despite the lack of predator efficacy data in the scientific literature, many producers of biological control agents are advertising them as ''an environmental alternative to chemical control.''

Many have suggested that biological control agents should be thoroughly tested for efficacy before they are sold to consumers (Hagler and Cohen [1991](#page-9-0); Hoy et al. [1991;](#page-9-0) Parrella et al. [1992](#page-9-0); Flint et al. [1995](#page-9-0); O'Neil et al. [1998;](#page-9-0) Silvers et al. [2002;](#page-10-0) van Lenteren et al. [1997](#page-10-0), [2003](#page-10-0); Vasquez et al. [2004](#page-10-0)). Specifically, studies are needed to evaluate the fitness and efficacy of these predators (Roush [1990\)](#page-10-0). If predators are improperly used or their effectiveness is misrepresented to the public, the biological control industry could suffer a loss of consumer confidence.

Here the results of two studies are provided that compared the predatory activity of commerciallypurchased convergent lady beetles, Hippodamia convergens Guèrin-Mèneville (Coleoptera: Coccinellidae), and laboratory-reared big-eyed bugs, Geocoris punctipes (Say) (Hemiptera: Lygaeidae), with their feral counterparts. The first study examined the feeding behavior of feral and commerciallypurchased H. convergens in single prey choice feeding arenas that contained either eggs of pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) or adults of silverleaf whitefly, Bemisia argentifolii Bellows & Perring (=B. tabaci [Genn.], strain B) (Hemiptera: Aleyrodidae). H. convergens was selected for this study because it is one of the most common predators sold to consumers for inundative biological control (van Lenteren [2003](#page-10-0)). Typically, the purchased beetles are mass-collected from their overwintering sites in the mountains of the northeastern United States and then sold to customers throughout the USA (Sherman [1938](#page-10-0); Hagen [1962](#page-9-0); Bennett and Lee [1989;](#page-9-0) Roach and Thomas [1991](#page-9-0)). This study was conducted to determine if the commercially-purchased beetles would feed like their feral counterparts on immobile pink bollworm eggs and mobile whitefly adults, prey items endemic to the arid southwestern United States. The second study compared the feeding behavior of feral and laboratory-reared G. punctipes in a multiple choice feeding arena that contained a cotton leaf disk infested with an abundance of whitefly eggs, nymphs, and adults. G. punctipes was selected for this study because it is one of the few predators that has potential for mass production on either host prey or artificial diet (Cohen [1985,](#page-9-0) [1992](#page-9-0), [2000](#page-9-0)). Additionally, two separate laboratory-reared strains of G. punctipes were available for this investigation. The laboratoryreared G. punctipes were descendents of individuals continuously reared in captivity for approximately five years $(>= 40$ continuous generations) on a strict pink bollworm egg and green bean diet. The two laboratoryreared strains of G. punctipes differed in that one strain had ''normal eye'' color while the second strain consisted solely of individuals containing a visible mutant ''red-eye'' color. This study was conducted to determine if the laboratory-reared individuals would feed as readily on whiteflies as feral big-eyed bugs after being reared in captivity for so many generations.

Materials and methods

Hippodamia convergens single diet choice bioassay

Test insects

Feral H. convergens adults were collected at various times throughout the year from an alfalfa field located 25 km NW of Tucson, Arizona, USA. Commercial H. convergens were purchased on the same week that field collections were made from Nature's Control (Medford, Oregon, USA), a supplier of beneficial insects. Each cohort was placed in separate communal rearing containers containing only a wet sponge for water. Each group was held in separate containers for no more than three days prior to their bioassay.

Single prey choice feeding arena

The behaviors exhibited by individual feral and commercially-purchased beetles were observed in one of two feeding choice arenas. Before each individual observation, an adult beetle was placed in a Petri dish and starved, but provided with water ad libitum for 24 h. A single beetle was then placed into a 3.5 cm Petri dish containing either 40 threeday-old pink bollworm eggs or 40 three-day-old adult

whiteflies. A total of 35 commercially-purchased and 41 feral beetles were observed feeding on pink bollworm eggs, and 24 commercially-purchased and 32 feral beetles were observed feeding on adult whiteflies. The behavior of each individual beetle was recorded continuously for 30 min. Preliminary observations of H. convergens behavior in the feeding arena revealed several distinct behaviors, therefore a behavioral ethogram (Lehner [1996\)](#page-9-0) was used and its components were programmed into The Observer®, a software program designed specifically for animal behavior research (Noldus Information Systems, Ver. 3.0, Leesburg, Virginia, USA). Descriptions of the behaviors recorded are given in Table 1. A new feeding arena was constructed after each 30 min observation.

Data analysis

The number of each prey type consumed and the time spent feeding, walking, resting, probing, and grooming by feral and commercially-purchased beetles were recorded. Differences in the mean number of prey consumed in a 30 min period and in the amount of time spent in each behavioral event between the feral and commercially-purchased H. convergens were first analyzed by a Student's t-test. In most cases, these data did not fulfill the necessary assumptions of the t-test (e.g., both non-transformed and transformed data were not normally distributed

Table 1 Description of the behavioral events recorded for feral and commercially-purchased Hippodamia convergens exposed to either pink bollworm eggs or whitefly adults in single prey choice feeding arenas

Observed behavior	Description of the behavior	
Walking	Lady beetle moving forward across the leaf surface	
Resting	Lady beetle standing motionless	
Grooming	Lady beetle making rapid movements with its fore or hind legs across its body surface and antennae	
Orienting	Lady beetle pivoting on the leaf without advancing in any particular direction	
Probing	Lady beetle probing a pink bollworm egg or a white fly adult, but not feeding	
Necrophagy	Lady beetle consuming a dead adult white fly	
Feeding	Lady beetle consuming a pink bollworm egg or a living adult whitefly	

and did not contain equal variances) (SigmaStat, Ver. 2.03, Chicago, Illinois, USA). In such cases, the nonparametric Mann-Whitney Rank Sum Test was used to identify significant differences between the two beetle treatments.

Geocoris punctipes multiple diet choice bioassay

Test insects

Feral G. punctipes adults were collected 24 h prior to observation from a cotton field located at the University of Arizona's Maricopa Research Center, Maricopa, Arizona, USA. The collections were made at various times between the months of July and September. Upon return to the laboratory, the feral big-eyed bugs were placed individually in 9.0-cm Petri dishes containing a wet sponge. Two separate and very distinct laboratory colonies of G. punctipes were available for testing that were easily distinguishable by their eye color. The ''normal-eyed'' individuals were descendants of individuals collected approximately 5 years $(>= 40$ generations) prior to this study from an alfalfa field located near Maricopa, Arizona, USA. The "red-eyed" individuals were descendents from a laboratory colony reared at the University of Arkansas, USA since the late 1980s (the prior rearing history is unknown). Both groups of bigeyed bugs were fed pink bollworm eggs and green beans for five years prior to this study.

Multiple prey choice feeding arena

The behaviors of individual feral and laboratory reared big-eyed bug adults were recorded continuously for 30 min each in a multiple prey-choice feeding arena containing a cotton leaf disk infested with whitefly eggs, nymphs (various instars), and adults as described by Hagler et al. [\(2004](#page-9-0)). Briefly, cotton plants, Gossypium hirsutum L. (cv 'Delta Pine 5415') were grown in 15.2-cm diameter pots in a greenhouse using standard cultural practices. Four- to five-week-old plants were infested with adult whiteflies on a weekly basis. When the plants were $\approx 8-9$ weeks old, a single cotton leaf containing numerous whitefly eggs and nymphs was removed from a plant and cut to fit exactly into the bottom of a 3.5-cm plastic Petri dish (the feeding arena). The number of whitefly eggs and nymphs were counted and the leaf was placed abaxial side up into the bottom of the feeding arena. Then, exactly 100 whitefly adults were introduced into the feeding arena and the Petri dish lid was placed over the top of the arena. A typical arena contained a 3.5-cm cotton leaf disk infested with 100 adult whiteflies (at a 1:1 sex ratio) and an average of 3,415 \pm 243 and 1,570 \pm 151 whitefly eggs and nymphs (various instars), respectively. A single big-eyed bug, held overnight without food, was then placed into the feeding arena and its behavior was continuously observed for 30 min under a dissecting microscope. A total of 30 individual bigeyed bugs were observed for each of the three G. punctipes treatment groups. Preliminary observations of G. punctipes behavior revealed several distinct behaviors, therefore a behavioral ethogram was used and its components were programmed in The Observer®. Descriptions of the big-eyed bugs behaviors recorded are given in Table 2. After each 30 min observation, the big-eyed bug was removed from the arena and replaced with another randomly selected big-eyed bug. No more than two predators were observed consecutively in the same arena. The feeding arenas were replaced daily with fresh plant and prey material.

Data analysis

The mean number of prey consumed, the number of plant feeding events, and the mean durations of the feeding (on whitefly and plant tissue), walking, resting, probing (on whitefly and plant material), and grooming events among the three cohorts of G. punctipes were recorded. None of the data recorded conformed to the assumptions of normality and/or equal variances needed for a proper data analysis by ANOVA. Therefore, a Kruskal-Wallis one-way ANOVA on the ranked data was used to identify significant differences in the various behaviors among the three treatment groups (SigmaStat Ver. 2.03). When a significant difference was detected among the treatments, a Dunn's multiple comparison test was used to identify the statistically different means.

Results

Hippodamia convergens single diet choice bioassay containing pink bollworm eggs

The commercially-purchased beetles consumed significantly more eggs (Mann–Whitney Rank Sum Test, $T = 1,557, n[commercial] = 35, n[feral] = 41, P =$ 0.03, Fig. [1a](#page-4-0)) and had a significantly faster feeding rate (prey handling time) than the feral beetles $(T = 223325, n[commercial] = 490, n[feral] = 525,$ $P < 0.001$ $P < 0.001$, Fig. 1b). On average, an individual commercially-purchased and feral beetle consumed 14.9 ± 2.0 and 13.8 ± 2.2 eggs every 30 min at a rate of

Table 2 Description of the behavioral events recorded for feral and laboratory-reared Geocoris punctipes exposed to a cotton leaf disk containing the various whitefly lifestages in a prey- and plant-choice arena

Observed behavior	Feeding choice on the various whitefly lifestages and cotton leaf disk	Description of the behavior
Walking		Big-eyed bug moving forward across the leaf surface
Resting		Big-eyed bug standing motionless
Grooming		Big-eyed bug making rapid movements with its fore or hind legs across its body surface and antennae
Orienting		Big-eyed bug pivoting on the leaf without advancing in any particular direction
Probing	Egg	Big-eyed bug probing a white fly egg, but not feeding
	Nymph	Big-eyed bug probing a white fly nymph, but not feeding
	Adult	Big-eyed bug probing a white fly adult, but not feeding
Feeding	Egg	Big-eyed bug consuming an egg
	Nymph	Big-eyed bug consuming a nymph
	Adult	Big-eyed bug consuming an adult
	Plant	Big-eyed bug feeding on plant tissue

Fig. 1 (a) Mean number of Pectinophora gossypiella eggs consumed by feral $(n = 41, 30 \text{ min})$ observations) and commercially-purchased $(n = 35, 30 \text{ min})$ observations) Hippodamia convergens. (b) Mean time of behavioral events recorded for feral and commercially-purchased Hippodamia convergens. The numbers inside the bars vertically adjacent to the x-axes are the total number of behavioral events recorded over the duration of the study. An asterisk or NS above each paired treatment indicates a significant or nonsignificant difference, respectively

23.4 \pm 1.1 and 27.2 \pm 0.9 s per egg, respectively (Fig. 1a, b). The mean time that the feral beetles spent probing $(T = 56,837, n[commercial] = 344, n[feral]$ $= 177, P < 0.001$, resting (T = 53,841, n[commercial] = 239, *n*[feral] = 218, *P* = 0.005), and walking $(T = 117,445, n[commercial] = 426, n[feral] = 297,$ $P<0.001$) was significantly longer (Fig. 1b) and there was no difference ($T = 37560$, n[commercial] = 204, n [feral] = 190, $P < 0.97$) in the times that each beetle group spent grooming.

Hippodamia convergens single diet choice bioassay containing adult whiteflies

The mean number of adult whiteflies consumed per individual beetle was significantly higher ($t = 3.18$, $df = 54$, $P = 0.003$, Fig. [2](#page-5-0)a), and the feeding time was significantly shorter $(T = 108,876, n[commer$ cial] = 222, *n*[feral] = 566, *P* < 0.001) for the feral beetles (Fig. [2b](#page-5-0)). On average, an individual feral and commercially-purchased beetle consumed 17.7 ± 1.9 and 9.1 ± 1.9 adult whiteflies every 30 min at a rate of 13.0 ± 0.3 and 20.4 ± 1.0 s per whitefly, respectively (Fig. [2](#page-5-0)a, b). The mean time spent probing $(T = 85,124, n[commercial] = 266, n[feral]$ $= 614, P < 0.001$), resting (T = 45,996, n[commercial] = 371, n [feral] = 153, $P \lt 0.001$), and grooming $(T = 33,530, n[commercial] = 184, n[feral] = 273,$ $P < 0.001$) by the feral beetles was significantly longer than that of their commercially-purchased counterparts (Fig. [2](#page-5-0)b). There was no significant difference in the amount of time the two groups of beetles spent walking $(T = 406,061, n[commercial] = 729, n[feral] = 605,$ $P = 0.751$) and feeding $(T = 340, n$ [commercial] = 17, n [feral] = 19, $P = 0.438$) on dead whiteflies (necrophagy).

Geocoris punctipes multiple diet choice bioassay containing the various whitefly lifestages

The *G. punctipes* were reticent to feed on whitefly eggs with only four whitefly eggs consumed over the duration of the study, all by the red-eyed mutant strain (Fig. [3](#page-6-0)a). There were no significant differences Fig. 2 (a) Mean number of Bemisia argentifolii adults consumed by feral $(n = 32,$ 30 min observations) and commercially-purchased $(n = 24, 30 \text{ min})$ observations) Hippodamia convergens. (b) Mean time of behavioral events recorded for feral and commercially-purchased Hippodamia convergens. The numbers inside the bars are the number of recorded observations for each behavioral event. An asterisk or NS above each paired treatment indicates a significant or nonsignificant difference, respectively

in the mean number of whitefly nymphs consumed per individual ($H = 2.8$, df = 2, $P = 0.249$) among the three treatment groups (Fig. [3a](#page-6-0)). However, the normal-eyed and red-eyed strains consumed two to three times more adult whiteflies than the feral strain $(H = 20.5, df = 2, P < 0.001; Fig. 3a)$ $(H = 20.5, df = 2, P < 0.001; Fig. 3a)$ $(H = 20.5, df = 2, P < 0.001; Fig. 3a)$. The mean feeding duration on whitefly nymphs was not significantly different among the three treatment groups $(H = 2.7, df = 2, P = 0.248; Fig. 3b)$ $(H = 2.7, df = 2, P = 0.248; Fig. 3b)$ $(H = 2.7, df = 2, P = 0.248; Fig. 3b)$. However, the feral strain had a significantly longer prey handling time on adult whiteflies $(H = 22.161, df = 2)$, $P \, < 0.001$) (Fig. [3b](#page-6-0)). The non-feeding behaviors exhibited by each cohort of G. punctipes revealed that the red-eyed G. punctipes spent significantly less time grooming ($H = 12.413$, df = 2 $P = 0.002$), resting $(H = 27.023, df = 2, P < 0.001)$, and walking $(H = 24.561, df = 2, P < 0.001)$ than the feral and normal-eyed *G. punctipes* (Fig. [3](#page-6-0)c). The mean duration of orienting was not significantly different between the three strains $(H = 2.594, df = 2)$, $P = 0.237$.

The occurrence of probing was rare on whitefly nymphs and adults and almost nonexistent on eggs for the feral and normal-eyed G. punctipes (Fig. [3](#page-6-0)d). However, the red-eyed strain was frequently observed probing $(n = 60 \text{ probing events})$ whitefly eggs. The duration of probing events on whitefly nymphs $(H = 5.041, df = 2, P = 0.080)$ and adults $(H = 1.041, df = 1.004)$ 1.329, df = 2, $P = 0.515$) was not significantly different among the three groups of big-eyed bugs (Fig. [3](#page-6-0)d).

The number of plant feeding events exhibited by the three cohorts of G. punctipes were not significantly different with an average of less than two feeding events occurring every 30 min ($H = 5.952$, $df = 2$, $P = 0.051$; Fig. [4](#page-7-0)a). However, the duration of plant feeding events was significantly different among all three treatment groups with feral $>$ normal-eyed $>$ red-eyed, respectively ($H = 28.032$, $df = 2, P < 0.001$; Fig. [4](#page-7-0)b). Also, the mean duration of plant probing events was approximately 2.5 times longer for the feral G. punctipes than the normal-eyed

Fig. 3 (a) Mean number of *Bemisia argentifolii* eggs, nymphs, and adults consumed by feral, normal-eyed, and red-eyed $(n = 30, 30 \text{ min}$ observations for each treatment group) Geocoris punctipes. (b) Mean prey handling time of feral, normal-eyed, and red-eyed G. punctipes on B. argentifolii eggs, nymphs, and adults. (c) Mean time of behavioral events recorded for feral, normal-eyed, and red-eyed G. punctipes.

and red-eyed strains ($H = 14.631$, df = 2, P < 0.001; Fig. [4c](#page-7-0)).

Discussion

An ideal entomophage should feed rapidly and frequently on its targeted prey (Cohen [2000;](#page-9-0) De Clercq [2002](#page-9-0)). A major concern of the biological control industry is that commercially-purchased predators, whether they are mass-produced on artificial diet or mass-collected from the field, may not feed as readily on targeted prey items as their feral counterparts. In the first part of this study, the feeding activity of commercially-purchased H. convergens was compared with their feral counterparts endemic to the southwestern desert in the USA. The purpose of this study was to determine if the purchased beetles which were collected from their cool

(d) Mean probing time of feral, normal-eyed, and red-eyed G. punctipes on B. argentifolii eggs, nymphs, and adults. The numbers inside the bars vertically adjacent to the x-axes are the total number of behavioral events recorded over the duration of the study. Bars followed by the same letter are not significantly different

overwintering sites in Oregon (Sherman [1938;](#page-10-0) Hagen [1962;](#page-9-0) Bennett and Lee [1989](#page-9-0); Roach and Thomas [1991\)](#page-9-0), shipped to Arizona, and stored for several days in a refrigerator would feed as readily as their feral counterparts on prey items endemic to the arid southwestern USA (e.g., pink bollworm eggs and adult whiteflies). Results reported here revealed that the commercially-purchased H. convergens adults were significantly better predators than feral beetles on pink bollworm eggs, but not on adult whiteflies in single diet choice arenas. The laboratory results obtained for beetles feeding on whitefly were in sharp contrast to research results yielded from a field study designed to assess the feeding behavior of massreleased H. convergens. In that study, a molecular gut content analysis revealed that a higher proportion of mass-released beetles contained whitefly remains in their gut than the feral beetles (Hagler and Naranjo [2004\)](#page-9-0). In short, it appears that commercially-

Fig. 4 (a) Mean numbers of plant feeding events exhibited by feral, normal-eyed, and red-eyed ($n = 30, 30$ min observations for each treatment group) Geocoris punctipes. (b) Mean plant feeding duration of feral, normal-eyed, and red-eyed G. punctipes on the cotton leaf tissue. (c) Mean plant probing

purchased beetles retained their ability to prey on pink bollworm eggs and at varying levels on whiteflies without any preconditioning to the targeted prey or environment.

This study indicates that commercially-purchased adult H. convergens retain their innate ability to feed on selected prey. However, there are still certain limitations that restrict their efficacy as inundative biological control agents in open-field situations. Previously, their poor performance has been attributed mainly to the fact that they do not remain long at their target site because of their tendency to disperse (Hagen [1962](#page-9-0); Kieckhefer and Olsen [1974;](#page-9-0) Rankin and Rankin [1980](#page-9-0); Davis and Kirkland [1982;](#page-9-0) Flint et al. [1995;](#page-9-0) Driestadt and Flint [1996](#page-9-0); Obrycki and Kring [1998\)](#page-9-0). For example, Hagler and Naranjo ([2004\)](#page-9-0) showed that only 3,642 of the \approx 540,000 marked adult H. convergens released $(\approx 0.7\%)$ were recaptured. Of these, 2,427, 883, and 332 were recaptured 3, 8, and 15 days after release, respectively. The steady decline in recovery rates of marked beetles with each subsequent sampling date

time of feral, normal-eyed, and red-eyed G. punctipes on the cotton leaf tissue. The numbers inside the bars vertically adjacent to the x-axes are the total number of behavioral events recorded over the duration of the study. Bars followed by the same letter are not significantly different

after release support the findings of other studies showing that H. convergens typically leave their release sites or die within days after release (Kieckhefer and Olsen [1974](#page-9-0); Davis and Kirkland [1982](#page-9-0); Driestadt and Flint [1996\)](#page-9-0). This study and others (Hagler and Naranjo [2004](#page-9-0)) show that commerciallypurchased adult beetles will likely prey on targeted pests, but methods (e.g., perhaps mechanical or cultural) are needed to entice the very mobile adult life stage to remain at their point of release in order for effective biological control to be achieved. For example, a flightless laboratory strain of Harmonia axyridis Pallas (Coleoptera: Coccinellidae) has been identified as a potential biological control candidate for aphids (Tourniaire et al. [2000\)](#page-10-0). Perhaps a flightless, less mobile strain of H. convergens could be selected for and then mass-produced. Flightless H. convergens could provide much more effective control of targeted pest species.

The two cohorts of G. *punctipes* that were reared continuously in the laboratory on only pink bollworm eggs and green beans for over five years $(>=40$ continuous generations) offered a unique opportunity to study their feeding behavior with their desertadapted (feral) counterparts. The study was conducted to determine if the laboratory reared strains would lose their innate ability to feed on alternate prey items after being conditioned to feed on pink bollworm eggs and green beans for so long. The results revealed that both groups of laboratoryreared bugs fed significantly more on adults and had significantly shorter handling times than their feral counterparts. These data support previous research that showed that G. punctipes reared for 60 continuous generations on a meat-based artificial diet can resume ''normal'' predation on a wide variety of prey types (Hagler and Cohen [1991](#page-9-0); Cohen [2000](#page-9-0)). Moreover, both groups of laboratory reared big-eyed bugs spent less time feeding on cotton leaf tissue.

An interesting aspect of the current study was that two distinctive laboratory strains of G. punctipes were tested. The original colony consisted of individuals that had ''normal eyes'' and the second colony had individuals that had ''red eyes.'' Insect eye color mutations have been reported for a wide variety of insects including P. gossypiella (Bartlett and Lewis [1978\)](#page-9-0), Drosophila melanogaster Meigen (Lindsley and Grell [1968](#page-9-0)), Ceratitis capitata (Wiedemann) (Saul [1982\)](#page-10-0), Dacus dorsalis Hendel (McCombs and Saul [1989](#page-9-0)), Anastrepha fraterculus (Wiedemann) (Yamada and Selivon [2001](#page-10-0)), and Lygus lineolaris (Palisot de Beauvois) (Snodgrass [2002](#page-10-0)). Snodgrass [\(2002](#page-10-0)) determined that there were no significant differences in egg production and egg viability between a normal-eyed and a red-eyed mutant strain of L. lineolaris that was reared continuously in the laboratory for over five years $(>= 40$ generations). Results from this study showed that redeyed G. punctipes were more voracious whitefly predators and less likely to feed on plant material than both the feral and normal-eyed G. punctipes. These results suggest that both strains of laboratoryreared G. punctipes merit further testing under field conditions. In the future, we plan to release laboratory-reared G. punctipes in the field and then compare their feeding profiles with feral bugs via molecular gut content analyses (Hagler and Naranjo [1996,](#page-9-0) [2004\)](#page-9-0). The red-eye strain of G. punctipes will be perfectly suited for such a study because the red eye mutation can be used to differentiate the released

predators from their feral counterparts (Hagler and Jackson [2001\)](#page-9-0).

This study shows that both commerciallypurchased lady beetles and laboratory-reared bigeyed bugs retain their ability of feed on selected prey items. However, it is emphasized that laboratory tests such as this one are just the first step toward evaluating predaceous biological control agents. There are several drawbacks associated with studies conducted in confined laboratory arenas which could lead to erroneous estimates of predation. First, the prey population in each arena was higher than the predators would encounter in nature. Second, the enclosed arenas eliminate the ability of mobile prey to escape predation. Finally, these generalist predators were not provided with any alternate prey choices. In short, the results yielded from this study are only the first step toward assessing predation. The encouraging results suggest that more complex studies are warranted to further validate the efficacy of these predators in greenhouse or open field situations.

In summary, although dozens of companies sell arthropod predators for use as augmentative biological control agents, relatively little effort has been devoted to the critical assessment of the biological control candidates (O'Neil et al. [1998](#page-9-0)). Moreover, as mass rearing technology improves, there will likely be more predators produced on artificial diets via large-scale automated systems (Cohen [1985](#page-9-0), [2000](#page-9-0); De Clercq and Degheele [1993;](#page-9-0) De Clercq et al. [1998a](#page-9-0); Grundy et al. [2000](#page-9-0); Rojas et al. [2000](#page-10-0); Reeve et al. [2003](#page-9-0); van Lenteren [2003;](#page-10-0) Grenier and De Clercq [2003\)](#page-9-0). Determining if mass-produced predators feed satisfactorily on targeted pests and in comparison to feral insects is of paramount importance for the development of a viable and trustworthy quality assessments which are needed for inundative biological control programs. Clearly vigorous testing standards are needed to determine if predators might become domesticated or lack the conditioning necessary to hunt, capture, and feed on prey items under field conditions (Hagler and Cohen [1991](#page-9-0); Hagler and Naranjo [2004\)](#page-9-0). Laboratory studies like the one presented here along with thorough field studies of predation and dispersal (Hagler and Naranjo [2004](#page-9-0)) are just two types of studies needed to validate the use of augmentative biological control agents for pest control. Additionally, further quality

control standards are needed to precisely assess predator fitness rearing conditions (De Clercq et al. 1998b; Cohen 2000), and shipping conditions (O'Neil et al. 1998) to assure viable biological control agents.

Acknowledgements I thank Wendy Cummings, Christine Durand, Melissa Erickson, George Green, Scott Machtley, and Erik Stone for technical assistance. I am grateful to Mary Louis Flint, Paul Grundy, Debbie Hagler and Marshall Johnson for reviewing earlier versions of this manuscript. Special thanks go to Steve Naranjo and Becci Burke for supplying the laboratoryreared Geocoris punctipes used in this study.

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