Does spruce budworm (Lepidoptera: Tortricidae) rearing diet influence larval parasitism?

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Abstract—Artificial diet is commonly used to rear the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in the laboratory. While its effect on spruce budworm performance is relatively well studied, no information exists about the influence of rearing diet on larval parasitism. In this study, spruce budworm larvae reared in the laboratory on artificial diet or balsam fir, *Abies balsamea* (Linnaeus) Miller (Pinaceae), foliage were introduced in the field to compare parasitism. Additionally, a laboratory choice test was conducted with the larval parasitoid *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae). No significant influence of spruce budworm rearing diet on parasitism in the field was found. However, in the laboratory, *T. rostrale* attacked significantly more foliage-fed larvae. We conclude that even if initial differences in parasitism may exist between diet-fed and foliage-fed larvae in the laboratory, spruce budworm larvae reared on artificial diet can be used in field studies investigating parasitism of wild spruce budworm populations without concern that the food source would affect parasitism.

Résumé—Dans la présente étude, des larves de la tordeuse des bourgeons de l'épinette (TBE), *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), ont été élevées en laboratoire sur nourriture artificielle ainsi que sur du feuillage de sapin baumier, *Abies balsamea* (Linnaeus) Miller (Pinaceae), pour ensuite être introduites en forêt afin de comparer leur parasitisme. Par ailleurs, un test de choix en laboratoire a été effectué avec le parasitoïde *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae). Aucune influence significative de la diète sur le parasitisme n'a été démontrée sur le terrain. Cependant, un nombre significativement plus grand de larves élevées sur le feuillage ont été attaquées par *T. rostrale* en laboratoire. Malgré la préférence pour les larves élevées sur le feuillage en laboratoire, nous concluons que des larves élevées sur la diète artificielle peuvent être utilisées dans la détermination des taux de parasitisme sur le terrain sans risque de biaiser les résultats obtenus.

The eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is commonly reared on artificial diet (McMorran 1965) for both laboratory and field experiments. Certain differences exist between foliage-fed and artificial diet-fed larvae, such as higher survival of larvae, increased pupal weight, adult longevity, fecundity and development times (McMorran 1965). Poirier and Borden (2000) observed that foliage-fed larvae did not respond to the oral exudates of artificial diet-fed larvae, whereas exudates from foliage-fed larvae had a deterrent effect on the feeding behaviour of both foliage-fed and artificial diet-fed larvae. Several studies used spruce budworm larvae reared on artificial diet for exposure in the field to investigate parasitism and parasitoid behaviour in endemic budworm populations (Doucet and Cusson 1996a, 1996b; Fidgen and Eveleigh 1998; Fidgen *et al*. 2000; Cusson *et al*. 2002).

It is well known that variation in the food consumed by herbivores can change their chemical composition and affect their susceptibility to predator attack (reviewed by Price *et al*. 1980). For instance, several plant compounds such as toxins assimilated by Lepidoptera can

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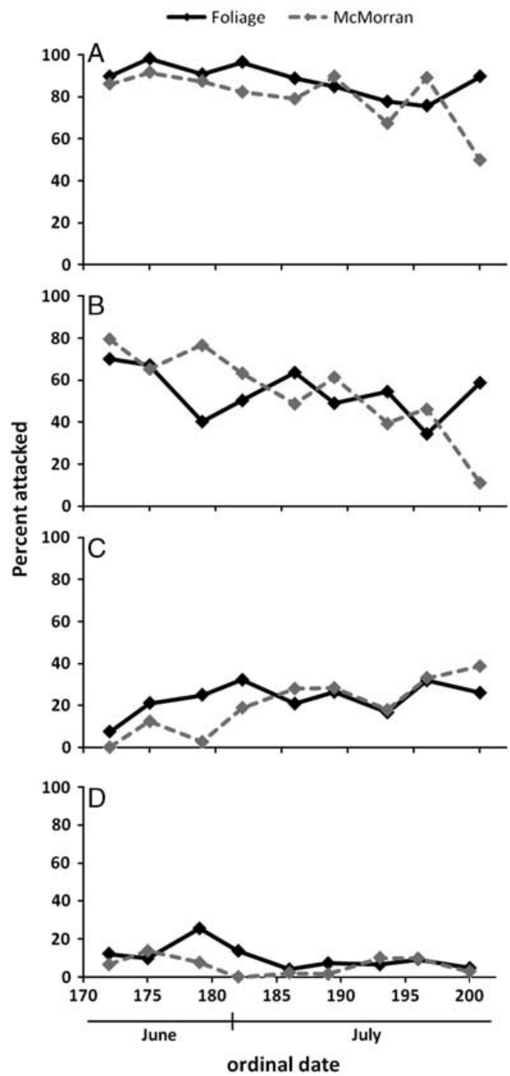
alter parasitoid performance (reviewed by Ode 2006). However, few studies have dealt with influences of artificial diets compared to natural diets of Lepidoptera on parasitism (but see Song *et al*. 1997; Havill and Raffa 2000). So far only the effect of host diet on parasitism of spruce budworm eggs had been tested (Song *et al*. 1997) but no attempt has been made to test if larval parasitoids of the spruce budworm are influenced by the artificial diet ingested by their host. Therefore, both field and laboratory experiments were conducted to test the use of diet-fed spruce budworm larvae for parasitism studies.

The field experiment was conducted in summer 2011 in the context of a larger study on the influence of partial cutting on parasitism (Seehausen 2012), in Laval University's Montmorency experimental forest (47°20′N, 41°30′W). This boreal forest is located in the Laurentian Mountains, 70 km north of Quebec City, Québec, Canada, in a balsam fir (*Abies balsamea* (Linnaeus) Miller; Pinaceae) – white birch (*Betula papyrifera* Marshall; Betulaceae) forest. Four plots, each ~4 ha in size, were located in unaltered, mature forests. Plot elevations ranged from 650 to 800 m on a north-eastern slope.

Diapausing second instar spruce budworm larvae were obtained from the Canadian Forest Service, Sault Ste. Marie, Ontario, Canada, and reared at 23 °C and a 16-hour photoperiod on either antibiotic-free artificial diet (modified from McMorran 1965) or foliage of balsam fir harvested in the study area. Foliage was stored at 4 °C for not longer than one week in buckets filled with water and offered to larvae as small branches in water-filled tubes. Branches in rearing containers were changed twice a week to provide larvae with fresh food. Larvae were held in plastic containers with food *ad libitum* until the desired instar for the experiment was reached (7–20 days).

To compare parasitism rates of budworm larvae, fourth to sixth foliage-fed and diet-fed instars were implanted twice a week from 21 June to 19 July 2011 in each of the four plots. Before implantation, larvae were held in small plastic containers without food for 24 hours at 4 °C in growth chambers. They were transported to the study area in cooling boxes and installed on current-year balsam fir shoots at eye level in groups of five larvae with the same treatment distributed on two to five young trees. Trees and branches were marked with coloured flags to facilitate recovery of larvae after exposure. Every group was randomly distributed in each plot and at least 15 m apart from each other. To synchronise the implantation of the larvae with their natural occurrence and the occurrence of the parasitoids in the study area, a model of spruce budworm seasonality (Régnière *et al*. 2012) was used. Larvae were recovered at nine dates after a

Fig. 1. Mean rates of (A) overall attack; (B) attack by *Tranosema rostrale*; (C) attack by *Elachertus cacoeciae*; and (D) attack by other parasitoid species, as a function of time (ordinal date) in 2011 (black: foliage-fed larvae; dashed grey: diet-fed larvae).



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seven-day exposure period (Fig. 1). Shoots containing the exposed budworms were cut so that immature stages of parasitoids (larvae or cocoons) hidden in the foliage in the vicinity of their host were also collected. After transport to the laboratory in a cooling box, larvae were placed in individual containers on artificial diet, reared at room temperature, and checked three times a week for parasitoid emergence. The proportion of parasitised recovered larvae was analysed using logistic regression, with treatments (diet) as fixed effects, plots as random effects, and time as repeated measures (PROC GLIMMIX, SAS Institute 2003). Repeated measures were needed because of the likely autocorrelation of the plots effect error. Individuals dying from causes other than parasitism were not included in the analysis.

A laboratory choice test was conducted in 2011 and 2012 with a total of 33 female specimens of the parasitoid *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) obtained from field implanted spruce budworm larvae in and near the study area. One foliage-fed and one diet-fed larva of the same instar (fourth or fifth) and size were placed at opposite sides in a 100 × 15 mm petri dish. Using an aspirator, one inexperienced wasp (a wasp that had never before parasitised a larva) was transferred to the petri dish for 10 minutes or until it had attacked one host larva. A larva was considered as attacked if the wasp's ovipositor was clearly inserted in its skin. If no parasitism occurred, the larvae were transferred to a new petri dish where the experiment was repeated using another inexperienced wasp. The choice test was analysed using a two-tailed sign test (PROC UNIVARIATE, SAS Institute 2003).

The overall parasitism rate in the field experiment was 86.2%, most of which was done by two species: *T. rostrale* and *Elachertus cacoeciae* (Howard) (Hymenoptera: Eulophidae). *Phytodietus vulgaris* Cresson (Hymenoptera: Ichneumonidae) (a probable determination) and a few unidentified individuals were also present (hereafter referred to as other species; Table 1; Fig. 1). No effect of larval diet on parasitism was found, whether overall or by species (Table 1). Overall parasitism decreased significantly throughout the season ($F_{8,19.67} = 2.94$; $P = 0.0244$), although this effect was not significant when parasitism was analysed separately for each parasitoid species (Fig. 1). These changes over time reflect the different phenology of each species, *T. rostrale* being more active early in the summer, and *E. cacoeciae* later (J. Régnière, unpublished data).

In the choice test with *T. rostrale* females, spruce budworm larvae fed on balsam fir foliage were chosen more than twice as often (70%, 23/33) as diet-fed larvae (30%, 10/33; $P = 0.0351$). While the sample sizes in these tests are small, the difficulty of obtaining sufficient biological material precluded access to larger number of female parasitoids. We hypothesise that *T. rostrale* uses olfactory cues from the budworm's host plant to find its host, such as volatiles liberated by feeding larvae or their frass, and therefore find or recognise foliage-fed larvae more readily. It is possible that in the first few hours of exposure in the field, foliage-fed larvae are also attacked more often than diet-fed larvae by *T. rostrale* females. However, an exposure period of seven days as described above would mask such an effect as the diet-fed larvae feed on foliage.

Table 1. Percent parasitism (mean and SEM, $n = 4 \times 9$) and statistical analysis of parasitism of foliage-fed ($n = 325$) and diet-fed ($n = 347$) spruce budworm larvae.

Species	% parasitism of foliage-fed larvae	% parasitism of diet-fed larvae	*F*	df	*P*-value
Tranosema rostrale	55.75 ± 3.69	59.04 ± 3.57	0.38	1, 46.19	0.5409
Elachertus cacoeciae	17.28 ± 2.75	17.69 ± 2.73	0.01	1, 24.96	0.9084
Other species	9.29 ± 1.64	5.92 ± 1.30	2.40	1, 48.80	0.1275
Total parasitism	88.70 ± 2.11*	83.93 ± 2.37	2.03	1, 46.16	0.1611

*Two larvae were multiparasitised by *T. rostrale* and *E. cacoeciae*; they were excluded from the species-specific analysis but included in total parasitism.

We conclude that implanting in the field of spruce budworm larvae reared on antibiotic-free McMorran (1965) artificial diet can be used without concern that the food source used in rearing would affect parasitism rates.

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