

Seasonal Parasitism and Host Instar Preference by the Spruce Budworm (Lepidoptera: Tortricidae) Larval Parasitoid *Tranosema rostrale* (Hymenoptera: Ichneumonidae)

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Abstract

The seasonal pattern of parasitism by a parasitoid can be influenced by many factors, such as interspecific competition and host instar preference. We conducted field and laboratory experiments to describe the seasonal pattern of parasitism of spruce budworm *Choristoneura fumiferana* (Clemens) larvae by *Tranosema rostrale* (Brischke), and to investigate whether this pattern can be explained by interaction with other parasitoid species, or by host instar preference. Larval survival, developmental time, sex ratio, and adult size of *T. rostrale* developing in different host instars were also measured to further assess the potential importance of host instar on parasitoid life history. Parasitism by *T. rostrale* increased over the season, reaching the highest rate during the fourth-instar larva, and then decreased again until the sixth-instar. At the same time, parasitism by another parasitoid, *Elachertus cacoeciae* (Howard), increased over the season, and multiparasitism with *T. rostrale* suggests potential competition between these two parasitoids. *Tranosema rostrale* showed no host instar preference when third- to sixth-instar larvae were exposed simultaneously in a manipulative field experiment. The proportion of females emerging from spruce budworm larvae increased over the season; however, no difference in sex ratio was observed in the manipulative field experiment. Only male pupal development time and adult size were marginally increased in fifth-instar spruce budworm larvae. We conclude that *T. rostrale*'s seasonal phenology or competition with *E. cacoeciae*, but not host instar preference, were possibly responsible for the observed seasonal pattern of parasitism.

Key words: *Choristoneura fumiferana*, multiparasitism, *Elachertus cacoeciae*, sex ratio

The spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) is one of the most destructive forest defoliators in North American conifer forests. This univoltine insect undergoes six larval instars per year and feeds on balsam fir *Abies balsamea* (L.) Miller (Pinaceae) and several spruce species (Greenbank 1963). Parasitism plays an important role as a mortality factor in the population dynamics of spruce budworm (Royama 1984, Régnière and Lysyk 1995, Régnière and Nealis 2007). The community composition of parasitoid species changes with the population density of spruce budworm (Eveleigh et al. 2007). In low-density populations, parasitism is an important factor in keeping population levels low over many years (Régnière et al. 2013). One of the most important parasitoids in low-density spruce budworm populations is *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae), a larval koinobiont endoparasitoid that can reach parasitism levels of over 90% (Cusson et al. 1998; Seehausen et al.

2013, 2014). Surprisingly, the influence of this parasitoid as a mortality factor in outbreaking spruce budworm populations is relatively low (McGugan and Blais 1959, J. R. unpublished data). Cusson et al. (1998) studied the basic biology of *T. rostrale*, and patterns of seasonal parasitism by this and related parasitoid species have been described in several studies (Cusson et al. 1998, 2002; Fidgen and Eveleigh 1998; Seehausen et al. 2013, 2014), although the underlying factors influencing the seasonal pattern of parasitism remain unknown.

Several factors can influence the seasonality of parasitism including the physiological response to the environment, especially ambient temperature (Powell and Logan 2005, Visser and Both 2005) and photoperiod (Koštal 2011), which determine when and where adults are active and can successfully attack their hosts (Boivin 1994, Thomas and Blanford 2003, Hance et al. 2006). In a well-synchronized parasitoid–host relationship, other factors may also be

important such as multiparasitism (parasitism of an already parasitized host) and host instar preference that influence the seasonal pattern of parasitoid attack on host populations (Vinson 1998, Harvey et al. 2013).

Interspecific competition between parasitoids (e.g., multiparasitism) can have an important influence on successful parasitism and the seasonal pattern of parasitism (Lee and Pemberton 2007, Harvey et al. 2013, Mohammadpour et al. 2014). Multiparasitism is usually only advantageous for a species if it wins the competition, generally by killing the competitor, and successfully developing in or on the host (Godfray 1994, Harvey et al. 2013). Therefore, many “inferior” parasitoid species have developed mechanisms to distinguish parasitized from unparasitized hosts in order to avoid interspecific competition (van Alphen and Visser 1990, Mackauer 1990, Tamò et al. 2006).

Many parasitoids preferentially attack certain host instars (e.g., Liu et al. 1984, Hébert and Cloutier 1990, Fuester and Taylor 1991, Fidgen et al. 2000), but not all (e.g., Mackauer 1973, Hébert and Cloutier 1990, Fuester and Taylor 1991). Host size, which increases with instar and therefore over time as an insect ages, is often associated with host instar preferences in parasitoids. When a preference exists, parasitoids often attack the largest available host (e.g., Hébert and Cloutier 1990, Wen et al. 1995, Lin and Ives 2003). Several life history traits of parasitoids have been shown to improve with increasing host size, e.g., survival of offspring (Hébert and Cloutier 1990), fecundity (King 1987, Fidgen et al. 2000), and longevity (King 1987, Hardy et al. 1992, Fidgen et al. 2000). The latter two traits are closely related to an increased size in the resulting adult parasitoid, as adult size is often linked to host size. Differential sex allocation is also associated with host size (i.e., Host Quality Model) for several parasitoid species where daughters are preferentially deposited in larger (or higher quality) hosts (e.g., Charnov 1982, King 1987, Cloutier et al. 1991, Lampson et al. 1996, Fidgen et al. 2000).

In this study, we conducted a series of field and laboratory experiments to describe *T. rostrale*'s seasonal parasitism of spruce budworm larvae and to investigate if the temporal pattern of parasitism can be explained by interaction with other parasitoid species, host instar preference, or other factors such as its phenology. In addition, several life history traits related to *T. rostrale*'s overall performance in different host instars were measured (immature survival, development time, sex ratio, and adult size) to further assess the importance of host instar in the performance of this larval parasitoid.

Materials and Methods

The experiments were conducted in two field sites near Armagh (46° 46' N, 70° 39' W, 312 m) and Petit-lac-à-l'Épaulé, Quebec (47° 18' N, 71° 12' W, 725 m), henceforth called Armagh and Epaulé. The physical environment, vegetation, and climate for these sites were described by Lethiecq and Régnière (1988). Overwintering second-instar spruce budworm larvae were obtained from the Insect Production Service of the Canadian Forest Service (Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada). Unless otherwise stated, all parasitized and unparasitized spruce budworm larvae used in the experiments were reared on current-year balsam fir foliage in growth chambers at 20°C, 60% relative humidity, and a photoperiod of 16:8 (L:D) h daily. Postdiapause spruce budworm larval instars (second–sixth) were determined visually by keeping track of the number of molts, head capsule size, and color of the

cuticle. Because developmental polymorphism exists in this species (Schmidt and Lauer 1977), only larvae clearly belonging to the desired instar were used in the experiments.

Seasonal Parasitism and Sex Ratio

To study seasonal parasitism by *T. rostrale*, spruce budworm larvae were reared on artificial diet (McMorran 1965) until the desired instar and exposed to parasitoids in the two study areas twice a week for 7 d between May and July during 2011–2015. Individual larvae were placed on current-year balsam fir shoots in the lower canopy between 1.5 and 2 m above ground. Because spruce budworm populations were low in our study sites, it was impossible to determine the exact natural seasonality for different larval instars using direct field observations; therefore, we used the Spruce Budworm Seasonal Biology Model (Régnière et al. 2012) to predict the occurrence of different larval instars in the sites. Only one or two consecutive larval instars were exposed to parasitoids in the field during each exposure period. Individuals of both instars were placed in the field in a 50:50 ratio when the model indicated the occurrence of approximately equal frequencies of two successive larval instars in the area. After returning to the lab, larvae were reared on artificial diet at room temperature until either moth or parasitoid emergence. Seehausen et al. (2013) found no significant difference in parasitism between spruce budworm larvae reared on either foliage or artificial diet. Adult parasitoids were identified using keys provided by Bennett (2008), Cusson et al. (1998), Fernández-Triana and Huber (2010), Huber et al. (1996), and O'Hara (2005). After visual assessment of apparent parasitism by the ectoparasitoid *Elachertus cacoecciae* (Howard) (Hymenoptera: Eulophidae), host remains were dissected to quantify any potential multiparasitism by *T. rostrale* and *E. cacoecciae*. When a parasitoid larva was found in the host remains, it was identified as *T. rostrale* only when the morphology of the larva matched the description by Cusson et al. (1998) and Miller and Renault (1963). Sex ratio (% females) of *T. rostrale* over the season was determined by sexing all *T. rostrale* emerging from exposed spruce budworm larvae in 2013 and 2015 from both study sites ($n = 763$).

An additional experiment was conducted in 2015 to determine whether *T. rostrale* could actually parasitize second-instar larvae in the early spring, and if so, to compare parasitism rates between second- and third-instar larvae. The implantation method described above was also used for this experiment. In Armagh, about 100 larvae in each of second- and third-instar were placed at eye level on buds of balsam fir from 13–15 May 2015, when wild second-instar spruce budworm larvae were predicted to emerge from their hibernacula in the study area (Régnière et al. 2012). After a 2-d exposure, buds with signs of larval feeding were collected and larvae were reared on balsam fir foliage in individual plastic containers. In addition, about 300 second-instar larvae overwintering in cheese cloth were pinned at eye level on branches of three balsam fir trees from 13–21 May 2015. At the end of the 8-d exposure period, larvae that migrated to buds were collected and reared until moth or parasitoid emergence. Thereafter, 200 third-instar larvae were exposed to parasitoids in the same study area from 21–28 May 2015, when the occurrence of wild third-instar larvae was predicted. In Epaulé, about 300 overwintering second-instar larvae in cheese cloth were placed during the predicted occurrence of wild second-instar larvae from 26 May–3 June 2015 and collected as described above. During the following predicted presence of wild third-instar larvae in the study area, about 300 second-instar larvae were again exposed in cheese cloth, and also 200 third-instar larvae

were placed at eye level directly on balsam fir buds from 3–12 June 2015 to compare parasitism of second- and third-instar larvae. Collected larvae were reared under the conditions described above.

Host Instar Preference and Consequences on Life History Traits

Host instar preference by *T. rostrale* was examined in the field by placing one group of four third- to sixth-instar spruce budworm larvae on current-year shoots at eye level of one or two neighboring, young balsam fir trees. Between 40 and 60 groups of all four instars were placed per exposure period depending on availability of larvae in laboratory rearing. Three exposure periods were used in each study site: 9–11 June 2014, 1–4 July 2015, and 10–15 July 2015 in Armagh; 18–20 June 2014, 24–26 June 2015, and 3–6 July 2015 in Epaulle. Exposure periods lasted only 2–3 d so that larvae did not moult to the next instar during the exposure period. Collected larvae were placed individually in plastic containers and were reared on balsam fir foliage until moth or parasitoid emergence. Adult parasitoids were identified using taxonomic keys, and emerging *T. rostrale* were sexed to assess sex ratios.

To assess survival of *T. rostrale* immatures in different host instars, 47 third- to sixth-instar spruce budworm larvae were parasitized under laboratory conditions. Prior to exposure to *T. rostrale* females, larvae were reared in growth chambers on balsam fir foliage under the conditions described above. Parasitism took place by releasing one mated *T. rostrale* female into a 37-ml transparent plastic cage with a screened window on the top for ventilation. Each cage contained approximately one 8-cm-long balsam fir twig with one spruce budworm larva feeding for 24–48 h on the current-year shoots. The female was observed until an attack took place; a successful attack was defined as the insertion of the ovipositor into the larva's cuticle. Parasitized larvae were reared in the above described 37-ml plastic cages on balsam fir foliage inserted into glass vials containing water-saturated floral foam to keep the foliage fresh. Larvae were provided with fresh foliage ad libitum and checked daily for moth or parasitoid emergence. Parasitoids successfully developing until pupa and adult were counted and developmental times were recorded.

To investigate the effect of host instar on parasitoid size, it was first necessary to establish the relationship of hind tibial length and dry mass of *T. rostrale*. Thus, the length of each right hind tibia was measured (Fig. 1) for 206 males and 243 females using a digital microscope measuring system (Wild MMS-235, Wild Heerbrugg, Heerbrugg, Switzerland). The insects were then dried for 24 h at 60°C and weighed using an electronic scale. Subsequently, hind tibial length was used as an index of parasitoid size.

Statistical Analysis

Seasonal parasitism of spruce budworm larvae, parasitism in the host instar preference experiment, and sex ratio of *T. rostrale* emerging from the instar preference experiment were analyzed using logistic regression with the binomial distribution (PROC GLIMMIX, SAS Institute Inc. 2015). Larval instar, study site, and their interaction were introduced as explanatory variables in all cases. For seasonal parasitism, years were used as replication, and separate analyses were performed for the different parasitoid species. Parasitism at different dates of the 7-d exposure periods was treated as repeated measures with a banded main diagonal covariance structure (type=UN(1)) to account for autocorrelation between probabilities of parasitism. Because the interaction term in the model for

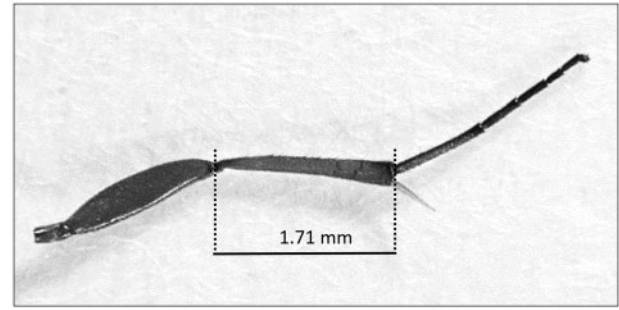


Fig. 1. Right hind leg of *T. rostrale*. Dotted lines indicate the points of measurement of hind tibial length in this study.

E. cacociae caused convergence problems, this particular analysis was only run with main effects. *Actia interrupta* was only present in sufficiently high numbers to conduct statistical analysis in the Armagh study site. For host instar preference, overall parasitism, parasitism by *T. rostrale*, and parasitism by all other parasitoid species combined, were analyzed separately. Because factors such as weather conditions influence parasitism (Porter 1983, Turnock et al. 1995, Wang et al. 1997), and the experiment was repeated at different dates, the date of the exposure period of host larvae was introduced as a random effect with a standard covariance structure (type=VC). The model containing the random effect was tested against the null model of complete independence based on the Residual Pseudo-Likelihood using the COVTEST statement. For parasitism other than by *T. rostrale*, data were analyzed without the random effect using the DSCALE option to account for overdispersion of the data (PROC GENMOD, SAS Institute Inc. 2015).

Seasonal changes in sex ratio were assessed using logistic regression with the binomial distribution and spruce budworm larval instar as explanatory variables. The DSCALE option was used to account for overdispersion of the data (PROC GENMOD, SAS Institute Inc. 2015). Fisher's Exact Test was used (PROC FREQ, SAS Institute Inc. 2015) to compare parasitism rates on second- versus third-instar larvae. The tibia length of parasitoids emerging from different host instars was analyzed using ANOVA (PROC GLM, SAS Institute Inc. 2015) with sex, larval instar, and the interaction of sex and instar as explanatory variables; Tukey's range test was used for the multiple comparisons of means. Larval and pupal survival of parasitoids in (or egressing from) different host instars was analyzed using separate Chi-square tests (PROC GENMOD, SAS Institute Inc. 2015), with host instar as an explanatory variable. The developmental time of parasitoids for different host instars was analyzed using nonparametric Wilcoxon Scores (Rank Sums) followed by a Kruskal-Wallis Test (PROC NPAR1WAY, SAS Institute Inc. 2015) because the data did not meet assumptions of normality and variance stability even if transformed to development rate; host instar was used as explanatory variable. Multiple comparisons of means were done using the Dwass, Steel, Critchlow-Fligner (DSCF) method, which is based on pairwise two-sample Wilcoxon comparisons (Dwass 1960, Steel 1960, Critchlow and Fligner 1991).

The relationship between hind tibia length and dry mass was analyzed using a generalized linear model with sex, dry weight, and an interaction term as explanatory variables (PROC GLM, SAS Institute Inc. 2015). A log-transformation of dry weight was performed because data did not meet assumptions of normality and variance stability.

Results

Seasonal Parasitism and Sex Ratio

Spruce budworm larvae (third- to sixth-instar) were parasitized by nine parasitoid species (Table 1). *Tranosema rostrale* and *E. cacoeciae* were the most frequent parasitoids in both study sites. *Actia interrupta* was more common in Armagh, although also emerging from a single host larva in Epaulé (Table 1). Over the 5-yr period, host instar (or the date of host implantation) had a significant influence on overall parasitism, and on parasitism rates of three separate parasitoid species, but not on parasitism by other species combined. Neither site nor the instar \times site interaction had a significant influence on parasitism rates (Table 2a); as a result, parasitism of different host instars over the season was pooled for both sites. Parasitism rates by *T. rostrale* increased from the third- to fourth-instar, and decreased in later instars. *Elachertus cacoeciae* did not parasitize third-instar larvae but parasitism rates on later instars steadily increased to a maximum in sixth-instar larvae. Parasitism by *A. interrupta* only occurred among larvae exposed as a mix of fourth- and fifth-instars and later, and was highest amongst larvae exposed as a mixture of fifth- and sixth-instars (Fig. 2). Multiparasitism by *T. rostrale* and *E. cacoeciae* occurred in all study sites and at all times during the season when both parasitoids were present but was always $<5\%$. Only one incidence of apparent multiparasitism by *T. rostrale* and *A. interrupta* occurred (Table 1). The proportion of females among *T. rostrale* adults emerging during the season was positively correlated with spruce budworm instar (third–sixth) exposed in the field according to the natural seasonal timing (Fig. 3; $F = 54.55$; $df = 1, 16$; $P < 0.0001$).

During the period of predicted occurrence of wild second-instar spruce budworm larvae, no parasitism of implanted second- ($n = 51$) and third-instars ($n = 19$) was observed in Armagh or Epaulé ($n = 34$ second-instars). However, 35% ($n = 23$) of third-instar larvae exposed at the time when natural third-instars could be expected in Armagh were parasitized by *T. rostrale*. In Epaulé, parasitism of second-instars by *T. rostrale* was significantly lower (3.51%, $n = 57$) than of third-instars (11.86%, $n = 59$, $P = 0.0306$) at the time that natural third-instars were occurring. While only *T. rostrale* attacked third-instars on either site, one second-instar larva was parasitized by *Enytus montanus* (Ashmead) (Hymenoptera: Ichneumonidae) during this period in Epaulé.

Host Instar Preference and Consequences on Life History Traits

Tranosema rostrale was by far the dominant parasitoid in both Armagh 42.14% \pm 6.65 SEM and Epaulé 44.40% \pm 21.43 SEM. The only other parasitoid species present in both study sites during this experiment was *E. cacoeciae*, although it was generally more common in Epaulé (14.21% \pm 13.67 SEM) than in Armagh (1.92% \pm 0.93 SEM). *Actia interrupta* (3.37% \pm 3.37 SEM) and *Phytodietus* sp. (1.26% \pm 0.79) were only present in Armagh. Neither parasitism by *T. rostrale*, nor by all other species combined, was significantly influenced by site, host instar, or their interaction (Table 2b; Fig. 4). The variance in parasitism by *T. rostrale* between the dates of exposure periods for host larvae (random effect) was significant ($\chi^2 = 81.14$; $df = 1$; $P < 0.0001$) and estimated as 94%. Mean overall parasitism in the field choice test was 50.75% (\pm 5.91 SEM; $n = 256$) in Armagh and 60.81% (\pm 28.54 SEM; $n = 307$) in Epaulé, and was significantly influenced by the interaction of site and host instar (Table 2b; Fig. 4), although there were no significant differences between the means. Host instar also significantly influenced overall parasitism as a main factor (Table 2b); comparisons of

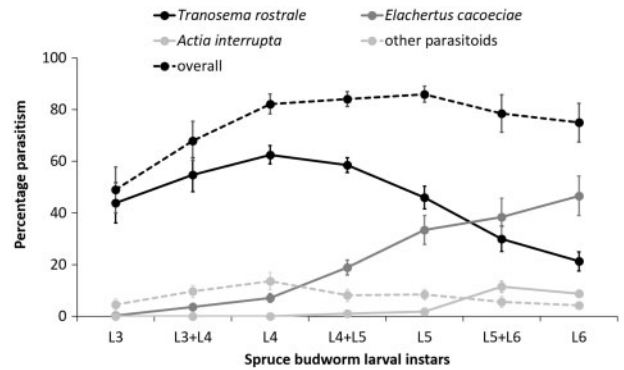


Fig. 2. Mean (\pm SEM) percentage parasitism of different spruce budworm larval instars over spring and summer in Armagh and Epaulé (Quebec, Canada) between 2011 and 2015 (pooled), by *T. rostrale*, *E. cacoeciae*, *A. interrupta*, and all other parasitoids.

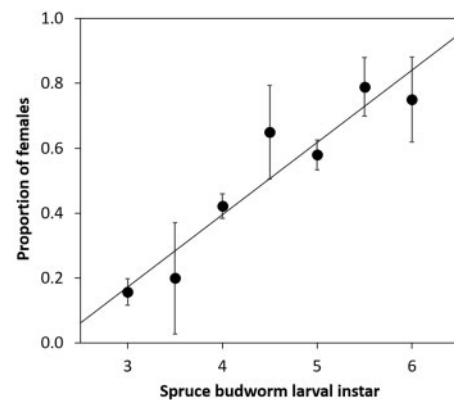


Fig. 3. Mean (\pm SEM) proportion of female *T. rostrale* emerging from spruce budworm larvae attacked in different instars (third to sixth) in the field at the time of natural occurrence in Armagh and Epaulé, Quebec, Canada ($n = 763$).

means showed that fifth-instar larvae were significantly more parasitized than third-instar larvae. Study site did not significantly influence overall parasitism. The random effect did significantly influence overall parasitism ($\chi^2 = 96.06$; $df = 1$; $P < 0.0001$), with its variance estimated as 99%.

The proportion of female *T. rostrale* emerging from spruce budworm larvae exposed to parasitoids during the field choice test (0.56 \pm 0.05) was not significantly influenced by host instar ($F = 2.24$; $df = 3, 17$; $P = 0.1211$). Significant positive correlations were found between the hind tibial length and insect dry mass for both *T. rostrale* males and females ($F = 620.07$; $df = 1$; $P < 0.0001$; $R^2 = 0.66$); however, females were heavier and had longer hind tibia than males (Fig. 5; $F = 332.93$; $df = 2$; $P < 0.0001$). Because the interaction term was not significant, it was removed from the analysis. The length of *T. rostrale* hind tibia was significantly influenced by sex ($F = 30.53$; $df = 1$; $P < 0.0001$), with female tibia being longer than male tibia. There was also a significant influence of host instar ($F = 7.28$; $df = 3$; $P = 0.0001$), with a significant instar \times sex interaction ($F = 3.78$; $df = 3$; $P = 0.0120$). The length of female hind tibia did not significantly change with host instar among females, but first increased from third- to fifth-instar and slightly decreased again in sixth-instar larvae for males (Fig. 6).

Survival and development time were only analyzed for males because only a single female emerged from the laboratory rearing.

Table 1. Overall percentage parasitism of third- to sixth-instar spruce budworm larvae by different parasitoid species in Armagh and Epaule, Quebec, Canada, 2011–2015

Parasitoid species	Armagh					Epaule				
	2011	2012	2013	2014	2015	2011	2012	2013	2014	2015
N	272	221	325	342	341	306	368	340	389	448
<i>Tranosema rostrale</i>	46.7	43.0	36.3	25.4	23.5	50.3	40.2	45.3	32.4 ^a	48.7
<i>Elachertus cacoeciae</i>	25.0	9.5	17.2	18.4	46.3	24.5	3.5	31.8	45.8	27.9
Multiparasitism by <i>T. rostrale</i> and <i>E. cacoeciae</i>	0.0	1.8	1.9	0.0	2.6	0.7	0.8	3.2	0.5	4.7
<i>Actia interrupta</i>	4.8	5.4	1.5	6.1	4.4	0.0	0.0	0.0	0.0	0.0
<i>Phytodietus</i> sp.	2.2	2.3	4.3	0.6	0.3	0.3	0.0	0.6	1.5	0.2
All other hymenopteran species ^b	5.2	14.0	6.2	4.7	4.4	8.8	11.1	7.9	5.9	1.3
Total	83.8	76.0	67.4	55.3	81.5	84.6	55.7	88.8	86.1	82.8

^aOne incidence of apparent multiparasitism with *A. interrupta* occurred.

^bIncludes one incidence of parasitism by *Agrypon prismaticum* (Norton) in each site, two incidences by *Smidtia fumiferanae* (Tothill) and *Exochus nigripalpis tectulum* Townes & Townes, respectively, and one by *Apanteles petrovae* Walley in Armagh, and several incidences by other nonidentified parasitoids in both study sites.

Table 2. Logistic regression analysis of (a) seasonal parasitism (2011–2015) and (b) host instar preference of *T. rostrale* and other parasitoid species attacking spruce budworm in Armagh and Epaule, Quebec, Canada

Parasitoid species	Host instar			Site			Host instar × Site		
	F	df	P	F	df	P	F	df	P
(a) Seasonal parasitism									
<i>Tranosema rostrale</i>	13.2	6,18.79	<0.0001	2.35	1,40.43	0.1333	1.4	6,18.79	0.2657
<i>Elachertus cacoeciae</i>	14.69	6,21.93	<0.0001	0.48	1,40.78	0.4924	–	–	–
<i>Actia interrupta</i>	3.35	6,26	0.0141	–	–	–	–	–	–
All other species	1.62	6,18.58	0.1979	0.11	1,41.77	0.7467	1.63	6,18.58	0.1932
Overall	3.73	6,18.63	0.0129	1.33	1,46.11	0.2556	0.48	6,18.83	0.8150
(b) Host instar preference									
<i>Tranosema rostrale</i>	0.72	3,16	0.5520	0.05	1,3.87	0.8401	0.75	3,16	0.5375
All other species	2.02	3,16	0.1523	0.00	1,16	1.0000	0.03	3,16	0.9940
Overall	3.97	3,16	0.0272	0.04	1,3.85	0.8511	3.50	3,16	0.0401

Survival of *T. rostrale* reared under laboratory conditions in spruce budworm larvae did not vary significantly by host instar, either from egg to pupa ($\chi^2=3.04$; df=3; $P=0.3849$) or from pupa to adult ($\chi^2=3.20$; df=3; $P=0.3614$). Host instar had no significant effect on the parasitoid's larval development time ($\chi^2=4.53$; df=3; $P=0.2096$), but affected the development time of pupae ($\chi^2=9.03$; df=3; $P=0.0289$). However, multiple comparisons of means revealed no significant differences in development times among host instars. Total development time was unaffected by host instar ($\chi^2=6.11$; df=3; $P=0.1066$).

Discussion

The experiments show conclusively that host instar preference does not influence the observed seasonal pattern of spruce budworm parasitism by *T. rostrale* in the two field sites. In addition, the host instar attacked by *T. rostrale* has little effect on indirect measures of fitness, such as survival, development time, and tibial length. The absence of specialization on a particular host instar may be one of the reasons why this parasitoid is so efficient as a mortality factor in low-density spruce budworm populations. The high parasitism rates of *T. rostrale* found in this study are remarkable (e.g., >50% in spruce budworm larvae exposed for only 2–3 d in the field) and were reported in other studies using the same sampling method (Seehausen et al. 2013, 2014). Parasitoids with a narrower seasonal

window and host instar preference would be less successful in low-density spruce budworm populations, perhaps explaining the change in parasitoid community composition with population density (Eveleigh et al. 2007).

The seasonal pattern of parasitism described in this study for a 5-yr period in two study sites confirm the findings from single- and two-year studies (Cusson et al. 2002, 1998; Seehausen et al. 2013, 2014). It can be assumed that the early increase in parasitism by *T. rostrale* is due to the phenology of the parasitoid, i.e., its early activity after diapause (Cusson et al. 1998). However, nothing is known about the overwintering life stage of *T. rostrale*. Cusson et al. (1998) reported that *T. rostrale* successfully attacked second-instar spruce budworm larvae in a laboratory choice test, albeit at very low frequency (1.33%). Our results from the field also suggest that second-instar larvae can be successfully parasitized; however, active adult *T. rostrale* females may not be present in the field at the same time as postdiapause second-instar larvae.

Concurrent with the decrease in parasitism by *T. rostrale* after the fourth-instar, parasitism by *A. interrupta* and *E. cacoeciae* increased. Multiparasitism of spruce budworm larvae by *A. interrupta* and *T. rostrale* has been shown by Cusson et al. (2002), but is generally too low to have much influence on seasonal parasitism by *T. rostrale*. Here, we report only one instance of apparent multiparasitism by *T. rostrale* and *A. interrupta*; however, we observed numerous cases of multiparasitism of spruce budworm larvae by *T. rostrale* and *E. cacoeciae*. No information is available about the

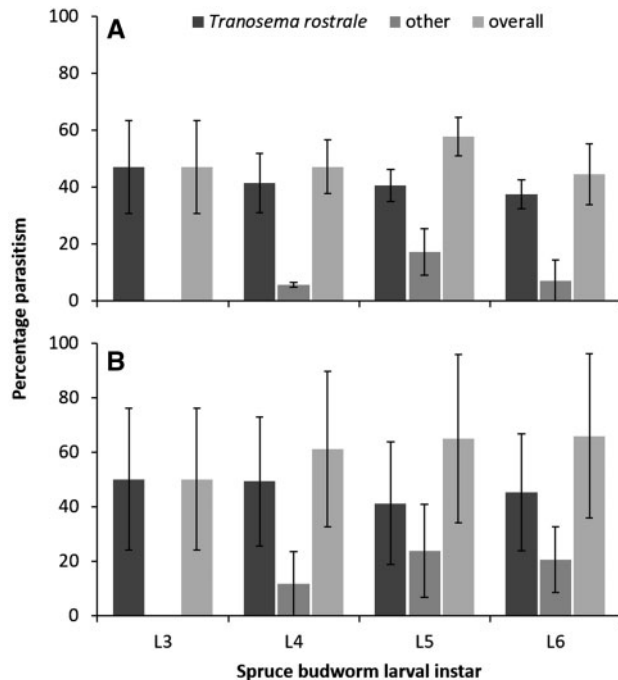


Fig. 4. Mean (\pm SEM) percentage parasitism (by *T. rostrale*, by all species other than *T. rostrale*, and overall) of third- to sixth-instar spruce budworm larvae simultaneously exposed to parasitoids in (A) Armagh ($n=256$) and (B) Epaule ($n=307$), Quebec, Canada, 2014 and 2015.

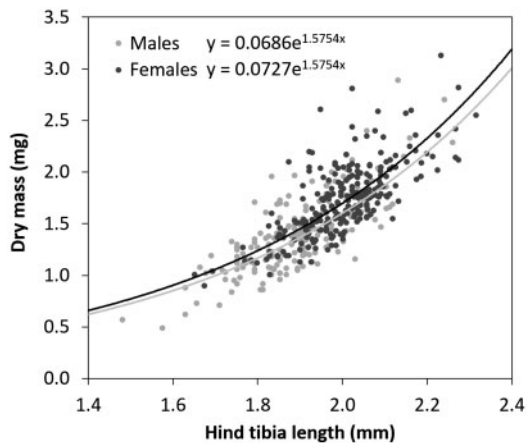


Fig. 5. Observed (circles) and predicted (lines) length of hind tibia (mm) as a function of dry mass (mg) for *T. rostrale* males (gray; $n=206$) and females (black; $n=243$) from Armagh and Epaule, Quebec, Canada. Data are back-transformed from log.

outcome of competition between these two species, although *E. cacoeciae* may have a competitive advantage over *T. rostrale* (Harvey et al. 2013) because it is an ectoparasitoid that paralyzes its host upon oviposition (Mills 1992). Future studies should investigate whether competition between these two species will influence their apparent seasonal patterns of occurrence.

Parasitoids are known to evaluate their host before laying an egg with the help of sensillae at the tip of their ovipositor (Arthur et al. 1969, 1972, Fisher 1971, Hegdekar and Arthur 1973). Thus, in addition to external traits such as host size, internal traits related to host quality may be assessed by the female at oviposition

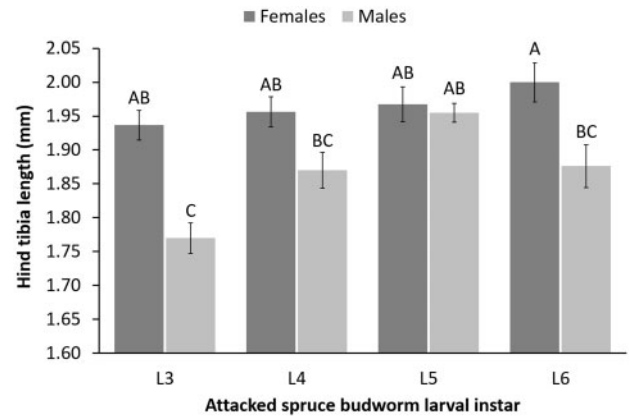


Fig. 6. Mean (\pm SEM) hind tibia length (mm) of *T. rostrale* male ($n=63$) and female ($n=87$) progeny emerging from spruce budworm larvae that were attacked during different instars (third to sixth), in Armagh and Epaule, Quebec, Canada, 2014 and 2015. Bars followed by the same uppercase letter are not significantly different at $P < 0.05$ according to post hoc multiple comparisons of means using Tukey's range test.

(e.g., nutritional value, ingested secondary plant compounds, presence of another parasitoid). For *T. rostrale*, avoiding multiparasitism may be more important than host size in terms of long-term fitness. The frequency of observed multiparasitism is relatively low when compared to parasitism by *T. rostrale* and *E. cacoeciae* alone, which may indicate the parasitoids' ability to avoid previously parasitized larvae. As well, host age may be more important than host size, especially when attacking sixth-instar larvae shortly before pupation (Doucet and Cusson 1996). Because we did not distinguish host age within an instar, this hypothesis is beyond the scope of the present study. Finally, *T. rostrale* is a generalist attacking several other lepidopteran species (Cusson et al. 1998), and therefore differences between host species may be more important for *T. rostrale* than host size, as it must be able to parasitize alternative hosts of different size and quality.

Our study demonstrates that at the beginning of the season, *T. rostrale* lays a higher proportion of male eggs when smaller hosts are present than later in the season when it lays a higher proportion of female eggs into larger hosts. The sex ratio was not affected by host instar in our choice tests, suggesting that some other factor may cause the observed seasonal shift in sex ratio. *Tranosema rostrale* is arrhenotokous, so fertilized eggs develop into females and unfertilized eggs into males. Increasing the probability of females being mated over the season could lead to the observed increasing proportion of daughters. The mean longevity of *T. rostrale* females reared under laboratory conditions at 20°C and feeding on sugar water was found to be 45 d (M.L.S., unpublished data), suggesting they could live long enough in the field to experience increased mating over their lifetime. Alternatively, females may be produced preferentially later in the season because of decreasing host densities as the season progresses (Comins and Wellings 1985, King 1987).

Tibial length of *T. rostrale* males from fifth-instars was significantly longer than that from third- and sixth-instars. Hind tibial length is a relatively common measure of parasitoid size (e.g., Waage and Ming 1984, Rosenheim and Rosen 1991). We found a significant positive correlation between hind tibia length and dry mass of *T. rostrale*, confirming tibia length as a general measurement of this parasitoid's size. However, only 66% of the variation in dry mass was explained by hind tibial length. Therefore, this measure has to be used with caution, and other measurements (e.g., wing length) may be a

better measure for dispersal or other flight-related factors (Harrison 1980, Malmqvist 2000). Generally, larger parasitoid males live longer (King 1987) and have a greater mating success than smaller ones (Grant et al. 1980, Charnov et al. 1981, Jones 1982); however, we found no relationship between male size and mating success in *T. rostrale* (M.L.S., unpublished data).

The influence of host instar on parasitoid survival and development time was measured for male parasitoids because only one female emerged. Male-biased sex ratios in laboratory-reared *T. rostrale* have been reported before (Cusson et al. 1998). Sex-specific survival is difficult to measure because currently, no method exists to sex *T. rostrale* immatures. We found that *T. rostrale* female pupae developed ~10% slower than males (M.L.S., unpublished data); however, there is no reason to assume that host instar has a sex-specific influence on development time of *T. rostrale*. Higher mortality of females may occur under natural conditions because of the longer pupal development time.

Given that *T. rostrale* shows no host instar preference when given the choice between all postdiapause instars, and that its performance in terms of survival, sex ratio, and total development time is unaffected by host instar, both suggest that the seasonal pattern of parasitism by this species is likely the result of other factors, such as its phenology (e.g., time of emergence from overwintering), competition with other parasitoids (e.g., *E. cacoeeciae*), or a combination of both. The field components of our study took place in two sites that vary considerably in environmental conditions (Lethiecq and Régnière 1988). It is possible that the phenology of *T. rostrale* and interspecific competition with other parasitoids could vary spatially and may affect local patterns of seasonal parasitism. To clarify more precisely what drives the seasonal pattern of parasitism by *T. rostrale*, future studies should investigate the phenology and voltinism of *T. rostrale*, the outcome of competition with other parasitoid species, and the pattern of seasonal parasitism in other parts of its distribution and on other host species.

Supplementary Data

The replication data underlying this research are freely available to the public through the Harvard Dataverse repository. The DOI for the dataset is: <http://dx.doi.org/10.7910/DVN/WO1XPG>.

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