

Receptor characterization of nontarget butterflies for risk assessment of biological control with the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae)

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Abstract—Receptor characterization (*i.e.*, identifying what will be affected by an activity) is the first step in a risk assessment of biocontrol agents for insects. Development of a representative list of species at risk, based on ecological vulnerability, enables host-range screening of potential biocontrol agents on a manageable group of nontarget insects. A database of 153 species was used to characterize the butterflies potentially at risk from an inundative release of the egg parasitoid *Trichogramma minutum* Riley. Risk criteria for butterfly species included North American, Canadian, and Ontario geographic distributions; oviposition phenology; number of generations per year; overwintering stage; host-plant preferences; and egg mass type and location. Ecological vulnerability lists of butterfly species were generated for northern and southern Ontario; areas where there have been recent experimental inundative releases of *T. minutum* for the suppression of forest pests. Based on the above criteria, 2 species and a maximum of 27 species would be potentially at risk, and thus requiring host-range testing if an inundative release were considered for northern and southern Ontario, respectively. The number of species on the ecological vulnerability list for southern Ontario could be reduced to 12 species depending on the specific geographic location in southern Ontario of the inundative release. The six criteria used for receptor characterization for *T. minutum*, associated primarily with host-habitat location and host-location, can also be used for other parasitoids. They are components of any target host's biology, and thus will affect the scale and impact of any parasitoid attacking eggs, larvae, or pupae. Additional criteria for receptor characterization may also be added that will relate to the specifics of a parasitoid's biology and are associated with host acceptance and host suitability. Development of ecologically based vulnerability lists should become standard practice in determining which nontarget species require host-range testing, for both inundative and classical biocontrol agents targeting insects, and for the potential impact of invasive species.

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Résumé—La caractérisation des récepteurs (*i.e.* la reconnaissance de ce qui sera affecté par une activité) constitue la première étape de l'évaluation des risques de l'utilisation d'agents de lutte biologique contre les insectes. L'élaboration d'une liste représentative des espèces à risque, basée sur leur vulnérabilité écologique, permet de faire un survol des espèces hôtes d'agents de lutte biologique éventuels au sein d'un groupe de taille raisonnable d'espèces d'insectes non ciblés. Une base de données de 153 espèces de papillons a servi à caractériser les espèces

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potentiellement à risque lors d'une libération en masse de *Trichogramma minutum* Riley. Parmi les critères d'évaluation des risques pour les espèces de papillons, il faut compter leur répartition en Amérique du Nord, au Canada et en Ontario, la phénologie de leur ponte, le nombre de générations qu'elles produisent par année, leur stade d'hiver, leurs préférences de plantes hôtes, leur type de masse d'oeufs et le lieu de leur ponte. Nous avons dressé des listes d'espèces de papillons du nord et du sud de l'Ontario en fonction de leur vulnérabilité écologique; ces deux régions ont été soumises récemment à des relâchements en masse de *T. minutum* pour supprimer des insectes ravageurs des forêts. D'après ces critères, 2 espèces du nord de l'Ontario et un maximum de 27 espèces du sud seraient potentiellement à risque et il faudrait faire des tests sur l'étendue des espèces hôtes avant de procéder à un relâchement massif d'agents de lutte. Le nombre d'espèces vulnérables de la liste peut se réduire à 12 dépendant du lieu géographique du sud de l'Ontario où se fait le relâchement en masse. Les six critères utilisés pour caractériser les récepteurs de *T. minutum*, critères associés surtout à la position géographique de l'habitat de l'hôte et à la position des hôtes, peuvent également servir avec d'autres parasitoïdes. Ce sont des composantes de la biologie de toute espèce hôte ciblée et, par le fait même, ils affectent l'impact et l'échelle d'action de tout parasitoïde qui s'attaque aux oeufs, aux larves ou aux nymphes. Des critères additionnels reliés aux caractéristiques particulières de la biologie des parasitoïdes et associés à la compatibilité avec l'hôte et à son acceptation peuvent aussi être utilisés. L'élaboration de listes d'espèces vulnérables sur la base de leur écologie devrait être une pratique courante pour déterminer quelles espèces non ciblées doivent être testées pour leur vulnérabilité aux agents de lutte biologique, tant classiques que de libération en masse, et pour évaluer l'impact potentiel des espèces relâchées en masse.

[Traduit par la Rédaction]

Introduction

Historically, classical biological control with insects has been considered an environmentally safe method of pest control (Huffaker and Messenger 1976; Caltagirone 1981). Cited benefits include the concept of re-establishing an ecological balance and subsequent control of an invasive species by its former natural enemies; safety for humans; cost effectiveness at the usually large scale affected by the invasive species; persistence of control once agents are established; absence of host resistance issues; and minimal nontarget impact (Wilson and Huffaker 1976; Waage 2001). Recently, some of these attributes such as persistence (*i.e.*, classical biological control agents once released cannot be recalled) and subsequent documentation of significant nontarget impacts by released biocontrol agents have been cited as pitfalls (Simberloff and Stiling 1996; Louda *et al.* 1997; Boettner *et al.* 2000; Henneman and Memmott 2001). An improved understanding of and emphasis on the importance of biodiversity and the conservation of endangered species has led some to argue against what in the past have been touted as biocontrol successes (Simberloff and Stiling 1996; Louda *et al.* 1997). Concerns about nontarget species have stimulated research in the development of improved methods to assess and insure the specificity of biological control agents selected for release (Follet and Duan 2000; Wajnberg *et al.* 2001). A critical aspect of these safety methods is striking a balance between the risks for nontarget species from the use of biological control *versus* the risks of allowing the continued proliferation of invasive species that may damage a different group of nontarget species. Risk assessment is an important tool that can assist in making decisions related to activities affecting nontarget species of concern.

Risk assessment has several definitions associated with environmental toxicology. For biological control, a useful definition is "a set of analytical techniques to estimate how much damage or injury can be expected as a result of an event" (Lonsdale *et al.*

2001). Risk assessment deals with the scientific issues associated with the risk of a particular activity and is part of a larger framework of risk management that includes social, economic, and policy aspects of decision making (Bourchier and McCarty 1995; Lonsdale *et al.* 2001). Both science and policy contribute to a final risk-management strategy to enable better decision making about natural resource issues.

As with the definitions, there are several frameworks for risk assessment depending on the activity to be assessed (*e.g.*, effects of chemical pollutants *versus* an activity like biological control). Lipton *et al.* (1993) expanded the National Academy of Sciences Framework (National Academy of Sciences 1983), used primarily for toxicology work, to include risk assessment for ecological activities (Lonsdale *et al.* 2001, Table 9.2). A key first step in any ecological risk assessment and the focus of the current paper is receptor characterization. Specifically, I address the question of which species are going to be affected by an activity such as biological control. The principal objective of this paper is to illustrate the development of an ecological vulnerability list of species, associated with a definable geographic area, that could be used for host-range screening or the subsequent stages of risk assessment as proposed by Lipton *et al.* (1993) and reviewed for weed biocontrol by Lonsdale *et al.* (2001). I use as a case study the potential risk to nontarget butterflies following the inundative release of the egg parasitoid *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), which is used to target forest pests such as the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). I have focused on nontarget butterflies because of the availability of data on their biology. Nontarget moths would be of equal or greater concern in the boreal forest, but their biology is not as well known.

Biocontrol agent

The egg parasitoid *T. minutum* has been studied for the inundative suppression of forest pests (Smith *et al.* 1990; Bourchier and Smith 1998). The objective of these releases has been to provide short-term suppression of a forest-pest population or to provide additional mortality that supplements natural mortality factors in a population. In the most recent large-scale program in forestry, 480 million *T. minutum* parasitoids were released on 30 ha near Black Sturgeon Lake (48°2'N, 88°5'W) in northern Ontario. The resulting mean rate of parasitism of spruce budworm egg masses by *T. minutum* was 68% in three 10-ha treatment plots *versus* less than 2% in the matching control plots (Bourchier and Smith 1998).

Pinto (1998) lists 49 host records for *T. minutum*, five species (two of which are unidentified) are butterflies and the remainder are sawflies and moths. Most *Trichogramma* species are reported to be generalists (Pinto and Stouthamer 1994). There is, however, significant variation of host preference and acceptance within and between *Trichogramma* species or lines (Hassan 1994; Bai *et al.* 1995) that ecologically limits the number of hosts which can be attacked in the field. Ecological host ranges of *Trichogramma* species are also further limited by habitat preferences, with some species being primarily arboreal and others preferring particular crop combinations (Nordlund 1994). Given their ease of handling, wide-scale historical application (Wajnberg and Hassan 1994; Smith 1996), and the variation between their potential (physiological) and realized (ecological) host ranges, *Trichogramma* species are good models to address questions concerning the nontarget impact of insect biocontrol. The results of which can be useful for assessing risks of both inundative and classical biocontrol systems.

TABLE 1. Distribution, phenology, host plants, egg type, egg location, overwintering stage, and abundance of Ontario butterfly species that are potential nontarget hosts for *Trichogramma minutum*.*

Family and species	Distribution**	Phenology†	Host‡	Egg type§	Egg class	Overwinter¶	Abundance#
Hesperiidae							
<i>Epargyreus clarus</i> (Cramer)	S	6					
<i>Urbanus proteus</i> (Linnaeus)	SM						
<i>Achalarus lyciades</i> (Geyer)	SM						
<i>Thorybes bathyllus</i> (JE. Smith)	S	6					
<i>Thorybes pylades</i> (Scudder)	B						
<i>Staphylus hayhurstii</i> (WH Edwards)	S	4	4H	2	4	L	R
<i>Erynnis icelus</i> (Scudder et Burgess)	B						
<i>Erynnis brizo</i> (Boisduval et Le Conte)	S	6					
<i>Erynnis juvenalis</i> (Fabricius)	B						
<i>Erynnis horatius</i> (Scudder et Burgess)	SM						
<i>Erynnis marialis</i> (Scudder)	S	5					
<i>Erynnis lucilius</i> (Scudder et Burgess)	B						
<i>Erynnis baptisiae</i> (Forbes)	S	4	4H	2	0	L	R
<i>Erynnis persius persius</i> (Scudder)	S	6					
<i>Erynnis persius borealis</i> (Cary)	N	6					
<i>Pyrgus centaureae</i> (Rambur)	N	6					
<i>Pyrgus communis</i> (Grote)	S	4	4H	2	3	L	R
<i>Pholisora catullus</i> (Fabricius)	S	3	4H	2	3	L	S
<i>Carterocephalus palaemon</i> (Pallas)	B						
<i>Ancyloxypha numitor</i> (Fabricius)	B						
<i>Oarisma garita</i> (Reakirt)	N	6					
<i>Thymelicus lineola</i> (Ochsenheimer)	B						
<i>Hylephila phyleus</i> (Drury)	SM						
<i>Hesperia comma</i> (Linnaeus)	B						
<i>Hesperia leonardus</i> Harris	B						
<i>Hesperia sassacus</i> Harris	B						
<i>Polites peckius</i> (W Kirby)	B						

TABLE 1 (continued).

Family and species	Distribution**	Phenology [†]	Host [‡]	Egg type [§]	Egg class	Overwinter [¶]	Abundance [#]
<i>Euchloe ausonides</i> (Lucas)	N	6					
<i>Euchloe olympia</i> (WH Edwards)	S	6					
<i>Colias philodice</i> Godart	B						
<i>Colias eurytheme</i> Boisduval	B						
<i>Colias gigantea</i> (Strecker)	N	6					
<i>Colias pelidne</i> (Boisduval et Le Conte)	N	1	3	0	0	L	
<i>Colias interior</i> Scudder	B						
<i>Colias palaeno</i> (Linnaeus)	N	6					
<i>Zerene cesonia</i> (Stoll)	SM						
<i>Phoebis semnae</i> (Linnaeus)	SM						
<i>Phoebis philea</i> (Linnaeus)	SM						
<i>Eurema mexicana</i> (Boisduval)	SM						
<i>Eurema lisa</i> Boisduval et Le Conte	SM						
<i>Nathalis iole</i> Boisduval	SM						
Lycanidae							
<i>Feniseca tarquinius</i> (Fabricius)	B						
<i>Lycæna phlaeas</i> (Linnaeus)	B						
<i>Lycæna hyllus</i> (Cramer)	B						
<i>Lycæna epixanthe</i> (Boisduval et Le Conte)	B						
<i>Lycæna dorcas</i> W Kirby	B						
<i>Lycæna helliodes</i> (Boisduval)	B						
<i>Satyrium acadicum</i> (WH Edwards)	B						
<i>Satyrium titus</i> (Fabricius)	B						
<i>Satyrium edwardsii</i> (Grote et Robinson)	S	2	2	2	4	E	S
<i>Satyrium calanus</i> (Hübner)	S	2	2	2	3	E	S
<i>Satyrium caryaevorum</i> (McDunnough)	S	2	2	0	0	E	R
<i>Satyrium liparops</i> Le Conte	B						
<i>Callophrys grynea</i> (Hübner)	S	5					
<i>Callophrys augustinus</i> (Westwood)	B						

TABLE 1 (concluded).

Family and species	Distribution**	Phenology [†]	Host [‡]	Egg type [§]	Egg class	Overwinter [¶]	Abundance [#]
<i>Chlosyne nycteis</i> (Doubleday)	B						
<i>Chlosyne harrisii</i> (Scudder)	B						
<i>Phyciodes tharos</i> (Drury)	S	3	4H	1	2	L	S
<i>Phyciodes cocyta</i> (Cramer)	B						
<i>Phyciodes batesii</i> (Reakirt)	B						
<i>Euphydryas phaeton</i> (Drury)	S	1	4H	1	2	L	S
<i>Polygonia interrogatoriz</i> (Fabricius)	B						
<i>Polygonia comma</i> (Harris)	B						
<i>Polygonia satyrus</i> (WH Edwards)	B						
<i>Polygonia faunus</i> (WH Edwards)	B						
<i>Polygonia gracilis</i> (Grote et Robinson)	N	2	3	0	0	A	
<i>Polygonia progne</i> (Cramer)	B						
<i>Nymphalis vaualbum</i> (Denis et Schiffermüller)	B						
<i>Nymphalis antiopa</i> (Linnaeus)	B						
<i>Nymphalis milberti</i> (Godart)	B						
<i>Vanessa virginensis</i> (Drury)	B						
<i>Vanessa cardui</i> (Linnaeus)	B						
<i>Vanessa atalanta</i> (Linnaeus)	B						
<i>Junonia coenia</i> (Hübner)	SBM						
<i>Limenitis arthemis arthemis</i> (Drury)	B						
<i>Limenitis arthemis astyanax</i> (Fabricius)	S	1	2	2	3	L	S
<i>Limenitis archippus</i> (Cramer)	B						
<i>Asterocampa celtis</i> (Boisduval et Le Conte)	S	2	2	2	0	L	R
<i>Asterocampa clyton</i> (Boisduval et Le Conte)	S	2	2	1	2	L	R
<i>Enodia antihedon</i> AH Clark	B						
<i>Satyroides eurydice</i> (Linnaeus)	B						
<i>Satyroides appalachia</i> (RL Chermock)	S	2	4G	0	0	L	S
<i>Megisto cymela</i> (Cramer)	B						

<i>Coenonympha tullia</i> Müller	B
<i>Ceryonis pegala</i> (Fabricius)	B
<i>Erebia mancinus</i> (Doubleday)	N
<i>Erebia discoidalis</i> (W Kirby)	N
<i>Oeneis macounii</i> (WH Edwards)	N
<i>Oeneis chryxus</i> Doubleday	B
<i>Oeneis jutta</i> (Hübner)	N
<i>Oeneis melissa</i> (Fabricius)	N
Danaidae	
<i>Danaus plexippus</i> (Linnaeus)	B

* Information is included for each species up to the parameter that was used to eliminate or keep the species on the vulnerability list. Species that have information in all fields are species that require further consideration of their biological attributes.

** Butterfly distribution: B, found in both northern and southern Ontario; N, northern Ontario only; S, southern Ontario only; SM, southern Ontario migrant; and SBM, southern Ontario migrant with periodic breeding populations.

† Flight-period phenology and generation code based on parasitoid release periods of 16–31 July (northern Ontario) and 1–15 July (southern Ontario): 1, midpoint of flight period occurs during parasitoid release period and species is univoltine; 2, midpoint of flight period occurs after parasitoid release period and species is univoltine; 3, midpoint of flight period occurs during parasitoid release period and species is multivoltine; 4, midpoint of flight period occurs after parasitoid release period and species is multivoltine; 5, midpoint of flight period occurs before parasitoid release period and species is univoltine; and 6, midpoint of flight period occurs before parasitoid release period and the species is multivoltine.

‡ Host plant: 1, coniferous; 2, deciduous; 3, shrub; 4G, grass and sedge; and 4H, herbaceous.

§ Egg type: 0, not reported; 1, egg mass; and 2, single egg.

|| Egg class: 0, not reported; 1, egg mass and location exposed; 2, egg mass and location hidden; 3, single egg and location exposed; and 4, single egg and location hidden.

¶ Overwintering stage: E, egg; L, larva; P, pupae; and A, adult.

Abundance in southern Ontario: S, secure found in many localities; and R, restricted or rare.

Nontarget butterfly dataset

The first priority when examining potential risks to a nontarget insect from released entomophagous biocontrol agents is to select a representative subset of the nontarget species of concern. This is because there are too many species to screen or rear in the laboratory. In addition, rearing methods have not been developed for many nontarget insects and this becomes a research area in itself, delaying any progress in screening potential biocontrol agents. Decision methods are needed for insect biocontrol, similar to those that have been developed for weed biocontrol (Marohasy 1998; Spencer 2000), to assist in the selection of appropriate nontarget species for use in host-range tests. There are, however, problems with the matching of lab-screening data on host range (*i.e.*, physiological host range) to what actually happens under field conditions (*i.e.*, ecological host ranges) in weed systems (Schaffner 2001). Interpreting physiological and ecological host ranges will be a critical issue for the future use of entomophagous biocontrol agents.

The butterfly dataset consisted of 153 species reported in Ontario (Holmes *et al.* 1991; Layberry *et al.* 1998). Taxonomic names and the list of butterfly species follow the treatment of Layberry *et al.* (1998). Additional aspects of the species biology were obtained from Scott (1986), Klassen *et al.* (1989), and Bird *et al.* (1995) (Table 1). Species were classified based on geographic distribution, oviposition phenology, number of generations per year, overwintering stage, host-plant type, and oviposition behavior (*i.e.*, single eggs *versus* clumped; exposed eggs *versus* hidden). Subspecies were included in the list only if they were recognized in Layberry *et al.* (1998) as unique to either northern or southern Ontario.

Ecological vulnerability criteria

Geographical distribution

Species distributions were classified on three scales. On a North American scale, distributions were scored as follows: **1**, both eastern and western North America; **2**, eastern North America only; **3**, western North America only; and **4**, continental United States of America only and potential migrant to Ontario.

On a Canadian scale, distributions were scored as follows: **1**, both eastern and western Canada; **2**, eastern Canada only; **3**, western Canada only; **4**, Alberta and British Columbia; **5**, Alberta only; **6**, Manitoba only; **7**, Ontario only; **8**, Maritime Provinces; and **9**, eastern Canada plus Manitoba. The division between eastern and western Canada was the Ontario–Manitoba border.

On an Ontario scale, where *T. minutum* releases have been conducted in the boreal forest (Smith *et al.* 1990; Bouchier and Smith 1998), distributions were scored as follows: **1**, both northern and southern Ontario; **2**, southern Ontario only; **3**, northern Ontario only; and **4**, migrant to southern Ontario. The division between northern and southern Ontario was the 40°F isotherm line (Holmes *et al.* 1991, p 4). The line starts slightly north of Sault Ste. Marie (46°31'N, 84 20'W), follows the north channel of Lake Huron along the edge of the boreal forest, excluding Algonquin Park, and across to the eastern border of the province.

Ontario distributions were used to assess the geographic vulnerability of a species within the province because this was the area of interest and locations of previous *T. minutum* releases (Table 1). North American and Canadian distributions were used to determine the rarity of a butterfly species at larger scales. For example, if a species was present within Ontario and common, but rare or uncommon across larger scales, this would have been suitable justification to include it on the ecological vulnerability list.

Flight period (oviposition) phenology and number of generations

Flight periods indicating oviposition phenology for each species were estimated from data provided in Layberry *et al.* (1998). The key question in terms of vulnerability is do the flight periods of nontarget butterflies overlap with that of the spruce budworm (*i.e.*, the main target in Ontario for which *T. minutum* would be released). The release period for *T. minutum*, based on flight periods of the spruce budworm, was the last 2 weeks of July (Julian date 197–211) in northern Ontario and the first 2 weeks of July (Julian date 183–197) in southern Ontario. Julian dates for the start and end of the flight period were rounded to the nearest week; “early” was matched to the 7th day of the month, mid-April was the recorded as the 15th day of April or Julian date 105, and “late” was matched to the 21st day of the month. Eggs were assumed to be potentially present throughout the flight period, however, vulnerability of a particular species was estimated by taking the mid-point of the flight period and comparing this date to the 2-week release period for *T. minutum* based on Bouchier and Smith (1998).

Phenology was combined with the number of generations per year as recorded from Layberry *et al.* (1998) to estimate an aggregate vulnerability ranking where 1 is highest vulnerability to an inundative release of *T. minutum* and 6 is the lowest vulnerability: **1**, midpoint of the flight period occurs during the parasitoid release period and the species is univoltine; **2**, midpoint of the flight period occurs after the parasitoid release period and the species is univoltine; **3**, midpoint of the flight period occurs during the parasitoid release period and the species is multivoltine; **4**, midpoint of the flight period occurs after the parasitoid release period and the species is multivoltine; **5**, midpoint of the flight period occurs before the parasitoid release period and the species is univoltine; and **6**, midpoint of flight period occurs before the parasitoid release period and the species is multivoltine.

Overwintering stage was considered a modifier of the ranking of oviposition phenology. If the flight period is early in the year, but the overwintering stage is an egg, these eggs likely are present during the *T. minutum* release period. Conversely, if the overwintering stage is an adult, the eggs are likely laid the following spring prior to a *T. minutum* application and are at a lower risk of attack.

Information for the remaining variables was not always available for some species. Details on specific host-plant preferences provide partial information that can be combined with other attributes such as egg type and egg location to determine the availability of nontarget host eggs to attack by *T. minutum*.

Host-plant type

The principal target of inundative releases in the boreal forest has been the spruce budworm. Previous work has demonstrated an affinity by *Trichogramma* species for a particular host that is related to its rearing host (Bergeijk *et al.* 1989; Bouchier *et al.* 1994; Corrigan and Laing 1994) and to the host's location in the habitat (Nordlund 1994). Thus, I discriminated among butterfly species by their principal host plants, assuming that a butterfly feeding on a conifer, like the target host (the spruce budworm), would more likely be found by the parasitoid than a species feeding on a deciduous tree. Similarly, I assumed that butterfly species feeding on trees would be more vulnerable than if they fed on shrubs or ground vegetation because *T. minutum* has been shown to parasitize target eggs more in the tops of trees. Attacks by *T. minutum* on sentinel egg masses increased with sentinel egg mass height in plantation spruce trees (approximately 6–7 m) (Smith 1988) and with location in the upper *versus* mid-crown locations in naturally collected spruce budworm eggs (Kemp and Simmons 1978; Jennings and Houseweart 1983). The ranking for host-plant type from highest (1) to lowest (4)

TABLE 2. Ecological vulnerability list of Ontario butterfly species that are potential nontarget hosts for *Trichogramma minutum*.

Location	Family and species	Key vulnerability factors
Northern Ontario	Nymphalidae <i>Speyeria cybele krautwurmii</i>	Unique subspecies to northern Ontario; oviposition after parasitoid application period; univoltine
	Lycaenidae <i>Lycaeides idas</i>	Oviposition within parasitoid application period and overwinters as an egg; univoltine
Southern Ontario	Hesperiidae	
	<i>Erymis baptisiae</i>	Restricted geographic range
	<i>Pyrgus communis</i>	Restricted geographic range; migrant but colonies persist for multiple years
	<i>Pholisora catullus</i>	Oviposition after parasitoid application period; multivoltine
	<i>Pompeius verna</i>	Restricted geographic range
	<i>Anatrytone logan</i>	Oviposition after parasitoid application period; univoltine
	<i>Poanes massasoit</i>	Restricted geographic range
	<i>Poanes viator</i>	Oviposition within parasitoid application period; multivoltine
	<i>Euphyes dion</i>	Oviposition within parasitoid application period; multivoltine
	<i>Euphyes dukesi</i>	Restricted geographic range; oviposition after parasitoid application period; univoltine
	<i>Euphyes conspicua</i>	Oviposition after parasitoid application period; univoltine
	<i>Euphyes bimacula</i>	Restricted geographic range
	<i>Staphylus hayhurstii</i>	Restricted geographic range
	<i>Polites origenes</i>	Oviposition within parasitoid application period; multivoltine
	<i>Wallengrenia egeremet</i>	Oviposition within parasitoid application period; multivoltine
	Papilionidae	
	<i>Papilio cresphontes</i>	Restricted geographic range
	Lycaenidae	
	<i>Satyrium edwardsii</i>	Overwinters as egg; univoltine
<i>Satyrium calanus</i>	Overwinters as egg; univoltine	
<i>Satyrium caryaevorum</i>	Restricted geographic range; overwinters as egg; univoltine	
<i>Everes comyntas</i>	Oviposition after parasitoid application period ; multivoltine	
<i>Lycaeides melissa</i>	Overwinters as egg; restricted geographic range, maybe extirpated from Ontario	
Nymphalidae		
<i>Speyeria cybele</i>	Oviposition after parasitoid application period; univoltine	
<i>Phyciodes tharos</i>	Oviposition within parasitoid application period; multivoltine	
<i>Euphydryas phaeton</i>	Oviposition within parasitoid application period; multivoltine	

TABLE 2. (concluded).

Location	Family and species	Key vulnerability factors
	<i>Limenitis arthemis astyanax</i>	Oviposition within parasitoid application period; univoltine, hidden egg mass in deciduous tree
	<i>Asterocampa celtis</i>	Restricted geographic range; oviposition after parasitoid application period; univoltine; lays egg mass in deciduous tree
	<i>Asterocampa clyton</i>	Restricted geographic range; oviposition after parasitoid application period; univoltine; lays hidden egg mass in deciduous tree
	<i>Satyrodes appalachia</i>	Oviposition after parasitoid application period; univoltine

vulnerability, based on the profile was as follows: **1**, coniferous host; **2**, deciduous host; **3**, shrub; and **4**, grass, sedge (4G in Table 1), or herbaceous host (4H in Table 1).

Egg type and exposure

Egg types were classified as either laid singly or in masses, with egg masses assumed to be most vulnerable to attack by *T. minutum* because once a parasitoid female found an egg mass they would often attack all eggs in the mass. For singly laid eggs it would be much harder for the parasitoids to attack a significant portion of the nontarget butterfly population.

Oviposition locations were classified as either open and exposed (*e.g.*, eggs laid on a needle such as the spruce budworm) or hidden (*e.g.*, eggs laid under bark scales). Nontarget eggs were ranked in the following egg classes going from highest to lowest vulnerability to attack by the *Trichogramma* species **1**, egg mass and location exposed; **2**, egg mass and location hidden; **3**, single egg and location exposed; and **4**, single egg and location hidden.

Building the ecological vulnerability list

There are 23 species that are unique to northern Ontario and 60 species that are unique to southern Ontario (Table 1). The remaining 70 species are eliminated from the vulnerability list because they are found in both geographic areas and in most cases are widely distributed outside Ontario. Information is included for each species (Table 1) up to the criterion that was used to eliminate or keep the species on the ecological vulnerability list (Table 2).

Northern Ontario

For the 23 species unique to northern Ontario, application of the phenology and generation criteria identifies 19 species that are ranked in the lowest vulnerability groups (5 and 6). These species have single (5) or multiple (6) generations that occur prior to the release of the *Trichogramma* species. These 19 species are eliminated from further consideration on the vulnerability list because the time when eggs would be available for *T. minutum* to attack did not coincide with the release period. Additionally, none of these species overwinters as an egg.

The remaining four northern species are *Lycaeides idas*, *Speyeria cybele krautwurmi*, *Polygonia gracilis*, and *Colias pelidne*. Two of these species require additional host-range testing after consideration of the biological attributes (host plant, egg class; Table 1). *Lycaeides idas* overwinters as an egg, with the oviposition period starting in

late July (Holmes *et al.* 1991); thus, eggs are likely present in the field at the time of *T. minutum* applications in northern Ontario. Its food plant is uncertain but is suggested to include the following shrubs: black crowberry (*Empetrum nigrum* L.; Empetraceae), dwarf billberry (*Vaccinium caespitosum* Michx.; Ericaceae), Labrador tea (*Ledum groenlandicum* Oeder; Ericaceae), and sheep laurel (*Kalmia augustifolia* L.; Ericaceae) (Holmes *et al.* 1991; Layberry *et al.* 1998). *Speyeria cybele krautwurmi* (unique subspecies to northern Ontario; Table 1) and the nominate species *Speyeria cybele* (unique to southern Ontario; Table 1) are both included as potentially vulnerable to *T. minutum* based on geographic and phenology criteria (Table 2).

Polygonia gracilis and *Colias pelidnae* are removed from the ecological vulnerability list for northern Ontario. *Polygonia gracilis* overwinters as an adult (Holmes *et al.* 1991; Layberry *et al.* 1998) and eggs are laid early in the spring before any *T. minutum* releases for budworm would be considered. The distribution of *C. pelidnae* in northern Ontario is along the shores of Hudson's Bay (Holmes *et al.* 1991) where *T. minutum* releases are highly unlikely because of the lack of commercial forest.

Southern Ontario

For the 60 species unique to southern Ontario, 18 species are classified as either seasonal migrants (SM; Table 1) or breeding migrants (SBM; Table 1). These species were removed from the vulnerability list because replenishment of their Ontario populations results from the migration of the species from the United States of America.

The phenology and generation criteria removed an additional 15 species that were ranked in the lowest vulnerability groups (group 5 and 6). These species had single (5) or multiple (6) generations that occurred before the release of *T. minutum* and none of these species overwintered as eggs. They were eliminated from the ecological vulnerability list (Table 2) because the time when their eggs would be available for *T. minutum* to attack did not coincide with the southern release period.

The remaining 27 species on the ecological vulnerability list for southern Ontario (Table 2) require the consideration of biological attributes to assess their risk from *T. minutum*. Data for all attributes were not available. Depending on the habitat and geographic location of a *T. minutum* release, potential interactions with any of the remaining 27 species would have to be considered and assessed prior to the release. Of the remaining 27 species, 11 species are restricted to specific habitats and locations in southern Ontario (Table 2) making them more vulnerable to a *T. minutum* release. Oak savanna and Carolinian habitats in the southwest of the province, along the shores of the Great Lakes, including Point Pelee, Walpole Island, and St. Williams are of particular importance as habitat for 5 of the 11 species: *Erynnis baptisiae*, *Asterocampa celtis*, *Asterocampa clyton*, *Euphyes dukesi*, and *Staphhylus hayhurstii*. A 12th species, the Karner blue (*Lycaeides melissa*), is rare and may be extirpated from the province (Holmes *et al.* 1991; Layberry *et al.* 1998). The remaining 15 species are reported to be "secure", with many occurrences in the province (Holmes *et al.* 1991).

Discussion

The relative importance of biological attributes (host plant, egg type, and egg location) of the nontarget species for parasitoid attacks is an area requiring additional experimentation, addressing *T. minutum* host-finding and host acceptance under field conditions. Host-finding is likely more important than host acceptance for nontarget impact. Many lab tests of host acceptance with several *Trichogramma* species indicate that once a female is placed on a host, she will often attempt to oviposit if she has eggs (Schmidt 1994; Bai *et al.* 1995). Of critical importance to nontarget impact is the

likelihood of the parasitoid even choosing to search on a host plant that does not contain the target-host eggs. None of the 27 species that are on the ecological vulnerability list for southern Ontario feeds on a coniferous tree (the host type of the target species spruce budworm). Only seven species are located in deciduous trees and thus would have eggs possibly located in the same strata of the forest as where mass-reared *T. minutum* are targeted. The remaining 21 butterfly species feed on grasses or herbaceous plants in a lower strata of the forest, and they would likely be less vulnerable to attacks from *T. minutum*, which tends to search at higher strata of the forest (Kemp and Simmons 1978; Jennings and Houseweart 1983; Smith 1988).

Cortesero *et al.* (2000) reviewed the importance of host-plant volatiles in recruiting parasitoids to actively search on a host plant containing potential hosts. Inundative releases with *T. minutum* will only be targeted at high-density populations of forest pests, such as the spruce budworm. The dominant plant chemical or host attractant in these stands will be from budworm feeding, thus making it more likely that *T. minutum* will find the target host rather than searching for nontarget hosts.

If the parasitoid finds a host plant containing eggs, complexity of the substrate will also influence host-finding and thus affect nontarget vulnerability. *Trichogramma minutum* attack rates on flour-moth eggs located on poplar were higher than on flour-moth eggs placed on balsam fir because poplar has a less complex surface than the balsam fir (Lukianchuk and Smith 1997). The location of the eggs on the host plant adds to the potential complexity for the searching parasitoid. If eggs are all located in a mass, once found it is a large resource compared with singly laid host eggs. *Trichogramma minutum* usually parasitizes multiple eggs within a spruce budworm egg mass (Smith *et al.* 1990). Similarly, if eggs are hidden [*e.g.*, oviposition under bud scales as for *Zeiraphera canadensis* Mutuura and Freeman (Lepidoptera: Tortricidae)], this may limit parasitism by *T. minutum* compared with parasitism rates if eggs were exposed (Ostiff and Quiring 1994). Combined egg type and location data were available for 13 of the 27 species. No species laid egg masses in the open, similar to the spruce budworm; three species laid single eggs in the open. The remaining 10 species laid hidden eggs, 6 in masses and 4 as single eggs (see Table 1 for species).

Orr *et al.* (2000) considered potential nontarget effects of *Trichogramma brassicae* in corn systems. They identified a list of 22 nontarget Lepidoptera (18 moths and 4 butterflies), based on field collections, whose flight periods overlapped with potential *T. brassicae* releases. A potential problem acknowledged by Orr *et al.* (2000) is that field collections may miss rare and potentially more vulnerable species. Listing all potential species that are in an area (Table 1) is a conservative approach which starts with the assumption that all species are initially vulnerable. Several of the species that are on the ecological vulnerability list (Table 2) are rare species, thus may be difficult to screen for parasitoid host range. By identifying species of concern based on ecological attributes, it is also possible to substitute common species for the host-range testing of rare species. The common species may be easier to rear, would lay eggs in a similar location to the rare species, and would occupy similar habitats.

Three of the four butterfly species that were trapped by Orr *et al.* (2000) (*Everes comyntas*, *Epargyreus clarus*, and *Colias eurythyeme*) were also on the initial Ontario list (Table 1). Orr *et al.* (2000) ranked these three species as poor-quality hosts based on oviposition tests in the lab that considered percent parasitism, percent emergence, sex ratio, and size of offspring. *Everes comyntas* is 1 of the final 27 species included in the southern Ontario vulnerability list (Table 2). *Colias eurythyeme* was eliminated from the ecological vulnerability list for southern Ontario because of its broad geographic range and *Epargyreus clarus* was eliminated because it had multiple broods of eggs starting before the *T. minutum* release dates.

The six criteria used for receptor characterization for *T. minutum* can also be used for other parasitoids. They are components of any target host's biology and thus will affect the scale and impact of any parasitoid attacking eggs, larvae, or pupae. These host criteria affect the specific stages of Doutt's generalized model of parasitoid behaviour where exposure (in risk terms) or successful parasitism is a function of (i) host-habitat location, (ii) host location, (iii) host recognition and acceptance, and (iv) host suitability (Doutt 1959). For *T. minutum*, which has a broad range of host acceptance when placed on eggs (Schmidt 1994; Bai *et al.* 1995), six of the criteria selected (geographic distribution, oviposition phenology, number of generations per year, overwintering stage, host-plant preferences, and egg-mass location) relate primarily to the host-habitat location and host-location stages. Only egg-mass type relates to the host-acceptance stage of successful parasitism. For risk assessments of other parasitoids, additional criteria for receptor characterization may be added that will relate to the specifics of their biology and may be associated with host acceptance and host suitability. Some examples include nontarget larval size for parasitoids that only accept host larvae above a threshold size or related instar (Godfray 1994) or the type and availability of alternative food sources, required by some foraging parasitoids to produce eggs, in the habitat of nontarget host (Lewis *et al.* 1998). Consideration of risk criteria associated with all stages of successful parasitism and aspects of host selection (Vinson 1998) will ensure better nontarget screening of biological control agents.

Much of the criticism that has been leveled at biological control has been targeted at classical biological control because if successful (establishment of the control agent) it is impossible to reverse (Simberloff and Stiling 1996; Louda *et al.* 1997). In this paper, I considered an inundative biocontrol program in which high release densities (*i.e.*, 12 million parasitoids per ha) are not sustained, and thus local impact on nontarget species is similar to that of a conventional insecticide targeting a specific stage of a pest. If an egg parasitoid was being considered for classical biocontrol, the ecological vulnerability list of species would be longer than for an inundative release, because the geographical area affected is larger. However, the species that are of concern for inundative release would be a starting point for a classical biocontrol program. After species of concern are identified the next step is to conduct prerelease host-range screening against species or their analogues for a classical biocontrol agent. In the case of an inundative release, nontarget species should be monitored in the field to compare predicted and realized host ranges.

If biocontrol is to remain available as an integrated pest management tool, it is likely that risk assessments for all biocontrol strategies will be required. The development of an ecological vulnerability list (Table 2) is the first step of a risk assessment for all types of activities associated with biological control. This includes assessing the risks of a classical biocontrol introduction, of a short-term inundative release, and of doing nothing. For the do-nothing scenario, the ecological vulnerability list would consist of the species that may be affected by the pest targeted for inundative biocontrol or affected by the invasive species targeted for classical biological control. Regardless of the proposed action, there will be a list of species affected. Consideration of ecological vulnerability criteria should become standard practice to determine which nontarget species require host-range testing, for both inundative and classical biocontrol agents targeting insects, and for the potential impact of invasive species. By basing the vulnerability list on ecological attributes, the number of species to be considered becomes manageable but still ensures that risks associated with biological-control choices are comparable and that when a decision is made, the risks are minimized.

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