

Observations on the life-history traits of the North American parasitoid *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) attacking *Agrilus planipennis* (Coleoptera: Buprestidae) in Ontario, Canada

Lucas E. Roscoe,¹ D. Barry Lyons, Sandy M. Smith

Abstract—Under laboratory and field conditions in Ontario, Canada, we explored the natural history of *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), an indigenous solitary endoparasitoid of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Adult *P. sulcata* emergence was ~20.2–23.9 days after host emergence at 21°C, with *P. sulcata* females emerging an ~5.4–5.8 days before males. Females also represented 61.8 and 67.0% of emerging adults in the two years sampled. Mean adult longevities for males and females of *P. sulcata* were 23.8 ± 1.10 and 28.9 ± 1.11 days at 21°C, respectively. Mean potential fecundity of *P. sulcata* at emergence was 55.7 ± 2.9 eggs per female. Based on adult emergence, parasitism at two sites varied from 11.7% to 34.4%. Adult parasitoids were first observed in the field in early June, with peak capture occurring in late June. Parasitism rates in *A. planipennis*-infested ash trees was not affected by tree height below 360 cm, whereas parasitism was not observed at heights > 360 cm. Our results suggest that although mass rearing will be challenging, observed parasitism rates in conjunction with temporal and spatial synchrony indicate that indigenous *P. sulcata* may be an important source of mortality for *A. planipennis* populations.

Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive pest of ash (*Fraxinus* Linnaeus; Oleaceae) that probably arrived in North America via contaminated shipping materials from its native range in Asia in the 1990s and went undetected until 2002 (Haack *et al.* 2002; Siegert *et al.* 2014). Since its arrival, *A. planipennis* has killed millions of ash trees in Canada and the United States of America and threatens to spread throughout planted and natural ranges of ash throughout North America (Poland and McCullough 2006). Deleterious ecological effects, such as canopy gap formation and shifts in biodiversity, are anticipated in infested areas (Gandhi and Herms 2010a, 2010b; Gandhi *et al.* 2014).

Economic costs related to *A. planipennis* management in Canada and the United States of America are projected to be CAD\$1.5 billion and US\$10.7 billion, respectively (Kovacs *et al.* 2010, 2014; McKenney *et al.* 2012). Various long-term management strategies, including chemical and biological control, have been proposed and executed (Herms and McCullough 2014). Insecticide treatments of individual trees in urban areas have been shown to be effective in preventing *A. planipennis* establishment (McCullough and Mercader 2012). Due to prohibitive costs associated with application, however, widespread use in high-density sites such as forests is not yet possible (Poland and McCullough 2006). In such areas, biological control using wasp parasitoids may be a more cost-effective and environmentally

Received 13 April 2015. Accepted 31 July 2015. First published online 16 March 2016.

L.E. Roscoe,¹ Forest Protection Limited, c/o Natural Resources Canada, Canadian Forest Service – Atlantic Forestry Centre, P.O. Box 4000, 1350 Regent Street, Fredericton, New Brunswick, E3B 5P7, Canada

D. Barry Lyons, Natural Resources Canada, Canadian Forest Service – Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada

S.M. Smith, Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario, M5S 3B3, Canada

¹Corresponding author (e-mail: l.roscoe@mail.utoronto.ca).

Subject editor: Véronique Martel

doi:10.4039/tce.2015.72.

sound management tactic (Poland and McCullough 2006; Bauer *et al.* 2008).

Surveys conducted in China shortly after the discovery of *A. planipennis* in North America identified three hymenopteran parasitoids – *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) – that attack the beetle; these have since been studied and reared, and all of which have been released in several states in the United States of America (Liu *et al.* 2003; Bauer *et al.* 2008; United States Department of Agriculture, Animal and Plant Health Inspection Service 2015). Two species, *O. agrili* and *T. planipennisi*, have been released in Canada (D.B.L., personal observation). Although surveys in 2003 showed natural enemy parasitism to be low (Liu *et al.* 2003), recent studies in Ontario and several states in the United States of America indicated that parasitism of *A. planipennis* by indigenous parasitoids has increased substantially (Duan *et al.* 2009, 2010, 2012; Lyons 2010). In southwestern Ontario, natural enemy surveys suggest that the koinobiont endoparasitoid *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) attacks *A. planipennis* regularly and may be a possible candidate for use as an augmentative biological control agent (Lyons 2010). *Phasgonophora sulcata* attacks native buprestids, including *A. anxius* Gory (Loerch and Cameron 1983), *A. liragus* Barter and Brown (Barter 1965), *A. bilineatus* (Weber) (Haack *et al.* 1981), and *Chrysobothris* Eschscholtz (Bouček *et al.* 1997), and is estimated to parasitise ~40% of *A. planipennis* in some sites in southwestern Ontario (Lyons 2010). Consequently, studies evaluating the potential effectiveness of this parasitoid as an augmentative biological control agent were recently initiated (Roscoe 2014).

For a parasitoid species to be used in a biological control programme, its basic natural history as it pertains to the target host must be determined (DeBach and Rosen 1991). Adult longevity, fecundity, emergence times, and flight period are essential for both insect production and evaluating parasitoid efficacy in the field (Huffaker and Messenger 1976). Although adult longevities, sex ratios, and emergence times for *P. sulcata* reared from *A. bilineatus* are known (Haack *et al.* 1981),

its interactions with *A. planipennis* are completely unknown. Parasitoid development is greatly influenced by the species from which it is reared (Quicke 1997; Arakawa *et al.* 2004), therefore it is essential that evaluations of *P. sulcata* as a biological control agent for *A. planipennis* are based on life-history aspects of individuals reared from the target host. Observations of temporal and spatial distributions of natural populations are also important, as temporal and spatial asynchrony of the adult parasitoid with preferred host stage can limit wasps from effecting appreciable amounts of mortality required for pest management (DeBach and Rosen 1991; Collier and van Steenwyk 2004). This information for *P. sulcata* does not exist, and must be acquired if the usefulness of *P. sulcata* as an effective natural enemy is to be established.

Herein, we analyse several life-history traits for *P. sulcata* in relation to *A. planipennis* in the laboratory and the field. We examined adult longevity, sex ratios, age-specific fecundity, and the presence of superparasitism and multi-parasitism in *P. sulcata* in specimens reared in the laboratory from *A. planipennis*, and observed flight periods and emergence times for *P. sulcata* collected from several sites over several years in southwestern Ontario. We also observed distributions of *P. sulcata* within infested trees and the immature development of both *P. sulcata* and *A. planipennis* during the season. These results will provide important information that will be useful in developing potential rearing programmes using *P. sulcata* and for determining the potential effectiveness of this indigenous natural enemy in areas affected by *A. planipennis*.

Methods

Emergence, parasitism, sex ratio, and longevity

To determine the emergence pattern of *P. sulcata* relative to its host *A. planipennis*, the sex ratio of emerging parasitoids, and the rate of parasitism at emergence, infested logs were collected from sites in southwestern Ontario in December 2009 and 2010 and returned to the laboratory in Sault Ste. Marie, Ontario. Green ash (*Fraxinus. pennsylvanica* Marshall) trees were felled in a plantation at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario

(latitude/longitude: 42.69467°N, 82.40414°W) in 2009. In 2010, green ash trees were felled in a private woodlot (42.65918°N, 81.59400°W) 4.4 km west of Dutton, Ontario. The main boles of the trees were cut into ~40 cm long bolts for ease of handling and shipping. Bolts were stored in a shipping container out of doors until March of the following year and then moved indoors into a controlled environment chamber at 4 °C until required. Periodically, samples of bolts were taken out of cold storage and housed at 21 °C with a 16:8 (light:dark) photoperiod to allow beetles and their parasitoids to emerge. The length of time to emergence at 21 °C and the sex of the adult insect were recorded. The percentage of parasitoids that were female and the percentage parasitism were determined.

To obtain adults for longevity determination, parasitoids were reared from ash bolts collected from five locations in Essex County, Ontario, Canada in 2008 (42.06297°N, 82.82562°W; 42.06576°N, 82.89115°W; 42.06257°N, 82.89026°W, 42.11445°N, 82.92274°W; 42.06290°N, 82.82612°W), and from both the W. Darcy McKeough Dam Floodplain and the Samia Golf and Curling Club, Samia, Ontario (42.99265°N, 82.39883°W) in 2009 and 2010. Following emergence from the log bolts, adults were kept in sex-specific groups in 355-mL plastic cups, with up to eight parasitoids per cup. Cups were covered with plastic lids with the centres removed and replaced with aluminum mesh screens to facilitate air circulation. Insects were held at 21 °C and a 16:8 (light:dark) photoperiod. These were parameters were used as they replicated the approximate environmental conditions at the southern Ontario sites from where the insects were collected. Parasitoids were provided with water from a 12-mL vial plugged with two cotton dental wicks and a strip of synthetic fabric, and a few drops of honey smeared onto the wire mesh of the lid. Water and honey were both replaced every 2–3 days. Parasitoids were observed every 1–2 days, and time from emergence to death was recorded. Parasitism was calculated by dividing the number of emerged wasps by the total number of beetles and wasps recovered.

Age-specific potential fecundity

Females of *P. sulcata* aged 0 (*i.e.*, newly emerged), 5, 10, 15, 20, 25, 30, 45, and 50 days

old reared largely from material retrieved from the W. Darcy McKeough Dam Floodplain site in 2010 were freeze-killed by holding them at –20 °C for 10 minutes. Between 11 and 15 females were examined for each age class. Parasitoids were dissected underneath a dissecting microscope. The abdomen was first removed from the female and then cut open dorsally using a pair of micro-scissors. The ovaries were removed from the abdomen using an insect pin and were placed on a glass microscope slide. A drop of 1% acetocarmine was applied to the ovaries using a bulb pipette. Acetocarmine stained immature eggs purple, whereas mature eggs remained white as their thicker chorions prevented absorption (Edwards 1954). After one minute of absorption, the ovaries were rinsed with saline solution on the microscope slide, and a cover slip was placed over them. All eggs undergoing reabsorption, identified by the presence of degenerating chorions, and mature eggs, identified by the presence of complete chorions, were counted to determine the total mature egg complement for each ovary. The length of five mature eggs from each female was also measured using an ocular micrometer attached to a compound microscope to determine their dimensions.

Flight period

Monitoring of the adult flight periods of *A. planipennis* and *P. sulcata* was conducted at the W. Darcy McKeough Dam Floodplain site in 2010. Sticky band traps consisted of 50-cm-wide bands of plastic shrinkwrap (Catalogue Number 498385, Staples, Business Depot, Markham, Ontario, Canada) wrapped about three times around the trunks of 30 ash trees, with a mean (\pm SE) diameter at breast height (DBH) of 11.7 ± 0.41 cm (size range 7.8–18.0 cm). The bottom edge of the band was approximately 1.4 m above the ground. Bands were coated with Pestick (Catalogue Number 4002, Phytotronics Inc., Earth City, Missouri, United States of America) applied by hand. Traps were deployed on 11 May 2010 and sampled on 1 June, 16 June, 28 June, 12 July, 26 July, 16 August, and 12 September, when they were removed. Adults of *A. planipennis* and *P. sulcata* were collected off the traps using forceps and placed into vials containing a solvent (Histo-Clear II, National Diagnostics, Atlanta, Georgia, United States of

America) to dissolve the Pestick. Upon return to the laboratory, vials were immersed in water in a heated ultrasonic cleaner (Model FS30H, Fisher Scientific Company, Ottawa, Ontario, Canada) to remove the glue from the insects. Insects were sexed and counted, then pinned or stored in vials containing 70% ethanol.

Within-tree and seasonal distributions of *Phasgonophora sulcata*

Within-tree and temporal distributions of *P. sulcata* were investigated at the W. Darcy McKeough Dam Floodplain site from 27 May 2010 to 28 August 2010. The site consisted of rows of green ash, silver maple (*Acer saccharinum* Linnaeus; Sapindaceae), and poplar (*Populus* Linnaeus; Salicaceae) planted along a central gravel road. Tree rows adjacent to the road were silver maple, the next four rows were ash and the last rows beyond were poplar. Trees were planted in 1984 at regular intervals on a north–south axis and contained 30 ash trees in each row. At the time of sampling, the mean (\pm SE) height of ash trees in the stand was 10.4 ± 0.3 m ($n = 29$), and mean (\pm SE) DBH was 10.8 cm ± 0.5 ($n = 24$). Mean (\pm SE) tree bark thickness at 30 cm was 6.0 ± 0.2 mm ($n = 29$).

On each sampling date, three trees that showed beetle signs and symptoms, such as epicormic shoots, canopy dieback, and “D”-shaped exit holes (de Groot *et al.* 2006), were selected and dissected. Infestations were confirmed by dissection. Selected trees were felled, and DBH recorded. Beginning at the base of the main trunk, trees were cut into eight 60-cm-long logs (labelled sequentially from “1” to “8”). Each log section represented a height level, beginning with height level “1” at 0–60 cm, and increasing in 60-cm increments to height level “8” at 420–480 cm. For each log, the top and bottom diameters were recorded, and each was debarked with a drawknife (Veritas Carver’s, Lee Valley Tools, Ottawa, Ontario, Canada) to collect all insects.

All *A. planipennis* and *P. sulcata* individuals from larval galleries and pupal chambers were collected from each log. Current-year *A. planipennis* and *P. sulcata* exit holes were also counted. A “current” exit hole was associated with a larval gallery that contained tightly packed, light-coloured frass and displayed very little or no callous tree tissue (Timms *et al.* 2006). “Old” exit

holes, containing loosely packed, dark frass and large amounts of callous tissue, were not counted. The determination of exit holes as either “current” or “old” was based on visual inspection.

All *A. planipennis* larval specimens were placed in 20-mL scintillation vials (Kimble Chase, Vineland, New Jersey, United States of America) containing 70% ethanol. Each vial was labelled with the date of collection and the log from which they were removed. Specimens from pupal chambers were placed in single wells of a 12-well culture plate (BD Biosciences, San Jose, California, United States of America), identified by log. All insects were classified and counted by log using the categories: *A. planipennis* larva (first, second, third, and fourth instar), pupa, or adult; and *P. sulcata* early/mid-instar larva, larva-pupa, and pupa-adult. Specimens designated “early/mid-instar larva” were retrieved from *A. planipennis* larval specimens, all of which were dissected with microscissors within three to five days after collection to determine if they contained a *P. sulcata* larva. Specimens were designated as “larva-pupa” and “pupa-adult” according to the degree of sclerotisation of the thorax and abdomen: (1) larva-pupa = no visual sclerotisation, and (2) pupa-adult = sclerotisation. Host larval instar was determined by measuring peristoma width using a micrometer (Loerch and Cameron 1983). All *P. sulcata* early/mid-instars were found within *A. planipennis* larvae, whereas larva-pupa and pupa-adult *P. sulcata* were all recovered from host pupal chambers.

To minimise any potential effects of time or multiple parasitoid generations on our calculations, spatial distributions were calculated using only trees sampled before peak adult emergence from 27 May to 28 June. Total parasitism within a height level was determined by dividing the sum of all *P. sulcata* recovered by the sum of all *A. planipennis* (parasitised and non-parasitised) recovered within each height level. To determine the seasonal distribution of *A. planipennis* and *P. sulcata*, all life stages for each species were converted to a proportion of the total number of insects by date. Current-year exit holes were included for within-tree analysis but were not taken into account for the temporal analysis. The proportions of superparasitised/multiparasitised hosts out of all parasitised hosts were also determined.

Statistics

All data analyses were conducted using SigmaPlot™ 12.0 (Systat Software Inc., San Jose, California, United States of America). To compare median emergence times between *A. planipennis* and *P. sulcata*, and between males and females of *P. sulcata*, Mann–Whitney rank sum tests were used. Longevity times for adults of *P. sulcata* that emerged from ash bolts collected in all three years (2008–2010) were pooled for analysis. The survival curves were compared using Kaplan–Meier survival analysis with a Gehan–Breslow test.

A one-way analysis of variance (ANOVA) was used to determine if the mean number of viable mature eggs was significantly affected by parasitoid age. Means were compared using post hoc Tukey tests. To determine if parasitoid age had an effect on the mean number of reabsorbed eggs, a Kruskal–Wallace one-way analysis of variance on ranks test was used, with means compared using Dunn's method. Spatial distributions of *P. sulcata* were compared against height using a Kruskal–Wallis one-way analysis of ranks. A significance level of $P < 0.05$ was assumed for all statistical results.

Results

Emergence, sex ratio, parasitism, and longevity

Totals of 604 and 1373 adults (including dead adults that were detected in rearing cages at the end of rearing periods) of *A. planipennis* emerged in 2010 and 2011 from the log bolts collected in 2009 and 2010, respectively. Mean (\pm SE) emergence times for the two years were 33.3 ± 0.18 days ($n = 596$) and 32.2 ± 0.21 days ($n = 1334$), respectively (Fig. 1). In 2010, 317 adults (101 males and 216 females) of *P. sulcata* emerged from the bolts, with a mean emergence time of 53.5 ± 0.30 days (49.6 ± 0.50 days for males ($n = 101$) and 55.4 ± 0.29 days for females ($n = 216$)) (Fig. 2). In 2011, 182 adults (60 males and 122 females) of *P. sulcata* emerged from the bolts with a mean emergence time of 56.1 ± 0.96 days (52.5 ± 1.62 days for males ($n = 50$) and 57.9 ± 1.14 days for females ($n = 95$)) (Fig. 2). The percentage of females emerging from 2009

and 2010 collections was 61.8% and 67.0%, respectively. Percent parasitism of *A. planipennis* by *P. sulcata* at emergence for the two years was 34.4% for log bolts collected in 2009 and 11.7% for log bolts collected in 2010. For both years, the median emergence date for *P. sulcata* was significantly later than the median emergence date for *A. planipennis* (2010, Mann–Whitney U statistic = 2356.5, $P < 0.001$; 2011, Mann–Whitney U statistic = 908.0, $P < 0.001$) (Fig. 1). Adults of *P. sulcata* displayed protandrous emergence, with the median male emergence time significantly earlier than the median female emergence time in both years (2010, Mann–Whitney U statistic = 3096.5, $P < 0.001$; 2011, Mann–Whitney U statistic = 1641.0, $P = 0.002$). Although mean emergence dates of *P. sulcata* for the two years were similar, emergence in 2011 was more protracted than emergence in 2010 (Fig. 2). The mean longevity times for males ($n = 150$) and females ($n = 295$) of *P. sulcata* were 23.8 ± 1.10 and 28.9 ± 1.11 days, respectively. The survival curves for males and females were not significantly different (Gehan–Breslow statistic = 3.693, $df = 1$, $P = 0.055$).

Potential and age-specific fecundity

Mean total mature egg complement was not significantly different from emergence (0 days) to 50 days old (one-way ANOVA, $P > 0.05$). Mean viable egg complement (\pm SE) for adult females of *P. sulcata* at emergence (0 days old) was 55.7 ± 2.9 eggs (Fig. 3). At 50 days, mean viable egg complement (\pm SE) (34.6 ± 1.67 eggs) was significantly less than that at emergence. This was significantly less than that at 25 days, but was not significantly less than egg loads at 30 days (Fig. 3).

Oosorption was not observed until parasitoids were 30 days old (Fig. 3). The mean number of reabsorbed eggs at 30 days was significantly less than the mean number at 45 (16.5 ± 1.5 eggs) and 50 days (15.9 ± 0.7 eggs) (Kruskal–Wallace analysis of ranks $P < 0.05$). There was no significant difference between egg complements at 45 and 50 days ($P < 0.05$). Mature eggs of *P. sulcata* adult females were sausage shaped and hymenopteriform. When viewed under a dissecting microscope, the eggs appeared to be smooth, white, and opaque. Mean (\pm SE) egg length was 417.7 ± 0.01 μm ($n = 30$). Eggs were approximately three times as long as they were wide.

Fig. 1. The cumulative emergence patterns, in the laboratory at 21°C, for adults of *Agrilus planipennis* and *Phasgonophora sulcata* from ash bolts collected in southwestern Ontario, Canada in December 2009 and 2010.

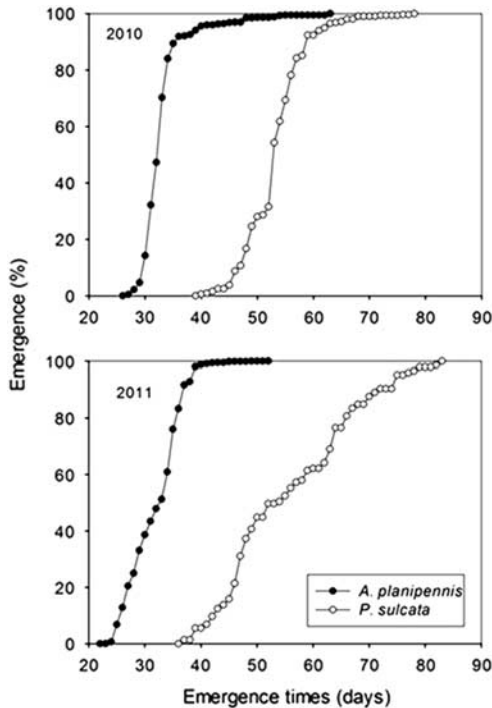
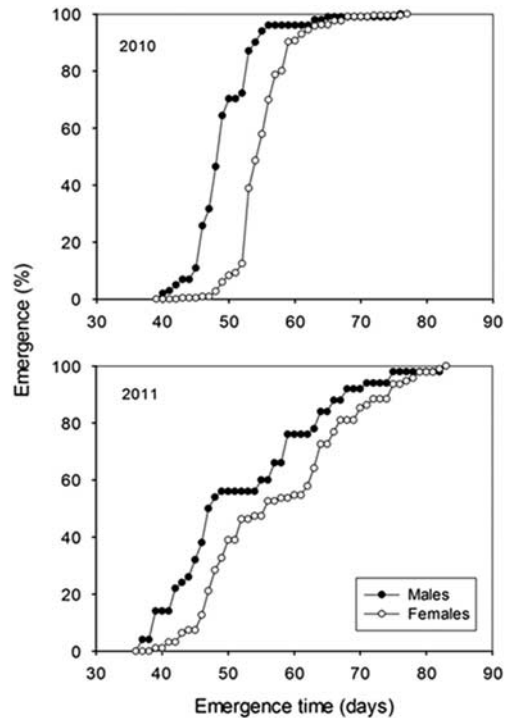


Fig. 2. The cumulative emergence patterns, in the laboratory at 21°C, for males and females of *Phasgonophora sulcata* from ash bolts collected in southwestern Ontario, Canada in December 2009 and 2010.



Flight period

The cumulative captures of *A. planipennis* and *P. sulcata* on sticky band traps are shown in Figure 4. A total of 687 adults (164 males and 525 females) of *A. planipennis* and 1170 adults (735 males and 435 females) of *P. sulcata* were captured on the traps. Based on these samples alone, parasitism rates would be estimated to be 63.0%, but these figures probably represent differential behaviour responses of males and females to the traps. In addition, the lower percentage of females (37.2%) of *P. sulcata* captured on the traps compared with males is very different than the proportion of females at emergence that we report here. The interpolated estimate of peak flight (50%) for *A. planipennis* (14 June) is 13 days earlier than the estimated peak flight of *P. sulcata* (29 June). Interpolated 95 percentile of the flight period was completed

by 8 July and 1 August for the host and the parasitoid, respectively. A few stragglers of each species were captured after 16 August.

Within-tree distribution of *Phasgonophora sulcata* and incidence of superparasitism

Parasitism by *P. sulcata* was observed at all heights up to 360 cm, but was not observed at the 360–420 and 420–480-cm height levels (Fig. 5). Parasitism was not significantly different across height levels up to 360 cm (Kruskal–Wallis one-way analysis of ranks H statistic = 1.34, $P = 0.931$, $df = 5$). Mean overall parasitism across all height levels was $23.1 \pm 2.80\%$. At the McKeough Dam in 2010, 1.6% of *A. planipennis* parasitised by *P. sulcata* were superparasitised ($n = 180$). Multiparasitism was never observed at any site.

Fig. 3. The effect of age on mature viable egg load (mean \pm SE) and number of eggs (mean \pm SE) undergoing oosorption in adult female *Phasgonophora sulcata*. Different letters indicate significant (one-way ANOVA, $P < 0.05$) differences between age categories. Capital letters represent significant differences between age categories for eggs undergoing oosorption, and lower-case letters represent significant differences between age categories for the mean number of mature viable eggs.

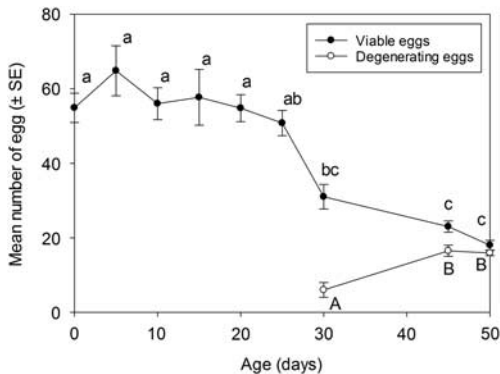
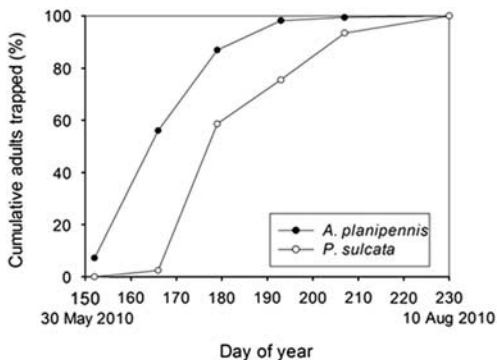


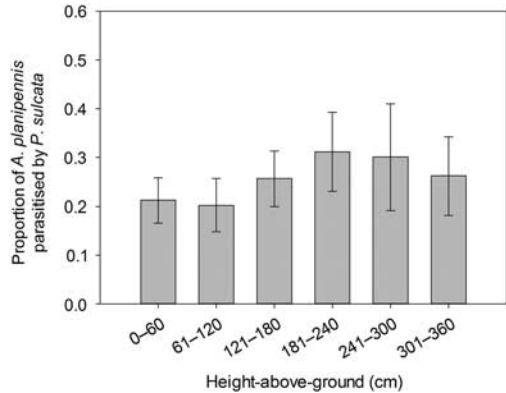
Fig. 4. The flight period of *Agrilus planipennis* and *Phasgonophora sulcata* as indicated by the cumulative capture of adults on sticky-band traps at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, Canada in 2010.



Seasonal distributions of *Agrilus planipennis* and *Phasgonophora sulcata*

On 27 May, 89.6% of *A. planipennis* were pupae residing in pupal chambers constructed in either the bark or sapwood (Fig. 6); the remaining 10.4% were second instars,

Fig. 5. Mean (\pm SE) proportion of *Agrilus planipennis* parasitised by *Phasgonophora sulcata* at different height levels above the ground in ash trees at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, Canada from 27 May to 29 June 2010.



third instars, or fourth instars that had overwintered as feeding *A. planipennis* larvae. By 6 June, many *A. planipennis* pupae had emerged as adults; *A. planipennis* remaining in trees on this date were either overwintered larvae or a few remaining pupae yet to emerge. These proportions remained unchanged until 12 July, when early instar progeny of the adult population emerging that year (2010) were first observed. From this point until the end of the summer, the proportion of early instar *A. planipennis* larvae decreased as they grew into third instars and fourth instars. On the final sampling date, 93.4% of *A. planipennis* were third instars or fourth instars, with the remaining proportion being first instars and second instars.

On 27 May, 90.0% of *P. sulcata* were found as larvae-pupae inside the *A. planipennis* pupal chambers (Fig. 7); the remaining were early instar endoparasitoids of the overwintering *A. planipennis* larval cohort. Pupa-adult *P. sulcata* were first observed on 6 June; they comprised 67.5% of the sampled population by 14 June. On 28 June, the progeny of the emerging *P. sulcata* were first observed as early instar parasitoids in *A. planipennis* larval hosts; similar proportions were also seen on 12 July. By 24 July, 80.4% of within-tree *P. sulcata* were early instar endoparasitoids of *A. planipennis* larvae; the remaining parasitoids were pupa-adult *P. sulcata* yet to emerge.

Fig. 6. Proportion of *Agrilus planipennis* within ash trees by sample date at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, Canada in 2010. The numbers above each sample bar represents the total number of *A. planipennis* individuals recovered from within sampled trees.

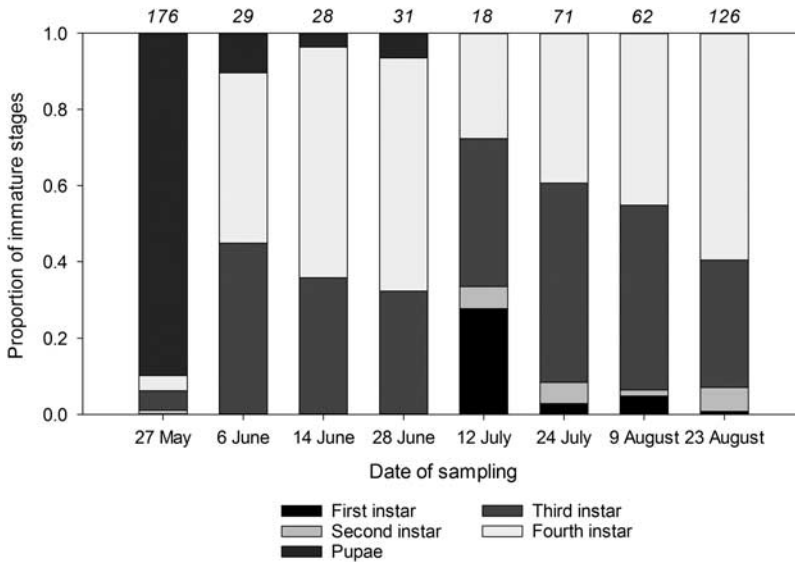
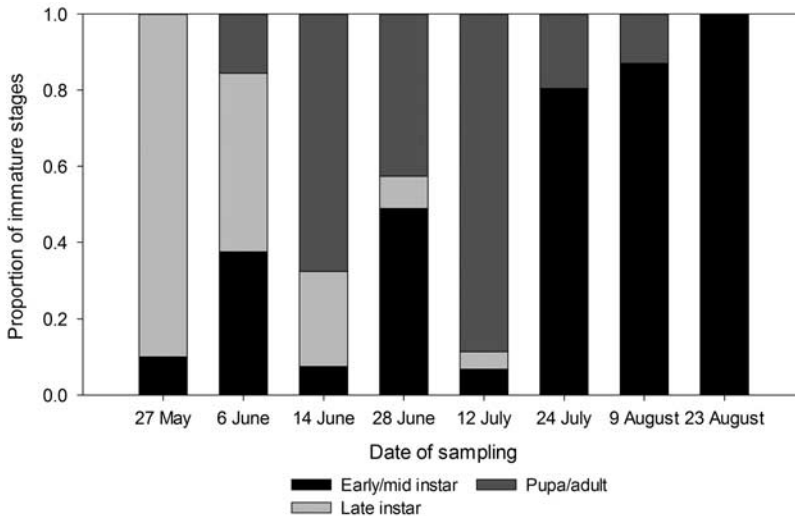


Fig. 7. Proportion of *Phasgonophora sulcata* within *Agrilus planipennis*-infested ash by sample date at the W. Darcy McKeough Dam Floodplain, Duthill, Ontario, Canada in 2010. The number above each sample bar represents the total number of *P. sulcata* individuals recovered from within sampled trees.



The early instar proportion continued to grow in subsequent sampling events until 23 August, when they comprised 100% of the within-tree *P. sulcata* population.

Discussion

We observed several life-history attributes of *P. sulcata* reared from *A. planipennis*, including

adult survival, sex ratios, emergence patterns, and age-specific fecundities. We also described the developmental cycle, within-tree distributions, and adult flight periods of naturally occurring populations. Our results represent some of the first detailed biological information on *P. sulcata* in relation to *A. planipennis*, as well as some of the first detailed documentation of a natural enemy attacking *A. planipennis* in Canada. This information will be useful in determining if *P. sulcata* is to be reared and potentially used as an augmentative biological control agent for *A. planipennis*.

Parasitism rates by *P. sulcata* at some sites, specifically the W. Darcy McKeough Dam Floodplain site, were comparable to classical biological control agents currently being used in the United States of America. In the native range of *A. planipennis*, parasitism by *T. planipennisi* and *S. agrili* is 22.4% and 30–50%, respectively (Yang *et al.* 2005; Liu *et al.* 2007). In release plots in Michigan, United States of America, parasitism by *T. planipennisi* was 21% in treatment plots (Duan *et al.* 2013a), whereas parasitism by *S. agrili* was 18% at one release site in Michigan in 2008 (Gould *et al.* 2009) but has rarely been recovered since (Duan *et al.* 2013a). Parasitism by another classical biological control agent, the egg parasitoid *O. agrili*, was 19% in treatment plots (Abell *et al.* 2014). As rates were similar or in some cases higher than those for classical biological control agents released in Michigan, and in some cases in China, it appears that *P. sulcata* could be extremely useful as an augmentative biological control agent. An important reason behind the substantial amounts of parasitism is the synchronisation of *P. sulcata* emergence with the availability of the preferred early instar host stage. At the W. Darcy McKeough Dam Floodplain, we observed that peak adult flight period was highest at the end of June just before the presence of early instars of *A. planipennis* in early July. As mean female longevity is approximately 30 days, and mean potential fecundity is still near its maximum levels at 10–15 days old, it appears that *P. sulcata* is both temporally and reproductively synchronised with the availability of the preferred early instar host larval stage. Protandrous emergence, whereby males emerged earlier than females, is also present, thus maximising the potential that females

will be mated and can produce female eggs when attacking hosts. Synchronisation between adult parasitoid emergence and availability of the preferred host stage ensures an interaction between the two insects and is critical for the success of a biological control programme (Huffaker and Messenger 1976; Collier and van Steenwyk 2004). Our emergence data are also consistent with emergence times of *P. sulcata* reared from North American *Agrilus* Curtis, such as *A. bilineatus*, which emerge ~20 days after host emergence (Haack *et al.* 1981). Synchronisation with native *Agrilus* is also important for sustaining natural enemy populations in areas where the target host is not present or if target host densities are low (DeBach and Rosen 1991; Van Driesche and Bellows 1996). It is possible that high *P. sulcata* densities associated with *A. planipennis* populations could result in adverse non-target effects on native buprestids, however, the host from which a parasitoid was reared from has been observed to influence host preference of the adult parasitoid (Storeck *et al.* 2000; Li *et al.* 2009). It may be possible that *P. sulcata* adults reared from *A. planipennis* could prefer the host which they were reared from over alternate hosts. Future studies analysing the non-target effects of *P. sulcata* on native buprestids are required to explore these potential effects.

Our results suggest that, whereas naturally occurring populations may parasitise large numbers of *A. planipennis*, mass rearing of *P. sulcata* may not be possible. Based on the solitary peak in capture of adult *P. sulcata* on 29 June and distributions in the development of immature stages thereafter, it is likely that *P. sulcata* is univoltine. Mass rearing of multi-voltine gregarious parasitoids is preferred due to the ability to generate large numbers of parasitoids from relatively few hosts over a short amount of time. For example, the Chinese parasitoids *S. agrili* and *T. planipennisi* possess generation times of ~25 and 28 days, respectively (Wang *et al.* 2007; Duan *et al.* 2011). Hosts parasitised by *T. planipennisi* can produce up to 122 individuals per host, each adult can be stored for up to 10 weeks (Duan *et al.* 2011). Based on these parameters, many thousands of these parasitoids can be produced and stored until required (United States Department of Agriculture, Animal and Plant Health Inspection Service 2012). Our results suggest that *P. sulcata* requires ~10 months to

complete one generation, with mass rearing being further hampered by the 1:1 ratio of parasitoid to host. Therefore, other means of insect production and dissemination should be investigated if *P. sulcata* populations are to be augmented. One possible method may be the attraction and/or retention of populations using attractive semiochemicals, several of which have already been observed to elicit behavioural responses in *P. sulcata* females (Roscoe 2014). This strategy has been effective in aggregating parasitoids to and increasing parasitism in areas of low natural enemy densities (Gross *et al.* 1975; Lewis *et al.* 1975; James 2003) and could be useful for increasing local *P. sulcata* population densities where required. Another possible strategy is the dispersal of logs containing parasitoids, a strategy that has been successfully used in the dispersal and establishment of parasitoids of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) (Haugen and Underdown 1990) in Australia. Studies investigating both methods of augmentation are currently underway.

Apart from heights above 360 cm where *A. planipennis* was rarely found (L.E.R., personal observation), we observed that *P. sulcata* parasitism was not statistically affected by tree height. Although parasitoid communities can vary across tree heights (Van Laerhoven and Stephen 2002), the most influential factor in parasitism of wood-boring hosts is bark thickness. Concealed hosts can only be accessed if the adult female parasitoid can penetrate the substrate in which the host resides (Quicke 1997). With the exception of species that forage through host-generated tunnels, parasitism rates will decrease if substrate thickness exceeds ovipositor length (Udayagiri and Welter 2000; Wang *et al.* 2009). Measurements of a number of ovipositors excised from *P. sulcata* adults showed that ovipositor length was ~6.4 mm, with the maximum length being 7.1 mm (Roscoe 2014). We observed here that mean bark thickness for trees at this site was 6.0 ± 0.2 mm. Therefore, as ovipositor length exceeds bark thickness, the lack of height effect observed in this study would be expected. Trunk bark thickness in ash trees correlates strongly with tree size (Timms *et al.* 2006), thus it is likely that parasitism by *P. sulcata* in the lower boles of larger trees will decrease as tree size increases. Spatial asynchrony could be a contributing factor

to lower amounts of *P. sulcata* parasitism observed at the Dutton site in 2010, where trees were considerably larger, and potentially at other sites of similar composition (Liu *et al.* 2003; Duan *et al.* 2009, 2013b). Within-tree studies similar to ours at other sites are necessary to confirm this. In sites containing trees similar in height (10.4 ± 0.3 m) and age (~26 years old) or smaller to those sampled here, *P. sulcata* parasitism should not be limited by bark thickness.

Although three exotic parasitoids in the United States of America have been studied and released for use against *A. planipennis*, little information concerning natural enemies in Canada exists. Our study represents one of the first detailed descriptions of the interactions between a natural enemy and *A. planipennis* in Canada. Although mass rearing is unlikely due to the long developmental cycle, the relatively high amounts of parasitism and synchrony between natural populations of *P. sulcata* and the preferred host stage are strong indicators of potential applicability. Although projects including the development of effective detection techniques using semiochemicals are currently underway, studies that determine the influence of stand-level characteristics such as density, species composition, and host density are required as *P. sulcata* parasitism greatly varies between sites (Liu *et al.* 2003; Duan *et al.* 2009, 2010; Roscoe 2014). Investigations into the compatibility of using *P. sulcata* in conjunction with other biological control agents are also necessary. Results from these studies will further contribute to predicting the usefulness of indigenous natural enemy populations in *A. planipennis* management in Canada and beyond.

Acknowledgements

The authors thank G. Jones (Natural Resources Canada, Canadian Forest Service) for technical assistance, and R. van Driesche, T. Smith, D.C. Darling, P. Kotanen, C. Simpson for commenting on an earlier version of the manuscript. The authors also thank S. White of the St. Clair Regional Conservation Authority for site assistance. We would also like to thank J. Lilley and C. Ortner for permission to cut trees on their properties. This work was funded by the Ontario

Ministry of Natural Resources, Natural Resources Canada, and the Faculty of Forestry, University of Toronto.

References

- Abell, K.J., Bauer, L.S., Duan, J.J., and Van Driesche, R. 2014. Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique. *Biological Control*, **79**: 36–42.
- Arakawa, R., Miura, M., and Fujita, M. 2004. Effects of host species on the body size, fecundity, and longevity of *Trissolcus mitsukurii* (Hymenoptera: Scelionidae), a solitary egg parasitoid of stink bugs. *Applied Entomology and Zoology*, **39**: 177–181.
- Barter, G. 1965. Survival and development of the bronze poplar borer *Agrilus liragus* Barter & Brown (Coleoptera: Buprestidae). *The Canadian Entomologist*, **97**: 1063–1068.
- Bauer, L.S., Liu, H., Miller, D., and Gould, J. 2008. Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America. *Newsletter of the Michigan Entomological Society*, **53**: 38–39.
- Bouček, Z., Halstead, J., Gibson, G., Huber, J., and Woolley, J. 1997. Chalcididae. Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera). National Research Council of Canada Research Press, Ottawa, Ontario, Canada.
- Collier, T. and van Steenwyk, R. 2004. A critical evaluation of augmentative biological control. *Biological Control*, **31**: 245–256.
- DeBach, P. and Rosen, D. 1991. *Biological control by natural enemies*. Cambridge University Press, New York, New York, United States of America.
- de Groot, P., Biggs, W.D., Lyons, D.B., Scarr, T., Czerwinski, E.J., Evans, H.J., *et al.* 2006. A visual guide to detecting emerald ash borer damage. Natural Resources Canada and Ontario Ministry of Natural Resources, Sault Ste Marie, Ontario, Canada.
- Duan, J.J., Bauer, L.S., Abell, K.J., Lelito, J.P., and Van Driesche, R. 2013a. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). *Journal of Economic Entomology*, **106**: 1145–1154.
- Duan, J.J., Bauer, L.S., Abell, K.J., and Van Driesche, R. 2012. Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. *BioControl*, **57**: 199–209.
- Duan, J., Fuester, R., Wildonger, J., Taylor, P., Barth, S., and Spichiger, S. 2009. Parasitoids attacking the emerald ash borer (Coleoptera: Buprestidae) in western Pennsylvania. *Florida Entomologist*, **92**: 588–592.
- Duan, J.J., Oppel, C.B., Ulyshen, M.D., Bauer, L.S., and Lelito, J. 2011. Biology and life history of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a larval endoparasitoid of the emerald ash borer (Coleoptera: Buprestidae). *Florida Entomologist*, **94**: 933–940.
- Duan, J.J., Taylor, P.B., Fuester, R.W., Kula, R.R., and Marsh, P.M. 2013b. Hymenopteran parasitoids attacking the invasive emerald ash borer (Coleoptera: Buprestidae) in western and central Pennsylvania. *Florida Entomologist*, **96**: 166–172.
- Duan, J.J., Ulyshen, M.D., Bauer, L.S., Gould, J., and Driesche, R.V. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). *Environmental Entomology*, **39**: 1513–1522.
- Edwards, R.L. 1954. The effect of diet on egg maturation and resorption in *Mormoniella vitripennis* (Hymenoptera, Pteromalidae). *Quarterly Journal of Microscopical Science*, **3**: 459–468.
- Gandhi, K.J.K. and Herms, D.A. 2010a. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests in eastern North America. *Biological Invasions*, **12**: 389–405.
- Gandhi, K.J.K. and Herms, D.A. 2010b. North American arthropods at risk due to widespread *Fraxinus* mortality caused by the alien emerald ash borer. *Biological Invasions*, **12**: 1839–1846.
- Gandhi, K.J.K., Smith, A., Hartzler, D.M., and Herms, D. A. 2014. Indirect effects of emerald ash borer-induced ash mortality and canopy gap formation on epigeic beetles. *Environmental Entomology*, **43**: 546–555.
- Gould, J., Fraser, I., Yang, Z., Wang, X., Mastro, V., and Williams, D. 2009. Update on EAB biocontrol: release and recovery of *Spathius agrili* and discovery of new potential biocontrol agents in Asia. *In Proceedings of the 20th USDA interagency research forum on invasive species 2009*; Annapolis, MD, 13–16 January 2009. *Edited by* K. McManus and K. Gottschalk. General Technical Report NRS-P-51. United States Department of Agriculture, Forest Service, Newtown, Pennsylvania, United States of America. Pp. 29. Available from <http://nrs.fs.fed.us/pubs/34230> [accessed 4 March 2015].
- Gross, H., Lewis, W., Jones, R.L., and Nordlund, D.A. 1975. Kairomones and their use for management of entomophagous insects: III. Stimulation of *Trichogramma achaeae*, *T. pretiosum*, and *Microplitis croceipes* with host-seeking stimuli at time of release to improve their efficiency. *Journal of Chemical Ecology*, **1**: 431–438.
- Haack, R., Benjamin, D., and Schuh, B. 1981. Observations on the biology of *Phasgonophora sulcata* (Hymenoptera: Chalcididae), a larval parasitoid of the twolined chestnut borer, *Agrilus bilineatus* (Coleoptera: Buprestidae), in Wisconsin. *Great Lakes Entomologist*, **14**: 113–116.
- Haack, R.A., Jendek, E., Liu, H., Marchant, K.R., Petrice, T.R., Poland, T.M., and Ye, H. 2002. The emerald ash borer: a new exotic pest in North America. *Newsletter of the Michigan Entomological Society*, **47**: 1–5.

- Haugen, D. and Underdown, M. 1990. Release of parasitoids for *Sirex noctilio* control by transporting infested logs. *Australian Forestry*, **53**: 266–270.
- Herms, D.A. and McCullough, D.G. 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. *Annual Review of Entomology*, **59**: 13–30.
- Huffaker, C.B. and Messenger, P.S. 1976. *Theory and practice of biological control*. Academic Press, New York, New York, United States of America.
- James, D.G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *Journal of Chemical Ecology*, **29**: 1601–1609.
- Kovacs, K.F., Haight, R.G., McCullough, D.G., Mercader, R.J., Siegert, N.W., and Liebhold, A.M. 2010. Cost of potential emerald ash borer damage in US communities, 2009–2019. *Ecological Economics*, **69**: 569–578.
- Kovacs, K.F., Haight, R.G., Mercader, R.J., and McCullough, D.G. 2014. A bioeconomic analysis of an emerald ash borer invasion of an urban forest with multiple jurisdictions. *Resource and Energy Economics*, **36**: 270–289.
- Lewis, W., Jones, R.L., Nordlund, D.A., and Sparks, A. 1975. Kairomones and their use for management of entomophagous insects: I. Evaluation for increasing rates of parasitization by *Trichogramma* spp. in the field. *Journal of Chemical Ecology*, **1**: 343–347.
- Li, L., Miller, D.A., and Sun, J. 2009. The influence of prior experience on preference and performance of a cryptoparasitoid *Scleroderma guani* (Hymenoptera: Bethyridae) on beetle hosts. *Ecological Entomology*, **34**: 725–734.
- Liu, H., Bauer, L.S., Gao, R., Zhao, T., Petrice, T.R., and Haack, R.A. 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. *Great Lakes Entomologist*, **36**: 191–204.
- Liu, H., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., Song, L., et al. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. *Biological Control*, **42**: 61–71.
- Loerch, C.R. and Cameron, E. 1983. Natural enemies of immature stages of the bronze birch borer, *Agrilus anxius* (Coleoptera: Buprestidae), in Pennsylvania. *Environmental Entomology*, **12**: 1798–1801.
- Lyons, D.B. 2010. Biological control of emerald ash borer. In *Workshop proceedings: guiding principles for managing the emerald ash borer in urban environments*, Burlington, Ontario, 18 November 2009. Edited by D. B. Lyons and T. A. Scarr. Ontario Ministry of Natural Resources and Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada. Pp. 29–35.
- McCullough, D.G. and Mercader, R.J. 2012. Evaluation of potential strategies to SLOW Ash Mortality (SLAM) caused by emerald ash borer (*Agrilus planipennis*): SLAM in an urban forest. *International Journal of Pest Management*, **58**: 9–23.
- McKenney, D.W., Pedlar, J.H., Yemshanov, D., Lyons, D. B., Campbell, K.L., and Lawrence, K. 2012. Estimates of the potential cost of emerald ash borer (*Agrilus planipennis* Fairmaire) in Canadian municipalities. *Arboriculture and Urban Forestry*, **38**: 81–91.
- Poland, T.M. and McCullough, D.G. 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. *Journal of Forestry*, **104**: 118–124.
- Quicke, D.L. 1997. *Parasitic wasps*. Chapman & Hall, London, United Kingdom.
- Roscoe, L.E. 2014. *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae): a potential augmentative biological control agent for the invasive *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in Canada. Ph.D. dissertation. Faculty of Forestry, University of Toronto, Toronto, Canada. Available from <https://tspace.library.utoronto.ca/handle/1807/68407> [accessed 15 March 2015].
- Siegert, N.W., McCullough, D.G., Liebhold, A.M., and Telewski, F.W. 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America. *Diversity and Distributions*, **20**: 847–858.
- Storeck, A., Poppy, G.M., van Emden, H.F., and Powell, W. 2000. The role of plant chemical cues in determining host preference in the generalist parasitoid *Aphidius colemani*. *Entomologia Experimentalis et Applicata*, **97**: 41–46.
- Timms, L.L., Smith, S.M., and De Groot, P. 2006. Patterns in the within-tree distribution of the emerald ash borer *Agrilus planipennis* (Fairmaire) in young, green-ash plantations of south-western Ontario, Canada. *Agricultural and Forest Entomology*, **8**: 313–321.
- Udayagiri, S. and Welter, S.C. 2000. Escape of *Lygus hesperus* (Heteroptera: Miridae) eggs from parasitism by *Anaphes iole* (Hymenoptera: Mymaridae) in strawberries: plant structure effects. *Biological Control*, **17**: 234–242.
- United States Department of Agriculture, Animal and Plant Health Inspection Service. 2012. Emerald ash borer, *Agrilus planipennis* (Fairmaire), biological control release and recovery guidelines. United States Department of Agriculture, Animal and Plant Health Inspection Service, Agricultural Research Service, Forest Service, Riverdale, Maryland, United States of America. Available from http://www.aphis.usda.gov/plant_health/plant_pest_info/emerald_ash_b/downloads/EAB-FieldRelease-Guidelines.pdf [accessed 16 March 2015].

- United States Department of Agriculture, Animal and Plant Health Inspection Service. 2015. Emerald ash borer program manual, *Agilus planipennis* (Fairmaire). United States Department of Agriculture, Animal and Plant Health Inspection Service, Riverdale, Maryland, United States of America. Available from http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/emerald_ash_borer_manual.pdf [accessed 16 March 2015].
- Van Driesche, R. and Bellows, T.S. 1996. Biological control. Chapman and Hall, New York, New York, United States of America.
- Van Laerhoven, S.L. and Stephen, F.M. 2002. Height distribution of adult parasitoids of the southern pine beetle complex. *Environmental Entomology*, **31**: 982–987.
- Wang, X., Yang, Z., Wu, H., Liu, S., Wang, H., and Bai, L. 2007. Parasitism and reproductive biology of *Spathius agrili* Yang (Hymenoptera: Braconidae). *Acta Entomologica Sinica*, **50**: 920–926.
- Wang, X-G., Johnson, M.W., Daane, K.M., and Yokoyama, V.Y. 2009. Larger olive fruit size reduces the efficiency of *Psytalia concolor*, as a parasitoids of the olive fruit fly. *Biological Control*, **49**: 45–51.
- Yang, Z-Q., Strazanac, J.S., Marsh, P.M., Van Achterberg, C., and Choi, WY. 2005. First recorded parasitoid from China of *Agilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae, Doryctinae). *Annals of the Entomological Society of America*, **98**: 636–642.