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ARTICLE

Introduction and establishment of biological control agents for control of emerald ash borer (*Agrilus planipennis*) in Canada

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Abstract
The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious pest of ash (*Fraxinus* spp.) (Oleaceae) in North America. Control of emerald ash borer is difficult in natural forest settings; therefore, a classical biological control programme is the most feasible management option for this invasive, nonnative insect. Here, we report the first Canadian release and establishment of parasitoids *Tetrastichus planipennisi* Yang (Hymeoptera: Eulophinae), *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), and *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae) in natural forests in Ontario, Quebec, and New Brunswick, Canada for the control of emerald ash borer. Releases of *T. planipennisi* were made from 2013 to 2019, *O. agrili* from 2015 to 2019, and *S. galinae* from 2017 to 2019. Trees from release sites were destructively sampled to rear out adult emerald ash borers and parasitoids 1–3 years after parasitoid release. Recoveries of *T. planipennisi* were made at 81% of release sites (13 of 16) 1–2 years after release, and *O. agili* were recovered from 29% of release sites (4 of 14) 1–3 years after release. *Spathius galinae* was not recovered. These data provide important information for the development and deployment of a successful biological control programme for the management of emerald ash borer in Canada.

Introduction

The emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is an invasive, phloem-feeding beetle that has killed tens of millions of ash trees, *Fraxinus* spp. (Oleaceae), in North America (Herms and McCullough 2014). The beetle was first detected in North America in 2002, when adults were reared from declining ash trees in Detroit, Michigan, United States of America and later in Windsor, Ontario, Canada (Cappaert *et al.* 2005; Siegert *et al.* 2014); however, it was likely present since the early 1990s (Siegert *et al.* 2014). The beetle has since been detected in 35 American states (Herms and McCullough 2014; United States Department of Agriculture 2021) and five Canadian provinces (Canadian Food Inspection Agency 2021). Control of emerald ash borer in North America is challenging because the insect's populations are difficult to detect at low densities (Ryall *et al.* 2011), their spread is stratified with both

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long-distance and short-distance dispersal (Liebhold and Tobin 2008), and although some species of native parasitoids are exploiting emerald ash borer (*e.g.*, Duan *et al.* 2011a; Roscoe *et al.* 2016), they have not prevented large-scale ash mortality. In urban areas, the removal of infested trees and the use of systemic insecticides are used to manage infestations. However, these tactics are effective only for removing small infestations, mitigating hazards of dead and dying trees, or, in the case of insecticides, for managing infestations in high-value trees and preserving the urban tree canopy (Sadof *et al.* 2017). These tactics have not often been deployed in natural forests because the size of the stands and economic cost of the tactics make management with tree removal and insecticides prohibitive for managing large infestations or large numbers of trees (Duan *et al.* 2018). Control in natural forest habitats in North America has therefore focused on the use of biological control agents and, in recent years, on the potential breeding of resistant ash trees (Koch *et al.* 2015; Wu *et al.* 2019) as the only feasible control options.

Since the insect's initial detection in Canada in 2002 near Windsor, Ontario, emerald ash borer populations have been detected elsewhere in Canada. The insect's range now includes most of southern Ontario and northern Ontario, including the Algoma district, Sudbury, and North Bay regions. The insect is also found throughout southern Quebec, to as far north as the City of Québec and St-Jean-Port-Joli, in New Brunswick along a corridor from Edmundston through Oromocto and Moncton, and in Nova Scotia in the community of Bedford within the Greater Halifax region. Two isolated populations have also established in Thunder Bay, Ontario and in Winnipeg, Manitoba (Canadian Food Inspection Agency 2021). In these regions and throughout much of eastern Canada, ash trees are an important component of urban and rural forests, comprising up to 30% of the urban forest canopy in many North American municipalities (Poland and McCullough 2006; Ball *et al.* 2007). The economic impact of emerald ash borer on Canadian urban areas alone was estimated at CAD $524 million (2010 currency rate) and up to $890 million when damage to backyard trees was included (McKenney *et al.* 2012). In the United States of America, the total damage caused by this insect has been estimated to be in the billions of dollars (Kovacs *et al.* 2010).

Hymenopteran parasitoids of emerald ash borer were identified in the beetle's native range of northeastern China and the Russian Far East (Liu *et al.* 2003; Yang *et al.* 2005, 2006; Zhang *et al.* 2005; Belokobylskij *et al.* 2012), and four species were subsequently released in the United States of America starting in 2007 (Gould *et al.* 2015): these were the larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Braconidae) (Yang *et al.* 2006) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang *et al.* 2005) and the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang *et al.* 2005). A third larval parasitoid, *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae) (Belokobylskij *et al.* 2012), was identified in the Russian Far East and was approved for release in the United States of America in 2015 (Duan *et al.* 2015). *Tetrastichus planipennisi* and *O. agrili* are established in many of the release sites in the northeastern, mid-Atlantic, and midwestern states and have spread naturally into new areas (Duan *et al.* 2013, 2018; Abell *et al.* 2014; Davidson and Rieske 2016; Jennings *et al.* 2016). *Spathius agrili* has not established in release sites in the northern United States of America (Ragozzino *et al.* 2020), perhaps due to the asynchronisation of adult parasitoid emergence with emerald ash borer larval development or because of climatic conditions at release site (Duan *et al.* 2019; Ragozzino *et al.* 2020). *Spathius galinae* has demonstrated better suitability to the climate of the northcentral and northeastern United States of America (Duan *et al.* 2018) and is established in release sites in Connecticut, Massachusetts, Michigan, and New York (Duan *et al.* 2019, 2020).

In Canada, Natural Resources Canada submitted petitions in 2013 to the Canadian Food Inspection Agency to introduce *T. planipennisi*, *S. agrili*, and *O. agrili* to Canada. The petitions for *T. planipennisi* and *S. agrili* were approved in 2013 (Canadian Food Inspection Agency 2021) but that for *O. agrili* was initially denied due to concerns that *O. agrili* might attack native species of *Agrilus*. A second petition for release of *O. agrili* was approved in

2015 (Canadian Food Inspection Agency 2022), in part because the release of *O. agrili* in the United States of America meant the insect would likely enter Canada on its own accord. The United States Department of Agriculture, Animal and Plant Health Inspection Service later withdrew recommendation for the release of *S. agrili* north of 40° latitude (Bauer *et al.* 2015), therefore excluding that species for release in Canada. A petition for the release of *S. galinae* was approved in Canada in 2017 (Canadian Food Inspection Agency 2022). The goal of the release programme when it was established in 2013 was to determine if *T. planipennisi*, *O. agrili*, and *S. galinae* could successfully establish and thrive in Canada.

Herein we document releases of *T. planipennisi* made from 2013 to 2019, *O. agrili* from 2015 to 2019, and *S. galinae* from 2017 to 2019. *Tetrastichus planipennisi* were recovered at 13 of 16 release sites one or two years after release. *Oobius agrili* were recovered from four of the 14 sites where sampling was completed, 1–3 years after release. *Spathius galinae* were not recovered. We also present preliminary assessments of the impacts of these parasitoids on Canadian emerald ash borer populations.

Materials and methods

Study sites

We released parasitoids from 2013 to 2019 at 26 sites located in Ontario (2013–2019), Quebec (2014–2019), and New Brunswick (2019; Tables 1–3; Fig. 1). One to seven new sites were established each year (Tables 1–3). These sites were selected based on criteria outlined by the United States Department of Agriculture (2012, 2019). Specifically, release sites were naturally forested areas of at least 16 ha (40 acres), with sufficient ash and emerald ash borer density to support an establishing parasitoid population. Some sites were smaller than 16 ha but were determined to have a high ash density and could act as corridors to other wooded areas with ash. At each study site, we identified a group of emerald ash borer–infested ash trees that were at least 100 m from any roadways, and within each group, we designated one tree as the epicentre. Three ash trees at least 4 cm around at 1.3 m above ground level (diameter at breast height) in each of the four cardinal directions around the epicentre tree were then selected as release trees, making for a total of 12 release trees at each site.

Source of parasitoids and field releases

We obtained parasitoids from the United States Department of Agriculture, Animal and Plant Health Inspection Service rearing facility in Brighton, Michigan, United States of America and the Natural Resources Canada, Canadian Forest Service, Insect Production and Quarantine Laboratories rearing facility at the Great Lakes Forestry Centre, Sault Ste. Marie, Ontario. The insects were provided as pre-emergent pupae (*T. planipennisi*), in small-diameter (10–15 cm × 20 cm) sticks cut from ash trees in which emerald ash borer larval hosts were reared, or within host eggs (*O. agrili*), or as live adults (*S. galinae*; see below). Parasitoids that were provided to us as pupae completed their development and emerged soon after deployment in the field (Jennings *et al.* 2016; Parisio *et al.* 2017), as explained below. *Tetrastichus planipennisi* from the Natural Resources Canada rearing facility were from colony Glfc:IPQL:Tpla01 (Roe *et al.* 2018). *Oobius agrili* were from colony Glfc:IPQL:Oagr01 (Supplementary material).

Tetrastichus planipennisi is a larval endoparasitoid. Adults emerge in late May and produce multiple generations per year (Duan *et al.* 2011b). Females parasitise late-instar emerald ash borer larvae, with up to 57 wasps produced per larva (Duan *et al.* 2011b). *Tetrastichus planipennisi* is reared in the laboratory by first infesting small-diameter (10–15 cm × 20 cm) ash sticks with emerald ash borer eggs and allowing the resulting larvae to develop to the third or fourth instar before exposing them to *T. planipennisi* in cages. The parasitised

Table 1. Estimated number of female *Tetrastichus planipennisi* released in Ontario, Quebec, and New Brunswick, Canada from 2013 to 2019.

Site name	Latitude	Longitude	Year						
			2013	2014	2015	2016	2017	2018	2019
Ontario									
Ausable Line	43.383	−81.543	6240						
Hay Swamp	43.356	−81.555	6070						
Brooke Line	42.844	−81.854	2820						
Wildwood Conservation Area	43.246	−81.057		1436	2703	2581			
Middleton-McConkey Tract	42.811	−80.623		3164	2703				
Silver Creek Conservation Area	43.680	−79.975		3316	2703				
Conroy Road	45.342	−75.609		3551	2703				
Metro Tract	44.311	−79.395			1281	2516	5069		
Wilmot Creek	43.914	−78.611					4984	4073	
Baxter Conservation Area	45.094	−75.631					4932	3904	
Monkland	45.248	−74.926					5151	3749	
Iroquois/Two Creeks	44.870	−75.263					2958	3889	
Renfrew	45.485	−76.689					5050	2798	
Fort St. Joseph	46.066	−83.946					3516†	3104†	
Lemoine Point Conservation Area	44.233	−76.612							9501
West Rocks Management Area	44.560	−80.956							9290
Potters Creek Conservation Area	44.140	−77.430							9225
South Bay Road	44.871	−79.775							9205
Total			**15 130**	**11 467**	**12 093**	**5097**	**31 660**	**21 517**	**37 221**

(*Continued*)

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Table 1. (*Continued*)

Site name	Latitude	Longitude	Year						
			2013	2014	2015	2016	2017	2018	2019
Quebec									
Gatineau Park	45.640	−75.946		3283	2703				
Bois de Liesse	45.505	−73.763			2703	2379			
Bois Summit	45.491	−73.606			1281	2385			
Jardin botanique	45.563	−73.564			2703	2486			
L'Assomption	45.820	−73.454					5030	3888	
City of Québec	46.790	−71.237						1500†	3285†
Drummondville	45.886	−72.524							3732†
Total				**3283**	**9390**	**7250**	**5030**	**5388**	**7017**
New Brunswick									
Siegas	47.212	−67.969							3227†
Grand Total			**15 130**	**14 750**	**21 483**	**12 347**	**36 690**	**26 905**	**47 465**

†Indicates all or part of release came from Canadian-reared populations from the Great Lakes Forestry Centre Insect Production and Quarantine Laboratories.

Table 2. Number of *Oobius agrili* released in Ontario, Quebec, and New Brunswick, Canada from 2015 to 2019. Site coordinates given in Table 1, except where noted.

	Year released				
Site name	2015	2016	2017	2018	2019
Ontario					
Wildwood Conservation Area	1500	1700			
Middleton-McConkey Tract	1500	1700			
Silver Creek Conservation Area	1500	1700			
Conroy Road	1500	1700			
Metro Tract		1700	1500		
Wilmot Creek			1400	1200	
Baxter Conservation Area			1400	1100	
Monkland			1500	1100	
Renfrew			1400	600	
Fort St. Joseph			1400	1260†	
Barrie*			3500		
Iroquois/Two Creeks				1000	
Lemoine Point Conservation Area					600
West Rocks Management Area					600
Potters Creek Conservation Area					600
South Bay Road					600
Total	**6000**	**8500**	**12 100**	**6260**	**2400**
Quebec					
Gatineau Park	1500	1700			
Bois de Liesse	1500	1700			
Bois Summit	1500	1700			
Jardin botanique	1500	1700			
L'Assomption			1500		
City of Québec			1000		1552†
Drummondville					1568†
Total	**6000**	**6800**	**2500**	**0**	**3120**
New Brunswick					
Siegas					1561†
Grand Total	**12 000**	**15 300**	**14 600**	**6260**	**7081**

*Urban release site: 44.358 N, –79.720 W.
†Indicates all or part of release came from Canadian-reared populations from the Great Lakes Forestry Centre Insect Production and Quarantine Laboratories.

Table 3. Number of *Spathius galinae* released in Ontario, Quebec, and New Brunswick, Canada from 2017 to 2019. Site coordinates given in Table 1.

Site name	Year		
	2017	**2018**	**2019**
Ontario			
Wilmot Creek		336	377
Baxter Conservation Area		345	376
Monkland	531	347	377
Iroquois/Two Creeks	210	310	417
Renfrew		340	380
Fort St. Joseph			419
Lemoine Point Conservation Area			200
West Rocks Management Area			183
Potters Creek Conservation Area			184
South Bay Road			189
Total	**741**	**1678**	**3102**
Quebec			
L'Assomption		357	
City of Québec			189
Drummondville			188
Total		**357**	**377**
New Brunswick			
Siegas			397
Grand Total	**741**	**2035**	**3876**

emerald ash borers are then consumed by the developing parasitoid larvae. In the present study, *T. planipennisi* were deployed in the field by hanging the sticks containing either pupae or pre-emergent adults on release trees within each release site. Each stick was hung approximately 1.5 m above the ground (Fig. 2A). We attempted to make six releases per year at each site: three in spring (from late May to early July) and three in summer (from mid-August to late September). The timing of releases was estimated from predicted emerald ash borer development, based on degree-days above a 10 °C threshold (DD_{10}). The first release at each year was made at 167 DD_{10} to target overwintering emerald ash borer larvae, with the second and third releases made two and four calendar weeks after the first phenological date. The fourth release date was made at 1000 DD_{10} to target the new-generation larvae, with the fifth and sixth releases occurring two and four weeks later. The number of *T. planipennisi* released was estimated by rearing a subsample of each lot of ash sticks and calculating the mean number of insects that emerged from each stick (Table 1).

Oobius agrili is an egg parasitoid, with two generations per year in its native range (Liu *et al.* 2007) and likely in the introduced range (Petrice *et al.* 2021a) as well, but the insect can be multivoltine in the laboratory (Yao *et al.* 2016). Each *O. agrili* female can parasitise up to 80 emerald ash borer eggs (Hoban *et al.* 2016), with one parasitoid egg laid per emerald ash borer egg. *Oobius agrili* was reared in the laboratory by collecting emerald ash borer eggs

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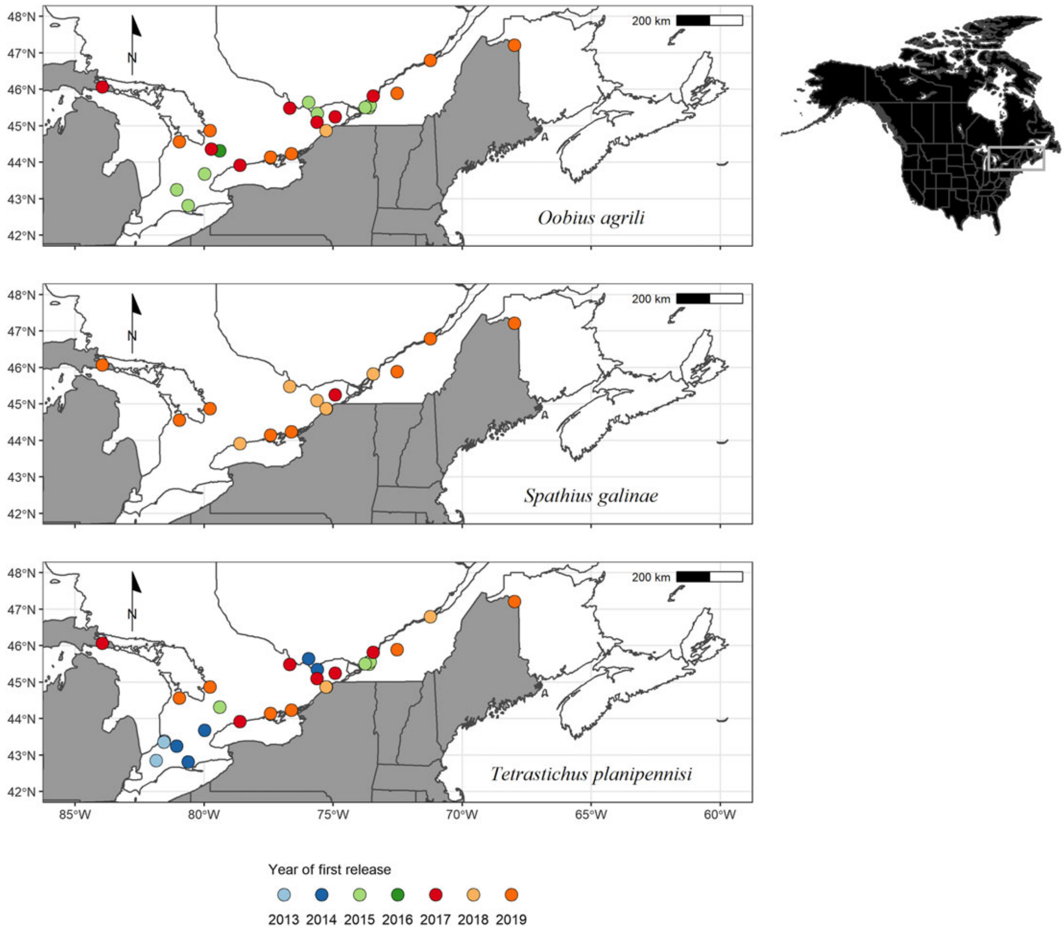

Fig. 1. Parasitoid release sites in Ontario, Quebec, and New Brunswick, Canada.

Fig. 2. Apparatuses used to deploy introduced biological control agents into ash (*Fraxinus* stands): **A**, *Tetrastichus planipennisi* are deployed as late-stage larvae or pupae in small ash sticks and **B**, *Oobius agrili* are deployed as immature stages inside parasitised emerald ash borer eggs on coffee filter paper inside a small plastic vial. Both sticks and vials are attached to trees inside release plots.

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from captive-reared emerald ash borer that were induced to oviposit on an artificial substrate (United States Department of Agriculture 2019). These eggs were then exposed to *O. agrili* adults in enclosures and allowed to mature in the laboratory. At maturity, approximately 100 parasitised emerald ash borer eggs were placed in a release device (Fig. 2B) and attached to release trees approximately 1.5 m off the ground. An initial release was made at each site timed to coincide with emerald ash borer oviposition (444 DD_{10}; United States Department of Agriculture 2019), with a second and third release made two and four calendar weeks after the first phenological date. All releases made in 2017 and earlier originated from the United States Department of Agriculture, Animal and Plant Health Inspection Services rearing facility and were likely nondiapausing individuals. Releases made in 2018 and 2019 originated from the Insect Production and Quarantine Laboratories and the United States Department of Agriculture, Animal and Plant Health Inspection Service (Table 2). *Oobius agrili* from the Insect Production and Quarantine Laboratories were from diapause stock, and those from the United States Department of Agriculture were assumed to be nondiapausing individuals (Table 2).

Spathius galinae is a gregarious larval ectoparasitoid that completes two to three generations per year (Duan *et al.* 2014). The parasitoid is produced in the laboratory using the same method as for *T. planipennisi*. Each emerald ash borer larva can support 8–16 larval *S. galinae* (Belokobylskij *et al.* 2012). Adult *S. galinae* are obtained by dissecting the ash sticks when the insects are in the pupal stage; they then complete development in emergence cages. We released *S. galinae* on an *ad hoc* schedule when sufficient numbers were provided to us by the United States Department of Agriculture, Animal and Plant Health Inspection Services rearing facility (Table 3). Adults were transported to release sites in sealed deli cups and were liberated on release trees.

Parasitoid recovery

Tree sampling. Ash trees (usually green ash, *Fraxinus pennsylvanica*) at each of the release sites were destructively sampled for parasitoids one, two, and three years after the final release. We typically cut four trees at a release site each time a site was sampled (some sites were sampled multiple times), although on one occasion, only two trees were sampled and on other occasions, five or six trees were sampled (Table 4). Some sites were also sampled more than once (Table 4). The trees we selected were alive, located near the epicentre of the release plot, showed signs of damage due to emerald ash borer (*e.g* epicormic shoots, woodpecker feeding holes, or bark splits), and were less than 25 cm (10 inches) in diameter at 1.3 m above ground level (*i.e.,* diameter at breast height or DBH, per United States Department of Agriculture 2019). The resulting samples ranged from 7.4 to 25.3 cm (mean ± 1 standard deviation: 16.6 ± 3.9 cm; Table 4). We then cut these trees into 40-cm-long sections (bolts) and transported them to the Great Lakes Forestry Centre. We sampled trees in the fall at or near the time of leaf abscission or in the spring before green-up. The bolts we collected in spring were placed into a rearing room (24–26 °C, 40–50% relative humidity, 16:8-hour light: dark photoperiod) shortly after collection; bolts collected in the fall were stored either outdoors in an unheated shipping container (Fick and MacQuarrie 2018) or in a controlled environment chamber (4 °C, 0:24-hour light:dark photoperiod) for several months before being brought into the laboratory and placed in the rearing room. In the rearing room, the bolts from an individual tree were placed in cardboard drums (1.3 m height × 40 cm diameter; Greif Lok-Rim Fibre Drums, Delaware, Ohio, United States of America) that were sealed on the bottom with a steel round ring and on the top with a steel lock ring that sealed on the plastic lid and had a modified funnel that emptied into a capture chamber. Each drum could fit 2–16 bolts, depending on the bolt diameters. One to 15 drums were required to rear all the bolts from a single tree. The drums were placed horizontally on a rack, and the capture chambers were examined for insect emergence (*i.e.,* emerald ash borer adults, *T. planipennisi*, *O. agrili*, *S. galinae*, and any native parasitoids) daily for a two-month period. At the end of

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Table 4. Mean emergence (± 1 standard deviation) of *Agrilus planipennis*, two introduced parasitoids – *Tetrastichus planipennisi* and *Oobius agrili* – and two native North American parasitoids – *Atanycolus* spp. and *Phasgonophora sulcata* – from samples of *Fraxinus pennsylvanica* taken between 2014 and 2019 at sites in Ontario and Quebec, Canada, where introduced parasitoids had been released. Mean (± 1 standard deviation) diameter at 1.3 m above ground level (DBH, diameter at breast height) is the average size of all sampled trees at a site in a given year. All samples were collected in October (unless indicated), and insects emerged the following January or February in the laboratory.

		Trees sampled	DBH	*A. planipennis*	Introduced parasitoids		Native parasitoids	
Year sampled	Site name	(n)	(cm)		***T. planipennisi***	***O. agrili***	***P. sulcata***	***Atanycolus* spp.**
2014	**Ontario**							
	Ausable Line	4	16.3 ± 3.5	158 ± 109	124 ± 215	*	20 ± 24	10 ± 17
	Brooke Line	4	12.85 ± 1.1	37 ± 22	10 ± 15	*	6 ± 5	10 ± 6
2015	**Ontario**							
	Conroy Road	4	16.1 ± 1.3	377 ± 254	10 ± 11	0	0	0.5 ± 1.0
	Middleton-McConkey Tract	4	19.8 ± 3.5	334 ± 276	5 ± 6	0	8 ± 14	13 ± 12
	Wildwood Conservation Area†	4	11.5 ± 4.2	29 ± 18	0	0	4 ± 7	21 ± 36
	Quebec							
	Gatineau Park	5	9.5 ± 1.6	82 ± 38	187 ± 142	0	0	0.6 ± 1.3
2016	**Ontario**							
	Conroy Road	4	18.4 ± 2.4	380 ± 77	41 ± 37	0	1 ± 2	104 ± 60
	Middleton-McConkey Tract	4	22.1 ± 3.8	450 ± 151	5 ± 4	0	3 ± 2	6.75 ± 3
	Wildwood Conservation Area†	4	14.25 ± 1.0	28 ± 20	13 ± 19	0	1 ± 3	5 ± 8
	Wildwood Conservation Area	4	15.8 ± 1.8	174 ± 35	107 ± 162	2 ± 4	9 ± 5	22 ± 26
2017	**Quebec**							
	Bois-de-Liesse	5	15.7 ± 5.0	2 ± 3	9 ± 21	0	0	0
	Bois Summit	4	18.2 ± 2.1	85 ± 48	36 ± 42	0	0	0.25 ± 0.50
	Jardin botanique	4	21.0 ± 3.6	69 ± 92	0	0	0	0
2018	**Ontario**							
	Metro Tract	4	17.3 ± 2.1	149 ± 107	0.25 ± 0.5	0	7 ± 14	5 ± 7

(*Continued*)

Table 4. (*Continued*)

Year sampled	Site name	Trees sampled (n)	DBH (cm)	*A. planipennis*	Introduced parasitoids: ***T. planipennisi***	Introduced parasitoids: ***O. agrili***	Native parasitoids: ***P. sulcata***	Native parasitoids: ***Atanycolus* spp.**
2019	**Ontario**							
	Baxter	4	19.5 ± 2.8	53 ± 33	4.25 ± 8.5	0.75 ± 1.50	0	6 ± 3
	Iroquois/Two Creeks	4	15.4 ± 1.3	18 ± 25	0	0	0	1 ± 2
	Metro Tract	4	17.6 ± 2.2	145 ± 116	0	0	5 ± 6	7 ± 11
	Monkland	4	17.3 ± 1.4	38 ± 34	0.25 ± 0.5	0.75 ± 0.50	0	0.75 ± 0.95
	Renfrew	4	13.8 ± 0.7	11 ± 10	10 ± 20	0	0	0
	Wilmot Creek	4	15.9 ± 1.4	37 ± 54	8 ± 16	1.75 ± 2.06	0.5 ± 1.0	5 ± 3
	Quebec							
	L'Assomption	4	19.2 ± 4.7	138 ± 130	0	0	0	13 ± 18
	Jardin botanique	2	21.4 ± 2.2	161 ± 65	0	0	0.5 ± 0.7	9 ± 9

*No sample;
†sampled in May 2014.

the two-month period, the drums were emptied, and the loose contents were collected and examined for the presence of any remaining insects.

Assessment of parasitism. All parasitoids emerging from the ash bolts were identified using the United States Department of Agriculture (2019) keys. We then used the counts of emerged emerald ash borers and parasitoids from the tree samples (above) to estimate emergence rates of parasitoids in our sampled stands. We calculated the emergence rate as the total number of adult parasitoids emerging from all trees collected at a release site in a given year, divided by the total number of adult emerald ash borers emerging from the same trees. This measure was adopted as a proxy for the parasitism rate because *T. planipennisi* is a gregarious species, with multiple adults emerging from each parasitised emerald ash borer larvae. We calculated these emergence rates for *T. planipennisi* and for two native parasitoids, *Atanycolus* spp. (Hymenoptera: Braconidae) and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), which are both commonly recorded from emerald ash borer (Cappaert and McCullough 2009; Hooie *et al.* 2015; Gaudon and Smith 2020). We recovered insufficient numbers of *O. agrili* and *S. galinae* to perform an assessment for these species. We then regressed the emergence rate of the three species against time (in years) since the last release of *T. planipennisi* at all release sites that we had data for to determine if a relationship existed between the emergence rates of native and nonnative parasitoids and the time since *T. planipennisi* had been released. We completed these analyses in the R statistical computing environment, with functions in the stats library (R Core Team 2021).

Pan trapping. We used yellow pan traps (United States Department of Agriculture 2019) to sample for parasitoid recovery in 2014 at the Ausable Line, Brooke Line, and Hay Swamp release sites, one year after final parasitoid releases at these sites. Traps were deployed on 18 August 2014 on the 12 release trees at Ausable Line and Brooke Line but on only five trees at Hay Swamp due to the high ash mortality. Traps were sampled on 3 and 16 September 2014 and again on 1 October 2014. The contents were then returned to the lab and inspected for the presence of *T. planipennisi*.

Results

Releases

Ash bolts containing *T. planipennisi* were deployed from 2013 to 2019 at 26 sites in Ontario, Quebec, and New Brunswick (Fig. 1; Table 1). At 17 sites, we released *T. planipennisi* in two or three consecutive years, whereas at nine sites we released *T. planipennisi* only once (Table 1). Emerald ash borer eggs parasitised with *O. agrili* were deployed from 2015 to 2019 at 24 sites in Ontario, Quebec, and New Brunswick: 15 sites received releases of *O. agrili* in two years, whereas nine sites had only one release (Fig. 1; Table 2). Twenty-three of these sites were also sites that received releases of *T. planipennisi*. Adult *S. galinae* were released from 2017 to 2019 at 14 sites (Fig. 1; Table 3). The number of insects available for release was limited in each of the three years and the timing of shipments to Canada varied. As a result, releases in 2017 and 2018 occurred in June and September, whereas releases in 2019 occurred in late June, early July, and late August (depending on the site). Five sites received releases of *S. galinae* in either two or three consecutive years; nine sites had only one release.

Parasitoid recovery

Tree sampling. We recovered *T. planipennisi* adults from 81% (13 of 16) of the release sites we sampled (Table 4). The total number of adult *T. planipennisi* emerging from the sample trees ranged from 1 to 937 (from 0.25 to 187 adults/tree; Table 4). *Oobius agrili* adults were recovered from 29% (4 of 14) of the release sites as of 2019 (Table 4). However, the total number of adults emerging per location was low, ranging from 2 to 8 individuals (from 0.75 to 4 adults/tree). In 2019, we sampled six sites where *S. galinae* had been released between 2017 and 2019 (Table 3).

The native parasitoids *Atanycolus* spp. and *Phasgonophora sulcata* were not released in this study but were recovered in harvested trees. We recovered *Atanycolus* spp. from 87% (14 of 16) of the release sites we sampled (Table 4). The total number of *Atanycolus* spp. emerging ranged from 1 to 169 (from 0.25 to 104 adults/tree; Table 4). *Phasgonophora sulcata* adults were recovered from 50% (8 of 16) of the release sites we sampled, with the total number of adults ranging from 1 to 56 (from 0.5 to 20.8 adults/tree; Table 4). An unidentified Eulophid wasp was also recovered in rearings from harvested trees at some of the release-infested sites. A preliminary genetic characterisation of samples of this insect suggests it is related to wasps in the genus *Pediobius* (G. Kyei-Poku, personal communication), possibly *Pediobius chylizae* Gates and Shauff (Hymenoptera: Eulophidae) (Gates *et al.* 2005). No *T. planipennisi* adults were recovered from any of the yellow pan traps in 2014.

Assessment of parasitism. We observed no increase in the emergence rate (*i.e.,* parasitism rate) by *T. planipennisi* in samples from older release sites compared to younger release sites ($F_{1,86} < 0.001$, $P = 0.99$; Fig. 3). However, emergence rates did decrease slightly for both *Atanycolus* spp. ($F_{1,86} = 3.895$, $P = 0.05$) and *P. sulcata* ($F_{1,86} = 7.156$, $P < 0.05$; Fig. 3). The average emergence rate was 2.48 adult *T. planipennisi* per adult emerald ash borer, but this estimate was highly variable, ranging from a low of 0.001 to a high of 53 *T. planipennisi* per adult emerald ash borer, with the highest variability in emergence rates occurring at sites sampled two years after final release (Fig. 3). The observed emergence rates were much lower for the two native parasitoid species, with rates of 0.232 *Atanycolus* spp. and 0.103 *P. sulcata* for each adult emerald ash borer that emerged (Fig. 3).

Discussion

This study documents the first releases and recovery of *T. planipennisi* and *O. agrili* for the control of emerald ash borer in Canada. Unfortunately, *S. galinae* was not detected in our survey, perhaps owing to the lower sampling effort relative to efforts for *T. planipennisi* and *O. agrili*. In the first six years of the programme (2013–2019), over 174 000 *T. planipennisi*, 51 000 *O. agrili*, and 6600 *S. galinae* were released at emerald ash borer–infested sites across southern Ontario, Quebec, and New Brunswick, Canada. Two of the three parasitoids were recovered in harvested trees in emerald ash borer–infested release sites within three years after release. Release and establishment of natural enemies in Canada is an important tool in emerald ash borer management and the most feasible option for the control of the insect in natural forest settings.

Early establishment of *T. planipennisi* appears high: at 81% of the sites sampled (13 of 16 sites), adults were recovered from trees harvested 1–2 years after parasitoid release. In the United States of America, *T. planipennisi* has established in at least 23% of all release sites established before 2019 (MapBioControl 2022), but because not all release sites have been examined for parasitoid establishment, this estimate may be conservative. Jennings *et al.* (2016) also reported that establishment and dispersal of this parasitoid were successful in the state of Maryland, United States of America. *Oobius agrili* recovery was lower than that observed with

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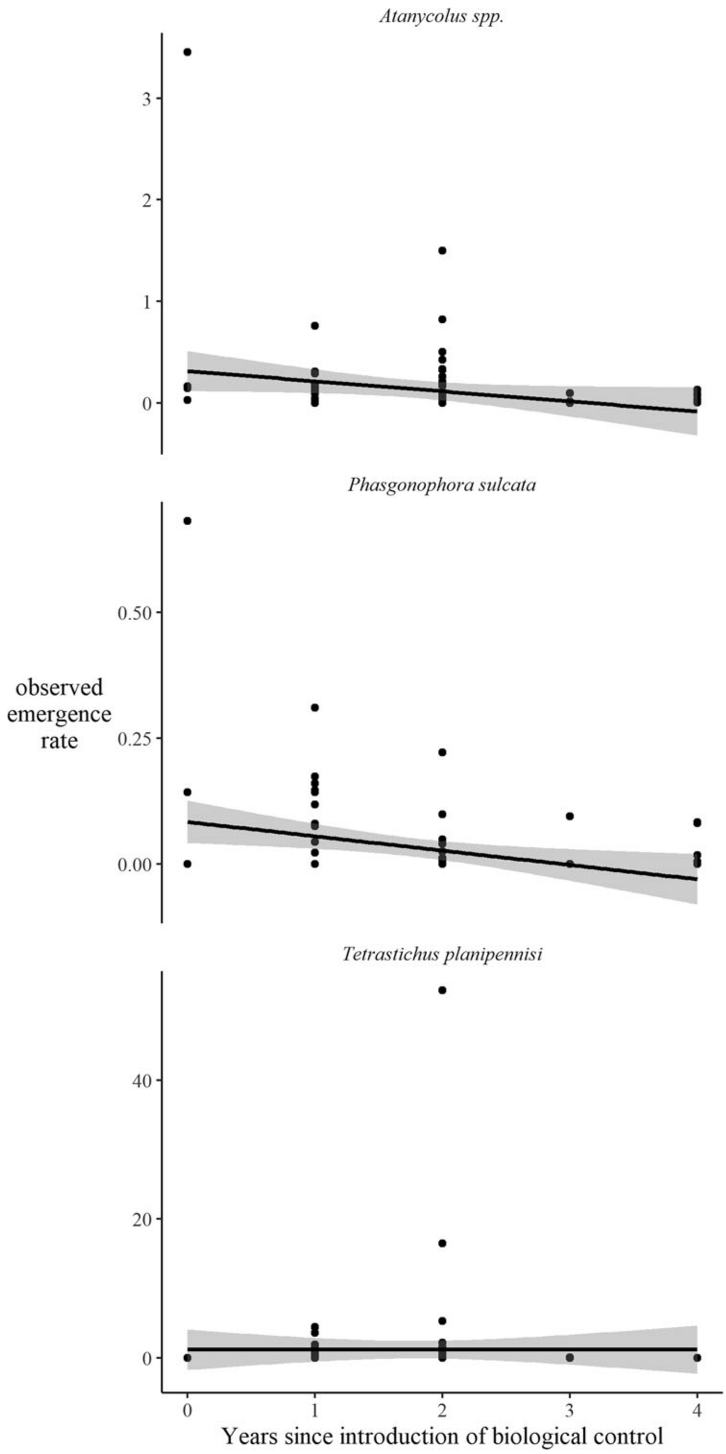

Fig. 3. The rate of emergence from *Agrilus planipennis* by two native parasitoids – *Atanycolus* spp. and *Phasgonophora sulcate* – and one introduced parasitoid – *Tetrastichus planipennisi* – from zero to four years after release of *T. planipennisi* into stands located in Ontario and Quebec, Canada. Not all stands were sampled in all years (Table 4), and regressions reflect trends across all sampled sites.

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T. planipennisi, with 29% of sites sampled (4 of 14) demonstrating adult recovery in trees harvested 1–3 years after release. Established populations of the egg parasitoid have been reported in the United States of America in at least 10% of all release sites established before 2019 (MapBioControl 2022). Sampling for *S. galinae* was done only one year after release and yielded no adult parasitoids. Duan *et al.* (2019, 2020), in contrast, reported recovery of *S. galinae* at all study sites in Connecticut, Massachusetts, Michigan, and New York state, United States of America, but those sites were sampled two years after release.

The number of adult parasitoids recovered for all three species released was low in the present study, considering the large number released over multiple years. A study by Jennings *et al.* (2016) that sampled more than 400 trees in a *T. planipennisi* recovery study reported that 60 adults were recovered from rearing, whereas most of the 1856 larvae, pupae, and adults were recovered though peeling of the bark from sample trees. This suggests that rearing of log bolts may be adequate for detection of the presence of adult parasitoids but does not provide a sufficiently accurate estimate of the number of parasitoids. Similarly, a study by Petrice *et al.* (2021b) determined that sampling for *O. agrili* by using yellow pan traps or by sifting parasitised emerald ash borer eggs from bark yielded more parasitoids compared to rearing the parasitoids from bark collected from infested trees. Duan *et al.* (2019) measured the impact of *S. galinae* two years after release by debarking trees and examining the exposed emerald ash borer larvae for evidence of parasitism. Our results suggest that in Canada, additional sampling of trees infested with emerald ash borer will be necessary to determine if the parasitoids have permanently established.

The emergence rate of *T. planipennisi* did not increase over the length of the study, but we did observe a slight decrease in the emergence of native parasitoids. This suggests that *T. planipennisi* may have quickly established its effective parasitism rate at our release sites within the first or second year of the release programme. The slight decrease in the emergence of the two native species is difficult to interpret but may suggest that some displacement of native species by the introduced species could be occurring. These estimates are also highly variable (Fig. 3), suggesting that other methods (*e.g.*, Rutledge *et al.* 2021) may be more effective than relying on emergence from infested trees for determining the true impact of introduced and native parasitoids.

Assessment of establishment from these initial releases is an important preliminary step towards developing and optimising an emerald ash borer biological control management programme in Canada. More research is necessary to refine the methods used to establish parasitoids within Canadian ash stands and to continue to evaluate the initial and permanent establishment of *T. planipennisi*, *O. agrili*, and *S. galinae*. The present study suggests that these methods have been successful for *T. planipennisi*; however, the evidence for establishment of *O. agrili* is weaker, with only about one-quarter of sites showing establishment. No evidence suggests that *S. galinae* became established after release. In addition, more work is required to determine the rate of spread of these parasitoids, their impact on emerald ash borer populations in Canada, and subsequent recovery of ash forests in Canada. Studies from the United States of America have shown that both *S. galinae* and *T. planipennisi* lower emerald ash borer populations, but these results have not been seen in Canada. Doing so will aid in determining if the emerald ash borer release programme in Canada is successful in reducing the impact of emerald ash borer.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.4039/tce.2022.32.

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