

Use of biological control against arthropod pests in Canadian greenhouse crop production

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Abstract: Greenhouse horticultural production currently represents an important and growing sector of Canada's food and plant production systems. Since 2006, the value of greenhouse vegetable crops in Canada exceeds that of field grown crops, signaling an important shift in the way food is cultivated in the country. While many factors have contributed to this change, a major area of innovation includes the discoveries and advances made in the development of commercial greenhouse production systems as well as the integration of biological control strategies for sustainable pest management. With this focus, this review offers a brief overview of the Canadian greenhouse industry, including a descriptive list of commonly used biological control organisms, as well as the role Canadian research has played in the development of these agents. We also address the threats that Canadian greenhouse producers face by invasive pests and the complications these have created for the commercialization of novel biological control agents. This information may serve as a guide for the development of parallel technologies and tools in other parts of the world where greenhouse production is expanding.

Key words: greenhouse; protected crops; invasive pests; beneficials; biocontrol agent release; global trade regulations

Introduction

Over the past century, the diversity and quantity of plant production in the greenhouse environment has steadily increased, giving it a greater role in global food production. The capacity of greenhouses to conserve radiant energy and protect plants from climatic events, such as low temperatures, snow, hail and wind has facilitated year-round crop production in regions where it would not have been possible otherwise. In Canada, nearly 6000 operations representing a total of over 14.3 million square meters of greenhouse production area generated over \$ CDN160 million in exports in 2014 (Statistics Canada, 2015). Under these glass, Plexiglas or polyethylene structures, crops are predominantly grown in hydroponic systems although some soil is used for organic vegetable or ornamental plant production. In Canada, vegetable crops represent about half of the total greenhouse area with the remainder being for horticultural purposes such as ornamental plant and tree nursery production. The main greenhouse vegetable crops include tomatoes, sweet peppers

and cucumbers, and the country is the largest producer of greenhouse tomatoes in North America (Cook & Calvin, 2005). More than half of the produce from these three crops is exported, with a value of \$ CDN730 million (Statistics Canada, 2015). In 2014, the volume of greenhouse production increased at an annual rate of approximately 0.9% likely attributed to increases in both production area and efficiency (Statistics Canada, 2015). However, as with other intensive greenhouse producing countries over the last century, the increasing scale of greenhouse plant production has led to new challenges. Following global trends, these challenges have been met by incremental structural, logistical and pest management innovations since the 1930s, which have led to the steady increase in area, output, and crop value. Production has also enhanced the capacity of greenhouse intensive countries to export a greater variety of food and ornamental crops year-round, and notably when it was previously not possible. However, these greater levels of trade have also increased the risk of introducing new pests or non-n-

ative beneficial organisms, rendering biosecurity an important issue not only in Canada, but also in all countries where greenhouse production is expanding.

Pest management for all crops require key practices such as prevention through sanitation, monitoring, physical exclusion and use of decision making tools. A number of biological control agents serve as a first line of defence to maintain pest populations below economic thresholds (Vincent *et al.*, 2007). Major pests of greenhouse-grown sweet peppers include aphids (fam. Aphidoidea), whiteflies (fam. Aleyrodidae), thrips (*Frankliniella* spp.), and spider mites (fam. Tetranychidae). Tomatoes are injured by pests including aphids, mites, whiteflies, thrips, and various species of caterpillars. Cucumbers grown in greenhouses in Ontario and British Columbia have to continuously deal with widespread occurrence of two-spotted spider mites (*Tetranychus urticae*), western flower thrips [*Frankliniella occidentalis* (Pergande), Thysanoptera: Thripidae], and whiteflies. In Ontario, aphids are also a yearly occurrence. All biological agents for use on sweet pepper crops are also applied against these pests on cucumbers. Important arthropod pests of greenhouse grown lettuce include aphids, leafminers (from orders of Lepidoptera, Symphyta and Diptera), cabbage loopers (*Trichoplusia ni*), fungus gnats (superfam. Sciaroidea), and whiteflies. Each of these species can be controlled or suppressed through biological control in combination with other suppressive production practices (Vincent *et al.*, 2007).

In this review, we use Canada's greenhouse industry as an example to first examine existing greenhouse pest concerns and the types of biological control innovations that have been developed to reduce pressure from these pests. We also discuss the importance of developing biosecurity protocols that take into account the risk of new crop pest introductions as plant material is increasingly traded between countries. Furthermore, as global climate changes, the risk that such pests may adapt to their new host range may further exacerbate the urgency of mitigating such biological threats and is discussed in concluding remarks.

Greenhouse biological control

Greenhouse vegetable production is an intensive agricultural practice often involving the continuous growth of crop plants on a vertical system unseen under typical field cropping conditions. Greenhouse crops often represent a monoculture creating very favourable conditions for pest arthropods or plant pathogens to thrive on, sometimes to the point of decimating the crop. While greenhouse structures generally limit pest introduction, greenhouse plants are sometimes vulnerable when transported from propagation facilities to production sites, which sometimes are located in different countries. Although conditions are generally more controllable than in the field, many factors contribute to the susceptibility of greenhouse plants to diseases and pests. For one, crops are cultivated year-round and it is therefore possible for field pests to invade when outdoor conditions become less favourable, or for them to overwinter despite limitations existing under field conditions. The high moisture levels often typical in greenhouses can favour plant diseases. With the ability of pests to establish rapidly under these ideal environmental conditions, greenhouse operators worldwide have adopted integrated pest management (IPM) for the mitigation of exogenous crop threats. Generally, IPM is seen as a safer alternative than the reliance on chemical pesticides and more compatible with the maintenance of environmental and human health (Barratt *et al.*, 2017).

One of the most important strategies in IPM is the use of biological control agents (Vincent *et al.*, 2007), which is not a new concept. In Europe, lacewings (Neuroptera) were released in greenhouses for the control of aphids as early as 1734 (Pilkington *et al.*, 2010). Biological control has also become a central component of commercial greenhouse vegetable and ornamental plant production in Canada, spurred on by increased incidence of insecticide resistance, environmental and health concerns, as well as the increased use of bumblebees for crop pollination (Murphy *et al.*, 2014; Shipp *et al.*, 2007; Summerfield & Grygorczyk, 2014; van Lenteren, 2007). For exam-

ple, nearly all greenhouse vegetable growers and 75% of ornamental growers in Ontario use biological control for the control of major pests including thrips, aphids, spider mites and whitefly throughout some or all of the production cycle (Summerfield & Grygorczyk, 2014).

Necessity is the mother of invention: Development of biological control for Canadian greenhouses

In Canada, as in other greenhouse production intensive countries, research and innovation in the field of biological control have been important components to the sustainable management of pest threats on crops. Productivity has thrived thanks to strong government and industry partnerships that have led to the rapid development and adoption of newly developed agents and strategies (Shipp *et al.*, 2007). The greenhouse whitefly parasitoid *Encarsia formosa* Gahan is one of the first agents to be mass produced in Canada (McLeod, 1939). While this early success temporarily failed due to the widespread availability of organochlorine and organophosphate insecticides, the prevalence of pesticide-resistant pests in the 1970s led to renewed interest in mass rearing and application of parasitoids and predators in North America and Europe (McClanahan, 1972; van Lenteren, 2000, 2007). In the 1980s, the successful mass production and use of *E. formosa* in Canada was followed by joint government and grower organization initiatives to commercialize the production of multiple agents including *Phytoseiulus persimilis* (Athias-Henriot), the non-diapausing predatory aphid midge, *Aphidoletes aphidimyza* Rodani (Diptera: Cecidomyiidae) as well as the pyrethroid resistant strain of the predatory mite, *Amblyseius fallacis* Garman (Acarina: Phytoseiidae) (Thistlewood *et al.*, 1992).

In 2002, a five-year federal research initiative, the Natural Sciences and Engineering Research Council (NSERC) Biocontrol Network, was created to help develop novel tools and strategies for the control of pests in greenhouses and tree nurseries (Schwartz *et al.*, 2007). The network aimed to improve the understanding of major pests and diseases, and to support the

commercial production of more biological control agents in Canada. Its activities yielded a considerable number of accomplishments that continue to be important to the sector today. Some of these include the creation of a Tier 1 Canada Research Chair in Biocontrol, the publication of over 250 refereed scientific papers and more than 70 books or book chapters. The network also fostered a strong involvement in bringing biopesticides into the Minor Use and Risk Reduction Prioritisation Process at Agriculture and Agri-Food Canada (AAFC).

In the following sections, we list the main biological control agents that have potential or are currently used in Canadian greenhouses to manage mainly arthropod pests. These agents include predators, parasitoids, and microorganisms. Where relevant, we detail the involvement of Canadian research in their development.

Arthropod biological control organisms

Predators

Feltiella acarisuga Vallot (Diptera: Cecidomyiidae) is a predatory gall midge and an efficient predator of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse vegetable crops (Wardlow & Tobin, 1990). Larvae prey on mites and can effectively control them on tomato, pepper and cucumber (Gillespie *et al.*, 1998). *F. acarisuga* larvae can consume up to 50 *T. urticae* eggs in a day, a high rate of predation relative to other spider mite predators such as *Neoseiulus californicus* (McGregor) and *Amblyseius swirskii* (Athias-Henriot) (Xiao *et al.*, 2013). Adults may feed on nectar, which can prolong their survival on the crop. This species is particularly interesting for northern latitude greenhouse operations during winter, since *F. acarisuga* can survive prolonged exposure at 2 °C in diapause and emerge when favourable temperatures return (Gillespie & Quiring, 2002).

Lady beetles

Hippodamia convergens Guérin-Ménéville (Coleoptera: Coccinellidae) or the convergent lady beetle is a generalist lady beetle species common across North America. While both larvae and adults are effi-

cient predators, especially of aphids, they also feed on other biological control agents as well as members of its own species when pest populations are low (Agarwala & Dixon, 1992). These beetles are not recommended on short season crops due to their propensity to enter diapause under shorter photoperiods (Michaud & Qureshi, 2006). As this species is generally field collected and not mass-produced, microsporidia and other pathogens may be present in shipments, which can jeopardize the health and survival of this predator relative to colony reared individuals (Bjørnson, 2008).

Delphastus catalinae LeConte (formerly *D. pusillus*) (Coleoptera: Coccinellidae) can establish and control whiteflies on multiple crops including peppers, tomatoes, cucumbers and ornamentals such as poinsettia. It preys on all stages of whitefly, with a preference for eggs and nymphs (Liu & Stansly, 1999). Females are voracious and do not reproduce unless they eat up to 100 prey per day (Parker *et al.*, 2008). It can recognize and avoid parasitized whiteflies, which presents an advantage in greenhouses that use parasitic wasps as biocontrol agents (Hoelmer *et al.*, 1994). It has been mass-reared and employed for several years but the presence of trichomes or hairs on plants decreases its efficiency (Liu *et al.*, 1993).

Stethorus punctillum Weise (Coleoptera: Coccinellidae) originates from Europe and has been unintentionally introduced into Canada (Gordon, 1985). It is a voracious spider mite predator consuming hundreds of mites per day on cucumber and pepper plants. Methods for its mass-rearing have been developed by AAFC in Ontario, and Applied Bio-nomics Ltd in British Columbia (Raworth, 2001). While the predator establishes well on pepper and cucumber, it does not do so on tomato, even in the presence of spider mite infestations, which may be due to trichomes on tomato stems, which impede its movement (Putman, 1955).

Generalist predatory bugs

Orius insidiosus Say (Hemiptera: Anthocoridae) is a widespread generalist predator in North America including several Canadian provinces, except British

Columbia (Henry, 1998). Schmidt *et al.* (1995) developed an economically feasible mass-rearing method based on the use of wax moth (*Ephestia kuehniella* Zeller) eggs as a food and bean pods as a water source and oviposition substrate. *O. insidiosus* has been used for controlling western flower thrips on greenhouse pepper and cucumber crops in Canada since the 1990s (Shipp *et al.*, 2002). In contrast, it cannot provide economically feasible control of this pest on tomato, despite high release rates, due to its low reproductive rate on tomato or its hindered searching behaviour due to trichomes on leaves and stems (Shipp & Wang, 2003).

Dicyphus hesperus Knight (Hemiptera: Miridae) is an omnivorous generalist predator of soft-bodied insects and is available in the USA and Canada for use on greenhouse vegetable crops. It is a prime example of how an endemic organism can be developed for biocontrol without the need for the importation of exotics (Gillespie *et al.*, 2007). As with other species of this family, including the European native *Macrolophus caliginosus* Wagner and *Dicyphus tamaninii* Wagner (Alomar & Albajes, 1996; Gabarra *et al.*, 1988), plants play an important role in its nutrition and as an oviposition substrate (Gillespie *et al.*, 2012). Females lay their eggs in plant tissue and both nymphs and adults can feed on host plant tissues, which may help them overcome periods of prey scarcity. However, they can also at times cause blemishes to fruit when prey are scarce. *D. hesperus* displays a variable response to photoperiod, an important consideration when using it to control whiteflies and spider mites under short season conditions (Gillespie & Quiring, 2005).

Aphidoletes aphidimyza Rond (Diptera: Cecidomyiidae) is a small, mosquito-like fly whose larvae are avid predators of many aphid species. It is widely distributed in North and South America, Asia, Europe, and Africa (Yukawa *et al.*, 1998). It was adopted as a biological control agent in commercial greenhouses in Europe, starting in Finland in 1978 (Markkula *et al.*, 1979), then in Canada by 1983 (Gilkeson & Hill, 1986). Its ability to seek out aphids

is greater than that of *Aphidius* spp. parasitoids. Diapause is facultative and varies among populations from different geographical areas (Havelka, 1980). Due to the propensity of *A. aphidimyza* to diapause on early season crops, Gilkeson and Hill (1986) were able to select populations with much reduced diapause. Information on diapause traits has allowed the development of highly successful commercial rearing techniques for this species.

Parasitoids

Parasitoids have been used in greenhouses for decades and are effective against various insects such as whiteflies and aphids (van Lenteren, 2007). Of the species known to attack *Bemisia tabaci* alone, 34 species of *Encarsia*, 12 species of *Eretmocerus*, two *Amittus* species and one species of each *Signiphora* and *Methycus* have been documented, and yet knowledge of the biological characters for most of these remain largely unknown (Qui *et al.*, 2004). With this perspective in mind, here we present information on only three of the most important parasitoids used in Canadian greenhouses to control whitefly and aphids.

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is one of the most commonly applied biological control agents and is used for the control of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), on tomato, cucumbers and ornamentals (Costello *et al.*, 1984; van Lenteren & Woets, 1988). First employed commercially in Europe in the 1920s, it is now used in multiple countries such as Russia, North America and Asia (van Lenteren, 1995). In Canada, mass production of *E. formosa* started in the early 1970s at the Agriculture Canada Harrow Research Station (McClanahan, 1972) and is now produced at Applied Bio-Nomics Ltd in British Columbia. With industry support, parasitoids are supplied to local greenhouse vegetable growers. Commercially available *E. formosa* are distributed as parasitized whitefly pupae that have been brushed off leaves and glued onto cardboard strips (Hoddle *et al.*, 1998).

Eretmocerus eremicus Rose and Zolnerowich (Hymenoptera: Aphelinidae) is an effective parasitoid

of the silverleaf whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), found throughout North America, and efficiently parasitizes the greenhouse whitefly *T. vaporariorum* (Greenberg *et al.*, 2000). *E. eremicus* has a higher temperature tolerance than *E. formosa*, making it ideal for warmer growing conditions.

Aphidius colemani Viereck (Hymenoptera: Braconidae) is a polyphagous aphid parasitoid thought to have originated from India or Pakistan but is now widely established including the Americas, Europe, and Australia (Stary, 1975). In North America, it is one of the most important parasitoids of the melon aphid, *Aphis gossypii* Glover (also known as cotton aphid) (Van Steenis & El-Khawass, 1995), which is a pest of numerous greenhouse vegetable and ornamental crops including peppers, tomatoes, cucumbers and cut flowers. It can also attack and contribute to control of the green peach aphid, *Myzus persicae* Sulzer, another major crop pest in North America (Bilu *et al.*, 2006).

Phytoseiid mites (Acari: Phytoseiidae)

The commercial production of over 20 species of phytoseiid mites globally represents an important control against major pests such as thrips, whiteflies, and two-spotted spider mites (Zhang, 2003). Phytoseiids can be classified into one of four lifestyle types, which define their nature: 1, specialist predators of web spinning mites; 2, selective predators of Tetranychidae; 3, generalist predators; and 4, pollen feeding generalists (McMurtry *et al.*, 2013). The species most commonly produced for biocontrol include *P. persimilis*, *A. swirskii*, *Neoseiulus cucumeris* (Oudemans), *Neoseiulus fallacis* (Garman) and *Amblydromalus limonicus* (Garman & McGregor). *P. persimilis* (Type 1) is a highly predacious and rapidly developing mite and a specialist predator of the two-spotted spider mite, *T. urticae*, a major pest of multiple greenhouse crops which is resistant to multiple pesticides (Helle, 1962; Van Leeuwen *et al.*, 2009). The nymphs and adults feed on all stages of the prey and an adult can eat 5–20 prey/day (Hoffmann & Frodsham, 1993).

N. cucumeris (Type 2) is one of the most commonly reared and economically important species used

for the control of western flower thrips (Gillespie, 1989). Its release is mainly by slow release sachets (also called controlled release sachets or breeding systems) that take 4–6 weeks to release the predators from a small breeding colony (Buitenhuis *et al.*, 2014; Shipp & Wang, 2003). The congeneric *N. fallacis* (Type 2) was first studied and developed as a biological control organism at AAFC (Hardman & Thistlewood, 2002). However, more recently developed phytoseiid biological control agents including *A. limonicus* and *A. swirskii* (both Type 3) have also proven to be excellent for controlling thrips. *A. swirskii* is an efficient predator of whitefly, thrips and other pests, and being a generalist, is increasingly used in commercial greenhouses (Symondson *et al.*, 2002).

Type 3 generalists, such as *N. cucumeris*, *A. swirskii* and *A. limonicus*, feed on several pests of different life stages including eggs. Type 4 phytoseiids (e.g., genera *Euseius* in McMurtry *et al.*, 2013) can also be generalists, but rely on pollen as a food source, which may need to be provided as a supplement. For these mites, plant feeding serves to improve long-term crop protection, often requiring only one application for multi-year pest control.

Laelapid predatory mites (Acari: Laelapidae)

Stratiolaelaps scimitus Wormersley (formerly *Hypoaspis miles*), *Gaeolaelaps gillesspiei* Beaulieu, and *Gaeolaelaps aculeifer* Canestrini (formerly *Hypoaspis aculeifer*) are ground-dwelling generalist predatory mites employed for the control of soft bodied arthropods and nematodes. Both genera are voracious predators of fungus gnat larvae and western flower thrips pupae and are often applied to greenhouse crops grown in soil such as ornamentals (Gillespie & Quiring, 1990; Gillespie *et al.*, 2002; Wright & Chambers, 1994). They offer an advantage over phytoseiid mites in their ability to dwell and survive as scavengers thus requiring fewer releases. In addition, laelapid predators can target thrips pupae that drop to the ground, a life stage that is unavailable to plant dwelling phytoseiids. They are best applied preventatively within the first few weeks of planting and prior to pest establishment.

Microorganisms

Bacillus thuringiensis* subsp. *kurstaki* and subsp. *israelensis (Bt), a Gram-positive bacterium, is the most successful microbial control agent commercialized to date. Numerous subspecies and strains produce highly host-specific crystal (CRY) protoxins upon sporulation. Their hosts include species within the orders Lepidoptera, Coleoptera, Diptera, Hymenoptera (Lacey *et al.*, 2015), as well as plant pathogenic nematodes from the genus *Meloidogyne* (Jurat-Fuentes & Jackson, 2012; Lacey *et al.*, 2015).

While most commercially-developed Bt is used against field crop and forestry pests, two subspecies are presently used against greenhouse pests. *B. thuringiensis* subsp. *kurstaki* is specific to members within Lepidoptera and has proven useful in controlling pests such as the cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), the European pepper moth, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) on sweet peppers (Buitenhuis *et al.*, 2013; Erlandson, 2013) and the tomato moth, *Autographa gamma* (L.) on tomatoes, peppers, roses, chrysanthemums and other ornamentals (Burgess, 2007). *B. thuringiensis* subsp. *israelensis* is specific to dipterans and is used in greenhouses to control fungus gnats (Diptera: Sciaridae) affecting bedding plants, ornamentals, vegetables and tree seedlings in propagation (Gillespie *et al.*, 2002). However, populations of cabbage loopers have developed resistance to the bacterial toxins after extensive use of *B. thuringiensis* subsp. *kurstaki* in greenhouses in British Columbia (Janmaat, 2007). Resistance appears to be related to changes in the efficiency of toxin binding to the host's midgut epithelium due to mutations of midgut membrane proteins (Wang *et al.*, 2007). This rapidly developed resistance underscores the need for diverse IPM strategies, especially in closed systems such as greenhouses.

Entomopathogenic fungi

Multiple entomopathogenic fungi are the basis of products developed for the control of greenhouse pests. Generally, these fungal pathogens reproduce via asexual

al spores (conidia) which attach to a host, germinate, and ramify within the host's haemocoel (Lacey *et al.*, 2015). The host succumbs due to competition for nutrients, mechanical disruption or toxins produced by the fungus. Once the host has succumbed, the fungus emerges, and under appropriate environmental conditions such as high humidity, produces conidiophores that give rise to new conidia. They are unique as they are the only pathogens that infect via direct penetration of the integument making them most suitable for control of plant sucking insects such as aphids and mealybugs.

Although these fungi have initially been developed strictly as microbial pesticides against insects and mites, recent discoveries have unearthed many special attributes such as endophytism, antagonism of plant pathogens, associations with the rhizosphere, and possibly even plant growth promotion (Vega *et al.*, 2009; Vidal & Jaber, 2015). These attributes are yet to be fully explored but may lead to many potential beneficial applications in greenhouses.

Beauveria bassiana Balsamo (Hypocreales: Cordycipitaceae) is one of the most common entomopathogenic fungus encountered worldwide. Although it infects over 700 host species (Meyling & Eilenberg, 2007), recent taxonomic revisions have shown that it belongs to a species complex with several new species being named (Rehner *et al.*, 2011). For the purposes of this review, we refer to *B. bassiana* sensu lato unless otherwise indicated.

Numerous products based on *B. bassiana* have been commercialized (Faria & Wraight, 2007). Products of interest for greenhouse growers include BotaniGard™ and Naturalis L™ with activity against whiteflies, thrips and a range of other pests including spider mites. In Canada, the *B. bassiana* strain GHA sensu stricto is registered as BotaniGard™ for use against multiple greenhouse pests and is available as a liquid emulsifiable suspension (ES) or wettable powder (22WP) (AAFC Biopesticide Database Directory). These products are registered for use on multiple ornamental and crop plants such as peppers, tomatoes and cucumbers. Numerous isolates are also endophytic,

providing host resistance to invertebrate pests (Vidal & Jaber, 2015) and even managing plant parasitic viruses (Jaber & Salem, 2014).

Isaria fumosorosea Wize (formerly *Paecilomyces fumosoroseus*) (Hypocreales: Cordycipitaceae) has been commercialized as numerous products for use against whiteflies, thrips, spider mites and aphids. It is used primarily in greenhouses as this environment can be manipulated to maintain optimal relative humidity levels that promote fungal growth (Faria & Wraight, 2007). Whereas most commercialized entomopathogenic fungal products are based on fungal conidia, *I. fumosorosea* products are based on blastospores produced in submerged culture, which are dehydrated and formulated as water dispersible powders or bran granules. Preferal™ is marketed against whiteflies, aphids, thrips, spider mites, psyllids, leaf miners, mealybugs, root weevils, thrip pupae, and rootworms. While not yet registered in Canada, this product is available in multiple European countries for use on tomato, cucumber, strawberries and ornamentals (AAFC, Biopesticide Database Directory). Simultaneous applications of *I. fumosorosea* and *D. hesperus* in tomato greenhouse microcosms against *T. vaporariorum* demonstrated an additive effect suggesting that the combination of generalist entomopathogenic fungi and generalist predators has the potential to cause increased pest mortality despite evidence of minimal interference (Alma *et al.*, 2007).

***Lecanicillium* spp.** (Hypocreales: Cordycipitaceae) are entomopathogenic fungi that consist of a multi-species assemblage once defined as a single species, *Verticillium lecanii*. This group now includes *L. attenuatum*, *L. lecanii*, *L. longisporum*, *L. muscarium*, and *L. nodulosum* (Goettel *et al.*, 2008). Members of this genus are commonly occurring pathogenic fungi known to infect aphids, thrips, Diptera, Homoptera, Hymenoptera, Lepidoptera, mites, and nematodes. They also include species with anti-phytoparasitic (Askary *et al.*, 1997) or anti-fungal properties (Verhaar *et al.*, 1997) offering the possibility of application for the simultaneous control of insect pest and plant pathogen such as aphids and powdery mildew in green-

house cucumber production (Kim *et al.*, 2007, 2008, 2010). These fungi are thus the basis of multiple commercially available crop protection products with each species potentially offering distinct host ranges. However, none have yet received registration in Canada for use against greenhouse pests. *L. muscarium* Petch is a species with a broad host range which includes whiteflies, aphids and mites, and commercialized as Mycotal™, a product for foliar application against whiteflies and thrips. Strains of *L. longisporum* have also been commercialized against aphids as Vertalec™ and whiteflies and thrips as Vertirril™ (Faria & Wraight, 2007).

Metarhizium spp. (Hypocreales: Clavicipitaceae) consist of a multi-species assemblage once defined as a single species, *M. anisopliae* Metch. They are cosmopolitan pathogens known to infect arthropods of multiple orders. Recent taxonomic revision has split the group into numerous species including *M. anisopliae* sensu stricto (Bischoff *et al.*, 2009).

Conidia of *M. brunneum* Petch, for instance, is the basis of products such as Met52®, which is registered in Canada for non-food use (AAFC, Biopesticide Database Directory), including application on ornamentals, shrubs, and forest and shade-tree seedlings. Met52 G provides consistent efficacy against all larval stages of the black vine weevil (*Otiorhynchus sulcatus* F.) (Ansari & Butt, 2013).

Recent discoveries have found *Metarhizium* isolates with strong rhizosphere associations with the ability to translocate nitrogen from parasitized insect hosts directly to plants and in return, plants providing a carbon source to the fungus (Behie *et al.*, 2012, 2017). Isolates have also been reported as endophytes with pest control properties. For instance, endophytic *Metarhizium* in bean plants negatively affect aphids (Akello & Sikora, 2012).

Nematodes

Entomopathogenic nematodes are unique in that they act as vectors of symbiotic entomopathogenic *Photorhabdus* spp. and *Xenorhabdus* spp. bacteria. Nematodes enter their host via the mouth, anus or injury and immediately release the bacterial symbionts by re-

gurgitation. The bacteria rapidly proliferate within the host's hemocoel, producing toxins. The nematodes also multiply, feeding not only on the bacteria but also on the degrading host tissues. Upon host death, many nematodes emerge as infective juveniles which then seek another host. They are safe to humans and are generally safe to other non-target organisms and consequently do not require pesticide registration safety requirements in many countries such as the United States and the European Union (Grewal *et al.*, 2005).

Steinernema feltiae Filipjev and **Steinernema carpocapsae** Weiser (Nematoda: Steinernematidae) can target multiple pest species and numerous products are available based on them. They have been used for control of fungus gnats in Canadian greenhouses (Gillespie *et al.*, 2002). Entonem®, a product based on *S. feltiae*, is available in Canada to control larvae of sciarid flies (Sciaridae) (soil application) and larvae (leaf application) and pupae (soil application) of thrips.

Viruses

Baculoviruses are the most common viruses infesting mostly lepidopterans and are very host specific with each species principally affecting only one species (Harrison & Hoover, 2012). They produce rod-shaped nucleocapsids containing circular double stranded DNA within occlusion bodies. When ingested, the occlusion bodies dissolve in the alkaline midgut releasing the virions, which then enter the midgut epithelial cells commencing a primary replication cycle. This is followed by a secondary systemic cycle of replication in a wide variety of tissues that ultimately results in host death and release of occlusion bodies to continue the life cycle. Their narrow host range makes them suitable for use in greenhouses where the conservation of other natural enemies is a priority as baculoviruses have no impact on predators and parasitoids (Cory & Evans, 2007).

Erlandson *et al.* (2007) report the presence of a baculovirus in cabbage looper populations in greenhouse vegetable crops in British Columbia. From these, large numbers of nucleopolyhedrovirus infected larvae were isolated and characterized as either TniS-

NPV or AcNPV. Bioassays for dose-response to these isolates demonstrated that while both isolates appeared to effectively infect 2nd and 4th instar cabbage looper larvae, AcMNPV was up to 10-fold more infectious to 5th instar than was TnSNPV. While these produced about five times more occlusion bodies per cadaver than AcNPV, both have good potential as biological control agents against this pest. Since this survey, AcMNPV has become the basis for a commercially available product (LoopexTM), which is registered in Canada and the US (AAFC, Biopesticide Database Directory).

Bumble bee vectoring for entomopathogen delivery

While bumblebees, *Bombus impatiens* Cresson (Hymenoptera: Apidae) have been commercially used for pollination of Canadian greenhouse crops for 20+ years, recent innovations have extended their use for vectoring of microbial agents to crops. Bumblebee vectoring of entomopathogens such as *Beauveria bassiana* can be used for the control of pests such as *Lygus lineolaris* (Palisot de Beauvois) and *F. occidentalis* in greenhouse crops (Kevan *et al.*, 2007). Kapongo *et al.* (2008) show that bees can vector both entomopathogens, such as *B. bassiana*, and phytoprotective agents, such as the conidia of *Clonostachys rosea* (Link), to combat grey mould (*Botrytis cinerea* [Pers.:Fr]). This combination of agents can kill 49% of whiteflies and suppress grey mould by 57% on flowers and 46% on leaves of a tomato crop. In sweet pepper, *L. lineolaris* mortality can be 73%. Bee Vectoring Technologies (Mississauga, Ontario) and Biobest Canada Ltd (Leamington, Ontario) have patented the vectoring system in 2012 and have rendered bee vectoring technologies commercially available.

Future areas of research and implications of the changing market and environment

Greenhouse horticultural research in Canada will continue to play an important role in adapting to changing standards on the use of pesticides for crop protection. Data of 2016 show that Canadian exports increased by another 5% with 51% of the vegetables exported being grown in greenhouses. The majority of the

greenhouse produce were destined for the United States with Japan and Taiwan of China being the other main importers of Canadian greenhouse vegetables (AAFC, 2017). More than ever, stringent international standards set by consumer market demand and government policy ban the use of some pesticides with the potential to leave a void in management options. Currently, the use of neonicotinoids in Canada is being reviewed or is being phased out (e.g., Ontario), which follows the recent ban in Europe stemming from a concern over pollinator health decline (Biozent, 2013). The development of new biological control methods will continue to be important for the industry to manage pest pressure all the while meeting current production practices.

The greenhouse vegetable industry is also diversifying into new crops such as strawberries, herbs and sprouts, mache, eggplant, and napa cabbage (AAFC, 2017). It is therefore essential to understand how biocontrol within greenhouses can support biosafety when plants are exported to other countries, to ensure that pests or biocontrol agents are not exported as well.

The indicators of changing food production practices in Canada include the implementation of international standards for safe greenhouse crop production intended to harmonize with current US Food and Drug Administration and USDA standards. Mandated by the Canadian Food Inspection Agency (CFIA), the Hazard Analysis Critical Control Point (HACCP) standards address biological, physical and chemical hazards that may jeopardize the safety of food. The greenhouse industry adopted HACCP standards as outlined by the CanadaGap Program led by the Canadian Horticultural Council with support of Agriculture and Agri-Food Canada. Since October 2015, greenhouse growers aiming to export to the United States must adhere to the Greenhouse Certification Program for Export of Greenhouse-grown plants to the United States (D-96-12, <http://www.inspection.gc.ca/industry-guidance/eng>). With growing concerns regarding pest management and health safety, the Canadian Food Inspection Agency (2017) published the *Greenhouse Vegetable Sector Biosecurity Guide*. This guide is in support of the National Volunta-

ry Farm-Level Biosecurity Standard for the Greenhouse, Nursery and Floriculture Sectors (Canadian Food Inspection Agency, 2017).

Mitigating global arthropod and microbial threats to the greenhouse industry

The Canadian greenhouse industry, like many others, will need to continuously adapt to changing agricultural and pest management landscapes. Most evident is the constant threat of new invasive crop pests, which require the industry to learn quickly how to manage pests from other affected areas. For instance, the brief occurrence in Canada in 2016 of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), demonstrates that country import restrictions are simply unable to restrain arthropods when plants are traded (Fernández *et al.*, 2017). The early life stages of this pest occur within the pepper fruits greatly limiting the capacity to detect their presence during importation (Fernández *et al.*, 2017).

Another example includes the tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), which has recently devastated tomato crops in many parts of the world (Chidege *et al.*, 2017; Gebremariam, 2015). This South American moth had initially decimated tomato crops both in its native South American range and in Europe, starting in Spain in 2006 (Urbaneja *et al.*, 2007). It has since spread throughout the Mediterranean Basin, the Middle East, and Africa and is currently expanding its range into Asia (Gebremariam, 2015). While it has yet to reach North America, its introduction could have devastating effects for the greenhouse and field tomato industries in Canada, Mexico and the United States. The threat of such a global pest therefore emphasizes the need for preparedness including investment in the development of monitoring strategies, biosecurity protocols, and reviews of potential methods and agents most likely to effectively manage such pests.

However, the risk of such pest introductions must be balanced with the benefits of importing biological control agents and plant material from other countries.

From a regulatory perspective, the greenhouse industry in Canada has benefitted enormously from the establishment of international organizations that have both improved the quality standards for biological control agents, as well as for the mitigation of inadvertent introduction of plant pest and adventive biological control agents. To protect the integrity of the international biocontrol industry, standards for the mass rearing and trade of biological control arthropods were established by the International Organization for Biological and Integrated Control (IOBC), the Association of Natural Biocontrol Producers (ANBP) in North America and the International Biocontrol Manufacturers Association (IBMA) in Europe (van Lenteren *et al.*, 2003). Furthermore, the International Plant Protection Convention (IPPC) established by the Food and Agriculture Organization of the United Nations (FAO) has provided an international regulatory structure for biological control producers which is adopted and regulated by signatories, including Canada. In addition, the Canadian Food Inspection Agency (CFIA) plays an important role in regulating the importation of both biocontrol agents into Canada as well as in mitigating the risk of inadvertent importation of plant pests and non-native biological control organisms. International coordination and knowledge transfer are also involved in minimizing the threat of exotic organism range expansion. The North American Plant Protection Organization (NAPPO) regulates trade standards in Canada, the United States and Mexico, which are modified and enacted by the plant protection organizations in each of these countries.

Regulatory hurdles in biological control

While regulatory standards established by these organizations exist to protect agricultural industries, these and others may actually hinder the global availability of biological control agents. Currently, many microbial control agents are available world-wide for use in greenhouses. However, stringent regulatory requirements and costs have precluded registration of many of them in Canada, not because they are unsafe, but principally because the expected benefits do not justify the cost of safety testing requirements under the Cana-

dian Pest Regulatory Agency. Regulations addressing importation of living organisms can also hamper the importation of biocontrol agents from outside of Canada or the export of produce "contaminated" with living biocontrol agents. There is clearly a continued need for research to address these shortcomings to be able to respond to new and challenging pest problems. It is with optimism that researchers and policy makers will continue to address these challenges in future, leading to the continued innovation of the Canadian greenhouse industry.

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