

TECHNICAL ARTICLE

Biocontrol in practice in Canadian floricultural greenhouses

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Abstract

The environment inside Canadian prairie greenhouses differs from greenhouses built in other northern latitude locations in terms of lighting, temperature, humidity, and photoperiod. Since the performance of biocontrol agents depends upon several interactive environmental variables, their effectiveness to control pests in a particular crop growing under certain climatic conditions does not directly translate to another crop or location. So, we analyzed research trials assessing the efficacy and compatibility of various biocontrol agents (*Amblyseius cucumeris*, *Amblyseius cucumeris*, *Phytoseiulus persimilis*, *Encarsia formosa*, *Aphidius colemani*, *Aphidius ervi*, and *Steinernema feltiae*) on key pests (Western flower thrips, two-spotted spider mites, greenhouse whiteflies, and aphids) of spring bedding plants grown in a commercial floricultural greenhouse. We analyzed several compatible combinations of biocontrol agents and observed a significant reduction in pest densities and plant damage symptoms as compared to untreated control plants. The results demonstrate that *P. persimilis* controlled two-spotted spider mites successfully in calibrachoa crop. The combination of *Amblyseius cucumeris* and *S. feltiae* resulted in significantly better control of Western flower thrips than the use of *Amblyseius cucumeris* alone in sweet potato vine plants. The application of *E. formosa* and *Amblyseius cucumeris* individually reduced greenhouse whiteflies on calibrachoa plants as compared to control, but their combination performed better resulting in a significantly lower number of whiteflies on plants. Another combination of *Aphidius colemani* and *Aphidius ervi* controlled green peach aphids and foxglove aphids effectively on the pansy crop. The biocontrol agents were effective for managing a variety of pests in a commercial greenhouse setting.

Keywords: biocontrol agents, controlled environment, pests, spring bedding plants.

Resumo

Biocontrole na prática em estufas canadenses de ornamentais

O ambiente interno das estufas das pradarias canadenses difere das estufas construídas em outros locais da latitude norte em termos de iluminação, temperatura, umidade e fotoperíodo. Uma vez que o desempenho dos agentes de biocontrole depende da interação de algumas variáveis ambientais, sua eficácia no controle de pragas em uma cultura específica que está crescendo sob certas condições climáticas não se traduz diretamente em outras plantas ou local. Portanto, foram analisados ensaios de pesquisa que avaliaram a eficácia e compatibilidade de vários agentes de biocontrole (*Amblyseius cucumeris*, *Amblyseius cucumeris*, *Phytoseiulus persimilis*, *Encarsia formosa*, *Aphidius colemani*, *Aphidius ervi*, e *Steinernema feltiae*) sobre pragas principais (tripes de flores ocidentais, dois ácaros aranha-pintados, mosca-branca de estufa e pulgões) em plantas de forração para primavera cultivadas em estufa de floricultura comercial. Foram analisadas várias combinações compatíveis de agentes de biocontrole e observou-se uma redução significativa nas densidades de pragas e sintomas de danos às plantas em comparação com plantas controle não tratadas. Os resultados demonstram que *P. persimilis* controlou com sucesso os ácaros aranha-pintados na cultura da calibrachoa. A combinação de *Amblyseius cucumeris* e *S. feltiae* resultou em um controle significativamente melhor de tripes de flores ocidentais do que somente o uso de *Amblyseius cucumeris* em plantas de videira de batata-doce ornamental. A aplicação de *E. formosa* e *Amblyseius cucumeris* reduziu individualmente as moscas-brancas em plantas de calibrachoa em comparação com o controle, mas sua combinação apresentou melhor desempenho, resultando em um número significativamente menor de moscas-brancas nas plantas. A combinação de *Aphidius colemani* e *Aphidius ervi* controlou efetivamente pulgões verdes e pulgões dedaleira na cultura do amor-perfeito. Os agentes de controle biológico foram eficazes no manejo de uma variedade de pragas em uma estufa comercial de ornamentais.

Palavras-chave: agentes de biocontrole, ambiente controlado, pragas, forrações para primavera

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Introduction

Canadian Prairie Provinces receive ample amounts of bright sunlight and longer day lengths as compared to other parts of the country where the greenhouse industry is mostly concentrated. Most prairie greenhouses use air inflated double polyethylene (due to continuous freezing and thawing of the ground) as covering material in contrast to glass, which is a material of choice in most other greenhouse growing regions. Due to these factors, the environmental conditions inside the greenhouses situated on the Canadian prairies are different from those located in other parts of Canada or Europe.

Canadian floriculture greenhouses produce a wide variety of crops, including bedding plants, cut flowers, perennials, and propagation material. Crops are produced on a large scale, with high species diversity in high-density greenhouse plantings. With diversity comes complications, such as ever-changing crop mixes, plant species susceptibility to various pests, the introduction of new crops that are potential hosts to pests, and high-quality standards imposed by the customers. Due to the importance of aesthetic quality of ornamental plants, even very small damage symptoms on leaves and petals are regarded as a reduction in the quality of these plants (Alipour et al., 2019). Plants arrive regularly from many different sources increasing the chances of pest introductions. There is also a continuous movement of plants from one growing site to another throughout the production cycle. Retail greenhouses, being open to the public, see variations in the sales of different plants; some plants sell quickly while others stay for relatively long periods. A longer stay increases the chances for pest incidence and development.

The susceptibility of plants to pests and the performance of biocontrol agents in the greenhouse depends on several environmental conditions like temperature, humidity, and sunlight (Brownbridge, 2017). Understanding how environmental factors interact with and affect the plants, pests, and their biocontrol agents, is essential for implementing a successful biological pest control program in the greenhouses, thus preventing a 'one-size-fits-all' approach. Most Canadian flower growers feel that the lack of connection to research or research into biological control options is one of the limiting factors in achieving adequate management of changing pest threats (Summerfield, 2019).

Our preliminary studies carried out in 2015-16 at the Assiniboine Community Colleges' (ACC) sustainable greenhouse located in Brandon, Manitoba revealed that the biocontrol agents were effective in significantly reducing the pest populations and damage to several ornamental and food crops. In the present study, we focused on key greenhouse pests viz. Western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), Greenhouse whiteflies *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), Green peach aphids *Myzus persicae* Sulzer (Hemiptera: Aphididae), Foxglove aphids *Aulacorthum solani* Kaltentbach (Hemiptera: Aphididae), and two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae), commonly found in

commercial greenhouse environments. These pests were chosen based on their high prevalence in the greenhouse as suggested by the greenhouse growers. Most of these pests were poorly controlled by chemical pesticides and had resulted in major losses of the crop in the past years. There are several biocontrol agents available in the market that are recommended by companies to use on an individual basis. However, many of these could work synergistically and could be effective for managing more than single pest species (Garriga et al., 2019). In general, there is a lack of scientific papers that deal with the outcome of biological control at field or greenhouse scale, as too often early lab trials are documented, while full-scale results stay with companies or growers. Therefore, the objective of the study was to assess the efficacy and compatibility of several biocontrol agents in a commercial floricultural greenhouse setting.

Materials and Methods

The experiments were conducted during the spring-summer season of years, 2017 and 2018 at Shelmerdine Garden Centre Ltd. located at Headingley, Manitoba, Canada (49°51' N, 97°22' W, 238 meters above the sea level). The average climatic parameters from 1981-2015 for the Winnipeg region are as follows: mean temperature varied from -13 °C to 25 °C; the frost-free days were in the range of 125 to 135. The region received annual sunshine hours of 2353; daylight hours ranged from 12 hours 53 minutes in March, 14 hours 40 minutes in April, 15 hours 30 minutes in May, 16 hours 02 minutes in June, 16 hours and 15 minutes in July, 15 hours and 10 minutes in August, and 14 hours 10 minutes in September. The average precipitation that the region received between May and September months was 389 mm. The average annual relative humidity was 69.6% and monthly average relative humidity ranged from 49% in May to 87% in February (Environmental Canada Weather Station ECCC-MS-C, Winnipeg).

Shelmerdine, the largest retail garden center in the province of Manitoba, had a gutter-connected greenhouse that was made up of aluminum and the covering material was composed of double-layer polycarbonate in the retail area, and air-inflated double polyethylene in the production area. Partitions within the greenhouse were created by erecting polycarbonate walls connected through the sliding doors, dividing the production area into 5 zones. The ebb and flow system of irrigation was used for irrigating bedding plant crops. The drip system of irrigation was used for the hanging baskets. The use of fertilizer largely depended upon the type, requirement, and growth stage of the crop, but in general, the fertilizer applied in the greenhouse were 15-0-15 and 20-8-20+micros. The mean air temperature fluctuated between 17.0 °C and 38.5 °C and the mean soil temperature ranged between 15.7 °C and 38.3 °C during the study period. The biocontrol agents were procured from Biobest Canada Ltd. through a local distributor, Evan-Spray & Chemicals Ltd., Winnipeg, Manitoba, Canada. The biocontrol agents used in the study include predatory

mites, viz. *Amblyseius cucumeris* Oudemans (Acari: Phytoseiidae), *Amblyseius swirskii* Athias-henriot (Acari: Phytoseiidae), *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae); parasitoid wasps viz. *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), *Aphidius colemani* Viereck (Hymenoptera: Braconidae), *Aphidius ervi* Haliday (Hymenoptera: Braconidae); and parasitic nematode, *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae) as biocontrol agents against the pests mentioned above.

Data analysis was conducted using analyses of variance (ANOVA) with CoStat ver. 6.45 (CoHort Software, USA). Differences between treatment means (\pm standard

deviation) were determined using Fisher's least significant difference at $P=5\%$. The experimental set up is described below:

Experiment 1: Efficacy of *P. persimilis* for the control of two-spotted spider mites (TSSM), *T. urticae*, on calibrachoa (*Calibrachoa x hybrida* 'Conga™ Deep Yellow').

Fifty plants of *Calibrachoa x hybrida* 'Conga™ Deep Yellow' planted in 8-inch pots were randomly selected for this experiment. The plants were at the vegetative stage for the first 7 weeks of study and were at the flowering stage thereafter (Table 1).

Table 1. Effect of *P. persimilis* on two-spotted spider mites (*T. urticae*) in calibrachoa

	Plant Growth Stage	¹ <i>P. persimilis</i>		² Control	
		TSSM per plant	*Plant Damage Rating	**TSSM per plant	***Plant Damage Rating
Week 1	8-10 leaves	0.40±0.20a	1.00a	0.60±0.20a	1.00a
Week 2	10-12 leaves	0.60±0.22a	1.00a	1.00±0.52a	1.00a
Week 3	10-12 leaves	0.80±0.10a	1.00a	0.60±0.31a	1.00a
Week 4	4-6 laterals	4.40±1.56a	2.00a	5.00±2.32a	2.00a
Week 5	6-10 laterals	6.20±2.45a	2.00b	6.60±3.00a	3.00a
*Week 6	8-10 laterals, 16-18 secondary laterals	16.80±3.33a	2.00b	15.00±5.21a	3.00a
*Week 7	8-10 laterals, 16-18 secondary laterals	16.00±4.30a	3.00a	17.20±4.88a	3.00a
Week 8	Flowering	13.20±2.67b	3.00b	18.20±6.77a	4.00a
Week 9	Flowering	5.20±2.00b	2.00b	25.00±6.77a	4.00a
Week 10	Flowering	4.00±1.00b	2.00b	35.00±7.67a	5.00a
Week 11	Flowering	4.20±2.80b	2.00b	40.00±8.98a	5.00a
Week 12	Flowering	3.80±2.33b	3.00b	56.20±9.89a	6.00a
Week 13	Flowering	4.00±2.22b	4.00b	78.00±10.09a	6.00a
Week 14	Flowering	6.20±1.98b	4.00b	99.80±15.67a	6.00a
*Week 15	Flowering	12.00±4.30b	4.00b	102.20±20.33a	7.00a
*Week 16	Flowering	11.80±3.34b	4.00b	110.00±24.54a	8.00a
Week 17	Flowering	7.20±2.33b	4.00b	120.60±24.00a	9.00a
Week 18	Flowering	7.20±2.00b	4.00b	134.40±36.66a	9.00a
Week 19	Flowering	3.20±1.00b	4.00b	126.00±20.99a	9.00a
Week 20	Flowering	2.80±1.34b	4.00b	144.40±40.00a	9.00a

¹*P. persimilis* =8 per m² during *weeks 6,7,15,16; ²Control=Untreated plants

**Means represent 2-year averages of 25 plants per treatment (2 treatments, 5 replications, 5 plants per replication, 50 plants in total)

***Plant damage rating: 1-9 scale (% leaf area affected): 1=0-5, 2=6-10, 3=11-20, 4=21-30, 5=31-40, 6=41-50, 7=51-60, 8=61-70, 9=>70

Number followed by the same letter are not significantly different in LSD at $P=0.05$

The experiment was laid out in the Randomized Complete Block Design with 5 replications and 5 plants per replication. All 5 production zones received plants from each replicate of every treatment. Two random groups of 25 plants each were created: 1) *P. persimilis* treatment group, 2) untreated control group. Each group of plants was

placed in 3 m² space inside a cage built with plastic pipes and covered with tightly woven breathable sheer fabric that did not allow the escape of insects. Two treatments were separated with 30 meters of space. Cages were placed close to the rest of the crop plants. The environmental conditions inside the cages were the same as the rest of the greenhouse.

P. persimilis mites mixed with bran were obtained in a sprinkle bottle delivery system. The mixture was spread evenly on the foliage of infested plants placed in close contact to promote dispersal of mites. The application was carried out at the rate of 8 mites per m² as recommended by the manufacturer during weeks 6 and 7, and again during weeks 15 and 16. Two-spotted spider mites on the plants were scouted using a hand lens of focal distance 2.5 cm, magnification 40X, and diameter 25 mm; every mite on all aboveground plant parts (leaves, stem, and flowers) was counted every week on the same day of the week, and averages were calculated. The initial number of TSSM at the start of monitoring (week 1) was less than 1 per plant. The damage symptoms on plants were recorded as percent leaf area affected using the following rating scale of 1 to 9: 1 (0-5%), 2 (6%-10%), 3 (11%-20%), 4 (21%-30%), 5 (31%-40%), 6 (41%-50%), 7 (51%-60%), 8 (61%-70%), 9 (>70%, pronounced stunting of the plant, severe leaf shedding, plant death).

Experiment 2: Efficacy of *Amblyseius cucumeris* and *S. feltiae* for the control of western flower thrips (WFT), *F. occidentalis*, on sweet potato vine (*Ipomoea batatas* ‘Marguerite’).

A total of 75 randomly selected mature plants of sweet potato vine (*I. batatas* ‘Marguerite’) planted in 10-inch pots were divided into 3 treatment groups of 25 plants each. The treatment groups were: 1) *Amblyseius cucumeris* + *S. feltiae*, 2) *Amblyseius cucumeris*, and 3) untreated control. The selected plants were at the 8-10 leaves stage at the start of monitoring (week 1) and grew to have dense foliage trailing through the sides of the pots from week 8 onwards (Table 2).

The layout and setup of the experiment were the same as experiment 1. *Amblyseius cucumeris* was applied as slow-release sachets staked into the growing medium using bamboo sticks. There was a small opening on sachets on one side for the gradual dispersal of mites. Each sachet

that was reported to last for 4 weeks contained 250 mites in a mix comprised of a food source for the reproduction of *Amblyseius cucumeris*. The release rate recommended by the manufacturer was 1 sachet per m², but due to the low number of WFT on our plants, we decreased the application rate to 1 sachet in 3 m² area. A package of *S. feltiae* containing 250 million nematodes was dissolved in 100 liters of water and applied at the rate of 500,000 nematodes per m² (manufacturer’s recommendation) by drenching growing media with the solution. A total of 5 applications (weeks 3, 7, 11, 15, and 19) of *Amblyseius cucumeris* and *S. feltiae* in a 20-week production cycle were carried out. The damage symptoms on plants were recorded using a rating scale of 1 to 9, the same as used in experiment 1. Weekly data on the pest populations was recorded by counting all the WFT insects both on the plants and the yellow sticky cards (BUG-SCAN®). Also, the foliage of each plant was shaken over a sheet of white paper and the number of dropped WFT (larvae and adults) were counted. These numbers were added to the WFT counts on the plants, and means were calculated. The yellow sticky cards cut into 4-inch squares were placed equidistantly among the crop; all WFT on both sides of the cards were counted and means were calculated. The initial number of WFT among treatments at the start of monitoring (week 1) was between 20.80 to 28.20 per plant and 113.40 to 130.00 per sticky card.

Experiment 3: Efficacy of *E. formosa* and *Amblyseius cucumeris* for the control of greenhouse whitefly (GW), *T. vaporariorum* on calibrachoa (*Calibrachoa x hybrida* ‘Chameleon®’).

The experiment was performed on calibrachoa (*Calibrachoa x hybrida* ‘Chameleon®’) planted in 8-inch pots. The plants were at the vegetative stage for the first 7 weeks of study and were at the flowering stage thereafter (Table 3).

Table 2. Effect of *Amblyseius cucumeris* and *S. feltiae* on western flower thrips (*F.occidentalis*) in sweet potato vine

	Plant Growth Stage	¹ <i>A.cucumeris</i> + <i>S.feltiae</i>			² <i>A.cucumeris</i>			³ Control		
		WFT (larvae + adults) per plant	*WFT (larvae + adults) per sticky card	***Plant Damage Rating	**WFT (larvae + adults) per plant	***WFT (larvae + adults) per sticky card	***Plant Damage Rating	**WFT (larvae + adults) per plant	***WFT (larvae + adults) per sticky card	***Plant Damage Rating
Week 1	10-12 leaves	28.20±9.83a	129.40±24.01a	5.00b	20.80±10.00b	113.40±29.02b	5.00a	25.60±13.33ab	130.00±43.54a	6.00a
Week 2	10 -12 leaves	24.60±6.78b	120.00±30.00b	5.00c	19.60±8.08b	88.40±12.22c	5.00b	28.40±6.77a	144.40±23.33a	6.00a
*Week 3	12-15 leaves	14.20±4.03b	109.40±11.11b	5.00c	14.40±7.80b	75.40±10.04c	5.00b	32.00±9.89a	150.00±33.33a	6.00a
Week 4	14-15 leaves	12.80±5.78b	68.20±9.90b	4.00b	15.80±10.99b	55.60±16.76c	5.00a	56.80±11.11a	170.20±49.90a	6.00a
Week 5	16-18 leaves	4.20±1.98c	43.00±15.00b	4.00b	11.60±6.68b	30.60±8.08c	3.00b	40.20±5.67a	200.00±30.00a	7.00a
Week 6	16-18 leaves	5.20±3.74b	32.20±10.00b	3.00b	7.20±4.00b	20.40±4.55c	3.00b	45.60±12.32a	211.40±68.90a	7.00a
*Week 7	16-18 leaves	3.40±2.00b	20.00±8.88b	3.00c	5.80±2.40b	25.20±9.87b	3.00b	50.00±7.78a	220.00±35.60a	7.00a
Week 8	18-20 leaves	1.80±0.90b	22.40±10.00b	3.00c	4.20±3.00b	19.40±7.01b	3.00b	62.40±4.09a	225.00±60.90a	7.00a
Week 9	18-20 leaves	1.00±0.50b	18.80±6.67b	3.00b	6.20±3.59b	27.00±6.66b	3.00b	76.00±18.09a	244.40±89.00a	8.00a
Week 10	18-20 leaves	0.80±0.14b	8.80±3.90b	2.00c	6.80±5.05b	20.00±7.08b	3.00b	78.00±7.86a	270.20±45.50a	8.00a
*Week 11	20-22 leaves	1.20±1.00b	10.00±4.50b	2.00b	6.22±2.34b	17.60±10.09b	2.00b	82.20±10.02a	280.20±67.78a	8.00a
Week 12	20-22 leaves	5.20±2.56b	5.00±3.00b	2.00c	6.80±3.45b	12.60±4.56b	3.00b	85.60±14.34a	275.80±45.00a	8.00a
Week 13	20-22 leaves	3.80±1.88b	2.20±1.20b	2.00c	4.80±2.40b	15.20±6.89b	3.00b	90.00±17.87a	288.40±66.90a	8.00a
Week 14	20-22 leaves	2.40±1.90b	10.00±3.43b	2.00c	6.10±4.04b	15.40±5.90b	2.00b	77.20±12.89a	300.00±90.09a	8.00a
*Week 15	24-26 leaves	5.60±3.34b	10.80±5.55b	2.00c	8.08±3.33b	10.20±6.76b	3.00b	82.20±17.89a	302.20±77.00a	9.00a
Week 16	24-26 leaves	3.80±1.00b	2.00±1.00b	2.00c	3.20±1.44b	8.80±5.65b	3.00b	88.80±16.89a	299.00±52.20a	9.00a
Week 17	24-26 leaves	4.80±3.89b	4.00±3.40b	2.00c	4.80±2.67b	14.00±4.40b	3.00b	89.60±15.55a	324.80±89.98a	9.00a
Week 18	24-26 leaves	1.00±0.20b	3.20±2.20b	2.00c	6.80±2.88b	14.40±9.98b	3.00b	91.00±26.78a	284.40±39.99a	9.00a
*Week 19	24-26 leaves	1.20±0.22b	3.00±1.23b	2.00b	6.50±1.78b	15.60±13.33b	3.00b	85.40±17.89a	331.00±80.01a	9.00a
Week 20	24-26 leaves	1.40±0.50b	1.30±0.42b	2.00c	3.20±0.76b	13.00±8.00b	3.00b	106.00±43.00a	334.40±55.23a	9.00a

¹*Amblyseius cucumeris* + *S.feltiae* treatment = *Amblyseius cucumeris* @ 1 sachet/3 m² + *S. feltiae* @ 1 million per 2m² during *weeks 3, 7,11,15,19

²*Amblyseius cucumeris* treatment @ 1 sachet/3m² during *weeks 3, 7,11,15,19; ³Control=Untreated plants

**Means represent 2-year averages of 25 plants per treatment (3 treatments, 5 replications, 5 plants per replication, 75 plants in total)

***Means represent 2-year averages of 25 card counts per treatment (3 treatment, 5 replications, 5 cards per replication (75 cards in total)

***Plant damage rating: 1-9 scale (% leaf area affected): 1=0-5, 2=6-10, 3=11-20, 4=21-30, 5=31-40, 6=41-50, 7=51-60, 8=61-70, 9=>70

Number followed by the same letter are not significantly different in LSD at P=0.05

Table 3. Effect of *E. formosa* and *Amblyseius cucumeris* on greenhouse whitefly (*T. vaporariorum*) in calibrachoa

	Plant Growth Stage	¹ <i>E. Formosa</i> + <i>A.swirskii</i>				² <i>A.swirskii</i>				³ <i>E. formosa</i>				⁴ Control			
		*GW adults /plant	*GW nymphs / plant	*Parasitized GW nymphs /plant	**Plant damage rating	*GW adults /plant	*GW nymphs / plant	*Parasitized GW nymphs / plant	**Plant damage rating	*GW adults /plant	*GW nymphs /plant	*Parasitized GW nymphs / plant	**Plant damage rating	*GW adults /plant	*GW nymphs /plant	*Parasitized GW nymphs / plant	**Plant damage Rating
Week 1	8-10 leaves	4.60±2.33a	1.80±0.20c	0.00b	1.00a	3.60±2.33b	2.80±0.99c	0.00	1.00a	4.20±1.50a	6.00±3.44a	0.00	1.00a	2.40±1.00b	4.00±1.45b	0.00	1.00a
Week 2	10-12 leaves	6.00±3.43a	1.00±0.20d	0.00b	1.00a	4.80±1.50b	3.80±1.78c	0.00	1.00a	5.40±3.22b	10.80±5.00a	0.00	1.00a	4.80±2.01b	6.00±2.67b	0.00	1.00a
Week 3	10-12 leaves	4.80±1.56b	2.60±1.10d	0.00b	1.00a	4.00±1.00b	5.60±2.33b	0.00	1.00a	6.00±3.87a	9.20±5.00a	0.00	1.00a	5.00±2.66b	7.80±3.11a	0.00	1.00a
Week 4	4-6 laterals	5.00±2.00c	1.60±0.89c	0.00b	1.00a	6.00±2.88b	3.80±1.98c	0.00	1.00a	9.00±4.55a	13.20±6.08b	0.00	1.00a	6.80±2.11b	18.60±6.54a	0.00	3.00a
Week 5	6-10 laterals	2.20±1.78c	2.00±1.11c	4.00±2.33a	1.00c	4.00±1.11c	4.00±2.02c	0.00b	1.00c	11.60±4.00b	13.60±5.99b	4.00±2.33a	2.00b	15.00±4.67a	20.00±5.11a	0.00b	4.00a
Week 6	8-10 laterals, 16-18 secondary laterals	2.20±0.56b	1.20±1.00c	2.20±1.23b	1.00c	3.80±2.22b	3.20±1.54c	0.00c	1.00c	13.00±5.65a	22.00±5.00b	9.20±3.33a	2.00b	15.00±5.90a	40.60±8.99a	0.00c	6.00a
Week 7	Flower bud development	3.00±1.23c	1.00±0.23d	3.80±2.44b	1.00c	4.00±2.00c	5.00±3.66c	0.00c	1.00c	15.20±4.78b	20.20±4.98b	11.80±4.90a	2.00b	22.00±5.33a	44.00±10.22a	0.00c	6.00a
Week 8	Flowering	2.40±1.00c	2.20±1.22c	3.00±2.56b	1.00c	4.60±3.13c	4.40±2.88c	0.00c	1.00c	13.80±4.00b	16.60±8.80±b	10.60±3.77a	2.00b	20.00±7.54a	48.00±13.33a	0.00c	6.00a
Week 9	Flowering	2.60±1.23c	1.80±1.00c	3.00±2.00b	1.00c	5.60±3.05c	3.60±1.67c	0.00c	1.00c	15.00±5.55b	17.80±4.00b	13.00±4.44a	2.00b	28.00±4.44a	40.00±10.34a	0.00c	6.00a
Week 10	Flowering	3.00±1.54c	1.20±0.76d	4.00±1.99b	1.00c	6.20±3.44c	4.20±2.33c	0.00c	2.00b	14.40±5.67b	10.00±3.99b	8.60±4.00a	2.00b	26.20±6.76a	53.00±15.01a	0.00c	7.00a
Week 11	Flowering	3.60±0.98c	1.80±0.86d	4.00±3.44b	1.00c	7.60±2.90c	5.60±3.44c	0.00c	2.00b	15.00±5.66b	14.60±5.00b	8.40±2.00a	2.00b	29.00±5.67a	65.00±15.32±a	0.00c	7.00a
Week 12	Flowering	4.80±1.34c	2.20±0.88c	2.00±0.99b	1.00c	4.20±3.44c	4.60±2.66c	0.00c	2.00b	13.20±4.87b	21.20±7.77b	10.60±4.44a	2.00b	30.00±8.22a	60.00±7.54a	0.00c	7.00a
Week 13	Flowering	1.60±0.76c	1.00±0.49d	2.60±1.22b	1.00c	5.00±2.30c	3.00±1.87c	0.00c	2.00b	14.00±4.33b	16.60±5.60b	13.20±5.65a	2.00b	27.00±5.66a	62.00±13.45a	0.00c	8.00a
Week 14	Flowering	2.00±1.03c	1.00±0.34d	3.20±0.80b	1.00d	4.00±1.99c	4.80±2.88c	0.00c	2.00c	17.00±4.00b	14.20±3.56b	11.00±3.44a	3.00b	28.00±a6.77	96.40±10.11a	0.00c	9.00a
Week 15	Flowering	4.00±2.22c	1.00±0.25d	4.00±2.11b	1.00d	4.00±2.76c	4.60±3.40c	0.00c	2.00c	15.00±5.55b	16.00±4.00b	12.20±5.43a	3.00b	30.00±7.10a	90.00±13.43a	0.00c	9.00a
Week 16	Flowering	2.00±1.01c	2.00±0.88c	4.40±3.45b	1.00d	3.60±2.02c	5.80±3.22c	0.00c	2.00c	13.60±5.44b	12.00±5.05b	12.80±4.00a	3.00b	26.00±8.89a	82.20±12.22a	0.00c	9.00a
Week 17	Flowering	3.20±1.89c	2.60±1.00c	3.00±1.99b	1.00d	3.60±c	4.00±c	0.00c	2.00c	15.00±b	23.60±b	8.40±a	3.00b	31.00±a	94.00±a	0.00c	9.00a
Week 18	Flowering	2.60±1.76d	3.80±1.34c	4.00±2.43b	1.00d	5.00±c	5.20±c	0.00c	2.00c	16.00±b	12.20±b	12.00±a	3.00b	33.00±a	106.00±a	0.00c	9.00a
Week 19	Flowering	2.00±0.97c	2.80±1.00c	4.60±2.00b	1.00d	4.20±c	3.60±c	0.00c	2.00c	12.80±b	15.00±b	9.40±a	3.00b	24.00±a	125.00±a	0.00c	9.00a
Week 20	Flowering	2.80±1.00c	1.00±0.50d	3.60±2.99b	1.00d	4.80±c	4.00±c	0.00c	2.00c	9.60±b	12.00±b	10.00±a	3.00b	20.00±a	120.80±a	0.00c	9.00a

¹*E. formosa* @ 3 per m² weekly + *Amblyseius cucumeris* @ 50 per m² biweekly; ²*E. formosa* @ 3 per m² weekly; ³*Amblyseius cucumeris* @ 50 per m² biweekly; ⁴Control: Untreated plants

*Means represent 2-year averages of 25 plants per treatment (4 treatments, 5 replications, 5 plants per replication, 100 plants in total)

**Plant damage rating: 1-9 scale (% leaf area affected): 1=0-5, 2=6-10, 3=11-20, 4=21-30, 5=31-40, 6=41-50, 7=51-60, 8=61-70, 9=>70

Number followed by the same letter are not significantly different in LSD at P=0.05

One hundred plants were randomly selected and grouped into 4 treatments consisting of 25 plants each. The treatment groups were: 1. *E. formosa* + *Amblyseius cucumeris*, 2. *Amblyseius cucumeris*, 3. *E. formosa*, and 4. untreated control. The layout and setup of the experiment were the same as experiment 1. *E. formosa* was received in paper cards containing pupae within a parasitized host; cards were placed in plant pots by staking into the growing medium. The rate of application used for *E. formosa* was 3 pupae per m² at weekly intervals as recommended by the manufacturer. *Amblyseius cucumeris* was applied at the rate of 50 mites per m² (instead of the manufacturer's recommended rate of 100 mites per m²) at biweekly intervals. Plants were placed in close contact with their foliage touching to promote mite dispersal. GW adults, nymphs, and parasitized nymphs on all the above-ground

plant parts were counted at the weekly interval, and means were calculated. The initial number of GW adults and nymphs at the start of monitoring (week 1) ranged from 2.40 to 4.60, and 1.80 to 6.00 per plant respectively. The damage symptoms on plants were recorded using a rating scale of 1 to 9, the same as used in experiment 1.

Experiment 4: Efficacy of *Aphidius colemani* and *Aphidius ervi* for the control of aphids (Green peach aphids -*M. persicae*, Foxglove aphids- *A. solani*) on pansy (*Viola x wittrockiana* 'Matrix® Citrus Mixture').

The pansy (*Viola x wittrockiana* 'Matrix® Citrus Mixture') crop was chosen to carry out this experiment due to its susceptibility to an aphid attack. The transplanted plants grew vegetatively until week 7 and flowered thereafter (Table 4).

Table 4. Effect of *Aphidius colemani* and *Aphidius ervi* on aphids (Green peach aphids – *M.persicae*, Foxglove aphids- *A.solani*) in pansy

	Plant Growth Stage	¹ <i>Aphidius colemani</i> + <i>Aphidius ervi</i>			² Control		
		Aphids per plant	**Parasitized aphids per plant	*Plant damage rating	**Aphids per plant	**Parasitized aphids per plant	***Plant damage rating
Week 1	5-8 leaves	15.40±5.00a	0.00	1.00a	11.00±4.55b	0.00	1.00a
*Week 2	5-8 leaves	16.00±5.78a	0.60±0.40a	1.00a	12.50±5.00b	0.00b	1.00a
*Week 3	8-10 leaves	18.20±7.65a	0.80±1.00±a	1.00a	20.40±4.44a	0.00b	1.00a
*Week 4	8-10 leaves	22.00±5.00b	1.50±1.01a	1.00a	26.00±6.05a	0.00b	1.00a
*Week 5	10-12 leaves	25.60±7.89a	3.20±0.33a	1.00a	22.00±4.44a	0.00b	1.00a
Week 6	12-15 leaves	20.20±10.00b	15.60±4.32a	1.00b	30.80±6.18a	0.00b	3.00a
Week 7	18-20 leaves	18.40±5.89b	17.00±7.87a	1.00b	42.00±10.09a	0.00b	3.00a
Week 8	20-25 leaves, flowering	16.60±7.77b	0.00	1.00b	56.60±12.20a	0.00	5.00a
Week 9	Flowering	6.60±2.88b	26.00±6.34a	1.00b	59.60±9.89a	0.00b	5.00a
Week 10	Flowering	5.50±2.00b	38.80±10.00a	1.00b	60.20±13.44a	0.00b	5.00a
Week 11	Flowering	5.00±3.11b	52.20±12.00a	1.00b	88.80±15.67a	0.00b	6.00a
Week 12	Flowering	3.20±0.99b	48.80±8.88a	1.00b	102.00±26.76a	0.00b	7.00a
Week 13	Flowering	4.40±1.67b	60.00±11.12a	1.00b	120.40±23.09a	0.00b	7.00a
Week 14	Flowering	2.00±1.00b	62.00±12.00a	1.00b	122.40±17.89a	0.00b	7.00a
Week 15	Flowering	1.60±3.00b	68.40±9.98a	1.00b	158.00±32.34a	0.00b	8.00a
Week 16	Flowering	1.60±1.42b	68.20±10.89a	1.00b	162.00±22.34a	0.00b	8.00a
Week 17	Flowering	1.00±0.33b	72.20±18.90a	1.00b	179.80±39.89a	0.00b	9.00a
Week 18	Flowering	1.80±1.00b	70.00±11.11a	1.00b	169.80±35.56a	0.00b	9.00a
Week 19	Flowering	1.00±0.20b	62.40±8.88a	1.00b	180.80±25.67a	0.00b	9.00a
Week 20	Flowering	1.00±0.33b	72.00±13.43a	1.00b	188.80±32.22a	0.00b	9.00a

¹*Aphidius colemani* + *A. ervi* (mix) @ 3 per m² during *weeks 2,3,4,5

²Control=Untreated plants

**Means represent 2-year averages of 25 plants per treatment (2 treatments, 5 replications, 5 plants per replication, 50 plants in total)

***Plant damage rating: 1-9 scale (% leaf area affected): 1=0-5, 2=6-10, 3=11-20, 4=21-30, 5=31-40, 6=41-50, 7=51-60, 8=61-70, 9=>70

Number followed by the same letter are not significantly different in LSD at P=0.05

Fifty randomly selected plants were divided into 2 treatment groups of 25 plants each. The treatment groups were: 1) *Aphidius colemani* + *Aphidius ervi*, 2) untreated control. The setup and layout of the experiment were the same as experiment 1. A mixture of *Aphidius colemani* and *Aphidius ervi* wasps were received as pupae within the aphid golden mummies, ready to emerge as adults. We used the recommended rate of application of 3 pupae per m². Although the manufacturers recommended applying *Aphidius* mix at weekly intervals, we only applied the mix during weeks 2, 3, 4, and 5. Every aphid and parasitized nymph on all above-ground plant parts was counted every week and means were calculated. The initial number of aphids during week 1 was 11.00 and 15.40 per plant in the treated and control treatments respectively. The damage symptoms on plants were recorded using a rating scale of 1 to 9, the same as used in experiment 1.

Results and Discussion

Experiment 1: Efficacy of *P. persimilis* for the control of two-spotted spider mites (TSSM), *T. urticae*, on calibrachoa (*Calibrachoa x hybrida* 'Conga™ Deep Yellow').

P. persimilis controlled TSSM in pansies successfully, and we were able to achieve positive results with only four applications of *P. persimilis* in 20 weeks. The TSSM remained suppressed and numbers remained low for most of the study period. After the first application of *P. persimilis* in week 6, 21.43% reduction in TSSM was observed in week 8, which further plunged to 69.05% in week 9. Significantly higher numbers of TSSM (144.40 per plant in week 20) were recorded in untreated plants in comparison to treated plants (Table 1). Untreated plants had a high level of damage symptoms from week 8 onwards. Graying or yellowing of the leaves was observed from week 5 onwards, and necrotic spots developed on leaves by week 8. Flowers showed browning and withering of the petals. All plant parts were covered by silky webbing material secreted by mites. Towards the end of the trial, plants displayed a stippled-bleached effect with most of the leaves turning yellow, gray, or bronze, followed by defoliation. No visible damage on *P. persimilis* treated plants were observed through most of the growing cycle of the crop.

The reasons for high efficacy for *P. persimilis* may have been the high relative humidity (RH-50%-80% range) and high plant density in our greenhouse. *P. persimilis* eggs are highly sensitive to constant low humidity, with only 20% of the eggs hatch at constant low relative humidity conditions. Under variable humidity conditions (as in our case), eggs can compensate for water loss during periods of higher humidity (Hesran et al., 2019). Close plant contact also improves the dispersal ability of *P. persimilis*. (Pundt, 2014). The temperature in our greenhouse fluctuated throughout the trial period and was higher than 35 °C on hot and sunny days in June and July which resulted in a surge in TSSM during summer. Abo-Elmaged et al. (2020) reported that temperature, relative humidity, and plant age

play an important role in the TSSM infestation than any other abiotic and biotic factors. Although the development time for *P. persimilis* is shorter than for spider mites (5 days at 30 °C, 9 days at 20 °C, and 25 days at 15 °C), at high temperatures above 30 °C TSSM develops at a much faster rate and *P. persimilis* may be unable to match its reproductive abilities (Pundt, 2014).

Experiment 2: Efficacy of *Amblyseius cucumeris* and *S. feltiae* for the control of western flower thrips (WFT), *F. occidentalis*, on sweet potato vine (*Ipomoea batatas* 'Marguerite').

The predator/parasitoid complex, *Amblyseius cucumeris* and *S. feltiae* resulted in an overall reduction of the WFT population by 95.04% on plants (1.40 mean number of WFT per plant) and 99.00% on sticky card (1.30 mean number of WFT per sticky card) from their starting population in a 20-week trial period. We observed the time taken for the first significant population reduction of WFT was 4 weeks for the *Amblyseius cucumeris* + *S. feltiae* treatment (Table 2). After application to the soil, it probably took time for the nematodes to reach the depth at which WFT pupated, and during this period, some thrips reached the adult stage, escaping from the effect of *S. feltiae*. Once in contact, *S. feltiae* can enter its host in multiple ways through mouth, anus, and spiracles (Chergui et al., 2019). *S. feltiae* infests WFT's at pre-pupae and pupal stage in the soil (Buitenhuis and Shipp, 2005), while *Amblyseius cucumeris* feeds on all above-ground life stages of WFT, except pupae (in soil) and eggs (inside leaf tissue) (Sarwar, 2016). This explains why our results for WFT management were better when we used *Amblyseius cucumeris* and *S. feltiae* together, as the combination provided better coverage for all stages of WFT.

The positive effect of the first application of *Amblyseius cucumeris* (alone) was observed during week 6 where we noticed a sharp decrease in WFT on plants by 65.38% and on sticky cards by 82.01% in comparison to WFT numbers during week 1. During the last week of trial, we recorded an average of 3.20 WFT per plant (84.62% lower than the starting population) and 13.00 WFT per sticky card (88.54% lower than the starting population) with this treatment (Table 2). The occasional decline in the performance of *Amblyseius cucumeris* could be due to suboptimal temperature and humidity in the greenhouse on which the success of predation depends, and also due to the mode of its release in the crop. The environmental stresses can adversely affect the performance of slow-release sachets of predatory mites (Shimoda et al., 2017). The emergence patterns of *Amblyseius cucumeris* from the sachets in the greenhouse could be highly variable even under ideal climatic conditions (Buitenhuis and Brommit, 2014). Mikawa et al. (2020) installed the predator release system in the greenhouse found approximately 1 month is necessary for the distribution of the released *N. californicus* on the leaves. Another reason for *Amblyseius cucumeris* to be less effective could be the low density of prey (Delisle et al., 2015). In our experiments, the damage symptoms on the plants were significantly higher on

control plants in comparison to treatment using *Amblyseius cucumeris* alone or in combination with *S. feltiae*. Severe damage occurred in control plants throughout the 20 weeks resulting in unmarketable plants. Leaves of untreated plants developed brownish silvery speckling and white patches. Growing points of some plants became distorted (Table 2).

We did not use *S. feltiae* alone as a biocontrol agent in our experiment. Since the level of WTF control is affected by the concentration of nematodes applied in the medium (Saito and Brownbridge, 2016), the cost of application of *S. feltiae* alone at the rate appropriate to regulate WFT populations with the desired mortality rates of greater than 80% was too expensive (Arthurs and Heinz, 2006).

Experiment 3: Efficacy of *E. formosa* and *Amblyseius cucumeris* for the control of greenhouse whitefly (GW), *T. vaporariorum* on calibrachoa (*Calibrachoa x hybrida* ‘Chameleon®’)

A reasonable level of parasitism was seen with the use of *E. Formosa* in our trial. However, using this parasitoid alone was ineffective in bringing the GW densities below the threshold levels of 3-4 nymphs per plant. Research shows that weather conditions (humidity, wind speed, and temperature) affect the foraging behaviour of parasitoids (Vosteen et al. 2020). Both flying ability (Van Roermund and Van Lenteren, 1995) and walking activity (Netting and Hunter, 2000) of *E. formosa* are affected at temperatures below 18 °C, lowering the parasitoid’s ability to find and parasitize GW. The temperature during our experiment dropped below 18 °C for a few days in March which explains why we did not record any signs of parasitism until week 5 in our experiments. The past research explained the influence of light intensity and daylength on feeding and oviposition activity of aphelinid parasitoids, *E. formosa*, and *Eretmocerus eremicus*. Both biocontrol agents parasitized significantly more whiteflies under the simulated summer (i.e., high light intensity [82.0-83.6 W m⁻²], long day length [L 16:D 8 h], 24 °C) treatment than the winter (i.e., low light intensity [10.8- 11.1 W m⁻²], short day length [L 8:D 16 h], 20° C) treatment (Zilahi-Balogh et al., 2006). The long daylength of [L 16:D 8 h] is generally observed in June and July months in Manitoba, whereas most of the production of bedding plants is done from March to May. The inability to achieve a higher parasitism rate in our study might also be due to the stronger influence of fluctuating temperatures than that of day length.

The GW adult and nymph densities recorded with the *Amblyseius cucumeris* treatment were significantly lower than those in the *E. formosa* treated, and also in untreated control plants. There were only minor weekly increases of GW nymphs per plant ($P>0.05$); starting population of 2.80 nymphs per plant was the lowest number observed and 5.80 nymphs per plant was the highest observed in week 16 (Table 3). The benefit of using *Amblyseius cucumeris* in flowering crops is that this predatory mite can develop and reproduce feeding on non-prey food sources such as pollen, which allows populations of the predator to build upon plants before the pests are present and to persist in the crop during periods when prey is scarce or absent (Nemati

et al., 2019). High-density close planting of bedding plants also facilitated the dispersal of the *Amblyseius cucumeris* in our greenhouse resulting in the success of this biocontrol treatment. An additional advantage of using *Amblyseius cucumeris* in the greenhouses is its effectiveness as a predator for western flower thrips along with whiteflies.

Although *Amblyseius cucumeris* alone also proved to be an effective predator for GW, however, due to less consistent temperatures and relative humidity in the greenhouse during the growing cycle, it was still unable to bring the GW densities below threshold levels. That is why we included *E. formosa* alongside *Amblyseius cucumeris*, as an added layer of protection to parasitize GW nymphs that escaped the predation of *Amblyseius cucumeris*. The combination treatment of *E. formosa* and *Amblyseius cucumeris* proved to be a significantly better treatment for control of GW in the crop than the other three treatments. This treatment brought the GW densities below threshold levels and helped maintain a high quality of plants in our study (Table 3).

As far as plant damage is concerned, severe damage symptoms were observed from week 6 onwards in the untreated control plants. Yellowing, malformation, and premature fall of leaves occurred in almost all of the plants. In contrast, no visible symptoms were found on the plants treated with *E. formosa* + *Amblyseius cucumeris* treatment. The use of *E. formosa* alone resulted in having a few plants with slight damage; the quality of plants was not premium, but they were marketable.

Experiment 4: Efficacy of *Aphidius colemani* and *Aphidius ervi* for the control of aphids (Green peach aphids- *M. persicae*, Foxglove aphids- *A. solani*) on pansy (*Viola x wittrockiana* ‘Matrix® Citrus Mixture’)

We started our trial with a high initial population of aphids in the greenhouse. We carried out the first application of the *Aphidius* mix during the second week of the trial period and continued the application of this mix for the next 3 weeks. Aphid densities displayed linear growth from week 1 to 5. This could be attributed to the defense strategies adopted by older and larger aphids to protect themselves from parasitoids by kicking, dropping, shaking their body, and running away (Vorburger, 2018). From week 6 to 8, the aphid density declined slowly, but from week 9 onwards it plummeted sharply. A steady increase of parasitized aphids per plant was noticed through to the 20-week trial period, resulting in a mean of 72.00 parasitized aphids per plant. A linear increase in the number of aphids per plant was observed from week 1 (11.00 per plant) to week 20 (188.80 aphids per plant) resulting in an escalation of 94.17% in untreated plants. (Table 4). The manufacturer’s recommendation was to apply the *Aphidius* mix every week. However, in our case, we were able to establish the self-sustaining parasitizing cycle of *Aphidius* spp. on aphids just with four successive weekly applications. There are many possible reasons for the successful control of aphids by the *Aphidius* mix in our study. Firstly, *Aphidius colemani* is very efficient in adapting itself to respond to changing host densities.

It exhibits a type III functional response on low-density patches (linear increase in parasitism with increasing host density, until a maximum is reached) while exhibiting a type II functional response (decreasing parasitism with increasing host density) at higher aphid densities (Prado et al., 2015). Secondly, adults of *Aphidius* spp. are extremely strong flyers and females can fly 16 meters within 24 hours after release in search of aphids (Langhof et al., 2005, Jennifer et al., 2018). Thirdly, *Aphidius* spp. are not impacted by short day length, so it is possible to use them year-round at optimum temperatures. Additionally, adult parasitoids of *Aphidius* spp. often consume flower nectar for carbohydrates and other nutrients (Goelen et al., 2018); such resource provisioning by plants can benefit parasitoid life-history traits and parasitism. Lastly, *Aphidius* spp. is adaptive and tolerant to abiotic factors like low relative humidity. There were no differences in foraging abilities (residence times, time allocation, or oviposition success) for *A. rosae* when exposed to low humidity in the field of 40% critical relative humidity (Fink and Volkl, 1995).

Conclusions

Overall, in our study, the biocontrol agents were as effective as chemical pesticides for managing a variety of pests in a commercial greenhouse setting. The growers informed us that they used 70% less chemical pesticides than in previous years without observing any deterioration in the quality of plants. So far, no information is documented about how climatic conditions like that of Canadian prairies affect the greenhouse environment, and how the latter influence pest management strategies for greenhouse pests. Therefore, until now, the only option for our growers was to rely on recommendations on biocontrol use from greenhouses located in other parts of Canada or Europe where climatic conditions are not similar to ours. The results of our study demonstrate that there was no adverse effect of prairie environmental conditions on the efficacy of biocontrol agents. We tried many different combinations of biocontrol agents and conclude that the combinations of tried biocontrol agents worked well together without interfering with one another to reduce efficiency. The information presented in the study could be of great value to floricultural greenhouse growers in Canada and elsewhere in the world where similar climatic conditions prevail. We anticipate that the success of this program will encourage other greenhouses to grow their crops using more sustainable and eco-friendly practices. Furthermore, the present study could create possibilities for growing food in the greenhouses for remote and isolated communities of the north where food insecurity is extremely high.

Author Contribution

PS: obtaining funds through a research grant, arranging an industry partner to carry out this research in a commercial greenhouse, planning and designing of research experiments, data analysis and interpretation, and writing of the manuscript. **TN:** data collection, and maintaining a regular communication with industry partner.

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