Quality Assessment of Biological Control Agents in a Greenhouse Production System[1]

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Abstract The quality (number of live individuals) of biological control agents, such as parasitoids and predators, is important to ensure the success of biological control programs. Shipment quality of slow-release sachets containing the predatory mite, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), and release cards containing the whitefly parasitoids, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae), was determined from a distributor/supplier in 2023. Slow-release sachets containing predatory mites were inserted into a mold of clay placed on a 7.6 $\times$ 12.7 cm yellow sticky card. The number of adults that emerged and were captured on the yellow sticky card was recorded. Release cards containing parasitized pupae of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), or sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), were placed into a 225-mL Mason jar and a 2.5 $\times$ 2.5 cm yellow sticky square was attached to the lid underside. The number of adults captured on the yellow sticky square was recorded. The numbers of *N. cucumeris*, *E. formosa*, and *E. eremicus* adults that emerged from slow-release sachets or release cards varied depending on shipment date. The mean ($\pm$ SEM) number of adults on the final assessment date was between 156.2 $\pm$ 12.9 and 410.0 $\pm$ 24.2 for *N. cucumeris*, 47.8 $\pm$ 2.2 and 52.6 $\pm$ 1.2 for *E. formosa*, and 40.0 $\pm$ 5.0 and 75.2 $\pm$ 9.4 for *E. eremicus*. Greenhouse producers should assess whether biological control agent shipments are viable when released into greenhouse production systems.

Key Words laboratory, parasitoids, predators, slow-release sachets, release cards

Quality assessment (number of live individuals) of commercially available biological control agents, including parasitoids, predators, and entomopathogenic nematodes, is important when implementing a biological control program (Williams and Cloyd 2005). After releasing biological control agents into greenhouses, greenhouse producers should determine if the individuals received from distributors/suppliers are viable (Cloyd 2016, Parrella 1999, van Lenteren and Nicoli 2004, Williams and Cloyd 2005) by sampling the biological control agent products (e.g., slow-release sachets or release cards). Biological control agents of inferior quality may lead to a false presumption that biological control is not effective in managing insect and/or mite pest populations when pest populations remain at plant damaging levels, even

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after releasing biological control agents (Losey and Calvin 1995, Lundgren and Heimpel 2003, O'Neil et al. 1998, Steward et al. 1996). Hence, the success of a biological control program depends on the quality of the biological control agents released (Cloyd 2016, van Lenteren et al. 1980). Furthermore, to ensure continued business from customers, distributors/suppliers must properly package biological control agents to provide protection from exposure to extremes in temperature and relative humidity encountered during shipping (Van Driesche and Heinz 2004, van Lenteren and Woets 1988, Vasquez et al. 2004).

The quality of biological control agents is not always associated with the distributor/supplier but instead may be related to shipping, which is beyond the control of the distributor/supplier (Waddington 1993). For example, exposure to unfavorable environmental conditions, such as temperature and relative humidity, can affect the number of live biological control agents received by a greenhouse producer (Buitenhuis et al. 2014, Cloyd 2016, van Lenteren 2003). The quality of biological control agents can vary depending on packaging of products from distributors/suppliers (Caamano et al. 2008, Fernández and Nentwig 1997). In addition, the quality of the same biological control agent can differ depending on the commercial distributor/supplier (Herrick and Cloyd 2021). Therefore, the quality of biological control agents purchased from distributors/suppliers should be assessed after releasing them into greenhouse production systems to determine if enough live individuals are present to maintain pest populations below plant damaging levels (Cloyd 2016).

Neoseiulus cucumeris (Oudemans) (Acari: Phytoseiidae) is a predatory mite of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Beard 1999, Gillespie 1989, Shipp and Wang 2003). *Neoseiulus cucumeris* is commercially available in slow-release sachets that contain eggs, larvae, nymphs, and adults mixed with a bran carrier (Cloyd 2023a, 2024; Pochubay et al. 2015; Shipp and Wang 2003). Slow-release sachets are placed among greenhouse grown crops (Cloyd 2024). Adults emerge from the slow-release sachets through perforated holes and then disperse among crops within the greenhouse over 4 to 6 weeks (Midthassel et al. 2014, Shipp and Wang 2003).

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) and *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) are parasitoids of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Berndt and Meyhöfer 2008, Vafaie et al. 2021, van Lenteren et al. 1996). The parasitoids are commercially available as release cards with a predetermined number of parasitized whitefly pupae attached to each release card (Cloyd 2023b,c).

There is minimal information in the scientific literature on the quality of biological control agents received from distributors/suppliers that are released into greenhouse production systems. Therefore, the objective of the study was to assess the quality of shipments of the predatory mite, *N. cucumeris*, and the whitefly parasitoids, *E. formosa* and *E. eremicus*, received from a distributor/supplier throughout the growing season in a greenhouse production system in 2023 so that the greenhouse producer could determine if sufficient numbers of viable individuals were present to maintain insect pest populations below plant damaging levels.

Materials and Methods

The study was conducted during the growing season of 2023 that involved the production of bedding plants (e.g., ornamentals and vegetables), chrysanthemum (*Tanacetum* × *grandiflorum* Thunberg), and poinsettia (*Euphorbia pulcherrima* Willdenow ex Klotzsch) at 5-H Greenhouses (Wamego, KS). There were 8 shipments of the biological control agents received by 5-H Greenhouses. All shipments of the biological control agents were ordered and obtained from Plant Products USA Inc. (Clinton, MI), which is the company that had been used previously. The slow-release sachets (SRS) were shipped in a box with brown paper and the release cards (RC) were shipped in a Styrofoam container with ice packs and brown paper. Upon receipt by the greenhouse producer, 5 SRS and 5 RC were randomly selected from each box and placed into sealed plastic containers [15 × 15 × 5 mm (length × width × height)]. The sealed plastic containers were stored in a refrigerator set at 3.3°C until they were collected within approximately 24 h. After collecting the SRS and/or RC, they were transported to a laboratory in the Department of Entomology at Kansas State University (Manhattan, KS). The laboratory conditions were 19.8 to 22°C and 50 to 60% relative humidity under constant light. The SRS containing *N. cucumeris* were used during production of the bedding plant crops. The RC containing *E. formosa* were used on a tomato, *Solanum lycopersicum* L., crop and the RC containing *E. eremicus* were used during production of the poinsettia crop.

The study was set up as a completely randomized design. Boxes containing 1,000 SRS of the predatory mite, *N. cucumeris*, were received on 3 and 26 March, 26 April, 11 May, and 20 July 2023. Boxes containing 100 RC of *E. formosa* were received on 18 January 2023, and 100 RC of *E. eremicus* were received on 15 September and 25 October 2023. Each RC associated with *E. formosa* had 50 parasitized greenhouse whitefly pupae per RC and each RC affiliated with *E. eremicus* had 100 parasitized sweetpotato whitefly pupae per RC.

Quality assessment of *Neoseiulus cucumeris*. Five SRS were randomly selected from each box of 1,000 to determine the quality of each shipment. The remaining SRS were placed among the bedding plant crops. A single SRS was inserted into a mold of clay (Crayola Air-Dry Clay: Crayola; Easton, PA) placed on a 7.6 × 12.7 cm yellow sticky card (Hummert™ International; Earth City, MO) (Fig. 1). The number of adults that emerged from the SRS and were captured on the yellow sticky card were counted using a dissecting microscope (Nikon SMZ1000; Model C-DSS115, Japan). The number of adults were counted on the yellow sticky card 5 to 27 d following initial preparation of the quality assessment.

Quality assessment of *Encarsia formosa* and *Eretmocerus eremicus*. Five RC were randomly selected from each box of 100 to determine the quality of each shipment. The remaining RC were placed among the tomato and poinsettia crops. A single RC, containing 50 (*E. formosa*) or 100 (*E. eremicus*) parasitized whitefly pupae per RC, was placed into a 225-mL clear glass wide mouth Kerr® SureTight™ Mason jar (Fig. 2). A 2.5 × 2.5 cm yellow sticky square was attached to the underside of the lid using double-sided sticky foam. The number of adults captured on the yellow sticky square were counted using a dissecting microscope (Nikon SMZ1000; Model C-DSS115, Japan). The number of *E. formosa* adults were counted on the yellow sticky square after 7, 13, and 17 d following initial preparation of the quality

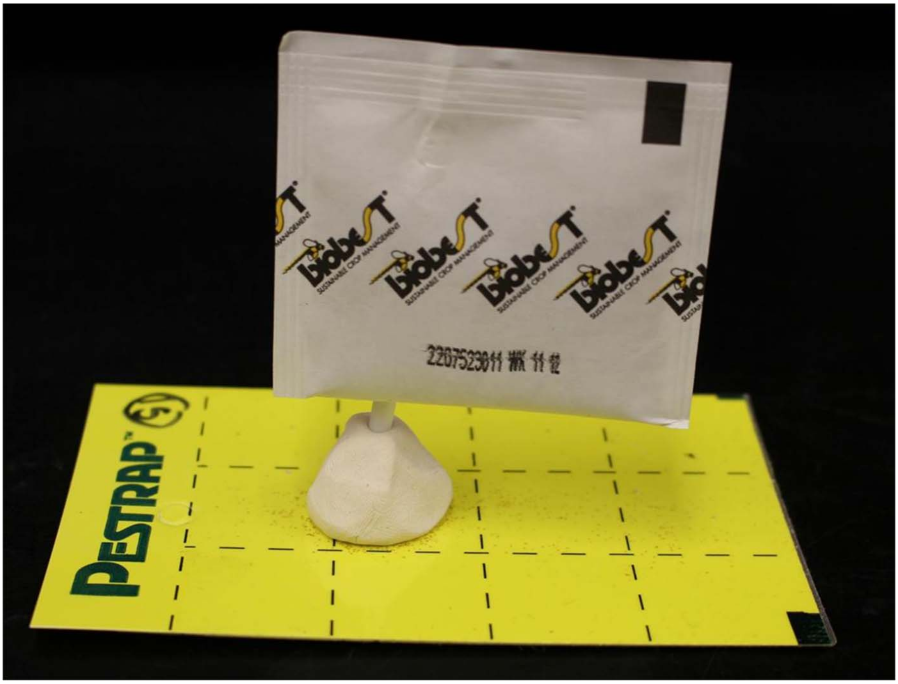

Fig. 1. Slow-release sachet containing *Neoseiulus cucumeris* adults, nymphs, larvae and eggs inserted into a mold of clay (Crayola Air-Dry Clay: Crayola; Easton, PA) placed on a 7.6 × 12.7 cm yellow sticky card (Hummert™ International; Earth City, MO).

assessment. The number of *E*. *eremicus* adults were counted on the yellow sticky square 8 to 24 d following initial preparation of the quality assessment.

Data collection and analysis. There were 8 shipments of the biological control agents. Costs associated with each of the biological control agent shipments was determined based on invoices from the distributor/supplier (Plant Products USA Inc; Clinton, MI). Data related to the number of *N. cucumeris*, *E. formosa*, and *E. eremicus* adults that emerged from the SRS and RC, and were captured on the yellow sticky card were summarized using descriptive statistics. Data from shipments 1 (SRS of *N. cucumeris*), 4 (SRS of *N. cucumeris*), 6 (RC of *E. formosa*), and 7 (RC of *E. eremicus*) were analyzed using a Student's *t* test (Microsoft Excel 2011). Data from shipments 2 (SRS of *N. cucumeris*), 5 (SRS of *N. cucumeris*), and 8 (RC of *E. eremicus*) were analyzed using an analysis of variance (SAS Institute 2012). Data associated with shipments 3 and 4 were not analyzed because only 1 box was received.

Results

Results associated with the SRS containing *N. cucumeris* are presented in Tables 1 to 5, and results from the RC affiliated with *E. formosa* and *E. eremicus* are presented in Tables 6 to 8.

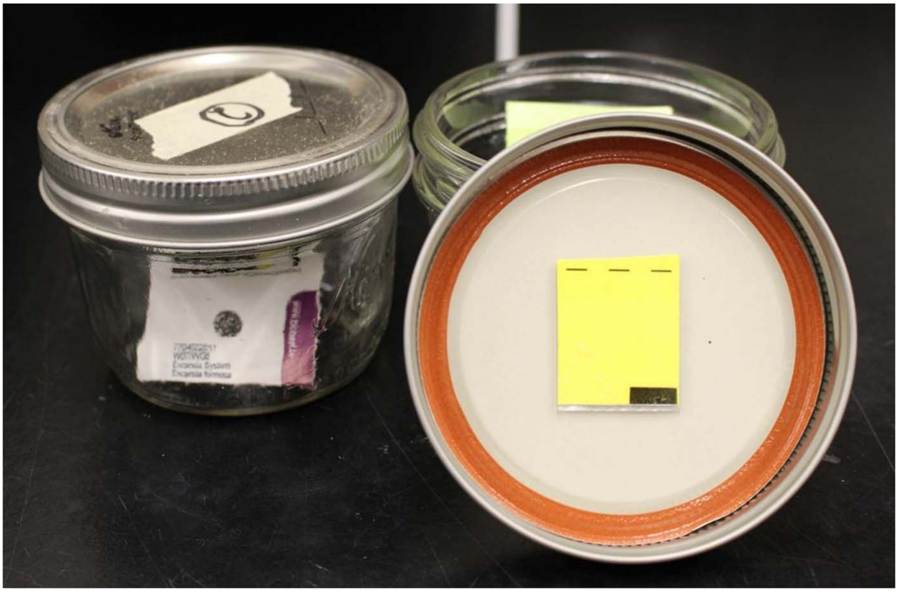

Fig. 2. Release card containing greenhouse whitefly, *Trialeurodes vaporariorum*, pupae parasitized by *Encarsia formosa* inside a 225-mL clear glass wide mouth Kerr® SureTight™ Mason jar and 2.5 × 2.5 cm yellow sticky square (Hummert™ International; Earth City, MO) attached to the underside of the lid.

Quality assessment of *Neoseiulus cucumeris*. There were significant differences in the number of *N. cucumeris* adults that emerged from the SRS from the different boxes associated with shipments 2 ($F = 39.29$; df = 2, 23; $P < 0.001$) and 4 [t(8) = −6.26; $P = 0.0002$), but not shipments 1 [t(28) = −0.18; $P = 0.85$] and 5 ($F = 2.46$; df = 2, 38; $P = 0.098$). For shipment 2, significantly more *N. cucumeris* adults emerged from the SRS affiliated with the second box [250.0 ± 10.8; mean ± standard error of the mean (SEM)] than the first (147.1 ± 9.2) and third (214.7 ± 2.9) boxes.

The number of *N. cucumeris* adults that emerged from the SRS varied depending on the time of year during the growing season. The mean and SEM of adults that emerged from the SRS on the final assessment date for all 5 shipments was between 156.2 ± 12.9 and 410.0 ± 24.2, and the range was between 132 and 469 (Tables 1 to 5).

Quality assessment of *Encarsia formosa* and *Eretmocerus eremicus*. There was no significant difference [t(28) = 0.71; $P = 0.48$] in the number of *E. formosa* adults that emerged from the RC affiliated with the different boxes for shipment 6. There were significant differences in the number of *E. eremicus* that emerged from the RC from the different boxes for shipment 7 [t(28) = −2.38; $P = 0.024$] and 8 ($F = 4.02$; df = 2, 30; $P = 0.028$). For shipment 7, significantly more *E. eremicus* adults emerged from the RC affiliated with box 2 (68.3 ± 5.4) than box 1 (53.7 ± 2.9). For shipment 8, significantly more *E. eremicus* adults emerged from RC associated with box 1 (47.0 ± 3.4) than box 2 (33.6 ± 3.2), but there

Table 1. Recorded count data, mean, and standard error of the mean (SEM) of *Neoseiulus cucumeris* adults that emerged from the slow release sachets (SRS) and were captured on a 7.6 $\times$ 12.7 cm yellow sticky card on 11, 21, and 27 March 2023. Slow-release sachets were prepared for quality assessment on 7 March 2023. There were 2 boxes of 1,000 with 5 SRS sampled from each box.

	11 March 2023 (5 d)*	21 March 2023 (15 d)	27 March 2023 (21 d)
	Box 1		
SRS Number			
1	33	288	320
2	90	246	367
3	82	268	286
4	84	263	294
5	65	225	284
Mean ± SEM	**70.8 ± 10.3**	**258.0 ± 10.6**	**310.2 ± 15.5**
	Box 2		
SRS Number			
1	49	250	316
2	46	390	392
3	51	305	350
4	45	285	297
5	30	250	264
Mean ± SEM	**44.2 ± 3.7**	**296.0 ± 25.7**	**323.8 ± 22.0**

* Number of days that predatory mites were counted on yellow sticky cards following initial preparation (7 March 2023).

were no significant differences in the number of *E. eremicus* adults that emerged from RC affiliated with box 1 (47.0 $\pm$ 3.4) and 3 (41.2 $\pm$ 3.7), and box 2 (33.6 $\pm$ 3.2) and 3 (41.2 $\pm$ 3.7).

The mean, SEM, and range of *E. formosa* adults that emerged from the RC associated with the 1 shipment of 2 boxes was between 52.6 $\pm$ 1.2 (range: 49 to 55) and 47.8 $\pm$ 2.2 (range: 42 to 53) (Table 6). The mean ($\pm$ SEM) of *E. eremicus* adults that emerged from the RC affiliated with the 2 shipments of 5 boxes was below the 100 indicated on the box from the distributor/supplier (40.0 $\pm$ 5.0 and 75.2 $\pm$ 9.4), with the range between 24 and 94 (Tables 7, 8).

The quantity, biological control agent, unit cost, total cost of biological control agent products, and approximate freight charge of the individual shipments (boxes) from the distributor/supplier are presented in Table 9.

Table 2. Recorded count data, mean, and standard error of the mean (SEM) of *Neoseiulus cucumeris* adults that emerged from slow-release sachets (SRS) and were captured on a 7.6 × 12.7 cm yellow sticky card on 10 and 21 April 2023. Slow-release sachets were prepared for quality assessment on 27 March 2023. There were 3 boxes of 1,000 with 5 SRS sampled from each box.

	10 April 2023 (15 d)*	21 April 2023 (26 d)
	Box 1	
SRS Number		
1	121	132
2	137	142
3	105	132
4	144	185
5	183	190
Mean ± SEM	**138.0 ± 13.1**	**156.2 ± 12.9**
	Box 2	
SRS Number		
1	204	290
2	245	298
3	212	291
4	223	234
5	240	263
Mean ± SEM	**224.8 ± 7.8**	**275.2 ± 11.9**
	Box 3	
SRS Number		
1	206	213
2	205	207
3	207	212
4	224	230
5	215	228
Mean ± SEM	**211.4 ± 3.6**	**218.0 ± 4.6**

* Number of days that predatory mites were counted on yellow sticky cards following initial preparation (27 March 2023).

Table 3. Recorded count data, mean, and standard error of the mean (SEM) of *Neoseiulus cucumeris* adults that emerged from slow-release sachets (SRS) and were captured on a 7.6 × 12.7 cm yellow sticky card on 3, 8, and 16 May 2023. Slow-release sachets were prepared for quality assessment on 28 April 2023. There was 1 box of 1,000 with 5 SRS sampled from the box.

	3 May 2023 (6 d)*	8 May 2023 (11 d)	26 May 2023 (19 d)
		Box 1	
SRS Number			
1	210	250	313
2	250	252	293
3	285	310	319
4	211	230	299
5	237	264	312
Mean ± SEM	**238.6 ± 13.9**	**261.2 ± 13.3**	**307.2 ± 4.8**

* Number of days that predatory mites were counted on yellow sticky cards following initial preparation (28 April 2023).

Discussion

Quality assessment is important to confirm that commercially available biological control agents, such as parasitoids and predators, are alive when released into greenhouse production systems. The current study demonstrates that the quality, based on the number of live biological control agents from a distributor/supplier, can vary during the growing season. Greenhouse producers do not know the number of individuals that should emerge from SRS and RC. Consequently, if not enough individuals emerge, then the biological control agents will not manage insect pests below plant damaging levels (Losey and Calvin 1995).

Distributors/suppliers are responsible for providing live individuals of biological control agents (O'Neil et al. 1998). However, distributors/suppliers have no control of the shipping process, which can affect the quality of biological control agent shipments. Therefore, greenhouse producers should conduct quality assessments after releasing biological control agents by following the procedures described in our study for SRS and RC.

Studies show there are issues associated with biological control agent shipments received from distributors/suppliers, such as number of individuals that emerge (Herrick and Cloyd 2021, Losey and Calvin 1995, Lundgren and Heimpel 2003, Steward et al. 1996, Vasquez et al. 2004) and survival of biological control agents (Kim et al. 2001, O'Neil et al. 1998). In the current study, the number of individuals that emerged from the SRS and RC varied during the growing season depending on the shipment date.

Most of the predatory mites should have emerged from the SRS after 3 to 4 weeks, and all the predatory mites should have emerged from the SRS after 6

Table 4. Recorded count data, mean, and standard error of the mean (SEM) of *Neoseiulus cucumeris* adults that emerged from slow-release sachets (SRS) and were captured on a 7.6 × 12.7 cm yellow sticky card on 16 May and 6 June 2023. Slow-release sachets were prepared for quality assessment on 11 May 2023. There was 1 box of 1,000 with 5 SRS sampled from the box.

	16 May 2023 (6 d)*	**6 June 2023 (27 d)**
	Box 1	
SRS Number		
1	222	451
2	212	330
3	232	396
4	288	469
5	222	404
Mean ± SEM	**235.2 ± 13.5**	**410.0 ± 24.2**

* Number of days that predatory mites were counted on yellow sticky cards following initial preparation (11 May 2023).

weeks (Midthassel et al. 2014, Shipp and Wang 2003). It is important that sufficient numbers of *N. cucumeris* adults emerged from the SRS to manage western flower thrips populations below plant damaging levels because *N. cucumeris* only feeds on the first-instar larvae (Shipp and Wang 2003, Shipp and Whitfield 1991). Hence, if there is any delay in emergence or if not enough *N. cucumeris* adults emerged from the SRS, then management of western flower thrips populations will be compromised. There are supposed to be 1,000 predatory mites in each SRS (Shipp and Wang 2003). However, the number *N. cucumeris* adults that emerged from the SRS during the assessment period (d) and were captured on the yellow sticky cards was well below 1,000 with means (± SEM) between 156.2 ± 12.9 and 410.0 ± 24.2 for all 5 shipments on the final assessment date. The invoices from the company (Plant Products USA Inc.; Canton, MI) indicated that there were "250/sachet." However, what does the "250/sachet" mean? Is this the number of predatory mites per SRS? Is this the number of adults? What about the number of eggs, larvae, and nymphs? There were several instances in which over 250 adult predatory mites emerged from the SRS (Tables 1 to 5). Consequently, distributors/suppliers need to provide information to greenhouse producers on the approximate number of predatory mites that should emerge from each SRS.

The SRS contain eggs, immatures (larvae and nymphs), and adults of *N. cucumeris* mixed with a bran carrier. In addition, the SRS contain bran mites, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), as a temporary food source (Buitenhuis et al. 2014, Kim et al. 2001). The bran mites emerged from the SRS and were captured on the yellow sticky card, thus

Table 5. Recorded count data, mean, and standard error of the mean (SEM) of *Neoseiulus cucumeris* adults that emerged from slow-release sachets (SRS) and were captured on a 7.6 × 12.7 cm yellow sticky card on 28 July, and 4 and 11 August 2023. Slow-release sachets were prepared for quality assessment on 21 July 2023. There were 3 boxes of 1,000 with 5 SRS sampled from each box.

	28 July 2023 (8 d)*	4 August 2023 (15 d)	11 August 2023 (22 d)
		Box 1	
SRS Number			
1	155	233	354
2	149	212	333
3	146	201	310
4	170	262	363
5	157	232	305
Mean ± SEM	**124.4 ± 4.1**	**228.0 ± 10.4**	**333.0 ± 11.5**
		Box 2	
SRS Number			
1	240	331	411
2	201	254	383
3	164	197	331
4	215	251	403
5	173	204	359
Mean ± SEM	**198.6 ± 13.8**	**247.4 ± 23.9**	**377.4 ± 14.6**
		Box 3	
SRS Number			
1	219	255	410
2	283	284	428
3	192	213	317
4	227	344	426
5	268	303	419
Mean ± SEM	**237.8 ± 16.6**	**279.8 ± 22.0**	**400.0 ± 21.3**

* Number of days that predatory mites were counted on yellow sticky cards following initial preparation (21 July 2023).

Table 6. Recorded count data, mean, and standard error of the mean (SEM) of *Encarsia formosa* adults that emerged from release cards (RC) and were captured on a 2.5 × 2.5 cm yellow sticky square on 27 January, and 2 and 6 February 2023. Release cards were prepared for quality assessment on 21 January 2023. There were 2 boxes of 100 RC per box with each RC containing 50 parasitized greenhouse whitefly, *Trialeurodes vaporariorum*, pupae per RC. Five RC were sampled from each box.

	27 January 2023 (7 d)*	2 February 2023 (13 d)	6 February 2023 (17 d)
	Box 1		
RC Number			
1	16	49	49
2	21	55	55
3	28	54	54
4	20	50	50
5	17	55	55
Mean ± SEM	**20.4 ± 2.1**	**52.6 ± 1.2**	**52.6 ± 1.2**
	Box 2		
RC Number			
1	22	47	47
2	17	41	42
3	26	49	53
4	16	53	53
5	17	42	44
Mean ± SEM	**19.6 ± 1.9**	**46.4 ± 2.2**	**47.8 ± 2.2**

* Number of days that adults were counted on yellow sticky squares following initial preparation (21 January 2023).

increasing the difficulty in accurately counting the number of *N. cucumeris* adults. Approximately 30% of the mites that were captured on the yellow sticky card were bran mites, which may have resulted in an overestimation of the number of *N. cucumeris* counted.

The mean (± SEM) of *E. formosa* adults that emerged from the RC on the final assessment date was 52.6 ± 1.2 and 47.8 ± 2.2, which was near the 50 indicated on the box from the distributor/supplier. However, the mean (± SEM) of *E. eremicus* adults that emerged from the RC on the final assessment date was below the 100 (40.0 ± 5.0 to 75.2 ± 9.4) indicated on the box from the distributor/supplier. Consequently, additional RC were purchased and placed among the poinsettia crop to

Table 7. Recorded count data, mean, and standard error of the mean (SEM) of *Eretmocerus eremicus* adults that emerged from release cards (RC) and were captured on a 2.5 × 2.5 cm yellow sticky card on 25 September, and 4 and 9 October 2023. Release cards were prepared for quality assessment on 16 September 2023. There were 2 boxes of 100 RC per box with each RC containing 100 [parasitized sweetpotato whitefly, *Bemisia tabaci*, pupae pe RC. Five RC were sampled from each box.

	25 September 2023 (10 d)*	4 October 2023 (19 d)	9 October 2023 (24 d)
	Box 1		
RC Number			
1	42	52	52
2	33	47	47
3	41	58	60
4	51	68	72
5	50	65	68
Mean ± SEM	**43.4 ± 3.2**	**58.0 ± 3.9**	**59.8 ± 4.6**
	Box 2		
RC Number			
1	71	85	94
2	81	93	93
3	54	66	70
4	55	77	77
5	25	42	42
Mean ± SEM	**57.2 ± 9.5**	**72.6 ± 8.8**	**75.2 ± 9.4**

* Number of days that adults were counted on yellow sticky squares following initial preparation (16 September 2023).

avoid having to deal with high numbers of whiteflies, which would have affected marketability and salability. Losey and Calvin (1995) also found that the number of *Trichogramma* spp. females associated with each RC was slower than what was indicated on the container from the distributor/supplier.

We collected 5 samples of SRS and RC from each shipment (box or boxes). However, 5 samples may not have been sufficient to accurately estimate the quality of the biological control agent shipments (O'Neal et al. 1998). Nonetheless, significant differences were found despite the small sample size. Moreover, the study attempted to simulate what would be practical for a greenhouse producer to conduct quality assessments of biological control agents.

Table 8. Recorded count data, mean, and standard error of the mean (SEM) of *Eretmocerus eremicus* adults that emerged from release cards (RC) and were captured on a 2.5 × 2.5 cm yellow sticky square on 4, 11, and 18 November 2023. Release cards were prepared for quality assessment on 28 October 2023. There were 3 boxes of 100 RC per box with each RC containing 100 parasitized sweetpotato whitefly, *Bemisia tabaci*, pupae per RC. Five RC were sampled from each box.

	4 November 2023 (8 d)*	11 November 2023 (15 d)	18 November 2023 (22 d)
		Box 1	
RC Number			
1	28	49	49
2	25	58	58
3	37	60	60
4	28	43	44
5	41	62	63
Mean ± SEM	**31.8 ± 3.0**	**54.4 ± 3.6**	**54.8 ± 3.5**
		Box 2	
RC Number			
1	22	36	39
2	31	53	55
3	28	44	44
4	16	37	38
5	16	22	24
Mean ± SEM	**22.6 ± 3.0**	**38.4 ± 5.1**	**40.0 ± 5.0**
		Box 3	
RC Number			
1	30	44	48
2	20	52	53
3	21	37	39
4	35	62	63
5	19	47	48
Mean ± SEM	**25.0 ± 3.1**	**48.4 ± 4.1**	**50.2 ± 3.9**

* Number of days that adults were counted on yellow sticky squares following initial preparation (28 October 2023).

Table 9. Quantity, biological control agent, unit cost, total cost of biological control agent products, and approximate freight charge associated with the shipments (boxes) of each biological control agent (*Neoseiulus cucumeris*, *Encarsia formosa*, and *Eretmocerus eremicus*) related to the slow-release sachets (SRS) and release cards (RC) received from the distributor/supplier (Plant Products USA Inc.; Canton, MI).

Quantity	Biological Control Agent	Unit Cost	Total Cost	Freight Charge
2 Boxes*	SRS (*Neoseiulus cucumeris*)	$114.76	$229.52	$166.92
3 Boxes	SRS (*Neoseiulus cucumeris*)	$114.76	$344.28	$250.18
1 Box	SRS (*Neoseiulus cucumeris*)	$114.76	$114.76	$124.49
1 Box	SRS (*Neoseiulus cucumeris*)	$114.76	$114.76	$72.98
3 Boxes	SRS (*Neoseiulus cucumeris*)	$114.76	$344.28	$192.42
1 Box**	RC (*Encarsia formosa*)	$28.60	$28.60	$82.68
2 Boxes***	RC (*Eretmocerus eremicus*)	$76.06	$152.12	$61.78
3 Boxes	RC (*Eretmocerus eremicus)*	$76.06	$228.18	$61.78
			$1,556.50	**$1,013.23**

* Boxes associated with the slow-release sachets (SRS) of *Neoseiulus cucumeris* contained 1,000 SRS per box.
** Boxes associated with the release cards (RC) of *Encarsia formosa* contained 100 RC per box.
*** Boxes associated with the release cards (RC) of *Eretmocerus eremicus* contained 100 RC per box.

A major expense when purchasing biological control agents is the freight charge because biological control agents must be shipped overnight to ensure survival upon receipt. Nonetheless, the freight charge can sometimes be higher than the actual cost of the biological control agents (Williams and Cloyd 2005). The freight charge can be a deterrent in implementing a biological control program, especially when shipments are scheduled to arrive weekly. In our study, the total freight charge for all 8 shipments of the biological control agents was approximately $1,013.23, which is $543.27 less than the total cost ($1,556.50) of the biological control agents.

In conclusion, our study demonstrates that greenhouse producers should conduct quality assessments to determine that biological control agents purchased from a distributor/supplier are viable when released among greenhouse grown horticultural crops. The quality (number of live individuals) of biological control agents is important to ensure the success of biological control programs. If a quality assessment indicates that not enough live individuals are emerging from the SRS or RC, then the distributor/supplier should be contacted immediately to receive a refund or a new shipment of biological control agents.

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