Life-History Traits and Temperature-Dependent Performance of *Tranosema rostrale* (Hym.: Ichneumonidae), a Parasitoid of Low-Density Spruce Budworm (Lep.: Tortricidae) Populations

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Faculty of Forestry
University of Toronto

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Abstract

The eastern spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) is one of the most important outbreaking defoliator in conifer forests of eastern North America. In low-density populations, the larval endoparasitoid *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) is known as an important mortality factor, but relatively little information is available about the factors influencing its ability to keep spruce budworm populations low. A series of studies were conducted to investigate *T. rostrale*’s: 1) reproductive biology and behaviour; 2) seasonal pattern of parasitism and host instar preference; 3) developmental, survival, and reproductive response to temperature; 4) ability to abrogate spruce budworm’s immune response at high temperatures; and 5) spatiotemporal biology in response to changing temperature. Three traits of the parasitoid’s reproductive biology contribute to its successful parasitism: its lack of a pre-mating and preoviposition period, the rapid maturity of its eggs soon after emergence despite being synovigenic, and its efficacy in host searching and oviposition behaviour. As *T. rostrale* does not prefer to attack any one particular host instar, its seasonal pattern of parasitism is likely influenced by its phenology or competition with the ectoparasitoid *Elachertus cacoeciae* (Howard)
(Hymenoptera: Eulophidae). Overall performance by *T. rostrale* was severely reduced at high temperatures. The negative correlation between the parasitoid’s survival and rearing temperatures above 20°C was associated with a downregulation of *T. rostrale*’s polydnavirus gene transcription and an enhancement of the accumulation of host immunity gene transcripts. An individual-based simulation model incorporating the parasitoid’s response to temperature allowed insight into unknown facts about its seasonal biology, such as phenology, voltinism, and overwintering strategy. The findings improve our understanding of the influence of temperature on spruce budworm population dynamics via its parasitoids, and our ability to predict pest outbreaks, which is important to develop more effective pest management strategies under scenarios of changing climate.
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Chapter 1
General Introduction

Parasitoid Natural History and Terminology

When I am asked what a parasitoid is, I usually answer using Godfray's (1994) analogy:

“Do you know the movie ‘Alien’ from the late 1970s? There is one scene, where an alien larva enters the body of a human, feeds on him, and leaves the body later, killing the guy. That’s a quite good description of a parasitoid, only that in my studies, it is usually an insect, which feeds and develops in or on another insect.”

From this description, most people understand what it is I study, and are often amazed, although a little startled, by the actual existence of such life-history traits in living organisms.

In the scientific literature, a parasitoid is defined by the feeding of its immature stage on or in a living host (usually an arthropod); this feeding eventually kills the host towards the end of the parasitoid’s development. Parasitoids are usually holometabolous (their life cycle can be divided into four stages, egg, larva, pupa, and adult), larvae require only a single host to complete their development, and adults are free-living (Godfray 1994). Most parasitoids belong to the taxonomic order of Hymenoptera (i.e., the wasps), considerably less to Diptera, and a few species to the orders Coleoptera (beetles), Lepidoptera, Neuroptera, and Trichoptera (Godfray 1994).

In general, parasitoids oviposit (lay their eggs) in, on or in the vicinity of their host. Some species are ovoviviparous, i.e., instead of laying eggs, their larvae hatch inside the mother and are deposited on or near the host, subsequently searching and penetrating it. Parasitoids developing inside the host are called endoparasitoids as opposed to ectoparasitoids, which live externally on the host and feed on it by burying their mouthparts into the cuticle. Ectoparasitic development of early parasitoid instars, and a subsequent change to development inside the host, is known for some species, but is rare. In addition, parasitoids can be categorized by the host life stages they attack and develop in or on: i.e., egg, larval, pupal, and adult parasitoids. Some parasitoid species attack one life stage, but continue to develop in another. In this case, the terms egg-larval or larval-pupal parasitoid are often used.
If a parasitoid feeds alone on a host, it is called *solitary*, whereas *gregarious* parasitoids feed together with their siblings on the same host; the number of siblings in a single host can range from two to several thousands. Oviposition by a solitary species of more than one egg in or on a host by one or several females of that species is termed *superparasitism*. If two or more species oviposit in one host, it is referred to as *multiparasitism*. *Hyperparasitism* on the other hand is parasitism of a parasitoid, and this may occur in a host following multiparasitism or outside of the host for ectoparasitoids, eclosed larvae or parasitoid pupae.

*Koinobionts* are parasitoids that allow hosts to continue development and remain mobile after parasitization whereas, *idiobionts* immobilize and arrest the development of their host by injecting venom either before oviposition or along with the egg. Parasitoids can also be categorized by their reproductive strategy as either *synovigenic* or *pro-ovigenic*. Synovigenic females typically emerge with no or only a few mature eggs and continue to develop more over their reproductive lifetime while pro-ovigenic females emerge with their full egg complement ready to oviposit. However, synovigeny has become viewed as a continuous gradient (Jervis et al. 2001). To describe this gradient, an index is used that ranges from 0 (emergence with no mature oocytes) to 1 (emergence with all oocytes mature) and is calculated by dividing the number of mature eggs at emergence by lifetime fecundity.

**Influence of Temperature on Parasitoids and Parasitoid-Host Interactions**

Insects are poikilotherms (cold-blooded), and as such their metabolic rate, as well as many life history traits, (*e.g.*, survival, development, and reproduction) depend on ambient temperature (Régnière & Powell 2013). Temperature can also affect interactions between insects at different trophic levels, and alter the dynamics of their populations (Fleming & Volney 1995; Hance et al. 2007; Gray 2008). Because of the importance of parasitoids as mortality factors for many insects, the influence of temperature on overall parasitoid performance has been generally well studied.

Probably the most obvious influence of temperature is on the mobility of parasitoids. Studies on the influence of temperature affecting several *Trichogramma* (Hymenoptera: Trichogrammatidae) species have shown that flight initiation and inhibition are critically dependent on ambient temperature (Fournier & Boivin 2000; Forsse et al. 1992). This effect may be closely related to reduced mobility, that in turn leads to longer patch time allocations (time spent in an area searching for hosts; Amat et al. 2006), handling times for parasitoids, as well as to decreased search, attack, and parasitism rates (Menon et al. 2002; Zamani et al. 2006). However, this relationship is not
necessarily linear; for example, both Reznik et al. (2009) and Watt et al. (2016) found strong non-linear temperature-dependent parasitism rates in Hymenoptera.

Countless studies have looked at the development time of insects in relation to temperature, including parasitoids (e.g., Spanoudis & Andreadis 2012; Appiah et al. 2013; Watt et al. 2016). In early studies, development times or development rates (1/development time) within a certain temperature range were described by a linear relationship to determine degree-day and base temperature requirements for parasitoids (Nealis & Fraser 1988). However, the response of insect development time to temperature is generally not linear because at extremely high temperatures, insects need longer for development, and at extremely low temperatures, their development time may decrease more slowly than at moderate temperatures. Therefore, non-linear models are now more frequently used to describe temperature-dependent development of poikilothermic organisms including insects (Logan et al. 1976; Lactin et al. 1995; Brière et al. 1999; Régnière 2012a).

Some parasitoid species require a post-emergence window in which to develop their eggs and attack hosts; this time lag is termed the preoviposition period. It has been shown that temperature affects the duration of the preoviposition period for parasitoids, with increasing temperature generally leading to a reduction in time (Wang et al. 1999; Reznik et al. 2009). Temperature can also affect the fecundity of parasitoids. Typically, fecundity increases with rearing temperature until an upper threshold, after which it decreases again (Chen et al. 2015; Watt et al. 2016). As a general rule, ectotherms reared at low temperatures are larger than those developing at high temperatures (Ray 1960; Atkinson 1994; Angiletta & Dunham 2003; but see Walters & Hassal 2006). Larger parasitoid females usually produce more eggs than smaller ones (e.g., King 1987; Ellers et al. 1998; Fidgen et al. 2000) and thus, there is a negative correlation between temperature and lifetime fecundity. Exposure of parasitoids to extreme temperatures has also been shown to severely affect parasitoid fecundity. For example, when immature stages of several species were reared at low temperatures, adult fecundity was severely reduced, depending on the temperature and in length of time of the exposure (Foerster et al. 2004; Bayram et al. 2005; Levie et al. 2005; Pandey & Johnson 2005). Finally, exposure of adult parasitoids to extreme low temperatures has been shown to lead to a reduction in fecundity of up to 80% (Uçkan & Gülel 2001; Bayram et al. 2005).
Along with the positive correlation between temperature and parasitoid metabolic rate, there are numerous examples showing an inverse relationship between adult parasitoid longevity and temperature (e.g., Nealis & Fraser 1988; Wang et al. 1999; Spanoudis & Andreadis 2012). Exposure of immatures (Jalali & Singh 1992; Rundle et al. 2004; Pandey & Johnson 2005) or adult parasitoids (Foerster & Doetzer 2006) to extreme cold temperatures has also been shown to reduce adult longevity.

Extreme temperatures can cause increased mortality of immature parasitoids, leading to the typical unimodal relationship of temperature and parasitoid survival (e.g., Spanoudis & Andreadis 2012; Chen et al. 2015). However, many insects are well adapted to cold temperatures, as they naturally reside in terrestrial regions where winters are harsh and minimum temperatures fall well below the freezing point. One way insects deal with these conditions is to enter diapause, a physiological state of dormancy that can be induced by an interaction of low temperature and shortened photoperiod (reviewed by Saunders 2014). In this case, parasitoids may be in a somewhat unique situation because they can enter diapause inside their hosts and thereby take advantage of the host’s own diapausing strategy; i.e., in the egg or larval stage (Brown 1946a; 1946b; Ellers & Van Alphen 2002), or as a pupa or adult in concealed places within the environment (Hébert et al. 1989; Numata 2011). Even in diapause, parasitoids and other insects are vulnerable to extreme cold temperatures, and this can lead to the development of diverse strategies to avoid freezing mortality. Some parasitoids have developed physical strategies for freeze avoidance, e.g., by accumulating glycerols to lower the temperature at which their tissues freeze and die, the so-called supercooling point (Sullivan et al. 1977; Turnock & Bilodeau 1992; Pullin 1994). Another strategy for many is termed freeze tolerance (Bale 1993; Vernon & Vannier 2002), wherein some parasitoid species can actually survive the formation of ice crystals in their tissues (Humble & Ring 1985; Humble 2006).

Temperature-driven simulation models can use the relation between temperature and physiological life history traits such as development rate, survival, and fecundity to forecast phenology, distribution, and ultimately insect dynamics influenced by ambient temperatures (Mols & Diederik 1995; Safranyik et al. 1999; Bentz et al. 2010; Régnière et al. 2012a; 2012b; Régnière et al. 2014). Phenology models are often used as a tool in pest management (e.g., Nealis et al. 2001; Régnière & Bentz 2007; Régnière et al. 2007) and can also include trophic interactions of pest insects with parasitoids (Drummond et al. 1985; Mols & Diederik 1995; de Souza et al. 2009). In general, two
kinds of phenology models for insects can be distinguished: *cohort* - and *individual-based models*. In cohort-based models, insects are modeled as groups that enter a stage at a given time, age within that time, then survive the stage with a certain probability and move to the next stage. Many insect species have been modeled with this approach (*e.g.*, Curry et al. 1978; Logan 1988). Individual-based models however, randomly assign specific traits to individuals of a modeled group of insects, allowing the individual to develop at its own pace. These individual traits are drawn from an experimentally established distribution of deviation from a mean for each life stage. Using this approach, complex behaviours can be modeled in a relatively simple way (Régnière et al. 2012a), and advances using this technique, such as combinations of cohort- and individual-based models (Parry & Evans 2008; Yurk & Powell 2010), can improve the computation process and precision of the models.

Parasitoids sometimes respond differently to temperature than their hosts, leading in some cases to a mismatch between the host and its natural enemies, which in turn may lead to an escape of the host from its enemies, and thus potentially to a pest outbreak (Fleming & Volney 1995). Such escapes may occur spatially, resulting in what has been termed ‘thermal refuges’ for hosts from parasitoids, *enemy-free space* or a spatial mismatch (*e.g.*, Klok et al. 2003; Hance et al. 2007; Jeffs & Lewis 2013; Karban et al. 2015). Escapes may also occur temporally, resulting in a phenological mismatch or asynchrony (*e.g.*, Godfray 1994; Strohm et al. 2001; Jeffs & Lewis 2013). For example, the optimum temperature for development of spruce budworm exceeds the optima for some of its natural enemies, including the parasitoid *Apanteles fumiferanae* Viereck (Hymenoptera: Braconidae) (Fleming 1996 and references therein). Thus, it is conceivable that spruce budworm may survive better under a scenario of climate warming because of decreased attack by natural enemies (Fleming 1996). In other cases, lower temperatures may mean that the host can escape from its parasitoids, *i.e.*, the larva of the autumnal moth *Epirrita autumnata* (Lepidoptera: Geometridae) remains active at low temperatures while its parasitoids cease activity. In this case, parasitoid activity increases at high temperatures and high parasitism rates lead to a decrease in host population densities (Virtanen & Neuvonen 1999).

Successful parasitism may also decrease with increasing temperature due to an improvement in the host’s immune defense. The efficiency of encapsulation of parasitoid eggs by the host is generally enhanced as temperatures increase (Blumberg 1991; Fellowes et al. 1999). In addition, the activity of the enzyme phenoloxidase, which is involved in the formation of melanin sealing
off foreign bodies from the host’s internal environment, is known to be affected by temperature, with a positive correlation between enzyme activity and temperature, up to an upper threshold varying between 20 and 50°C, depending on the species (Lockey & Ourth 1992; Hara et al. 1993; Cherqui et al. 1996; Zufelato et al. 2004).

The Role of Parasitoids in the Dynamics of Spruce Budworm Populations

Natural enemies are known to play an important role in the outbreak dynamics of many forest insect pests (Nealis 1991). Consequently, understanding the relationship between an insect pest and its natural enemies is critical to successfully managing forest pests. Parasitoids have received considerable attention in their role as natural mortality factors and as biological control agents in integrated pest management programs (e.g., Waage & Hassell 1982; Smith 1993; MacQuarrie et al. 2016). In forestry, biological control traditionally includes the introduction of new natural enemies (classical biocontrol), inundative or inoculative releases (augmentation), and the enhancement and protection of indigenous natural enemies (conservation) (Pschorn-Walcher 1977; Nealis 1991; Smith 1993; Wallace 1995; MacQuarrie et al. 2016). The latter approach relies on support of indigenous natural enemies that are associated with forest pests in complex food webs, and that contribute to maintain their host populations below economically damaging levels. It is important to study these native natural enemies throughout the entire population cycle of their host pests in order to better understand conditions that lead to natural control, and to predict when and where pests may escape this control and reach outbreak levels (Nealis 1991). This emphasizes the need to break new ground in forest pest management and to study not only the relationship of pest insects and their natural enemies in outbreak situations, but also when pest population levels are low.

In northeastern North America, the most important forest insect pest is the eastern spruce budworm, Choristoneura fumiferana (Clemens) (Lepidoptera: Tortricidae). During its last major outbreak (1968-87), spruce budworm caused moderate to severe defoliation in a total area of >50 million hectares of forest in Canada (NFD 2016) and caused losses of over 500 million cubic metres of wood fibre in Québec’s forests alone through tree mortality and growth reduction (Coulombe et al. 2004).

Tree ring (Blais 1954; 1965a; Morin et al. 1993; Boulanger & Arseneault 2004; Simard et al. 2011; Boulanger et al. 2012) and sediment (Simard et al. 2006) analyses give strong evidence that spruce budworm outbreaks have occurred in northern forest ecosystems for at least 8500 years, and
recurrently over the past four centuries. Together with more recent defoliation surveys (Kettela 1983; Simpson & Coy 1999), these analyses show that spruce budworm outbreaks occur in a more or less regular periodicity of 30–40 years in the conifer forests of eastern North America (e.g., Royama 1984), and in somewhat shorter intervals, in the west (Shore & Alfaro 1986; Burleigh et al. 2002). A multitude of practical and theoretical studies were conducted during the last major outbreak of spruce budworm to identify factors influencing its dynamics, particularly during population decline. Many theories have been developed, discussed, and in some cases, dismissed (reviewed by Pureswaran et al. 2016).

Two main theories have been developed to explain spruce budworm dynamics; one is often referred to as the double-equilibrium theory (Clark et al. 1979) and the other as the oscillatory theory (Royama 1984). The double-equilibrium theory suggests that spruce budworm populations are dominated by two density-dependent equilibria; a lower equilibrium maintained by high mortality through natural enemies (predators, parasitoids and diseases), and a higher equilibrium set by food availability. According to this theory, outbreaks can develop (shift from the lower to the higher equilibrium) when the spruce budworm population growth rate exceeds the predation rate due to favourable conditions (mature forests and warm, dry weather) and the spread of outbreaks may occur by migration of moths out from these epicentres (Clark 1978). In contrast, Royama's (1984) oscillatory theory hypothesises that spruce budworm subpopulations undergo basic oscillations in population density determined by density-dependent mortality factors including parasitoids and diseases. In this theory, a secondary fluctuation in spruce budworm populations caused by density-independent fluctuations in immigration and emigration of moths (which are highly correlated with meteorological conditions) acts above the basic oscillation and causes the subpopulations to increase and decrease in synchrony. Using a simple time-series model, Royama (1984) showed that the combination of primary and secondary fluctuations can create an oscillation cycle similar to that observed for spruce budworm populations. This theory aligns with the idea of the Moran effect (correlated density-independent disturbances that bring independently oscillating local populations into synchrony, Moran 1953) and was shown again in a spruce budworm population survey, where egg recruitment rates in different regions brought outbreaks into unison across New Brunswick (Royama 2005).

Several studies suggest that the synchrony of spruce budworm population outbreaks is more complex than proposed in either of these two theories, and that other processes need to be
considered (Régnière & Lysyk 1995; Williams et al. 2000). Gray (2008) explicitly investigated the relationship between climate and spruce budworm outbreak characteristics and found that 54% of the spatial variability in outbreaks can be explained by a matrix of six climate variables, forest composition, and spatial location.

A more recent in-depth study of a spruce budworm outbreak near Black Sturgeon Lake, Ontario, analysed the ecological mechanisms explaining observed population changes from the beginning of the outbreak in 1983 to its collapse in 1997 (Nealis & Régnière 2004a; 2004b; Régnière & Nealis 2007). This work revealed the importance of density-related trophic interactions that vary in order and strength of influence over the time of the outbreak. More specifically, the findings showed that host-tree feedback influenced spruce budworm survival via both annual and cumulative defoliation. The influence of natural enemies, namely predators and parasitoids, however was also found to govern changes in budworm population density during the collapse of the outbreak, mainly through the mortality of late immature instars (Régnière & Nealis 2007). This study, together with new findings about increasing spruce budworm populations in the province of Québec (Béchard et al. 2014), challenge the oscillatory theory in particular, and suggest that a multitude of complex factors are involved in spruce budworm population dynamics that cannot be explained by any one theory alone (see also Pureswaran et al. 2016).

Despite exhaustive work to identify drivers explaining the epidemic phase of spruce budworm population cycles, comparatively little has focused on low-density populations (also called endemic). One factor shown to keep spruce budworm populations at low densities is a mate-finding Allee effect (Régnière et al. 2013). This study showed that there is a non-linear positive relationship between spruce budworm population density and mating success; i.e., at low population densities, females have difficulty in attracting and successfully mating with males whereas at higher densities, mating success increases until almost 100%. An ongoing, long-term study near Armagh and Petit-Lac-à-l’Epaule, Québec, suggests that high predation pressure by a number of generalist parasitoids constitutes an additional strong Allee effect on endemic spruce budworm populations (J. Régnière, unpublished data). This sustains population levels below an Allee threshold, and appears only to be overcome by an immigration of male moths and egg-bearing females, which both lead to an increase in overall mating success (Régnière et al. 2013). A higher mating success may then result in a higher population growth rate for spruce budworm populations that may exceed the high mortality inflicted by generalist parasitoids.
The above description makes clear that natural enemies play a crucial role in spruce budworm population dynamics, especially during the collapse of outbreaks (Régnière & Nealis 2007) and at low population densities (Blais 1965b; Miller & Renault 1976; Seehausen et al. 2014; J. Régnière unpublished data). While diseases (Bird & Whalen 1954; Thompson 1960; Perry & Régnière 1986) and predators (Morris et al. 1958; Crawford et al. 1983; Jennings & Houseweart 1984; Crawford & Jennings 1989) have been identified as natural enemies in the spruce budworm system, parasitoids seem to play the most important role as mortality factors at varying spruce budworm population densities (McGugan & Blais 1959; Blais 1960; Miller 1963; Régnière & Nealis 2007).

As of 2010, 122 species of parasitoids had been identified attacking all immature spruce budworm life stages (Fernández-Triana & Huber 2010). Interestingly, the community composition of parasitoids has been shown to drastically change with spruce budworm population density (Eveleigh et al. 2007). For example, two of the most common spruce budworm specialists, *A. fumiferanae* and *Glypta fumiferanae* (Viereck) (Hymenoptera: Braconidae), are known to attack spruce budworm larvae primarily during rising or at high populations (e.g., McGugan & Blais 1959) whereas *Meteorus trachynotus* Viereck (Hymenoptera: Braconidae), *Smidtia fumiferanae* (Tothill) (Diptera: Tachinidae), and *Actia interrupta* Curran (Diptera: Tachinidae) are all known to be most abundant during declining spruce budworm populations (Dowden et al. 1948; Jaynes & Drooz 1952; McGugan & Blais 1959; Blais 1960; Miller 1963). Generalist parasitoids such as *Elachertus cacoeciae* (Howard) (Hymenoptera: Eulophidae) and *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) both seem to contribute the greatest mortality only at low spruce budworm densities (Eveleigh et al. 2007; Johns et al. 2013a; b; Seehausen et al. 2013; 2014; Béchard et al. 2014). Parasitoid community composition also seems to change spatially across the geographical distribution of the spruce budworm. For example, in New Brunswick *E. cacoeciae* is considered the most important parasitoid at low-density spruce budworm populations while *T. rostrale* plays only a minor role there (Eveleigh et al. 1994; 1997; 2007). In contrast, the latter is by far the most important species in Québec while *E. cacoeciae* plays only a secondary role in terms of parasitism (Cusson et al. 1998a; Johns et al. 2013b; Seehausen et al. 2013; 2014).

**The Biology of Tranosema rostrale** (Brischke) (Hym.: Ichneumonidae)

Parasitism rates of low-density spruce budworm populations by *T. rostrale* can exceed 90% (Cusson et al. 1998a; Seehausen et al. 2013; 2014), making this species one of the main
contributors to the above mentioned predation Allee effect that keeps spruce budworm populations low over long periods of time. Despite its importance, little information is available on the general biology and ecology of this parasitoid. Existing knowledge gaps around key life-history traits of *T. rostrale* that contribute to its efficacy as a mortality factor of spruce budworm populations must be filled. The following review addresses aspects of *T. rostrale* biology that are needed to better understand what drives spruce budworm parasitism by this species.

**Taxonomy**

*Tranosema rostrale* (Fig. 1.1) was first described by Brischke (1880) as *Limneria rostralis* in western and eastern Prussia (now mainly Germany and Poland). All described specimens were found around the city Danzig (now Gdańsk, Poland), however, no description of the exact location is given. Also, no host species were described for the parasitoid, likely because it was caught as an adult in the field and not reared from a host. Most other parasitoid species in the same genus are described to parasitize lepidopterans in the family Tortricidae, *e.g.*, *Tortrix* sp. (Brischke 1880). *Tranosema rostrale* was later described by various authors, under multiple names, and in many locations (Table 1.1).


**Distribution**

Carlson (1979) described the distribution of *T. rostrale* in North America with northern limits ranging from Québec (east) to British Colombia (west), and southern limits from northern New York through to northern Michigan, northern Minnesota, western Montana, and western Washington. Unfortunately, no reference is made for the source of these data. Available scientific literature on *T. rostrale* describes the species in six countries of central Europe, six northern states of the USA, and six provinces in Canada (Table 1.1) suggesting a broad Holarctic distribution (Fig. 1.2). In Canada, this species has been most frequently identified from locations in Quebec, possibly attributed to the high number of spruce budworm parasitoid studies carried out here. It is
possible that the parasitoid is also present in other countries and regions, however, the literature from some (e.g., Russia) is difficult to access and surveys are often done in areas of economic or biological interest, such as orchards or old-grown forest stands.

**Hosts Species**

A total of 19 host species can be identified for *T. rostrale* from the literature, from which all but two belong to the lepidopteran family Tortricidae (Table 1.1). In Europe, 12 hosts are known, from which 11 are tortricids and only one belongs to the family Gelechiidae (*Recurvaria leucatella* Clerck). Most of these tortricids are considered pests in apple orchards (Janssen 1959; Evenhuis & Vlug 1983; Blommers et al. 1988; Kienzle et al. 1997), however, some have also been collected on fir (Mills & Kenis 1991) or oak trees (Zwölfer & Kraus 1957). In North America, eight host species of *T. rostrale* have been mentioned in the literature (Table 1.1) with one other potential host (*Malacosoma californica pluvialis* (Dyar); Lepidoptera: Lasiocampidae) (Carlson 1979). Of these eight, all but one (*Dysstroma citrata* (L.); Lepidoptera: Geometridae) are in the family Tortricidae and either feed on conifers (*C. fumiferana* and *C. occidentalis* Freeman) or have been collected from different deciduous trees, including apple. Interestingly, of all these hosts, only *C. fumiferana* was found to be heavily parasitized by *T. rostrale*.

In North America, *T. rostrale* is known to be an important parasitoid of low-density spruce budworm populations in Québec where parasitism by this species can exceed 90% locally (Cusson et al. 1998a; Seehausen et al. 2014), underlining its influence on spruce budworm mortality and its contribution in keeping populations low over long periods of time (Régnière et al. 2013). This impact appears to decrease as spruce budworm populations increase (Miller 1963; Eveleigh et al. 2007; J.R. unpublished data), however it is unclear why this may be the case, suggesting further work is needed.

**Seasonal Biology**

The basic biology of *T. rostrale* in Québec has been described by Cusson et al. (1998a). Adult parasitoids become active in the field at the end of May when they parasitize postdiapause spruce budworm larvae. Laboratory trials confirm that *T. rostrale* can parasitize all postdiapause spruce budworm larval instars (2nd – 6th), however, in the field only parasitism of 3rd- to 6th-instar larvae has been observed (J. Régnière unpublished data). It has been shown that in Québec, spruce budworm larvae are attacked by *T. rostrale* between the end of May and the end of July, with a peak during the mid- to end of June when spruce budworm is in the 4th or 5th instar (Cusson et al.
Inside its host, the parasitoid larva undergoes three instars, egressing from 5th- or 6th-instar spruce budworm larvae (Cusson et al. 1998a). Upon egression, larvae start to spin cocoons on the foliage and adults emerge in late June to early July. Paradis and LeRoux (1965) reported adult *T. rostrale* emerging from *Archips argyrospilus* (Walker) (Lepidoptera: Tortricidae) between 21 June to 2 July in the Montréal area suggesting a very similar rate of development on this alternative host. One or two additional generations are suspected in Québec.

Despite exhaustive efforts to find potential *alternate* hosts *T. rostrale* attacks after ist development in spruce budworm, to date only *alternative* hosts have been identified in the field which the parasitoid attacks at the same time as the spruce budworm (see above). Successive rearing on spruce budworm larvae in an outdoor insectary in Québec City has demonstrated the potential for almost three parasitoid generations (Cusson et al. 1998a) with the last parasitoid cocoons being formed in early October, albeit without adult emergence. While these findings, combined with those showing early parasitism activity in the spring, suggest that *T. rostrale* may be overwintering in the pupal stage (or more unlikely as an adult), this hypothesis has not yet been confirmed. In earlier studies, *T. rostrale* was reported to oviposit in prediapause spruce budworm larvae (1st and 2nd instars) and to emerge the following spring from older larvae (Coppel 1947; Wilkes et al. 1948; Miller 1963). This early appearance of adult parasitoids in the spring, combined with the fact that extensive rearing of overwintering spruce budworm larvae has not yielded a single *T. rostrale* (Cusson et al. 1998a), suggests that the parasitoid either overwinters as a pupa, as an adult, or uses a different overwintering strategy that involves an alternate host species.

One spring and one summer generation of adult *T. rostrale* have been observed in German apple orchards, where the summer generation coincides with the occurrence of *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae) larvae in the field (Kienzle et al. 1997). Evenhuis and Vlug (1983) reported the presence of adult *T. rostrale* attacking *Archips rosana* (L.) (Lepidoptera: Tortricidae) in apple orchards from The Netherlands between 14 June and 26 July, and also found one hibernating specimen emerging on 7 May the following year. Unfortunately, no further details on this hibernation were provided. In a later Dutch study, Blommers (1994) hypothesized that *T. rostrale* spent the autumn and winter in another host (possibility as a larva) after attacking *A. rosana* during the summer generation.
Reproduction
The courtship behaviour of *T. rostrale* was described by Cusson et al. (1998a) and involves male wing fanning in front of the female for successful coupling, a behaviour that suggests the involvement of pheromones. In laboratory trials, *T. rostrale* has occasionally been observed to mate more than once, and that the second mating is longer than the first. The importance of these multiple matings in terms of reproductive success is not known and warrants further investigation (Cusson et al. 1998a). Under laboratory conditions, *T. rostrale* offspring exhibit a strong male bias, regardless of host instar or size, however, in the field, the sex ratio (males:females) decreases over the season. Whether this is a seasonal phenomenon or related to changes in host size (many parasitoid species are known to prefer laying female eggs in larger hosts; King 1987) remains unknown.

Similarly, little is known about *T. rostrale*’s host searching and oviposition behaviour. Because parasitism in low-density spruce budworm populations is normally very high in the field, it can be assumed that the searching efficiency of *T. rostrale* females must be good. In addition, the parasitoid’s searching efficiency appears to be independent of host density as similar rates were found in trees planted with either 50 or one larva per tree in a manipulative field experiment (Cusson et al. 1998a). Spatial differences in parasitism rates of *T. rostrale* have also been shown at the tree level, with higher parasitism at eye level than at the mid-crown of balsam fir trees (Cusson et al. 1998a).

Multi- and Hyperparasitism
In the spruce budworm system, it is expected that multiparasitism will occur because numerous parasitoid species attack spruce budworm larvae, even during the same instar. Multiparasitism by *T. rostrale* and the tachinid fly *Actia interrupta* Curran (Diptera: Tachinidae), a species also frequently encountered in low-density spruce budworm populations, has been reported from the field, and its outcome has been studied in laboratory experiments (Cusson et al. 2002). In general, *A. interrupta* appears to have a competitive advantage over *T. rostrale*, however, multiparasitism in the field is not high enough to explain the characteristic decline in rates of *T. rostrale* parasitism when apparent parasitism (measured as the proportion of emerged parasitoids to emerged adult spruce budworm) by *A. interrupta* increases. At least one additional parasitoid species of low-density spruce budworm populations, *E. cacoeciae*, is also known to increase in apparent parasitism as *T. rostrale* parasitism decreases (Fidgen et al. 2000; Seehausen et al. 2013; 2014).
As an idiobiont ectoparasitoid (Mills 1992), *E. cacoeciae* has the potential to outcompete *T. rostrale* when multiparasitism occurs (Harvey 2013), and therefore it may be an important factor in shaping the seasonal pattern of apparent spruce budworm parasitism by *T. rostrale*. However, multiparasitism of spruce budworm larvae by *T. rostrale* and *E. cacoeciae* in the field or in the laboratory has not been investigated and warrants further study.

In North America, no hyperparasitism of *T. rostrale* has been reported although this may be simply an artefact of sampling. *Tranosema rostrale* parasitism is usually assessed by implanting spruce budworm larvae in the field and collecting them the following week for rearing in the laboratory (Seehausen et al. 2013). In this way, parasitized host larvae would unlikely be exposed for a sufficient period of time (more than one week) to detect hyperparasitoids, and *T. rostrale* cocoons would rarely be sampled in the field, thus significantly reducing the likelihood of finding hyperparasitoids.

In Europe, several species of hyperparasitoids are known for *T. rostrale*. Zwölfer and Kraus (1957) reported four species emerging from *T. rostrale* cocoons in France; three ichneumonids, *Gelis albipalpus* (Thomson), *Itoplectis maculator* (Fabricius), and *Mesochorus sylvarum* Curtis (=*M. giberius* (Thunberg)), and one unidentified chalcid wasp. *Mesochorus sylvarum* is also known as a hyperparasitoid from parasitoids attacking *C. murinana* (Hübner) in France and Switzerland (Mills & Kenis 1991). Interestingly, this species is also described as a parasitoid from several tortricid moths in North America (Krombein et al. 1979; Bennett 2008) and as a hyperparasitoid of *Glypta fumiferanae* attacking spruce budworm in New Brunswick (Miller 1960). In addition, *M. sylvarum* has been reared from low-density spruce budworm populations in Vermont, USA (Hanson 1982) and in Québec, Canada (Blais 1965b), where *T. rostrale* is also present. Because *M. sylvarum* is an obligate hyperparasitoid (Bennett 2008), it is highly likely that it is a hyperparasitoid of *T. rostrale* in North America.

**Effects of Parasitism on the Host**

Many braconid and ichneumonid endoparasitoids have evolved a strategy to evade the host’s immune response by injecting a virus along with their egg at oviposition (Strand & Burke 2014). These polydnaviruses are considered obligate symbionts of the parasitoids and replicate only in the ovaries of female wasps. In the parasitoid’s host, these virions infect the host tissues and express polydnavirus genes that induce pathologies, including a reduction in the host’s immune response (Doucet & Cusson 1996a; Asgari et al. 1997; Shelby et al. 2000; Pruijssers & Strand
2007; Suderman et al. 2008), developmental arrest of the host (Dover et al. 1987; Doucet & Cusson 1996b; Soller & Lanzrein 1996; Beckage 2012), and mobilization of host protein reserves for use by the parasitoid (Thompson & Dahlman 1998; Nakamatsu et al. 2001; Pruijssers et al. 2009).

*Tranosema rostrale* females are known to transfer a polydnavirus (the *T. rostrale* ichnovirus or “TrIV”) to their hosts that can prolong the final instar of spruce budworm larvae (Doucet & Cusson 1996b), reduce hemocyte (blood cell) count, and lower the activity of enzymes (phenoloxidase) involved in the melanisation of foreign objects in the hemolymph, such as parasitoid eggs (Doucet & Cusson 1996a). Both viral gene expression (Béliveau et al. 2000; 2003; Rasoolizadeh et al. 2009a; 2009b; Djoumad et al. 2013) and function (Cusson et al. 2000; Doucet et al. 2008; Djoumad et al. 2013) of TrIV have been studied.

**Objectives and Thesis Outline**

The overall goal of this dissertation was to investigate key life history traits and the influence of temperature on the performance of *T. rostrale* to determine conditions that will influence its efficacy in controlling low-density spruce budworm populations. Each of the following five data chapters (Chapters 2-6) address unique aspects of this host-parasitoid system. Immediately following the Introduction (Chapter 1), I explore the general reproductive biology of *T. rostrale* (Chapter 2) and follow this with a study examining the pattern of parasitism under field conditions (Chapter 3). Chapter 2 is descriptive and provides new information about this parasitoid more generally whereas Chapter 3 specifically compares the seasonal parasitism by *T. rostrale* to that of other parasitoids simultaneously attacking spruce budworm. In this case, I tested the hypotheses that host instar preference by *T. rostrale* and multiparasitism of spruce budworm larvae by *T. rostrale* and *E. cacoeciae* are possible drivers underlying seasonal patterns of observed parasitism.

In Chapter 4, information from the previous chapters is used to rear *T. rostrale* under constant laboratory conditions and study the effect of temperature on parasitoid development, survival, and reproduction. Chapter 5 goes on to investigate the influence of temperature on two related factors contributing to parasitoid survival, namely the performance of *T. rostrale*’s polydnavirus and response of spruce budworm’s immune system. Two specific hypotheses were tested here: (1) that high temperatures reduce the performance of the polydnavirus through a depression of viral gene expression that limits its effectiveness in abrogating the host immune response; and (2) that high temperatures enhance the spruce budworm’s immune system, enabling more effective
encapsulation and melanisation reactions as a result of a rise in the expression of immunity-related genes.

The results from Chapter 4 on temperature-dependent development, survival, and fecundity were then used to build an individual-based simulation model in Chapter 6 that gives insights into the seasonal biology of *T. rostrale* under changing temperatures. In this case the objective was to predict the seasonality (phenology) of *T. rostrale*, and specifically the spatiotemporal occurrence of different life stages and parasitism under field conditions. In addition, the model was developed to allow for hypotheses that would address the overwintering strategy and temperature-dependent performance (e.g., fitness) of *T. rostrale* in different locations across North America. Finally, Chapter 7 integrates the findings of the data chapters and outlines the general conclusions of the dissertation. It concludes with contribution to research and suggestions for future work. An outline of the dissertation showing the main objectives of each chapter is presented in Fig. 1.4.

All of the studies presented in this dissertation are intended to give greater insight into the host-parasitoid relationship between the eastern spruce budworm and its native larval endoparasitoid, *T. rostrale*, and in particular, to expand our knowledge about the influence of temperature on this relationship. Ultimately, these findings will help improve our understanding as to how present and future climatic conditions will affect spruce budworm dynamics via its parasitoids. Most importantly, they will improve our ability to predict pest outbreaks and to develop for more effective pest management strategies under scenarios of changing climate.

With the exception of the General Introduction (Chapter 1) and the General Conclusions and Future Research (Chapter 7), this dissertation is written in self-contained papers that are either under review (Chapters 4, 5), published (Chapters 2, 3) or will be sent to scientific journals (Chapter 6). As a consequence, some repetition may occur, especially in the Introduction and Material and Methods sections of each chapter. The journal where the paper was sent to or is intended to be sent to, as well as the authors that contributed to each are mentioned in a footnote following the respective title. As first author, I was the main originator of conception and design, data acquisition and analysis, and final interpretation, as well as the writing of each chapter.
Table 1.1: Locations where *Tranosema rostrale* has been found throughout (A) Europe and (B) North America between 1880 and 2016 as reported in the literature. Where available, the year(s) of the discovery, the scientific name used, and the host attacked are also provided.

<table>
<thead>
<tr>
<th>Location</th>
<th>Coordinates (Decimal Degrees)</th>
<th>Year(s)</th>
<th>Name</th>
<th>Host</th>
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<td>Guebwiller</td>
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<td>1990</td>
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<td>Mills &amp; Kenis 1991</td>
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<td>1983-85</td>
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<td><em>T. arenicola</em></td>
<td><em>Pandemis heparana</em></td>
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<td>1994</td>
<td><em>T. rostralis</em></td>
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**Note:**
- The years indicate the range of years during which the species were collected.
- The coordinates are given in degrees, minutes, and seconds.
- The species names and their subspecies are categorized based on the referenced studies.
- The references are cited for each location to provide a source for the documented findings.
<table>
<thead>
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<td><em>C. fumiferana</em></td>
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<td><em>C. occidentalis</em></td>
<td>Wilkes et al. 1948</td>
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<td>Mount McLean</td>
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<td><em>Horogenes cacoeciae</em></td>
<td><em>C. occidentalis</em></td>
<td>Wilkes et al. 1948</td>
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<td><em>T. rostrale</em></td>
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<td><em>T. rostrale</em></td>
<td><em>C. fumiferana</em></td>
<td>Cusson et al. 1998</td>
</tr>
</tbody>
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*CNC* Canadian National Collection; *Gl* Belongs to the family Gelechiidae; *Gm* Belongs to the family Geometridae
Fig. 1.1: Tranosema rostrale rostrale (Brischke) (Hymenoptera: Ichneumonidae) adult female.
Fig. 1.2: Recorded distribution of the holoform *Tranosema rostrale rostrale* (Brischke) (Hymenoptera: Ichneumonidae) and of two subspecies, *T. r. albula* and *T. r. scaponigrum*, based on an extensive review of scientific literature between 1880 and 2016 (see Table 1.1 and Chapter 1).
Fig. 1.3: Outline of the dissertation showing objectives of each chapter, the main subjects addressed (boxes), and how they are connected.
Chapter 2
Reproductive Biology and Behaviour of *Tranosema rostrale* (Hym.: Ichneumonidae), a Parasitoid of Low-Density Spruce Budworm (Lep.: Tortricidae) Populations

Abstract

*Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) is an important parasitoid of low-density spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) populations. In order to understand what makes this parasitoid effective in attacking endemic spruce budworm populations, we conducted a detailed laboratory study on its reproductive biology and behaviour including mating behaviour, potential fecundity, longevity, host searching, and oviposition behaviour. We found that the occurrence of mating increases with the number of males present in a cage but was almost 10× lower when females had mated previously. Females were able to mate multiple times with different males in one breeding session, and mating lasted about 4× longer when the same male mated with the same female for a second time. Dissections of *T. rostrale* oviducts showed that it is synovigenic, emerging with about 17% (9.0 ±1.4 SEM eggs) of its lifetime egg load; most of its subsequent eggs are developed during the first three days after emergence at 20°C. Sugar, but not pollen, significantly increased parasitoid longevity about 6× for males and 11× for females compared to only water. Spruce budworm larvae, silk from larvae, and damaged balsam fir foliage triggered probing in *T. rostrale* females significantly more often than larval feces. The sequence of the parasitoid’s behaviours leading to successful attack is described as antennation, probing, insertion of the ovipositor, oviposition, and subsequent disinterest or resuming of the sequence. Defense mechanisms of the host larva such as vigorous movements, biting, and/or regurgitation and behavioural countermeasures by *T. rostrale* are described in detail.

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Introduction

One of the most important outbreaking insects in boreal coniferous forests of eastern North America is the spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (USDA Forest Service 2009; NFD 2013). It reaches outbreak levels in more or less regular intervals of 30-40 years (Blais 1965a; Morin 1994). Several factors influencing its population dynamics have been identified, one of which are natural enemies, in particular parasitoids (Royama 1984; Régnière & Lysyk 1995; Régnière & Nealis 2007). Spruce budworm parasitoid communities change in terms of species occurrence and relative importance as mortality factor depending on their host’s population densities and geographic location (Eveleigh et al. 2007). It is thus important to not only study parasitoids attacking spruce budworm during outbreaks, but also those causing high mortality at low spruce budworm population densities. This will help to better understand conditions that lead to natural control, and to predict when and where pests may escape such control and reach outbreak levels (Nealis 1991).

*Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) is an important parasitoid of low-density (endemic) spruce budworm populations. Parasitism by this species can exceed 90%, inflicting considerable mortality on spruce budworm larvae (Cusson et al. 1998a; Seehausen et al. 2014), and helping to maintain populations at low levels for many years (Régnière et al. 2013). Besides spruce budworm, *T. rostrale* attacks several other tortricid species and is believed to have one or two additional generations per year on unknown alternate hosts (Cusson et al. 1998a). Like most parasitoid wasps, *T. rostrale* is arrhenotokous, with fertilized eggs developing into female wasps and unfertilized eggs becoming males (Flanders 1965; Quicke 2014). Although some aspects concerning the basic biology of *T. rostrale* have been described (Cusson et al. 1998a), several key life history traits related to its reproductive biology and behaviour (e.g., mating, female fecundity, oviposition behaviour) remain unknown. This limits our understanding of the role *T. rostrale* plays in spruce budworm population dynamics and our ability to manipulate it for further laboratory experiments.

Previous work has shown that female *T. rostrale* can mate at least twice in their lifetime (Cusson et al. 1998a), but it is unclear whether females mate with multiple males or whether male competition and mate guarding is important in this process (Thornhill 1984). Similarly, it is unknown whether *T. rostrale* is proovigenic (full egg complement upon adult emergence) or synovigenic (mature eggs during their adult life), yet these oogenesis strategies are important in
determining essential life-history traits such as preoviposition period, oviposition, and foraging habits that ultimately determine parasitoid fitness (Flanders 1950; Jervis & Kidd 1986). For synovigenic species, the longer a female lives, the more eggs she can produce and the more hosts she can attack. Although it is well established that feeding (either on the host (Jervis & Kidd 1986) or on carbohydrate and protein sources such as flower nectar, pollen, or honeydew (Jervis et al. 1993)) increases parasitoid longevity (Syme 1975; Berndt & Wratten 2005; Wäckers et al. 2008), it is not clear whether different diets influence the longevity of *T. rostrale*, nor how they may affect biological traits determining parasitoid fitness.

Successful parasitism by *T. rostrale* is a function of a number of factors, including the parasitoid’s ability to locate hosts and effectively overcome their defenses during attack. Many parasitoids locate their hosts in the environment through volatiles emitted by the host itself or from its host plant (Godfray 1994). Because *T. rostrale* is a parasitoid that successfully attacks low-density spruce budworm populations, it is likely that it has a very effective host location mechanism (Cusson et al. 1998a), although the olfactory cues for this are unknown. Once a host is found, host defenses influence the attack and oviposition success of a parasitoid. Such host defenses can be behavioural (*e.g.*, concealed feeding, commensalism, violent movements) or morphological (*e.g.*, thick cuticle, hairs) or physiological (*e.g.*, immune response); in either case, parasitoids have co-evolved countermeasures that overcome mechanisms that hinder their success (reviewed in Godfray 1994). In this study, we investigated the behavioural host defenses and countermeasures by *T. rostrale*.

In this laboratory study, we examine key life-history traits of *T. rostrale* that could play a role in its success as a parasitoid attacking low-density spruce budworm populations. Through behavioural and experimental observations, we describe: mating behaviour, fecundity, longevity, host-searching behaviour, oviposition behaviour of the parasitoid. The overall goal is to provide insight into the successful reproduction of *T. rostrale* in order to better understand how this parasitoid reaches such high parasitism levels during endemic spruce budworm populations (Cusson et al. 1998a). In practice, the information can be used to rear this parasitoid under laboratory conditions for further experiments.

**Material and Methods**

Adult *T. rostrale* were obtained by implanting laboratory-reared spruce budworm larvae into two field sites in Quebec near Armagh (46°46’ N, 70°39’ W, 312 m) and Petit-lac-à-l’Épaule (47°18’
N, 71°12’ W, 725 m). The physical environment, vegetation, and climate for these sites is described by Lethiecq and Régnièr (1988). A detailed description of the sentinel implantation technique is provided by Seehausen et al. (2013). After recovery from the field, implanted spruce budworm larvae were placed individually into 37-ml plastic cups in a growth chamber at 20°C and a L16:D8 h diel period, and checked daily until parasitoid or moth emergence.

**Mating Behaviour**

One virgin or mated female and one to three males were introduced into a meshed sleeve cage (24×41×32 cm) at room temperature and placed next to a window providing natural light. Males were aged between <24 h and 25 days, and females between <24 h and 36 days. The number and duration of couplings were recorded in 469 trials. Individuals were separated when no mating occurred after a 30 min observation period. To investigate whether females were mono- or polyandrous, one female was released into a cage with three color-marked males. We used blue, yellow, and red non-toxic paint (SCHOLA Inc., Marieville, Quebec) to mark males on their thorax. When mating occurred, the color code of the male was noted to test whether a specific color biased the probability of mating. Mating behaviour was observed and the number of couplings with one or more males and duration of mating were recorded for 97 trials. Male and female age, as well as the length of the hind tibia of males, were recorded to test the influence of age and male size on the occurrence of coupling. To test whether females would re-mate, a total of 71 females were presented within 24 h after the first coupling to one (n=20), two (n=29) or three (n=22) males, and the success and number of couplings were recorded.

**Potential Fecundity**

True lifetime fecundity of parasitoids is difficult to measure under laboratory conditions because they rarely lay the same number of eggs as in the field. However, potential fecundity can be measured, e.g., by counting the number of eggs produced and matured by parasitoid females (egg load). To measure potential fecundity for *T. rostrale* over time, virgin females were held without hosts for 2, 5, 7, 10, 15 or 20 days at 20°C and a L16:D8 h diel period, and provided with a 20% sucrose water solution. At the end of each time period, 10 individuals were frozen and dissected in a saline buffer solution under a binocular microscope. Female reproductive organs were isolated, ovarioles were separated from the two lateral oviducts, and mature eggs in the oviducts were counted.
**Longevity**

Virgin and unfed *T. rostrale* males and females were transferred within 24 h of emergence into 237-ml transparent plastic cages (height: 10 cm; diameter: 4.8) with a screened window on the top for ventilation. Cages were placed into a growth chamber at 20°C, 70% relative humidity, and a L16:D8 h diel period. They were offered one out of four different diets *ad libitum*, provided through soaked cotton rolls inserted into glass vials placed at the bottom of the cage. The diets were tap water, 20% sucrose solution as a source of carbohydrates, water containing 1% of commercially available multifloral pollen mix (Prince-Leclerc & Ass., Saint-Agapit, Quebec) as a source of protein, vitamin C and iron (sprayed on the cotton roll to make the pollen available to parasitoids), or a mix of 20% sucrose solution and 1% pollen. Diet vials were replaced every two days until the death of the parasitoid. Replicates of 15-18 males and 15 females were followed for each diet. Parasitoids were checked daily for survival.

**Short Distance Host-Searching Behaviour**

Naïve mated and fed females aged between 11 and 24 days (mean 17.25) were placed into the 237-ml transparent plastic cages described above in a closed box lit from below to avoid light from any other particular direction. A small window at the side of the box, facing away from the light sources in the room, allowed the female to be observed. Five cues related to the host (*C. fumiferana*) were presented in random order to each of 20 females. The host cues were: (1) an unparasitized 5th-instar spruce budworm larva feeding on balsam fir (*Abies balsamea* (L.) Miller) foliage in its feeding tunnel, containing silk and feces (hereafter referred to as ‘whole host system’); (2) an unparasitized 5th-instar larva; (3) silk from a spruce budworm larva rolled on a piece of paper; (4) fresh feces from spruce budworm larvae; (5) balsam fir foliage with feeding damage from a spruce budworm larva and carefully cleaned of other cues (feces and silk) using a microscope; and (6) no cue as a control. Two behaviours related to host location and recognition were evaluated: antennation and probing. Antennation was defined as bringing antennae together in front of the head and drumming them against the presented cue or surface. Probing was defined as bending of the abdomen, extracting the ovipositor from its shield and pushing it forward several times while walking over the substrate. Cues were presented on the bottom of the plastic cage for 5 min, or until both antennation and probing occurred. Control females were observed for 5 min in the cage with no host cues. The number of samples triggering one or both behaviours and the time until the two behaviours occurred were analyzed.
**Oviposition Behaviour**
A total of 135 mated (n=98) and virgin (n=37) *T. rostrale* females of different ages were released individually 1-33 times during their adult life into 237-ml transparent plastic cages (described above) containing an 8-cm balsam fir twig with one 5th-instar spruce budworm larva having fed at least 24 h on the shoot. Females were observed 5 min or until an attack took place. A successful attack was defined as the insertion of the ovipositor through the larva’s cuticle. Female behaviour prior to the attack was noted, such as the position of ovipositor insertion into the host larva (dorsal, lateral or ventral; anterior, mid- or posterior section of the larva; observed for a subsample, n=248, attacked in- and outside of the feeding tunnel), and the age of the female (n=930). Defensive host behaviour before and during the attack was also noted. The success of oviposition was evaluated by dissecting 58 5th-instar larvae after the attack to determine whether an egg had been laid.

**Statistical Analysis**
Success of coupling, occurrence of multiple matings, occurrence of antennation and probing, and success of attack were all analyzed using binomial logistic regression (PROC GLIMMIX; SAS Institute Inc., 2015). To analyze success of coupling, the number of males, previous mating status (mated, virgin), and female and male age were used as explanatory variables. The occurrence of multiple matings was related to the number of males present in the cage. The effect of the host cues, and of their order of presentation, on the frequency of antennation and probing were determined in separate analyses. Females were introduced into the model as repeated measures because all cues were presented to each female. Females were also taken as repeated measures in the analysis of attack success, because each female was tested several times during the experiment. The explanatory variable in this case was female age.

Analyses of variance (ANOVA) were used to analyze mating duration, longevity, potential fecundity, and time before host-searching behaviour occurred (PROC GLM; SAS Institute Inc., 2015). Two separate mating time analyses were performed: one where the sequence of matings (1-5) was the explanatory variable, and one specifically for mating duration of color-coded males where the sequence of mating (first and second) and the identity of the male (same, different) were the explanatory variables. Mating durations were log transformed in the first analysis to meet the assumption of residual normality. For the analysis of longevity, the explanatory variables diet, sex, and an interaction term were used, and longevity was rank-transformed because of non-normality of residuals. Female age was the explanatory variable of potential fecundity. Two separate analyses
were performed for time until antennation and probing (log transformed), with host cue as explanatory variable and females used as repeated measures.

Separate chi-square tests were used to analyze the influence of male color code (blue, yellow, red) and male size class (small, medium, large) on the probability of coupling, and the frequency with which different body parts of host larvae were attacked by the parasitoid (PROC FREQ; SAS Institute Inc., 2015).

In all analyses, differences between means of significant effects were tested using Tukey’s range test and all requirements of residual dispersion were met for all models unless otherwise stated.

Results

Mating Behaviour

Occurrence of coupling significantly increased from 42 to 57% with the number of males present in the cage ($F_{1,399}=8.56; P=0.0036$). Occurrence of coupling was almost ten times lower among previously-mated females ($F_{1,399}=29.32; P<0.0001$). Male ($F_{1,399}=0.72; P=0.3963$) or female age ($F_{1,399}=0.53; P=0.4675$) had no influence on the success of coupling.

About 25% of females mated more than once during the experiments ($n=184$). The mean number of matings per female during one mating session was 1.66 ($\pm 0.13$ SEM, maximum 18 times over 4 h 48 min). The number of males present in the cage did not significantly influence the occurrence of multiple matings with one female ($F_{2,181}=0.02; P=0.9815$). From the experiment with differently colored males, 24% ($n=54$) of females mated more than once, most of those with different males (69%; $n=13$). Four females mated twice with the same male. Male hind tibia length (size) did not significantly influence success of coupling ($\chi^2=2.5336; P=0.2817$). Color coding of males did not significantly influence the probability of mating ($\chi^2=0.059; P=0.9710$).

Mating duration varied significantly between first (or only) and subsequent matings in multiple mating series (Fig. 2.1; $F_{7,77}=3.22; P=0.0048$). The first mating was shortest (420 s $\pm 10$ SEM), the second was longest (999 s $\pm 97$ SEM), but duration decreased gradually with subsequent matings down to 563 s ($\pm 93$ SEM) after more than 5 matings in a single mating session. Results from the male-coloration experiment showed that mating durations were significantly influenced by an interaction between the individual male and the sequence in which males were mating with the female ($F_{3,18}=61.17; P<0.0001$). Mating duration was significantly longer when a female mated a
second time with the same male during one mating bout. However, when mating for a second time with a different male, mating duration did not differ from the first mating (Fig. 2.2).

**Potential Fecundity**
The oviducts of *T. rostrale* contained an average of 9.06 (±1.44 SEM) mature eggs within 24 h after emergence. This number increased significantly over the first three days after emergence (F\(_{6,68}\)=25.92; P<0.0001), and reached a mean maximum of 52.10 (±5.25 SEM) eggs after 7 days. No further increase or significant decrease was found thereafter until day 20 of adulthood (Fig. 2.3).

**Longevity**
Diet had a significant influence on longevity of *T. rostrale* (F\(_{3,118}\)=135.9; P<0.0001). Differences between males and females were small and non-significant (F\(_{1,118}\)=3.59; P=0.0607). There was no significant interaction between diet and sex (F\(_{3,118}\)=1.99; P=0.1185). Adult *T. rostrale* lived significantly longer on a 20% sucrose water solution, with or without 1% pollen, than on water alone or water with 1% pollen (Fig. 2.4).

**Short Distance Host-Searching Behaviour**
The order in which host cues were presented to females did not influence the probability of antennation (F\(_{1,113}\)=0.38; P=0.5401) or probing (F\(_{1,113}\)=3.34; P=0.0701). But the cues themselves influenced both the probability of antennation (F\(_{5,113}\)=3.57; P=0.0049) and probing (F\(_{5,113}\)=2.98; P=0.0144). The probability of antennation seemed unaffected by the presence or absence of host cues (none were significantly different from cue-free controls). However, significantly fewer females responded with antennation to feces than to silk, damaged foliage or the whole host system (Fig. 2.5A). No female responded with probing in the absence of host cues and the probability of probing was significantly lower in the presence of feces alone when compared to other cues, except foliage (Fig. 2.5B; F\(_{4,94}\)=3.10; P=0.0191). The duration before occurrence of the behaviour was not significantly influenced by host cues, whether for antennation (F\(_{5,72}\)=0.64; P=0.6689) or for probing (F\(_{4,45}\)=1.25; P=0.3045).

**Oviposition Behaviour**
*Tranosema rostrale’s* oviposition behaviour can be described as a sequence of five behaviours. (1) Antennation while searching for a host on the substrate (*e.g.*, foliage). (2) Probing while searching and antennating. Once *T. rostrale* gets closer to a host larva, both antennation and probing become
more frequent and insistent. (3) When the host is found by antennation, the parasitoid bends its abdomen under the thorax in front or deep into the foliage and pushes the ovipositor towards the host. When the larva is found by probing, the ovipositor is simply pushed forward under the cuticle of the host. (4) The oviposition itself only takes approximately 0.5-1.0 s, during which the parasitoid is immobile or tracks the host if it is attempting to escape. (5) Immediately after the attack, most females walk away but some resume oviposition behaviour and attack the same host again. Even if some host larvae “bleed” after oviposition, no host feeding has been observed in more than 1000 observations done during this study.

Spruce budworm larvae exhibited several defensive behaviours against attack by *T. rostrale*. When the larva was approached or touched by the parasitoid, it responded with one or a combination of the following behaviours: vigorous movements, biting, and/or regurgitation. In some cases, larvae fell out of their feeding tunnel on a silk thread when the parasitoid approached. While this may lead to avoidance of parasitism in some cases, *T. rostrale* females were observed following the fresh silk thread to the bottom of the cage through antennation, where they found and attacked the larva.

Significant differences were found in the frequency of parasitoid attacks on different positions of the host’s body (Table 2.1; $\chi^2_{6}=112.23; P<0.0001$). Dorsal attacks were more frequent (62.5%) than ventral (26.6%) or lateral (10.9%) attacks.

The overall success of attack (defined above) was 32% (n=943), and there was no difference between mated (32%; n=835) and virgin (32%; n=108) females. There was a significant positive correlation between the female’s age and the success of attack ($F_{1,928}=35.13; P<0.0001$). However, even newly-emerged females successfully attacked spruce budworm larvae. *Tranosema rostrale*’s eggs were found in 83% (n=58) of spruce budworm larvae dissected after successful attack.

**Discussion**

Our laboratory results show that female *T. rostrale* are more likely to mate when there are more males present in a cage. In nature, female parasitoids can attract several males that compete for mating (Goh & Morse 2010), however, it is unknown whether this occurs in *T. rostrale*. One factor known to play an important role for both intra- and intersexual selection in parasitoids is male size (Grant et al. 1980; Charnov et al. 1981; Jones 1982). While we did observe fights between males for a single female, we found no relationship between male size and mating success in *T. rostrale*.
Flight movements that take more space than provided by the cages in this experiment might be an important factor influencing mating success in the field.

The probability of mating was 10× higher for virgin *T. rostrale* females than for females that had mated on a previous day. However, among receptive *T. rostrale* females, 25% mated multiple times in one mating session, in most cases with different males. Polyandrous females (mating multiple times) are thought to be relatively rare among hymenopteran parasitoids, one mating being generally sufficient to provide enough sperm to fertilize all eggs (Gordh & DeBach 1978; van den Assem 1986; Ridley 1993; Godfray 1994).

To ensure paternity, males of some species have evolved morphological, physiological, and behavioural adaptations (Parker 1970; Knowlton & Greenwell 1984; Simmons 2001). Behavioural paternity enhancement mechanisms include prolonged mating duration, increased mating frequency (Thornhill 1984), post-copulatory interactions or restricting access to the female (called ‘mate guarding’) (Parker 1974; Gwynne 1984; Alcock 1994). When *T. rostrale* males mated a second time with the same female, mating duration was significantly increased. This prolonged mating for the second mating may be a post-copulatory ritual that prevents the female from mating with other males. In some parasitoid species, males remove the sperm from the precedent male before inseminating the female with their own (Simmons 2001). One indicator of sperm removal is that the mating time with the second male is considerably longer than with the first (Thornhill & Alcock 1983; Waage 1984). We found that in *T. rostrale*, mating duration is short the first time a male mates with a specific female, even if another male had previously mated with that female. Therefore, sperm of different males is most probably mixed in the female’s spermatheca.

Our results demonstrate that *T. rostrale* is synovigenic, with an index of approximately 0.17 at 20°C, meaning that its initial egg load at emergence is about 17% of its potential life time fecundity (Jervis et al. 2001). This rate is most likely overestimated, as females in our experiments were not allowed to lay eggs, which may reduce the total number of eggs produced during a female’s lifetime due to capacity limits in the female’s oviduct. Most egg production occurred within the first three days of emergence, after which no further increase in the number of eggs was observed. This plateau in potential fecundity may be due to capacity limits of the oviducts, once again because females were not allowed to lay eggs.

Adult parasitoids are known to feed on a number of food sources in their habitat such as nectar and pollen from flowers (Jervis et al. 1993). Several laboratory studies have shown that longevity
and fecundity of parasitoids are increased by access to flowers, nectar, honey or sugar-water (e.g., Syme 1975; Dyer & Landis 1996; Mathews & Stephen 1997, Fidgen & Eveleigh 1998). In T. rostrale, the addition of pollen to water as a source of protein, vitamin C, and iron did not increase longevity, but 20% sucrose water solution increased longevity almost 6× for males and more than 11× for females, highlighting the importance of carbohydrates for longevity of this species. Natural sources of sugar in forest habitats are diverse and include flowers in the understory or trees and honeydew from sap-sucking insects (Wäckers et al. 2008). It remains unknown whether the fecundity of T. rostrale is also increased by an availability of carbohydrate- or protein-based food.

It is well-established that parasitoids use chemical, visual, tactile and auditory cues from the host’s microhabitat and host plant, as well as indirect (derived from the activity of the host) and direct (derived from the host itself) cues from the host for host location (Vinson 1976, Godfray 1994). Volatile chemical cues are often used by the parasitoid for long-range host or host-habitat location, while contact chemicals and other cues are used for short-ranged host location (reviewed by Vinson 1976; Hilker & McNeil 2008). Our findings are restrained to short-range host location, as experiments were done in confined plastic cages. While for T. rostrale antennation was not triggered by a specific host cue, probing occurred most often when a female encountered direct cues from the host, i.e., silk and the host larva itself, but also when damaged foliage was found and less frequently when larval feces were encountered. As spruce budworm larvae are more or less concealed in their feeding tunnel, probing into the foliage triggered by direct or indirect host cues might be the most effective way for T. rostrale to find and oviposit into the host. Females were observed probing for several minutes into empty feeding tunnels, while the host larva was just outside of the tunnel, suggesting that visual cues might not be important for this species at short range. In the field, T. rostrale does not exhibit any host instar preference (Chapter 3). Therefore, it can be assumed that the host searching behaviour of T. rostrale we describe here for 5th-instar spruce budworm larvae is applicable to other instars.

Insects have developed numerous morphological and behavioural defense mechanisms against parasitoids (Gross 1993). The relatively short duration of oviposition for T. rostrale that we observed might be necessary for the parasitoid to avoid host defensive behaviours such as biting, spitting or escaping, as also described for other parasitoid species (Prop 1960, Tripp 1960, Goff & Nault 1974). A defense mechanism that T. rostrale has apparently learned to circumvent is the retreat of the larvae on a silk thread. We show that silk can be detected by the parasitoid and that
it triggers probing. Therefore, spruce budworm larvae “spinning-down” on a silk thread to escape parasitism can be easily tracked and parasitized by *T. rostrale*. Such behaviour has also been reported for other parasitoid species (Yeargan & Braman 1986; 1989).

Some parasitoid species oviposit in specific locations of their host, to avoid active removal of eggs (Herrobut 1968, Danks 1975, Martin et al. 1989), sclerotized host cuticles (Shaw & Huddleston 1991), or aggressive host defense behaviour. Spruce budworm larvae are most frequently attacked by *T. rostrale* in the dorsal part of the body. This may simply be related to the position of the host larva in its feeding tunnel relative to *T. rostrale* search behaviour. *Tranosema rostrale* crawls on top of the foliage when searching for hosts, probing in between balsam fir needles to find the larva. However, by ovipositing through the foliage, *T. rostrale* avoids aggressive host defense behaviour.

Mated and virgin *T. rostrale* females attacked spruce budworm larvae at similar rates. This behaviour is consistent with the biology of haplodiploid parasitoids, where arrhenotokous females lay viable eggs (male) without mating. We observed female wasps readily attacking hosts within 24 h of emergence, and the probability of attack increased with female age. These behaviours may also be related to the reproductive biology of *T. rostrale* because females emerge with mature eggs available for oviposition and can continue to develop more during their life time regardless of mating status. It appears therefore that *T. rostrale* has neither an obligatory nor facultative preoviposition period in captivity.

Our work provides critical insight into key reproductive biology and behaviour of *T. rostrale*. It is clear that a number of traits contribute to the success of this parasitoid in attacking low-density spruce budworm populations, namely: (1) its lack of a pre-mating or preoviposition period; (2) the relatively rapid maturity of its eggs soon after emergence despite being synovigenic; and (3) its efficacy in short distance host searching and oviposition behaviour that appears to successfully circumvent basic host defenses. The results reported here were obtained under laboratory conditions, using small experimental arenas, mating, host searching, and attack success in nature may differ. However, our work also includes advanced methodological insights for rearing this important spruce budworm parasitoid in the laboratory, including: (1) its mating success is increased when more males are present; (2) mating and oviposition can take place immediately after emergence; and (3) a sucrose water solution is sufficient to significantly increase parasitoid longevity, when compared to water only.
Table 2.1: Number and percentage of attacks by *T. rostrale* females at different body locations of 5th-instar spruce budworm larvae in the laboratory. Percentages followed by the same letter do not differ significantly at P < 0.05 according to *post hoc* comparisons with Tukey’s range test.

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<th>Location of attack</th>
<th>No. of attacks</th>
<th>Percentage</th>
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<tr>
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</tr>
<tr>
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<td>20.16 ab</td>
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<tr>
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<td>25.00 a</td>
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<tr>
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<tr>
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<td>4.03 c</td>
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<tr>
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</tr>
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<tr>
<td>Mid-section</td>
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<tr>
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<td>7.26 c</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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Fig. 2.1: Mean (± SEM) mating duration (seconds) for subsequent matings by *Tranosema rostrale* females in a single mating bout. Bars followed by the same letter do not differ significantly at P<0.05 according to *post hoc* comparisons with Tukey’s range test.
Fig. 2.2: Mean (± SEM) mating duration for the first and second subsequent mating of *Tranosema rostrale* females with two different males or the same male. Bars followed by the same letter do not differ significantly at P<0.05 according to post hoc comparisons with Tukey’s range test.
Fig. 2.3: Mean (± SEM) number of mature eggs in oviducts of *Tranosema rostrale* females (potential fecundity) as a function of female age (days).
Fig. 2.4: Mean longevity (± SEM; days) of *Tranosema rostrale* males (dark grey) and females (light grey) on different diets (water, water with 1% pollen, water with 20% sucrose, and water with 20% sucrose and 1% pollen). Bars followed by the same letter do not differ significantly at P<0.05 according to post hoc comparisons with Tukey’s range test.
Fig. 2.5: Mean (± SEM) probability of (A) antennation and (B) probing by *Tranosema rostrale* influenced by different cues from spruce budworm larvae. Bars followed by the same lower case letter do not differ significantly at P <0.05 according to post hoc comparisons with Tukey’s range test.
Chapter 3
Seasonal Parasitism and Host Instar Preference by the Spruce Budworm (Lep.: Tortricidae) Larval Parasitoid *Tranosema rostrale* (Hym.: Ichneumonidae)

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 4th-instar larva, and then decreased again until the 6th-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 3rd- to 6th-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 5th-instar spruce budworm larvae. It appears that the seasonal phenology of *T. rostrale* and not competition with *E. cacoeciae* or host instar preference drives the observed seasonal pattern of spruce budworm larval parasitism.

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Introduction

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 & Lysyk 1995; Régnière & 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 parasitize over 90% of a given spruce budworm population (Cusson et al. 1998a; Seehausen et al. 2013; 2014). Surprisingly, the influence of this parasitoid as a mortality factor in outbreaking spruce budworm populations is relatively low (McGugan & Blais 1959; J. Régnière unpublished data). Cusson et al. (1998a) 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. 1998a; 2002; Fidgen & 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 parasitoid’s physiological response to the environment, especially ambient temperature (Powell & Logan 2005; Visser & Both 2005) and photoperiod (Koštál 2011), which determine when and where adults are active and can successfully attack their hosts (Boivin 1994; Thomas & Blanford 2003; Hance et al. 2006). In a well synchronised 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 2013).

Interspecific competition between parasitoids (*e.g.* multiparasitism) can have an important influence on successful parasitism and the seasonal pattern of parasitism (Lee & Pemberton 2007; Harvey 2013; Mohammadpour et al. 2014). Multiparasitism can only be 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 2013). Therefore, many ‘inferior’ parasitoid species have
developed mechanisms to distinguish parasitized from unparasitized hosts in order to avoid interspecific competition (van Alphen & Visser 1990; Mackauer 1990; Tamò et al. 2006).

Many parasitoids preferentially attack certain host instars (e.g., Liu et al. 1984; Hébert & Cloutier 1990a; Fuester & Taylor 1991; Fidgen et al. 2000), but not all (e.g., Mackauer 1973; Hébert & Cloutier 1990a; Fuester & 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 may for example attack the largest available host (e.g., Hébert & Cloutier 1990a; Wen et al. 1995, Lin & Ives 2003). Several life history traits of parasitoids have been shown to improve with increasing host size, e.g., survival of offspring (Hébert & Cloutier 1990a), 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 (e.g., development after diapause). In addition, several life history traits related to T. rostrale’s overall performance in different host instars were measured (immature survival, developmental time, sex ratio, and adult size) to further assess the importance of host instar in the performance of this larval parasitoid.

Material and Methods
The field experiments were conducted in two sites near Armagh (46°46’ N, 70°39’ W, 312 m) and Petit-lac-à-l’Épaule, Quebec (47°18’ N, 71°12’ W, 725 m), henceforth called Armagh and Epaule. The physical environment, vegetation, and climate for these sites were described by Lethiecq & Régnière (1988). Overwintering 2nd-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% humidity, and with a 16-hour daily photoperiod. Post-diapause spruce budworm larval instars
(2\textsuperscript{nd}-6\textsuperscript{th}) were determined visually by keeping track of the number of molts, head capsule size, and colour of the cuticle. Because developmental polymorphism exists in this species (Schmidt & Lauer 1977), only larvae clearly belonging to the desired instar were used in the experiments.

\textbf{Seasonal Parasitism and Sex Ratio}
To study seasonal parasitism by \textit{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 (sentinel implantation) twice a week for seven days 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. 2012b) 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. (1998a), Fernández-Triana and Huber (2010), Huber et al. (1996), and O’Hara (2005). After visual assessment of apparent parasitism by the ectoparasitoid \textit{Elachertus cacoeciae} (Howard) (Hymenoptera: Eulophidae) using the morphological description of the parasitoid provided by Fidgen & Eveleigh (1998), host remains were dissected to quantify any potential multiparasitism by \textit{T. rostrale} and \textit{E. cacoeciae}. When a parasitoid larva was found in the host remains, it was identified as \textit{T. rostrale} only when the morphology of the larva matched the description by Cusson et al. (1998a) and Miller and Renault (1963). Sex ratio (% females) of \textit{T. rostrale} over the season was determined by sexing all \textit{T. rostrale} emerging from exposed spruce budworm larvae in 2013 and 2015 from both study sites (n=763; data from other years were not available).

An additional experiment was conducted in 2015 to determine whether \textit{T. rostrale} could actually parasitize 2\textsuperscript{nd}-instar larvae in the early spring, and if so, to compare parasitism rates between 2\textsuperscript{nd}- and 3\textsuperscript{rd}-instar larvae. The sentinel implantation method described above was also used for this
experiment. In Armagh, about 100 larvae in each of 2nd- and 3rd-instar were placed at eye level on buds of balsam fir from 13-15 May 2015, when wild 2nd-instar spruce budworm larvae were predicted to emerge from their hibernacula in the study area (Régnière et al. 2012b). After a 2-day 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 2nd-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-day exposure period, larvae that migrated to buds were collected and reared until moth or parasitoid emergence. Thereafter, 200 3rd-instar larvae were exposed to parasitoids in the same study area from 21-28 May 2015, when the occurrence of wild 3rd-instar larvae was predicted. In Epaule, about 300 overwintering 2nd-instar larvae in cheese cloth were placed during the predicted occurrence of wild 2nd-instar larvae from 26 May - 3 June 2015 and collected as described above. During the following predicted presence of wild 3rd-instar larvae in the study area, about 300 2nd-instar larvae were again exposed in cheese cloth, and also 200 3rd-instar larvae were placed at eye level directly on balsam fir buds from 3-12 June 2015 to compare parasitism of 2nd- and 3rd-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 3rd- to 6th-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 Epaule. Exposure periods lasted only 2-3 days 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 3rd- to 6th-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 237-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 to 48 hours 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 237-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 under the standard rearing conditions described above.

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. 3.1) 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 analysed 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 seven-day 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 *E. cacoceciae* caused convergence problems, this particular analysis was only run with main effects. *Actia interrupta* (see Results for more details) 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 analysed 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 analysed 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 (number of females / total) were assessed using logistic regression with the binomial distribution and spruce budworm larval instar as explanatory variable. 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 2nd versus 3rd-instar larvae. The tibia length of parasitoids emerging from different host instars was analysed 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 analysed 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 analysed using non-parametric 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 & Fligner 1991).

The relationship between hind tibia length and dry mass was analysed 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 (3rd- to 6th-instar) were parasitized by nine parasitoid species (Table 3.1). *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 Epaule (Table 3.1). Over the 5-year period, host instar (or the date of host implantation) had a
significant influence on overall parasitism, and on parasitism rates of three parasitoid species, but not on parasitism by other species. Neither site nor the instar×site interaction had a significant influence on parasitism rates (Table 3.2a); as a result, parasitism of different host instars over the season was pooled for both sites. Parasitism rates by *T. rostrale* increased from the 3rd to 4th-instar, and decreased in later instars. *Elachertus cacoeciae* did not parasitize 3rd-instar larvae but parasitism rates on later instars steadily increased to a maximum in 6th-instar larvae. Parasitism by *A. interrupta* only occurred among larvae exposed as a mix of 4th- and 5th-instars and later, and was highest amongst larvae exposed as a mixture of 5th- and 6th-instars (Fig. 3.2). Multiparasitism by *T. rostrale* and *E. cacoeciae* occurred in all study sites and throughout 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 3.1). The proportion of females among *T. rostrale* adults emerging during the season was positively correlated with spruce budworm instar (3rd-6th) exposed in the field according to the natural seasonal timing (Fig. 3.3; $F_{1,16} = 54.55; P<0.0001$).

During the period of predicted occurrence of wild 2nd-instar spruce budworm larvae, no parasitism of implanted 2nd- (n=51) and 3rd-instars (n=19) was observed in Armagh or Epaule (n=34 2nd-instars). However, 35% (n=23) of 3rd-instar larvae exposed at the time when natural 3rd-instars could be expected in Armagh were parasitized by *T. rostrale*. In Epaule, parasitism of 2nd-instars by *T. rostrale* was significantly lower (3.51%, n=57) than of 3rd-instars (11.86%, n=59, $P=0.0306$) at the time that natural 3rd-instars were occurring. While only *T. rostrale* attacked 3rd-instars on either site, one 2nd-instar larva was parasitized by *Enytus montanus* (Ashmead) (Hymenoptera: Ichneumonidae) during this period in Epaule.

**Host Instar Preference and Consequences on Life History Traits**

*Tranosema rostrale* was by far the dominant parasitoid in both Armagh 42.14% ± 6.65 SEM and Epaule 44.40% ± 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 Epaule (14.21% ± 13.67 SEM) than in Armagh (1.92% ± 0.93 SEM). *Actia interrupta* (3.37% ± 3.37 SEM) and *Phytodietus* sp. (1.26% ± 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 3.2b; Fig. 3.4). The variance in parasitism by *T. rostrale* between the dates of exposure periods for host larvae (random effect) was significant ($\chi^2 = 81.14; P<0.0001$) and estimated as 94%. Mean overall parasitism (by *T. rostrale* + all other species) in the field choice
test was 50.75% (± 5.91 SEM; n=256) in Armagh and 60.81% (± 28.54 SEM; n=307) in Epaule, and was significantly influenced by the interaction of site and host instar (Table 3.2b; Fig. 3.4), although there were no significant differences between the means. Host instar also significantly influenced overall parasitism as a main factor (Table 3.2b); comparisons of means showed that 5th-instar larvae were significantly more parasitized than 3rd-instar larvae. Study site did not significantly influence overall parasitism. The random effect did significantly influence overall parasitism (χ²=96.06; 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 ± 0.05 SEM) was not significantly influenced by host instar (F₃,₁₇=2.24; P=0.1211). Significant positive correlations were found between the hind tibial length and insect dry mass for both *T. rostrale* males and females (F₁=620.07; P<0.0001; R²=0.66), however, females were heavier and had longer hind tibia than males (Fig. 3.5; F₂=332.93; 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 (F₁=30.53; P<0.0001), with female tibia being longer than male tibia. There was also a significant influence of host instar (F₃=7.28; P=0.0001), with a significant instar×sex interaction (F₃=3.78; P=0.0120). The length of female hind tibia did not significantly change with host instar among females, but first increased from 3rd- to 5th-instar and slightly decreased again in 6th-instar larvae for males (Fig. 3.6).

Survival and development time were only analyzed for males because only one female emerged from the laboratory rearing. Survival of *T. rostrale* reared under laboratory conditions in spruce budworm larvae did not vary significantly by host instar, either from egg to pupa (χ²=3.04; P=0.3849) or from pupa to adult (χ²=3.20; P=0.3614). Host instar had no significant effect on the parasitoid’s larval development time (χ²=4.53; P = 0.2096), but affected the development time of pupae (χ²=9.03; P=0.0289). However, multiple comparisons of means revealed no significant differences in development times among host instars. Total development time (larval + pupal development time) was unaffected by host instar (χ²=6.11; 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 days 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-year period in two study sites confirm the findings from single- and two-year studies (Cusson et al. 1998a; 2002; 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. 1998a). However, nothing is known about the overwintering life stage of *T. rostrale*. Cusson et al. (1998a) reported that *T. rostrale* successfully attacked 2nd-instar spruce budworm larvae in a laboratory choice test, albeit at very low frequency (1.33%). Our results from the field also suggest that 2nd-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 2nd-instar larvae.

Concurrent with the decrease in parasitism by *T. rostrale* after the 4th-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 outcome of competition between these two species, although *E. cacoeciae* may have a competitive advantage over *T. rostrale* (Harvey 2013) because it is an ectoparasitoid that paralyses 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 & Arthur 1973; Obonyo
et al. 2011). Thus, in addition to external traits such as host size, internal traits related to host quality may be assessed by the female at oviposition (e.g., nutritional value, ingested secondary plant compounds, presence of another parasitoid). For *T. rostrale*, avoiding multiparasitism may be more important than host size for 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 6th-instar larvae shortly before pupation (Doucet & Cusson 1996b). 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. 1998a), 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 fertilised 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 days (Chapter 2), suggesting they could live long enough in the field to experience increased probabilities of mating over their lifetime. Alternatively, females may be produced preferentially later in the season because of decreasing host densities as the season progresses (Comins & Wellings 1985; King 1987).

Tibial length of *T. rostrale* males from 5th-instars was significantly longer than that from 3rd- and 6th-instars. Hind tibial length is a relatively common measure of parasitoid size (e.g., Waage & Ming 1984; Rosenheim & 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* (Chapter 2).

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 approximately 10% slower than males (Chapter 4), 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 post-diapause 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. cacoeciae*) or a combination of both. The field components of our study took place in two sites that vary considerably in environmental conditions (Lethiecq & 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.
Table 3.1: Overall percentage parasitism and multiparasitism of 3<sup>rd</sup> to 6<sup>th</sup>-instar spruce budworm larvae by different parasitoid species in Armagh and Epaule, Québec, Canada, 2011-2015.

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Armagh</th>
<th>Epaule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sampled larvae</td>
<td>272</td>
<td>221</td>
</tr>
<tr>
<td><em>Tranosema rostrale</em></td>
<td>46.7</td>
<td>43.0</td>
</tr>
<tr>
<td><em>Elachertus cacoeciae</em></td>
<td>25.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Multiparasitism by <em>T. rostrale</em> and <em>E. cacoeciae</em></td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Actia interrupta</em></td>
<td>4.8</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Phytodietus</em> sp.</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>All other hymenopteran species&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.2</td>
<td>14.0</td>
</tr>
<tr>
<td>Total</td>
<td>83.8</td>
<td>76.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>One incidence of apparent multiparasitism with *A. interrupta* occurred.

<sup>b</sup>Includes 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 non-identified parasitoids in both study sites.
Table 3.2: Logistic regression analysis of (a) seasonal parasitism (2011-2015) and (b) host instar preference of *Tranosema rostrale* and other parasitoid species attacking spruce budworm in Armagh and Epaule, Quebec, Canada.

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Host instar</th>
<th>Site</th>
<th>Host instar*Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F  df  P</td>
<td>F  df  P</td>
<td>F  df  P</td>
</tr>
<tr>
<td>(a) Seasonal parasitism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tranosema rostrale</em></td>
<td>13.2  6,18.79  &lt;0.0001</td>
<td>2.35  1,40.43  0.1333</td>
<td>1.4  6,18.79  0.2657</td>
</tr>
<tr>
<td><em>Elachertus cacoeciae</em></td>
<td>14.69  6,21.93  &lt;0.0001</td>
<td>0.48  1,40.78  0.4924</td>
<td>-   -   -</td>
</tr>
<tr>
<td><em>Actia interrupta</em></td>
<td>3.35  6,26  0.0141</td>
<td>-   -   -</td>
<td>-   -   -</td>
</tr>
<tr>
<td>All other species</td>
<td>1.62  6,18.58  0.1979</td>
<td>0.11  1,41.77  0.7467</td>
<td>1.63  6,18.58  0.1932</td>
</tr>
<tr>
<td>Overall</td>
<td>3.73  6,18.63  0.0129</td>
<td>1.33  1,46.11  0.2556</td>
<td>0.48  6,18.83  0.8150</td>
</tr>
<tr>
<td>(b) Host instar preference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tranosema rostrale</em></td>
<td>0.72  3,16  0.5520</td>
<td>0.05  1,3.87  0.8401</td>
<td>0.75  3,16  0.5375</td>
</tr>
<tr>
<td>All other species</td>
<td>2.02  3,16  0.1523</td>
<td>0.00  1,16  1.0000</td>
<td>0.03  3,16  0.9940</td>
</tr>
<tr>
<td>Overall</td>
<td>3.97  3,16  0.0272</td>
<td>0.04  1,3.85  0.8511</td>
<td>3.50  3,16  0.0401</td>
</tr>
</tbody>
</table>
Fig. 3.1: Right hind leg of *Tranosema rostrale*. Dotted lines indicate the points of measurement of hind tibial length in this study.
Fig. 3.2: Mean (±SEM) percentage parasitism of different spruce budworm larval instars over spring and summer in Armagh and Epaule (Quebec, Canada) between 2011 and 2015 (pooled), by *Tranosema rostrale, Elachertus cacoeciae, Actia interrupta*, and all other parasitoids.
Fig. 3.3: Mean (±SEM) proportion of female *Tranosema rostrale* emerging from spruce budworm larvae attacked in different instars (3rd to 6th) in the field at the time of natural occurrence in Armagh and Epaule, Québec, Canada (n=763). Statistics: F_{1,16}=54.55; P<0.0001; y=0.2224x-0.4938; R^2=0.712.
Fig. 3.4: Mean (±SEM) percentage parasitism (by *Tranosema rostrale*, by all species other than *T. rostrale*, and overall) of 3rd- to 6th-instar spruce budworm larvae simultaneously exposed to parasitoids in (A) Armagh (n=256) and (B) Epaule (n=307), Quebec, Canada, 2014 and 2015.
**Fig. 3.5:** Observed (circles) and predicted (lines) length of hind tibia (mm) as a function of dry mass (mg) for *Tranosema rostrale* males (grey; n=206) and females (black; n=243) from Armagh and Epaule, Quebec, Canada. Data are back-transformed from log.
Fig. 3.6: Mean (±SEM) hind tibia length (mm) of *Tranosema rostrale* male (n=63) and female (n=87) progeny emerging from spruce budworm larvae that were attacked during different instars (3rd to 6th), 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 posthoc multiple comparisons of means using Tukey’s range test.
Chapter 4
Developmental and Reproductive Responses of the Spruce Budworm (Lep.: Tortricidae) Parasitoid Tranosema rostrale (Hym.: Ichneumonidae) to Temperature

Abstract
The temperature-dependent development, immature survival, adult longevity, and potential fecundity of the endoparasitoid Tranosema rostrale (Hymenoptera: Ichneumonidae) parasitizing spruce budworm Choristoneura fumiferana (Lepidoptera: Tortricidae) larvae was investigated under laboratory conditions at several constant temperatures ranging from 5 to 30°C. Non-linear maximum likelihood modeling approaches were used to estimate changes in development, survival and longevity over all temperatures. In addition, we developed a general non-linear model describing potential fecundity of the parasitoid taking temperature-dependent oogenesis and oosorption into account, using discrete difference equations. In-host and pupal development rate of the parasitoid increased with temperature up to 25°C and decreased thereafter. Immature survival was highest below 20°C and rapidly decreased at higher temperatures. Adult longevity decreased exponentially with increasing temperature for both males and females. Highest potential fecundity was reached at 10°C. Considering survival and potential fecundity, the parasitoid seems to be best adapted to cool temperatures below 20°C. Simulations of the life-history traits under variable temperature regimes indicate that temperature fluctuations decrease survival and increase realized fecundity compared to constant temperatures. The developed temperature-dependent fecundity model can be applied to other non-host-feeding synovigenic parasitoids. The equations and parameter estimates provided in this paper can be used to build comprehensive models predicting the seasonal phenology of the parasitoid and spruce budworm parasitism under changing climatic conditions.

3 A version of this chapter is under review by the Journal of Insect Physiology: Seehausen, M.L., Régnière, J., Martel, V., & Smith, S.M. Developmental and reproductive responses of the spruce budworm (Lepidoptera: Tortricidae) parasitoid Tranosema rostrale (Hymenoptera: Ichneumonidae) to temperature.
Introduction

Insects are poikilotherms (cold-blooded) and as such, their metabolic, survival, developmental and reproductive rates all depend on ambient temperature (Régnière & Powell 2013). The understanding of the influence of temperature on insects is therefore crucial to understand changes in seasonal patterns of phenology (Powell & Logan 2005), distribution (Régnière et al. 2012b), and overall population dynamics (Kingsolver 1989; Gray 2008). Temperature can also affect interactions between insects at different trophic levels and can alter the dynamics of their populations (Fleming & Volney 1995; Gray 2008; Hance et al. 2008).

The influence of temperature on overall parasitoid performance is well studied, because they are an important mortality factor for many insects (e.g., reviewed by Hance et al. 2007). Temperature can affect not only their development time, survival, and longevity, but also their fecundity, a determinant of the number of hosts they can potentially attack (Lysyk 1998; Sagarra et al. 2000; Ris et al. 2004). Two general reproductive strategies can be distinguished in female parasitoids: pro-ovigeny, where females emerge with their full lifetime egg content, and synovigeny, where females emerge with only a few, if any mature eggs, and develop more during their adult life (Flanders 1950). Jervis et al. (2001) proposed that rather than two strategies, there is evidence for a continuum in ovigeny, from pro-ovigenic to extremely synovigenic species. Some synovigenic parasitoids have evolved two concurrent physiological processes contributing to their fecundity: egg production (oogenesis) and egg resorption (oosorption) (Jervis et al. 2001). The current understanding of egg resorption is that it acts as a buffer against environmental stochasticity (Richard & Casas 2009) by allowing the female to regain energy from her own eggs for important metabolic processes (Bell & Bohm 1975), and therefore to invest in future rather than immediate fitness. As poikilotherms, both egg maturation (Papaj 2000) and egg resorption (Bell & Bohm 1975; Barbosa & Frongillo 1979; Santolamazza-Carbone et al. 2008) are temperature-dependent in insects.

The spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is a major pest insect that is native to North America. It undergoes periodic outbreaks at irregular intervals, averaging 35-40 years, in boreal coniferous forests across eastern North America (Blais 1965a; Morin 1994), where it causes defoliation and mortality on balsam fir *Abies balsamea* (L.) Miller, and to a lesser extent on several spruce species including white, *Picea glauca* (Moench) Voss; red, *P. rubens* Sarg; and black, *P. mariana* (Miller) BSP (MacLean 1980). Parasitoids are prominent
among the several factors that influence spruce budworm population dynamics (Royama 1984; Régnière & Lysyk 1995; Régnière & Nealis 2007). The parasitoid community of the spruce budworm changes depending on its host’s density (Eveleigh et al. 2007), and at low population levels, parasitoids are the main mortality factor (Régnière et al. 2013).

The koinobiont synovigenic larval endoparasitoid *Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae) is a key mortality factor in endemic spruce budworm populations in Québec, Canada, where it can inflict mortality rates >90% (Cusson et al. 1998a; Seehausen et al. 2013; 2014). Little is known about *T. rostrale*’s seasonal history despite its importance in the dynamics of spruce budworm populations during the period between outbreaks. Attacks on spruce budworm start in late May while in the 3rd and 4th larval instars (Cusson et al. 1998a; Chapter 3). Although the actual duration of the parasitoid’s larval and pupal development in the field is unknown, the larva emerges from its host within a few weeks of oviposition, mainly when the host larva is in the 5th or 6th instar, and forms a silk cocoon on the foliage near the host’s cadaver (Cusson et al. 1998a). Adults emerge from the cocoon in early July and it is believed that the parasitoid has more than one generation per year in the locations where it has been studied in central Québec (Cusson et al. 1998a). Nothing is known of its overwintering habitat or life stage.

As a first step in addressing these knowledge gaps, we obtained basic information on *T. rostrale*’s developmental, survival, and reproductive responses to temperature. To this end, we reared the parasitoid in growth chambers at constant temperatures between 5 and 30°C and applied non-linear modeling approaches to estimate changes in development, survival, and longevity over all temperatures. To describe temperature-dependent fecundity of the parasitoid, we developed a new non-linear model. The results will eventually be used to build a seasonal biology model for this species.

**Material and Methods**

Adult *T. rostrale* were obtained by exposing laboratory-reared 3rd-6th-instar spruce budworm larvae to parasitoids on balsam fir trees in two study sites in Québec, near Armagh (46°46’ N, 70°39’ W, 312 m) and Petit-lac-à-Épaule (47°18’ N, 71°12’ W, 725 m) (Lethiecq & Régnière 1988), where parasitism rates by *T. rostrale* have been consistently high over the last 20 years (J.R., unpublished data). The different larval instars were exposed according to their natural time of occurrence, as determined by the Spruce Budworm Seasonal Biology Model (Régnière et al. 2012b). Host larvae were recovered after being exposed for one week and reared on either artificial
diet (McMorran 1965) or balsam fir foliage at room temperature until parasitoid or moth emergence. Adult parasitoids were identified using dichotomous keys (Cusson et al. 1998a; Bennett 2008).

**Stage-Specific Development Time and Survival**

Spruce budworm larvae were reared under laboratory conditions on balsam fir foliage and 5th-instar larvae were parasitized by *T. rostrale* as described in Chapter 2. This host instar was chosen because of its convenient size and development time. Survival and development time of *T. rostrale* does not differ between spruce budworm instars (Chapter 3). Immediately after parasitism, larvae were transferred into 237-ml transparent plastic containers with screened windows for ventilation and a twig of balsam fir foliage having at least three current-year shoots that were inserted into a glass vial with water. Within 1 h after parasitism, a total of 212 parasitized spruce budworm larvae were transferred into growth chambers at 11 constant temperatures, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30°C (14 to 26 larvae per temperature), representing the approximate range of temperatures encountered by the parasitoid under natural conditions in Québec (Lethiecq & Régnière 1988). Balsam fir foliage was renewed every 1-7 days, depending on rearing temperature, until parasitoid or moth emergence. Parasitoid sex was determined at emergence by the presence or absence of the clearly visible ovipositor. The temperature transfer method (Régnière et al. 2012a) was used to rear *T. rostrale* in-host (eggs and larvae) and pupae at the more extreme temperatures. At 5 and 7.5°C, insects were kept for 5 days at low temperature and then transferred to 15°C for 2 days. At 27.5 and 30°C, insects were kept for 2 days at high temperature and then transferred to 15°C for 5 days. The transfers were repeated until adult parasitoids or moths emerged. For all parasitoids, stage (in-host, or pupa) and sex specific development time and stage specific survival were recorded. Parasitized spruce budworm larvae dying from causes other than egressing parasitoid larvae were excluded from the analysis.

**Adult Longevity**

Adult parasitoids (64 females and 65 males) were transferred <24 h after emergence to the above described 237-ml transparent plastic containers and placed into growth chambers at 6 constant temperatures, 5, 10, 15, 20, 25, and 30°C. Instead of branches, the glass vials at the bottom of the container held cotton rolls soaked with a 20% sucrose water solution. Insects were provided *ad libitum* with the solution that was renewed every 1-4 days, depending on rearing temperature. All insects were observed daily and the day of death was recorded for each individual.
**Potential Fecundity**

A total of 269 virgin parasitoid females were transferred within 24 h of emergence into transparent plastic cages with a 20% sucrose water solution as described above. Subsequently, cages were randomly assigned to one of six growth chambers at 5, 10, 15, 20, 25 and 30°C. At all temperatures, about 10 females were dissected in a saline buffer solution under a binocular microscope after 5, 10, 15 and 20 days to count all eggs in the oviducts. The detailed methods to count eggs in *T. rostrale* oviducts are described in Chapter 3. Additional females were dissected after 3 and 7 days at 20, 25 and 30°C (again about 10 females at all temperatures). At 30°C, no females were dissected after 20 days because they did not survive beyond 15 days.

Temperature and humidity in all growth chambers were measured every 5 min with HOBO® data loggers (ONSET, U12-012). Mean relative humidity in the growth chambers was 73, 76, 89, 79, 82, 75, 71, 50, 64, 40, and 59% at 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30°C respectively. Because the temperature in growth chambers fluctuated slightly (± 2°C) during the experiment, stage-specific (in-host, pupa, and adult) mean temperatures for each individual were calculated and used for data analysis.

**Data Analysis**

**Stage-specific development time** - The developmental response of immature stages (eggs and larvae inside the host and pupae after egression) was described with the model of Sharpe and DeMichele (1977), as modified by Schoolfield et al. (1981). Parameters of this model refer to the thermodynamics of enzyme reactions, assuming the rate of the modeled process is controlled by a single enzyme:

\[
  r = \frac{(\rho + \rho_m) K}{298} \exp \left[ \frac{H_A}{R} \left( \frac{1}{298} - \frac{1}{K} \right) \right] + \exp \left[ \frac{H_L}{R} \left( \frac{1}{T_L} - \frac{1}{K} \right) \right] + \exp \left[ \frac{H_H}{R} \left( \frac{1}{T_H} - \frac{1}{K} \right) \right] \epsilon \quad \text{for } K > 273 \quad [1]
\]

where \( K \) is temperature in °Kelvin, \( R=1.987\times10^{-3} \text{ kcal K}^{-1} \text{ mol}^{-1} \) is the universal gas constant, \( \rho \) is the development rate at 25°C (298°K), \( H_A \) is the enthalpy of reaction activation (kcal mol\(^{-1}\)), \( T_L \) is the low temperature at which the 50% of the rate-controlling is inactive (°K), \( H_L \) is the change in enthalpy associated with low-temperature inactivation (kcal mol\(^{-1}\)), \( T_H \) is the high temperature at which 50% of the rate-controlling enzyme is inactive (°K), \( H_H \) is the change in enthalpy associated
with high temperature inactivation (kcal mol\(^{-1}\)), and \(\epsilon\) is a unit-less lognormal random variable with mean = 1 and variance = \(\sigma^2\). The set of parameters \(\{\rho, \rho_m, H_A, H_L, T_L, H_H, T_H, \sigma^2\}\) was estimated for each life stage, with \(\rho_m\) being the male differences with females. Eq. [1] was fitted to individual sex-specific development times, some including transfer treatments, using the method described by Régnière et al. (2012a) with SAS (PROC NLMIXED; SAS Institute Inc., 2015).

Adult longevity and the ageing rate of males and females were analysed using the same maximum likelihood approach based on individual development times. Longevity \(l\) was described as a function of temperature \(T\) with:

\[
l = \left[ e^{a+bT} \right]^{-1} \epsilon, \tag{2}
\]

where \(a\) and \(b\) are parameters for females, \(a_m\) and \(b_m\) are male differences with females, and \(\epsilon\) is a lognormal random variable with mean 1 and variance \(\sigma^2\), also a parameter to be estimated.

**Stage-specific survival** (number surviving relative to number starting the life stage) was analyzed as proposed by Régnière et al. (2012a), using the binomial distribution (PROC NLMIXED, SAS Inc. 2015). The approach assumes that survival \(s\) during the given life stage, under the experimental conditions, is given by:

\[
s = \left[ 1 + e^{-\beta_0 + \beta_1 T_1 + \beta_2 T_2} \right]^{-1} \left[ 1 + e^{-\beta_0 + \beta_1 T_1 + \beta_2 T_2} \right]^{-t_2}, \tag{3}
\]

where \(T_1\) and \(T_2\) are the two temperatures involved in transfer treatments, lasting a total of \(t_1\) and \(t_2\) days respectively, and \(\{\beta_0, \beta_1, \beta_2\}\) are parameters to be estimated. At temperature treatments not involving transfers, \(t_2\) is set to 0. This model was fitted by adding terms one at a time until further addition did not reduce the AICC. Using the resulting significant parameters, the life-stage’s daily survival function is then:

\[
s = \left[ 1 + e^{-\beta_0 + \beta_1 T_1 + \beta_2 T_2} \right]^{-1}, \tag{4}
\]

from which the stage-specific survival function was calculated over the duration of the life stage:
where \( r(T) \) is the development rate of the life stage at temperature \( T \) as provided by equation [1].

In the case of in-host survival, the right-hand side of equations [3]-[5] was multiplied by 0.828 to adjust for pseudo-parasitism (no oviposition during an attack of the host), as determined in Chapter 2.

**Fecundity** - To analyse the potential fecundity data, we posited that egg production \( P \) resulted from two simultaneous and opposite processes (King & Richards 1968; Richard & Casas 2009) in adult females of \( T. \ rostrale \): oogenesis \( O \) and egg resorption \( R \). We further postulated that resorption is not perfectly efficient at returning energy to the female for further egg production, through a constant that represents the loss of energy associated with egg resorption. Thus, in discrete difference-equation we define egg production as:

\[
P_t = P_{t-1} + O_t - \kappa R_t
\]

The rate of oogenesis \( (O_t) \) depends directly on temperature \( T \) (°C, with intercept and slope parameters \( a, b \)), is inversely proportional to the number of eggs already produced \( (P_{t-\Delta t}) \) relative to some maximum egg production \( P_{\text{max}} \), and is always \( \geq 0 \):

\[
O_t = \max \left[ \frac{P_{\text{max}} - P_{t-\Delta t}}{P_{\text{max}}} (a + bT), 0 \right]
\]

This formulation produces the typical diminishing-return (asymptotic) behaviour of egg accumulation, and suggests that as energy reserves are exhausted, the production of new eggs slows down.

The rate of resorption is also directly dependent on temperature (with intercept and slope parameters \( c, d \)), is directly proportional to the number of eggs already produced, and is also \( \geq 0 \):

\[
R_t = \max \left[ \frac{P_{t-\Delta t}}{P_{\text{max}}} (c + dT), 0 \right]
\]

This equation illustrates the idea that when energy reserves are high, there is little resorption, but as they drop, resorption increases.

In the absence of oviposition, eggs in the oviducts \( E \) accumulate according to:
with initial condition $E_0$, the average number of eggs contained in the oviducts of females at emergence. This value was estimated by dissecting emerging females, with $E_0 = 9.1 \pm 1.4$ SEM eggs/female (n=17; Chapter 2). The distribution of this number is near-lognormal with mean 1.97 and standard deviation 0.8 (Anderson-Darling=0.49, P=0.19, n=17). In equation [9], the inefficiency parameter is not used because we are describing the number of eggs, not the energy they represent.

When a female is allowed to lay all her eggs, the oviposition rate (eggs per day) is simply:

$$Ovi_t = O_t - R_t$$

[10]

Because the iterative nature of the fecundity model (discrete difference equations), parameters were estimated using Microsoft Excel’s Solver, minimizing the residual sum of squares (maximizing $R^2$) between observations and simulation output on corresponding days at corresponding nominal temperatures. Using trial and error, we also estimated the amount of variation in the value of $P_{max}$ among individuals by comparing observed variation with the width of the bands of predicted egg accumulation produced for females emerging, $E_0 = \bar{E}_0 \pm \sigma_{E_0}$ and $P_{max} = \bar{P}_{max} \pm \sigma_{P_{max}}$.

**Simulation of Survival and Fecundity at Constant and Variable Temperature**

We ran simulations of stage-specific survival and fecundity of the parasitoid under various temperature regimes to investigate the behaviour of our models and the effect of constant and variable temperature on its output. For simulations at variable daily temperature, we generated normally-distributed temperature time series with means of 5 to 30°C in steps of 1°C and a standard deviation of 5°C ($\sigma = 5$). We then calculated (a) expected in-host and pupal survival of average individuals (i.e., using mean parameter estimates) during their development, and (b) egg production, accumulation in non-ovipositing females, and oviposition in females laying all available eggs, again for the average individual ($E_0 = 9.1$ and $P_{max} = 143$) during its expected lifetime. All simulations were conducted on a daily time step.
Results

Stage-Specific Development Time
The unimodal nature of the thermal response of in-host and pupal development time was clearly displayed in our data, with the shortest development times of approximately 11 days inside the host and 9 days in the pupal stage occurring at about 25°C (Fig. 4.1). Above 25°C, development time rapidly increased again and development rate dropped to near-zero at about 35°C. Male pupae developed about 10% faster than females at all temperatures, but sex had no effect on in-host development (Fig. 4.1; Table 4.1). Equation [1], fitted to development times, described very well the responses of both life stages ($R^2=0.99$ and 0.97, for in-host and pupa, respectively; Table 4.1). The variation of individual development rates ($\sigma_e$) was small for in-host (0.085) and pupal development (0.090), and was well approximated by the lognormal distribution (Fig. 4.1).

Adult Longevity
Adult longevity decreased exponentially with increasing temperature for both male and female *T. rostrale* (Fig. 4.1). The parameter estimates of equation [2] are; $a=5.170 \pm 0.099$, $b=0.0707 \pm 0.0057$, and $b_m=0.0246 \pm 0.00494$. Parameter $a_m$ was not significantly different from zero and was dropped from the model. At all temperatures, females lived longer than males. The individual variation among our observations was unusually large ($\sigma_e=0.537$), because several individuals lived much shorter lives than expected, especially at the more extreme temperatures (Fig. 4.1). For this reason, variance parameter was halved for further use ($\sigma_e=0.268$).

Stage-Specific Survival
The best fitting survival model had two parameters for in-host ($\beta_0=7.025 \pm 0.616, \beta_1=-0.174\pm0.027$) as well as pupal development ($\beta_0=9.620 \pm 1.339, \beta_1=-0.239\pm0.058$). In-host survival (Fig. 4.2a) was lower than pupal survival (Fig 4.2b) at all temperatures, in large part because in-host survival included about 18% pseudo-parasitism (failure to lay an egg during attack). Survival was highest at lower temperatures until about 20°C, after which it rapidly decreased to near zero at temperatures >31°C for in-host (Fig. 4.2a) and 34°C for pupal development (Fig 4.2b). In temperature transfers at 30°C, only 11.8% (n=17) of individuals completed their development to the adult stage. Survival was greatly enhanced by the use of transfer treatments at extreme temperatures of 28, 7.5 and 5°C (50, 61, and 53%, respectively). Host mortality from causes other than parasitism was about 15% between 7 and 25°C, but about 30% at 5, 28 and 30°C.
Potential Fecundity

The observed relationship between temperature and number of eggs in the oviducts of T. rostrale females (potential fecundity) is described accurately by equation [9] ($R^2=0.959$; Table 4.2; Fig. 4.3). At 5°C the number of eggs increases linearly over time. With increasing temperature, the response becomes progressively non-linear, reaching a plateau of about 65 eggs after 10 days at 15°C. Above 20°C, the number of eggs increases quickly in the first three days, and reaches a maximum of about 50, 45, and 40 eggs at 20, 25, and 30°C, respectively. At these three temperatures, the number of eggs in oviducts decreases over time after this maximum is reached (Fig. 4.3). Variability in the number of eggs carried by females at any given time and temperature was high in these experiments. To generate equivalent variability among simulated females, we used the observed mean and variance of $E_0$ (log-normal distribution) to assign the initial number of eggs to females at emergence, and normally-distributed values of $P_{max}=143 \pm 30$ eggs female$^{-1}$. The dotted lines in Fig. 4.3 depict mean ± 1 SD on expected number of eggs among simulated females.

Simulation of Survival and Fecundity at Constant and Variable Temperature

Survival is predicted to be lower at variable temperature than at constant temperatures for both in-host and pupal survival (Fig 4.4). Simulated realised fecundity is highest around 10°C for both constant and variable temperatures (about 140 eggs), decreasing rapidly to <40 eggs at 30°C. In contrast to survival, simulated realised fecundity is always higher at variable temperature than at constant temperature (Fig. 4.4). In addition, the simulation of fecundity over time at variable temperature predicts that the highest oviposition rates occur in the first few days after emergence, especially at mean temperatures ≥10°C (Fig. 4.5). While both egg production and accumulated realised fecundity are predicted to steadily increase over time at temperatures ≤10°C, at higher temperatures egg production decreases after the initial peak, leading to a decrease of total realised fecundity (Fig. 4.5). Increased longevity at a mean temperature of 5°C and a very efficient egg production at 10°C lead to the highest predicted realised fecundity of females at these temperature regimes, compared to others.

Discussion

Based on the laboratory and simulated results here, the optimal temperature for development of T. rostrale was ca. 25°C, while the survival of immatures was highest at temperatures below 20°C. Adult longevity increased with decreasing temperature, and highest realised fecundity occurred at
around 10°C. Nevertheless, the overall fitness of *T. rostrale* appears to be maximized at temperatures below 20°C. In the two field sites where parasitoids for this study were collected, most of the development of *T. rostrale* in its spruce budworm host takes place in June (Cusson et al. 1998a; Chapter 3), a period during which mean daily temperatures range from 10 to 20°C in the two sites (Lethiecq & Régnière 1988). Thus, *T. rostrale* is well adapted to temperatures in these areas.

The impact of temperature on survival of spruce budworm larvae was investigated in several other studies, showing that it is around 95% at temperatures between 15 and 30°C (Weber et al. 1999; Régnière et al. 2012b). Here, we measured somewhat higher mortality at temperatures between 28 and 30°C. Thus, temperatures between 20 and 30°C negatively affect the survival of *T. rostrale*, but not that of its host in the same way. Several factors could be involved in the decreased survival of *T. rostrale* at high temperature, especially in-host. The immune system of the host may be more efficient (Fellowes 1999; Zufelato et al. 2004), or the polydnavirus that *T. rostrale* injects into the host at oviposition (Doucet & Cusson 1996a) may be less effective at disrupting the host’s immune system (Khafagi & Hegazi 2004). This warrants further inquiry.

Longevity of *T. rostrale* adults decreased with increasing temperature, as is commonly observed in insects (e.g., Papanikolaou et al. 2013; Andreadis et al. 2014; Cheng et al. 2015), and specifically for parasitoids (e.g., Spanoudis & Andreadis 2012; Chen et al, 2015; Watt et al. 2016). Females lived longer than males at all temperatures. *Tranosema rostrale* females are generally larger than males (Chapter 3) and may therefore have more metabolic resources available that are necessary to increase longevity (Ellers 1996). Larger female size may also explain a slightly longer development time in the pupal stage. Some particularly short-lived individuals were observed in our experiments. These individuals may have died from causes other than old age (e.g., handling accidents, disease or genetic conditions). These individuals were not eliminated from the analysis, and therefore probably artificially increased the variance of observed longevity.

The potential fecundity of *T. rostrale* at different temperatures shows a trade-off between an increasing rate of egg accumulation in the oviducts and a decreasing maximum egg load with increasing temperature. We posit that this decrease in egg load is caused by increasing egg resorption at higher temperatures, a hypothesis that was formalized in equation [6]. The simulation of realised fecundity over the lifespan of females indicates an additional trade-off between realised fecundity and adult longevity. Our results suggest that these trade-offs reach an equilibrium for *T.*
*T. rostrale* at 10°C, where a fast egg production, a high maximum egg load, and a long adult life maximize realised fecundity. Under field conditions, the realised fecundity of the parasitoid is dependent on host availability. Because the simulation of realised fecundity at variable temperature takes female longevity into account, an estimated temperature-dependent ovigeny index could be calculated for *T. rostrale* by dividing the initial egg load by the potential lifetime fecundity of the parasitoid (Jervis et al. 2001). The approximate index is 0.06, 0.06, 0.09, 0.18, 0.23, and 0.3 at 5, 10, 15, 20, 25, and 30°C, respectively. This finding underlines the importance of reporting the rearing temperature when an ovigeny index is calculated for a parasitoid or an insect in general.

The results of the fecundity simulations also suggest that *T. rostrale* has a Type-2 pattern of age-specific realised fecundity (Jervis et al. 2008): initial egg loads are relatively low and realised fecundity peaks in the first few days of the female’s life, after which it declines.

To our knowledge, the fecundity model developed in this study is the first temperature-dependent parasitoid fecundity model taking both oogenesis and oosorption into account. The model is based on current understanding of the two processes (Richard & Casas 2009), also taking the energy costs of egg resorption into account. It can be used to model temperature-dependent potential and predicted realised fecundity of non-host-feeding synovigenic parasitoids. Because of the non-linearity of the temperature responses of *T. rostrale*, it is important to take the effect of temperature variability into account to estimate life history traits under field conditions. This is one of the strengths of using non-linear functions to describe those temperature responses. The equations and parameter estimates presented in this paper can be used to model the seasonal pattern of phenology of *T. rostrale* in its natural habitat to predict the seasonality of this species which should be particularly useful to model the interaction of *T. rostrale* with spruce budworm under various climatic conditions.
Table 4.1: Parameter estimates for *Tranosema rostrale*’s temperature-dependent in-host and pupal development time with spruce budworm as host (equation [1]). Parameters refer to the enthalpies as well as activation and deactivation energies of enzymes. See Material and Methods and Schoolfield et al. (1981) for more details about the parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In-host</th>
<th>Pupa</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$</td>
<td>0.114</td>
<td>0.262</td>
</tr>
<tr>
<td>$\rho_m$</td>
<td>0.000</td>
<td>0.029</td>
</tr>
<tr>
<td>$H_A$</td>
<td>-6.149</td>
<td>-27.3</td>
</tr>
<tr>
<td>$H_L$</td>
<td>-34.4</td>
<td>-44.9</td>
</tr>
<tr>
<td>$T_L$</td>
<td>291.1</td>
<td>299.8</td>
</tr>
<tr>
<td>$H_H$</td>
<td>108.2</td>
<td>100.7</td>
</tr>
<tr>
<td>$T_H$</td>
<td>302.6</td>
<td>308.0</td>
</tr>
<tr>
<td>$\sigma_e^2$</td>
<td>0.082</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Table 4.2: Parameter estimates for *Tranosema rostrale*’s temperature-dependent potential fecundity (equation [9]) after rearing in 5th-instar spruce budworm larvae as hosts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>-2.736</td>
</tr>
<tr>
<td>$b$</td>
<td>0.956</td>
</tr>
<tr>
<td>$c$</td>
<td>-26.971</td>
</tr>
<tr>
<td>$d$</td>
<td>2.554</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>0.890</td>
</tr>
<tr>
<td>$P_{\text{max}}$</td>
<td>142.969</td>
</tr>
</tbody>
</table>
**Fig. 4.1:** *Tranosema rostrale* development and longevity data fitted to equations [1] and [2]. Left column: development time; middle column: development rate; right column: distribution of individual variation with the corresponding lognormal distribution. For (upper row) in-host (egg and larva) and (middle row) pupal development for (dotted line) males and (solid line) females at (open circles) constant temperature and (closed circles) temperature transfer treatments; and for (lower row) adult development (longevity and ageing rate) for (open circles) males and (closed circles) females.
**Fig. 4.2:** Survival data of immature *Tranosema rostrale* developing in spruce budworm larvae fitted to equations [3-5]. Upper panel: in-host (eggs and larvae) survival ($R^2=0.592$). Lower panel: pupal survival ($R^2=0.786$). Solid line: stage survival function. Closed symbols: observations (± SE of proportion), open symbols: values predicted from eq. [3]. Circles: constant temperature, squares: transfer treatments. Dotted line: daily survival function.
Fig. 4.3: Observed (circles: mean ± SD) and simulated (lines, equation [9]) accumulation of eggs in the oviducts of non-ovipositing *Tranosema rostrale* females at 5, 10, 15, 20, 25 and 30°C.
Fig. 4.4: Simulated probability of survival for *Tranosema rostrale* developing (upper panel) inside the host as an egg or larva, (middle panel) as pupae after egression from the host, at constant (closed symbols) and variable (open symbols) temperature. Lower panel: Simulated realized fecundity of female *T. rostrale* allowed to lay all available eggs each day of their life, at constant (closed symbols) and variable (open symbols) temperature.
Fig. 4.5: Daily output of the fecundity simulation for *Tranosema rostrale* under 6 variable temperature regimes with means 5, 10, 15, 20, 25 and 30°C (σ = 5). Grey full line: egg production \((P)\); Dotted line: cumulative oviposition; Black line: oviposition rate; Black line and black dots: daily temperature.
Chapter 5
High Temperature Induces Downregulation of Polydnavirus Gene Transcription in Lepidopteran Host and Enhances Accumulation of Host Immunity Gene Transcripts

Abstract
Endoparasitoids face the challenge of overcoming the immune reaction of their hosts, which typically consists of encapsulation and melanisation of parasitoid eggs or larvae. Some endoparasitic wasps such as the solitary *Tranosema rostrale* (Hymenoptera: Ichneumonidae) that lay their eggs in larvae of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), have evolved a symbiotic relationship with a polydnavirus (PDV), which in turn helps them suppress the host’s immune response. We observed an increase in mortality of immature *T. rostrale* with increasing temperature, and we tested two hypotheses about the mechanisms involved: high temperatures (1) hamper the expression of *T. rostrale* PDV genes and (2) enhance the expression of spruce budworm immunity-related genes. Dissections of parasitized spruce budworm larvae reared at 30°C revealed that most parasitoid eggs or larvae had died as a result of encapsulation and melanisation by the host. A qPCR analysis of *T. rostrale* PDV (TrIV) gene expression showed that the transcription of several TrIV genes in host larvae was downregulated at high temperature. On the other hand, encapsulation, but not melanisation, of foreign bodies in spruce budworm larvae was enhanced at high temperatures, as shown by the injection of Sephadex™ beads into larvae. However, at the molecular level, the transcription of genes related to spruce budworm’s melanisation process (prophenoloxidase 1 and 2) was upregulated at high temperature. Our results support the hypothesis that a temperature-dependent increase of encapsulation response is due to the combined effects of reduced expression of TrIV genes and enhanced expression of host immune genes. These findings may be applicable to other host-parasitoid systems implicating PDVs.

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4 A version of this chapter is under review by the Journal of Insect Physiology: Seehausen, M.L., Cusson, M., Régnière, J., Bory, M., Stewart, D., Djoumad, A., Smith, S.M., Martel, V. High temperature induces downregulation of polydnavirus gene transcription in lepidopteran host and enhances accumulation of host immunity gene transcripts.
Introduction

Encapsulation and melanisation, best studied in the Lepidoptera and the Diptera (Lavine & Strand 2002), are the two most common immunoreactions that insects mount against invaders such as viruses, spores of fungi and parasitoid eggs and larvae. Encapsulation is a cellular defense mechanism during which hemocytes (blood cells) spread and adhere to a foreign target (e.g., parasitoid egg), encasing it within a multi-layer cellular shell. As such, encapsulation builds a physical barrier between the foreign organism and the insect hemocoel, thereby contributing to the invader’s death (Lavine & Strand 2002). In contrast, melanisation is a humoral response (i.e., non-cellular) that results in the accumulation of heteropolymer melanin at wounding sites, in plasma or inside hemocytes, sealing off foreign bodies from the host’s internal environment (Sugumaran 2002; Kanost et al. 2004). In addition, melanin promotes a cytotoxic reaction against the intruder (Nappi & Christensen 2005). The formation of melanin is catalysed by the enzyme phenoloxidase (PO), which is present in the hemolymph or in hemocytes of insects as zymogen prophenoloxidase (PPO) and is brought into its active form by the prophenoloxidase activating enzyme (PPAE) (Cerenius & Söderhäll 2004). Cellular and humoral immune responses may also work together, as certain types of hemocytes have been found to release PPOs and PPAEs (Jiang et al. 1997; Müller et al. 1999).

To successfully complete their larval development, endoparasitoids must either abrogate or evade the immune reaction of their insect hosts and have evolved various counterstrategies to circumvent the host immune response. One widely studied countermeasure is the use of a viral gene delivery system that enables the transfer of virulence genes from the wasp to the caterpillar, in which viral gene expression results in a depression of its immune reaction. These viruses, known as polydnaviruses (PDVs), are obligate symbionts of some endoparasitic wasps. The family Polydnaviridae comprises two genera, namely the bracoviruses (BVs), which are associated with a subset of braconid wasps, and the ichnoviruses (IVs), which are found in two subfamilies of ichneumonid wasps (Strand & Burke 2014). PDVs are unique in that they constitute the only known virus taxon with a segmented dsDNA circular genome (Webb 1998; Tanaka et al. 2007). The virus replicates in the ovaries of female wasps, where it accumulates in the lumen of lateral oviducts. Virions are transmitted to the host during oviposition, along with parasitoid eggs. In the host, no viral replication takes place, but virions infect host tissues, express PDV genes, and induce pathologies that include suppression of the host immune response through alterations of hemocyte behaviour or hemocyte production (e.g., Asgari et al. 1996; Doucet & Cusson 1996a; Pruijssers &
Strand 2007; Suderman et al. 2008), and suppression of PO activity (Doucet & Cusson 1996a; Shelby et al. 2000). In addition, PDVs can also induce host developmental arrest (Dover et al. 1987; Doucet & Cusson 1996b; Soller & Lanzrein 1996; Beckage 2012) and mobilize host protein reserves for use by the parasitoid (Thompson & Dahlman 1998; Nakamatsu et al. 2001; Pruijssers et al. 2009).

Many key life-history traits in insects are influenced by ambient temperature because they are poikilothermic organisms, and this affects parasitoids and hosts alike (e.g., Hance et al. 2007). Temperature also affects the immune system of insects; for example, efficiency of encapsulation of parasitoid eggs is generally enhanced by a rise in temperature (Blumberg 1997; Fellowes et al. 1999). In addition, PO activity in insect hemolymph is positively correlated with temperature, up to an upper threshold varying between 20 and 50°C, depending on the species (Lockey & Ourch 1992; Hara et al. 1993; Cherqui et al. 1996; Zufelato et al. 2004). It has been hypothesised that high temperatures negatively influences PDV gene expression (Khafagi & Hegazi 2004), however, to our knowledge, no study has confirmed this hypothesis to date.

In recent work we showed that survival of the larval endoparasitoid Tranosema rostrale (Brischke) (Hymenoptera: Ichneumonidae) within its host, the spruce budworm Choristoneura fumiferana (Clemens) (Lepidoptera: Tortricidae), is negatively correlated with rearing temperatures above 20°C (Chapter 4). This parasitoid transmits a PDV (the T. rostrale ichnovirus or “TrIV”) to its host (Cusson et al. 1998b), for which both viral gene expression (Béliveau et al. 2000; 2003; Rasoolizadeh et al. 2009a; 2009b; Djoumad et al. 2013) and function (Doucet & Cusson 1996a; 1996b; Cusson et al. 2000; Djoumad et al. 2013; Doucet et al. 2008) have been characterized. As such, this host-parasitoid association provides an excellent model to study the effect of temperature on PDV gene expression and on the performance of the host immune system. Given our earlier results on temperature-dependent survival of T. rostrale, we hypothesised that high temperature: (1) reduces the performance of TrIV through a depression of viral gene expression that limits TrIV’s effectiveness in abrogating the host immune response, and (2) enhances the spruce budworm’s immune system, enabling more effective encapsulation and melanisation reactions as a result of a rise in the expression of immunity-related genes.
Material and Methods

Rearing and Parasitization of Spruce Budworm Larvae
Parasitoids were obtained from two study sites near Quebec City, QC, Canada, through implantation of spruce budworm larvae on balsam fir *Abies balsamea* (L.) Miller, Pinaceae, as described in detail in Chapter 2 and 4. Overwintering spruce budworm larvae obtained from the insect rearing facility of the Canadian Forest Service (Great Lakes Forest Research Centre, Sault Ste. Marie, ON, Canada) were reared on current-year balsam fir foliage in growth chambers at 20°C and under a 16 h photoperiod up to the 5th-instar. Larvae where then exposed to *T. rostrale* females for parasitization in plastic containers (Chapter 2; 4). Wasp and larvae were monitored until stinging was observed, and only stung larvae were used for subsequent experiments. Within 30 min after parasitization, spruce budworm larvae were transferred to 237-ml plastic containers featuring a top window screen for ventilation and provided balsam fir foliage for food. The containers were then placed at different temperatures for rearing.

Dissection of Parasitized Spruce Budworm Larvae
To identify the causes of in-host mortality of parasitoids at high temperature, spruce budworm larvae were placed in rearing chambers immediately after parasitization at either 20°C (n=10), where survival is known to be high (see Chapter 4), or 30°C (n=10), where they were reared for 5 days. Larvae were then immobilized by exposure to -20°C for 3-5 min and dissected under a microscope in a buffered saline solution. The developmental stage of the parasitoid (egg or larva) and its status (live, encapsulated or melanised) were determined visually.

Haemolymph Melanisation
As for the dissections, spruce budworm larvae were reared immediately after parasitization for 5 days at 20 (n=10) or 30°C (n=10). Haemolymph was collected from parasitized larvae by puncturing the dorsal cuticle with an insect pin and drawing hemolymph with a capillary tube. Placed on a Parafilm™ sheet, the hemolymph was then monitored at room temperature for a display of melanisation, as described in Doucet and Cusson (1996a). A change of haemolymph colouration from yellow-green to dark-brown was considered normal melanisation, whereas the maintenance of the original colour after 30 min of exposure to air was considered inhibition of melanisation.
RNA Isolation and qPCR

Immediately after parasitization, spruce budworm larvae were randomly assigned to three rearing temperatures, 10, 20, or 30°C, where they were reared for 6, 24, 72, and 120 h. At each temperature and time, 10 parasitized larvae were processed, for a total of 120 larvae in the experiment. These larvae were homogenized in 500 µL TRIzol reagent (Invitrogen Life Technologies) to isolate RNA. Larval cuticle debris were removed by centrifugation at 17,000 g for 5 min, and the supernatant was transferred to a new tube. RNA purification was performed according to the Direct-zol™ RNA MiniPrep Instruction Manual (Zymo Research Corp.), including an in-column DNase I digestion for 15 min at room temperature. Total RNA was quantified using a NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific Inc.). Three samples from each temperature and time treatment were chosen for the following steps based on the RNA concentration (160-900 ng µL⁻¹) and its ratio of absorbance at 260 nm and 280 nm (~2.0). Reverse transcription was performed according to the protocol described in the QuantiTect Reverse Transcription Handbook (Qiagen®), including elimination of genomic DNA in a 14 µL reaction volume containing 2 µL gDNA Wipeout Buffer, which was incubated for 2 min at 42°C. The reverse-transcription reaction was carried out in a 20 µL reaction volume containing 1 µL Quantiscript Reverse Transcriptase, 4 µL Quantiscript RT Buffer, 1 µL RT Primer Mix and 14 µL template RNA from the previous step. The master mix was held for 30 min at 42°C, followed by 3 min at 95°C to inactivate the reverse transcriptase. The cDNA reaction was then diluted in 180 µL 10 mM TRIS/HCl.

Quantitative Real Time PCR (qPCR) was performed using an Applied Biosystems 7500 Fast Real Time PCR machine. We used 96-well BrightWhite real-time PCR plates (Cat. BW-FAST, Primer Design, UK), with Applied Biosystems MicroAmp Optical Adhesive Film. Expression levels were measured for four TrIV genes (TrV1, TrFrep1, Ank2, Cys), Prophenoloxidases 1 and 2 (PPO1, PPO2), PPO1 activating enzyme (PPAE1) and two housekeeping genes (GAPDH and gTubulin). No-RT samples were also included to confirm the absence of genomic DNA in the samples. Primer pairs for TrIV genes were obtained from Rasoolizadeh et al. (2009a); for all other genes, primers were designed using OligoExplorer software (Gene Link). Two technical replicates were run for each sample containing 2 µL of cDNA (10 ng of converted RNA). PCRs were performed using the Quantitect SYBR Green PCR Kit (Qiagen) with 50 cycles of 95°C for 15 s, 60°C for 30 s, and 65°C for 90 s. Linear regression of efficiency (LRE) analysis developed for modelling qPCR amplification (Rutledge 2011) was used to determine absolute quantities of target molecules.
Lambda genomic DNA was used as a quantitative standard. Copy number results were normalized using the GeNorm algorithm (Vandesompele et al. 2002).

Samples with no polydnavirus-related RNA detected (4 samples, 11.1%) were replaced with new ones, assuming that these larvae had not been effectively parasitized.

**Injection of Sephadex™ Beads into Larvae**

To measure the immunoreaction (encapsulation and melanisation) of spruce budworm larvae towards foreign bodies at different temperatures, we injected Sephadex™ G25 beads into larvae. To this end, overwintered 2nd-instar spruce budworm larvae were reared on artificial diet (McMorran 1965) in 21 mL plastic cups (6 larvae per cup) at 20 and 30°C until the 6th instar. Injections of beads took place 3 and 2 days after the molt to the 6th instar at 20 and 30°C, respectively, so that measurements were taken at approximately the same physiological age in the two groups of larvae. Prior to injection, larvae were anesthetised with CO₂ for 1-2 min and fixed with metal clips to a wax pad. Three beads with a measured diameter between 100 and 200 µm were injected in 1 µL PBS into each larva using a 5 µL syringe (Model 7105 KHWG SYR, Knurled Hub NDL) and a 0.31 mm gage needle. The needle was inserted between two segments of the larva’s thorax and beads were injected right under the cuticle to avoid injury of the inner organs. After injection, a liquid bandage (New-Skin®, Prestige Brands Holdings Inc.) was applied to the wound to avoid bleeding and ejection of the beads. At both temperatures, dissections of larvae were carried out at 2, 4, and 6 h post-injection. To this end, larvae were immobilized by exposure to -20°C for 3-4 min, after which they were dissected in PBS under a microscope. Once isolated, pictures of encapsulated beads were taken under standardized light conditions with a digital camera (Dino-Lite AM7023B) mounted onto a microscope (Optiphot, Nikon). Using an image analysis software (DinoCapture), the surface of each encapsulated bead was measured by tracing a line around the capsule containing the bead and calculating the area within the line. The surface of encapsulation $S$ was measured for the three beads and averaged using $S = \left( \sum S_E - \sum S_B \right) / 3$, where $S_E$ is the surface of the encapsulated bead and $S_B$ is the surface of the bead prior to injection. Encapsulation was measured in a total of 79 larvae. In addition to encapsulation, the degree of melanisation of beads was measured by grading the colour and coverage of melanisation after injection, using a melanisation scale of 0 to 5 (Fig. 5.1), 0 and 5 denoting no change in colour and a dark brown colouration of the entire bead, respectively. An average melanisation index for each
larva was then calculated by taking the mean of the values from all three beads. Melanisation was measured in a total of 59 larvae.

**Statistical Analysis**

We used Fisher’s Exact Test to compare parasitoid mortality and haemolymph melanisation at 20 and 30°C (PROC FREQ; SAS Inc. 2016). Backward model selection using the Corrected Akaike Information Criterion (AICc) for selecting the best fitting model was used to analyse the influence of temperature ($T$) over time ($t$) on the expression of polydnavirus genes and genes related to spruce budworm immunity, as well as encapsulation and melanisation of Sephadex™ beads (PROC GLMSELECT; SAS Inc. 2016). The full model included the two independent variables ($T$ and $t$), their squares, and all two-way interactions. The best fitting model (Table 5.1) was then submitted to a multiple regression analysis (PROC GLM; SAS Inc. 2015). To meet the assumptions of normality and variance stability, all dependent variables related to gene expression were log-transformed.

**Results**

**Effect of Temperature on In-Host Mortality of T. rostrale and Melanisation**

*Tranosema rostrale* eggs or larvae were recovered in 19 out of 20 dissected larvae. The spruce budworm larva that had not been truly parasitized was excluded from the experiment. Significantly more parasitoids were found dead at 30°C than at 20°C ($P=0.0011$): 89% ($n=9$) of larvae were found dead (encapsulated and/or melanised as eggs or larvae) at 30°C compared to 10% ($n=10$) at 20°C. No hemolymph samples drawn from parasitized spruce budworm larvae reared at 20°C melanised at ambient air ($n=10$); however, 33% ($n=9$) of the samples from insects reared at 30°C melanised (Fisher’s Exact Test: $P=0.0867$).

**Effect of Temperature on TrIV Gene Expression**

Among the four TrIV genes whose transcript levels were quantified in this study, *TrV1* was by far the most highly expressed, followed by *TrFrep1, Ank2*, and *Cys* (Fig. 5.2). The time-dependent pattern of *TrV1, TrFep1* and *Cys* expression was generally parabolic (first increasing, reaching maximum expression after 72 h, and decreasing thereafter), and varied as a function of temperature (relatively low at 10°C, highest at 20°C, and again lower at 30°C) (Table 5.1A, Fig. 5.2). However, the expression of *TrV1* and *Cys* deviated slightly from this general pattern, as indicated by the significant interaction terms (Table 5.1A). *Ank2* transcript levels increased in a more linear fashion...
over time, a trend that was particularly apparent at 20°C (Fig. 5.2), which resulted in a significant $t \times T^2$ interaction (Table 5.1A).

**Effect of Temperature on the Spruce Budworm Immune Response**

There was a significant $t^2 \times T$ interaction with respect to the encapsulation surface of Sephadex™ beads (Table 5.1C). The extent of encapsulation increased at both temperatures over time; however, the increase was faster at 30°C, reaching 13,000 µm$^2$ 4 h after injection, as compared to ~10,000 µm$^2$ at 20°C (Fig. 5.3A). Melanisation intensity increased significantly over time, but was not affected by temperature (Table 5.1C). It reached a plateau just over 3 on the melanisation intensity index at both temperatures (Fig. 5.3B).

There was a significant $t^2 \times T^2$ interaction in the expression of PPO1 and PPO2 (Table 5.1B). PPO1 transcript abundance increased fastest at 30°C, up to the 72 h sampling point, after which it dropped slightly. In contrast, at 20°C PPO1 transcript levels first declined, reaching their lowest point at the 24 h sampling point, but increased thereafter. At 10°C, no significant change in PPO1 over time was observed (Fig. 5.4). The time-dependent pattern of PPO2 transcript abundance was very similar to that of PPO1, with the exception that its highest transcript levels were observed at the 120 h sampling point at 30°C (Fig. 5.4). Transcript abundance for the prophenoloxidase-activating enzyme, PPAE1, was neither influenced by time nor by temperature (Table 5.1B, Fig. 5.4).

**Discussion**

The data presented here provide support for the hypotheses we set out to test: enhanced encapsulation and melanisation of *T. rostrale* eggs and larvae in parasitized *C. fumiferana* hosts exposed to high temperature (30°C) are associated with reduced TrIV gene transcription and elevated expression of two spruce budworm immunity-related genes, as compared with hosts held at 20°C (Table 5.1; Figs. 5.2 and 5.4). However, the causal link between these two types of gene expression alterations and enhanced encapsulation/melanisation at high temperature is not yet firmly established, and the results presented here need to be interpreted in the light of prior observations on *T. rostrale-C. fumiferana* interactions.

Although TrIV has been shown to have a strong inhibitory effect on melanisation and PPO activity in *C. fumiferana*, its effect on the host cellular immune response appears weaker than that observed for other PDVs (Doucet & Cusson 1996a). Indeed, TrIV infection was shown to cause a significant reduction in hemocyte counts, but glass rods introduced into the hemocoel of *C. fumiferana* larvae
parasitized by *T. rostrale* were nonetheless encapsulated (while remaining unmelanized). Whether the artificial surface provided by the foreign object was, in part, responsible for its encapsulation is unclear, but *T. rostrale* eggs and larvae always escaped encapsulation (Doucet & Cusson 1996a). Thus, TrIV may provide protection against parasitoid encapsulation while allowing encapsulation of other foreign bodies. Whether this protection is the result of TrIV gene expression remains unclear as the presence of TrIV virions at the surface of *T. rostrale* eggs has been hypothesized to provide passive protection against encapsulation, presumably through a mechanism where the layer of virions at the egg surface prevents recognition of eggs as foreign (Cusson et al. 1998b). However, such a mechanism would not account for the ability of *T. rostrale* larvae to evade encapsulation. It therefore appears likely that the expression of some TrIV genes is required for the immature parasitoid to escape encapsulation and melanisation.

Which TrIV genes are responsible for abrogating the encapsulation/melanisation reaction against *T. rostrale* is currently unknown. The two TrIV genes that are most highly expressed in parasitized *C. fumiferana* larvae are *TrV1* and *TrFrep1* (Béliveau et al. 2000; Volkoff et al. 2002; Rasoolizadeh et al. 2009a; 2009b; Djoumad et al. 2013), but their exact function(s) remain uncertain. However, *TrV1* is strongly suspected of being responsible for the important developmental arrest observed in last-instar *C. fumiferana* larvae parasitized by *T. rostrale* (Béliveau et al. 2000; Béliveau et al. 2003; Djoumad et al. 2013). Although *TrV1* and *TrFrep1* transcript accumulation was depressed at 30°C relative to levels measured at 20°C (Fig. 5.2), the impact of temperature on transcript abundance was greater for the other two genes examined, *Ank2* and *Cys*, for which homologs have been shown in other host-parasitoid-PDV systems, to play a direct role in the inhibition of the encapsulation/melanisation reaction (Li & Webb 1994; Cui et al. 1998; Kroemer & Webb 2005; Gueguen et al. 2013) and developmental disturbance (Kroemer & Webb 2004). Although these genes were expressed at much lower levels than *TrV1* and *TrFrep1*, as shown earlier (Rasoolizadeh et al. 2009b), the strong effect of temperature on the accumulation of their transcripts suggests a role in the interaction between temperature, their expression and the encapsulation/melanisation response observed here. It must be pointed out that, for the present study, we sampled only four representative TrIV genes; the possibility remains that transcript abundance of other genes may have been more strongly affected than that of the four we selected. Data presented in Fig. 5.2 also show a strong suppression of TrIV gene transcript accumulation in parasitized *C. fumiferana* larvae held at 10°C, relative to values measured at 20°C. Such an effect
of low temperature on gene transcription was not unexpected given that most enzyme reactions involved in gene expression will be inhibited by low temperature. Interestingly, of the three temperatures examined here, 10°C provided the best conditions for *T. rostrale* survival in *C. fumiferana* (Chapter 4). Since low temperatures are also expected to considerably slow down enzymatic reactions involved in the host immune response (*e.g.*, see PPO1 and PPO2 transcript accumulation at 10°C; Fig. 5.4), we hypothesize that the reduced level of TrIV gene expression observed here at 10°C may be sufficient to prevent encapsulation and melanisation at this temperature.

To assess the impact of rearing temperature on the expression of host immune genes, we selected three *C. fumiferana* genes for which we had sequence data; all three are involved in the melanisation reaction. The expression of PPO1 and PPO2 was strongly enhanced by exposure of parasitized *C. fumiferana* larvae to 30°C, as compared to larvae held at 20°C, while accumulation of PPAE1 appeared little affected by temperature (Fig. 5.4). These results provide strong evidence that the transcription of some, but not all, immunity-related genes is stimulated by elevated temperatures, which could enhance the immune response mounted against parasitoids. Interestingly, the higher transcript abundance measured for PPO1 and PPO2 at 30°C, relative to 20°C, was not accompanied by a significant rise in the melanisation of Sephadex™ beads injected in unparasitized *C. fumiferana* larvae held at 30°C (Fig. 5.3B). However, comparisons between these two experiments must be made with caution as budworm larvae differed in their parasitism status (and, as a consequence, in their TrIV infection status), and the foreign body used to assess encapsulation and melanisation (Sephadex™ beads) had an artificial surface, generating strikingly different results from those reported here for immature parasitoids assessed through dissection. In fact, the results obtained for the Sephadex™ bead experiment (Fig. 5.3) point to a marginal impact of temperature (20°C versus 30°C) on the encapsulation and melanisation of such beads in *C. fumiferana*. These results suggest that, in the absence of TrIV, the *C. fumiferana* immune response is not strongly affected by temperature, at least not enough to account for the important temperature-related differences observed in the encapsulation and melanisation of *T. rostrale* immatures in parasitized hosts. Thus, the question of whether the strong accumulation of PPO1 and PPO2 transcripts in parasitized larvae held at 30°C (Fig. 5.4) is due to an inhibition of TrIV gene expression needs to be assessed. However, earlier work indicated that TrIV-dependent downregulation of PPO1 and PPO2 expression plays a minor role in the inhibition of melanisation in this host-parasitoid system (Doucet et al. 2008).
In conclusion, the present work points to a role of PDV and host immune gene expression in the success of immature parasitoid encapsulation and melanisation as affected by rearing temperature. Importantly, observed differences may be due to interactions between the virus and host immune genes, as suggested by the marginal impact of temperature on the encapsulation and melanisation of Sephadex™ beads introduced into unparasitized *C. fumiferana* hosts. Efforts to further explore these hypotheses will require the use of gene-silencing approaches such as RNA interference (RNAi) to assess which, if any, TrIV genes are required to abrogate the host immune reaction and, conversely, which host immune genes are required to mount it. It would also be interesting to determine whether the relationship between temperature and encapsulation/melanisation described here applies to other host-parasitoid-PDV systems; among the latter, those where PDVs have been shown to play a strong role in the inhibition of the host immune response may provide alternative models to test our hypotheses.
Table 5.1: Independent variables chosen by AICC-based backward model selection and their corresponding P-values explaining variation in levels of (A) TrIV gene-specific mRNA, (B) spruce budworm gene-specific mRNA, and (C) Sephadex™ bead encapsulation surface and intensity of melanisation.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Model</th>
<th>$R^2$</th>
<th>Time</th>
<th>$Time^2$</th>
<th>Temperature</th>
<th>$Temperature^2$</th>
<th>$Time \times Temperature$</th>
<th>$Time^2 \times Temperature$</th>
<th>$Time \times Temp^2$</th>
<th>$Time^2 \times Temperature^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) TrIV gene-specific mRNA</td>
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<td></td>
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<tr>
<td>$TrV1$</td>
<td>&lt;0.0001</td>
<td>0.64</td>
<td>0.0003</td>
<td>0.0192</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0059</td>
</tr>
<tr>
<td>$Rep1$</td>
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<td>0.55</td>
<td>0.0064</td>
<td>0.0421</td>
<td>0.0008</td>
<td>0.0023</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$Cys$</td>
<td>0.0001</td>
<td>0.59</td>
<td>0.0006</td>
<td>0.0022</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0455</td>
<td>-</td>
<td>0.0957</td>
<td>-</td>
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<td>$Ank2$</td>
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<td>0.75</td>
<td>0.0064</td>
<td>-</td>
<td>0.0128</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>0.0559</td>
<td>&lt;0.0001</td>
<td>-</td>
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<tr>
<td>B) Spruce budworm gene-specific mRNA</td>
<td></td>
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<td>PPO1</td>
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<td>0.46</td>
<td>-</td>
<td>0.0339</td>
<td>-</td>
<td>0.0783</td>
<td>0.0044</td>
<td>0.0020</td>
<td>0.0016</td>
<td>0.0009</td>
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<tr>
<td>PPO2</td>
<td>0.0081</td>
<td>0.39</td>
<td>-</td>
<td>0.0783</td>
<td>-</td>
<td>0.0362</td>
<td>-</td>
<td>0.0124</td>
<td>0.0101</td>
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</tr>
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<td>PPAE1</td>
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<td>0.15</td>
<td>-</td>
<td>0.0711</td>
<td>-</td>
<td>-</td>
<td>0.0971</td>
<td>0.0686</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C) Sephadex™ bead encapsulation and melanisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Encapsulation</td>
<td>&lt;0.0001</td>
<td>0.66</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Melanisation</td>
<td>0.0059</td>
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<td>0.0242</td>
<td>0.0681</td>
<td>-</td>
<td>-</td>
<td>-</td>
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**Fig. 5.1:** Scale for ranking melanisation intensity of Sephadex™ beads injected into spruce budworm larvae. This scale was used to assess speed of melanisation at two different rearing temperatures (20 and 30°C). Index values (0 to 5) provide an estimate of the degree of melanisation, which is based on colour (light to dark) and coverage level of bead melanisation after injection and ranges between 0 (no change in colour) and 5 (dark brown colouration of the entire bead).
Fig. 5.2: qPCR assessment of transcript abundance (mean ± SEM) for four TrIV genes (*TrV1, TrFrep1, Ank2, and Cys*) in spruce budworm larvae parasitized by *Tranosema rostrale* and reared at three different temperatures (10, 20, and 30°C). Each data point is the mean number of transcripts, as measured from three larvae, with two technical replicate run for each RNA extract.
Fig. 5.3: Effect of temperature on temporal pattern of (A) encapsulation surface (mean ± SEM; n=79) and (B) melanisation intensity (mean ± SEM; n=59) of Sephadex™ beads injected into spruce budworm larvae.
**Fig. 5.4:** Temporal mean (±SEM) transcription levels assessed by qPCR of three spruce budworm genes, prophenoloxidase (PPO) 1 and 2, and PPO activating enzyme (PPAE1) in spruce budworm larvae parasitized by *Tranosema rostrale* and reared at three different temperatures (10, 20, and 30°C). Each point is the mean number of transcriptions from three larvae and two technical replicates carried out on each RNA extract.
Chapter 6
Insights into the Spatiotemporal Biology of a Parasitoid Using a Temperature-Dependent Individual-Based Model

Abstract
Despite their importance as mortality factors in the population dynamics of many insects, the detailed biology and ecology of parasitoids often remains unknown. Knowledge gaps, such as their seasonal biology, voltinism, and overwintering strategies restrict the detailed study of most parasitoids. Ecological modeling approaches can help to fill these knowledge gaps by providing insight into life history traits that are difficult to study in the field or under laboratory conditions. We developed an individual-based model that takes the developmental, survival, and reproductive responses to temperature of one spruce budworm parasitoid, *Tranosema rostrale* (Hymenoptera: Ichneumonidae), into account in order to gain insight into its spatiotemporal biology across northern North America. The predicted phenology of the first generation closely matches observations from the field and accurately estimates the seasonal pattern of spruce budworm attack. The model forecasts 1-4 generations of the parasitoid and marked differences in temperature-dependent annual population growth rates across the spruce budworm’s distribution. The model offers two hypotheses that address the overwintering strategy of *T. rostrale* either as: (1) a pupa under the snow or (2) 10% through its immature development in a host that is exposed to air temperature. It also offers further predictions about the critical day lengths that induce diapause and the lethal lower temperature threshold for the parasitoid. Our approach permits further model expansion to improve our understanding about the dynamics of this parasitoid, but it can also be applied to other parasitoids or poikilothermic organisms.
Introduction

Parasitoids play an important role as natural mortality factor in population dynamics of numerous insects (Nealis 1991; Hawkins et al. 1997; Kidd & Jervis 1997) and as biological control agents for insect pests in diverse ecosystems (Waage & Hassell 1982; Régnière & Griffiths 1992; Van Driesche et al. 2008; MacQuarrie et al. 2016). Despite their obvious ecological importance, relatively little is known about the life history and distribution of many parasitoid species because their basic biology and ecology are poorly understood. Besides the more general uncertainties in taxonomy (LaSalle & Gauld 1991), distribution (Quicke 2012), and complex interactions over several trophic levels (e.g., Lewis et al. 2002; Eveleigh et al. 2007), specific knowledge gaps, such as their seasonal biology, voltinism, and overwintering strategies, restrict detailed understanding.

Ecological modeling can be used to gain insights into complex systems by simplifying relationships and taking only essential factors into account. Combining empirical data with modeling approaches can help to identify and close knowledge gaps that are otherwise difficult or impossible to approach under natural or laboratory conditions (e.g., Régnière & Griffiths 1992). Individual-based models (IBMs) have been gaining popularity over the last decades (Grimm 1999; DeAngelis & Grimm 2014; Stillman et al. 2015). IBMs are different from other modeling approaches because of their integral view on an organism’s life cycle, their consideration of variability among individuals of a population and the resources they exploit, and because populations are represented by integer numbers (Uchmański & Grimm 1996). What makes them interesting for ecologists is the simplicity of their structure, one that can be built on a few abstract concepts or theoretical constructs, and can include relatively simplistic data that simulate natural conditions (Régnière et al. 2015).

As poikilotherms, insects are strongly dependent on ambient temperature because it influences their key life-history traits, i.e., development, survival, and reproduction rate (Janisch 1932, Brown et al. 2004, Régnière et al. 2012a). Because of its importance relative to other factors, temperature is often used in models to better understand and predict insect changes in seasonal patterns of phenology (Powell & Logan 2005), distribution (Régnière et al. 2012b), and overall population dynamics (Flinn et al. 1992). While numerous temperature-driven models have been used as tools to model herbivore phenology in insect pest management (e.g., Nealis et al. 2001, Régnière & Bentz 2007, Régnière et al. 2007, Régnière et al. 2015), very few have been developed for

*Tranosema rostrale* (Brischke) (Hymenoptera: Ichneumonidae), is a larval endoparasitoid that plays an important role as a natural mortality factor in low-density spruce budworm *Choristoneura fumiferanae* (Clemens) (Lepidoptera: Tortricidae) populations. Parasitism levels by *T. rostrale* appear to be negatively density dependent. While parasitism rates > 90% were recorded repeatedly in central Quebec by this species in low-density spruce budworm populations (Cusson et al. 1998a, Seehausen et al. 2013, 2014), its importance as a mortality factor is low in other Maritime areas such as Fredericton, New Brunswick (Eveleigh et al. 1994, 1997) or at high host densities (Eveleigh et al. 2007). The parasitoid appears to have a Holarctic distribution, being also described as a parasitoid of several tortricids in central Europe (Zwölfer 1956, Zwölfer & Kraus 1957, Janssen 1959, Horstmann 1977, Evenhuis & Vlug 1983, Blommers et al. 1988, Mills & Kenis 1991, Kienzle et al. 1997). One subspecies is also described in Latvia (Ozols 1959) and another in Japan (Momoi 1968). While the parasitoid is present in North America at various locations between Alaska and Newfoundland, little is known about its impact on hosts across this range. Besides a negative influence of forest thinning on parasitism by *T. rostrale* on spruce budworm (Seehausen et al. 2014), nothing is known about the factors influencing parasitism rates at the landscape level.

The basic biology of *T. rostrale* in North America has been described (Cusson et al. 1998a), as well as details about its reproductive biology (Chapter 2) and its seasonal pattern of parasitism (Chapter 3). In Quebec, adult females are active in early spring when they attack post-diapause spruce budworm larvae and several other Lepidoptera. Upon egression, larvae pupate in silk cocoons on the foliage of their host’s food plant and adults emerge in late June to early July, after which the parasitoid is suspected of having at least one additional generation. However, it is not clear when adults of *T. rostrale* are active in spring, summer or autumn, or about the alternate hosts the parasitoid exploits following spruce budworm. Likewise, its overwintering stage and habitat are unknown.

In Chapter 4, the thermal responses of *T. rostrale* were described in terms of development time, longevity, survival, and fecundity. In this paper, these descriptions are used to develop an individual-based model of the parasitoid’s thermal responses to predict its seasonal biology and determine the relationship between natural temperature regimes and its fitness at the landscape
level. The model addresses the main knowledge gaps about *T. rostrale*, including its probable overwintering stage and habitat, phenology, voltinism, and fitness across North America.

**Material and Methods**

*Parasitoid Rearing and Parameter Estimation*

The insect rearing methods, analysis of data, and parameter estimates are all described in detail elsewhere (Chapter 4) and will only be presented here briefly as a general overview. Parasitoids were obtained by exposing spruce budworm larvae to parasitoids on balsam fir *Abies balsamea* (L.) Miller (Pinaceae) trees in two study sites in Québec, near Armagh (46°46’ N, 70°39’ W, 312 m) and Petit-lac-à-Épaule (47°18’ N, 71°12’ W, 725 m, hereafter Epaule). Parasitoid developmental, survival, and reproductive responses to temperature were determined in rearing chambers under several constant temperatures ranging from 5 to 30°C. Spruce budworm larvae feeding on balsam fir foliage were used as hosts for immature development.

The developmental response of parasitoid immatures to temperature was described using the Sharpe-Schoolfield model (Sharpe and DeMichele 1977, Schoolfield et al. 1981) separately for; (1) eggs and larvae inside the host (in-host) and (2) pupae after egression. These descriptions were based on individual development times, so that individual variability (distribution and variance) was described simultaneously by the method of Régnière et al. (2012a). Stage-specific survival of immatures at different temperatures was also analysed by the method of Régnière et al. (2012a) applying a non-linear mixed model with binomial distribution and two parameters. The effect of temperature on longevity of adult females was analysed with a maximum likelihood approach with a simple exponential equation and two parameters that also provide a description of the variability between individuals. To describe temperature-dependent female fecundity, we developed a general non-linear model using discrete difference-equations that describes egg production as two simultaneous and opposite processes, oogenesis and egg resorption (or oosorption). Variability of the parameters was also obtained and used to simulate individual variability of fecundity.

*Model Description*

The model developed in this study (see Fig. 6.1) is an IBM (see Régnière et al. 2015) that tracks a population of 1000 individuals, each with its own temperature-dependent traits (development, survival, longevity, and fecundity) randomly drawn from observed distributions of each trait. Development of the parasitoid starts on 1 January of a given year. Stage-specific immature
development of in-host stages ($R_t$) and of pupae ($R_p$) is based on the principle of non-linear development summation (Logan et al. 2003), which takes the non-linear relationship between temperature and insect development into account to accumulate development over small time steps (here 1 h) under a variable temperature regime. Stage-specific survival of in-host stages ($S_t$) and of pupae ($S_p$) is applied at each time step through temperature-dependent probability of survival. Death of an individual occurs when a random number drawn from a uniform [0,1] distribution exceeds the survival probability during the time step. When the adult stage is reached, parasitoids age at a temperature-dependent rate ($R_A$). Adult females emerge with a mean of 9.1 mature eggs (Chapter 2), and thereafter develop eggs based on temperature-dependent oogenesis ($O_G$) and oosorption ($O_S$) rates. The model assumes that oviposition is not restricted by host density or the female’s searching or handling time. Therefore, females lay all eggs available in their oviducts at each time step, and eggs constitute new individuals. Because the only source of mortality in the model is the temperature-dependent attrition rate derived from laboratory experiments, a constant rate of culling ($S_C$) is applied to all eggs laid by females before new individuals are created. This limits the number of individuals that must be kept track of by the model, and also provides for realistic potential intergeneration growth rates. This culling rate includes a sex ratio of 2:1 (males:females; Cusson et al. 1998a) so that the model only simulates females. Surviving female eggs then constitute the next generation. Individuals of several generations can coexist in the model. In simulating only female parasitoids, we assume that mating is not a limiting factor.

**Overwintering Strategy and Model Calibration**

The overwintering strategy of *T. rostrale* is unknown. Therefore, possible overwintering scenarios were explored in the model by arresting parasitoid development at the end of the year and starting it at the beginning of the following year at different parasitoid ages and different times of the season, in an attempt to find the best fit for predicting parasitism observed in the field. Two possible and biologically meaningful overwintering strategies of *T. rostrale* were identified using this approach: (1) the parasitoid diapauses as pupa under the snow or (2) it diapauses as egg or larva in a host somewhere on trees and therefore exposed to air temperature (Fig. 6.1). In Scenario 1, diapause is induced when the parasitoid enters the pupal stage and day length is below a critical threshold ($D_C$) past the summer solstice. Diapausing pupae do not develop until diapause requirements are satisfied, and we assume this occurs by 31 December. Thus, the individuals constituting the initial generation are considered to be all produced as non-diapausing young pupae.
on 1 January. Development of overwintering pupae starts once soil temperature reaches air temperature, some time after snowmelt (defined as ground snow water equivalent < 1 mm, as predicted from temperature and precipitation by BioSIM; Régnière et al. 2014). The soil surface takes some time after snowmelt before reaching air temperature (Régnière & Griffiths 1992), and this is mimicked in the model by using a warmup delay ($D_s$, in days) before pupal development resumes, at air temperature. In Scenario 2, parasitoid diapause is induced when a critical age ($a_D$) is reached and day length is below the critical threshold after summer solstice ($D_C$). In this scenario, the individuals constituting the initial population are all of age $a_D$ on 1 January. In both scenarios, diapause implies interrupted development and cold-hardiness of the parasitoid in the diapausing stage. Non-diapausing individuals are killed when temperature drops below a lethal temperature ($T_L$).

The value of $D_s$, the delay in soil warming after snowmelt in Scenario 1, was unknown. It was estimated by varying its value from 7 to 22 days, in 15 steps of 1 d each. Similarly, the age of entry into diapause ($a_D$) in Scenario 2 was estimated by varying the value of $a_D$ between 0 (just-laid egg) to 0.3 (30% through in-host development) in 12 steps of 0.025. The varying values were then submitted to the model to maximize the correlation between simulated and observed attack rates in Armagh and Epaule. For this, a large data set was used containing parasitism rates of spruce budworm larvae by *T. rostrale* for overlapping 7-day periods that were assessed twice a week over the season in two study sites for 8 years from 2008 to 2015. The observed seasonal patterns in each year and study site were correlated with the simulated average number of eggs laid over successive 7-day periods (centered), staggered twice a week to mimic the field observations. Because the model is stochastic, each simulation was replicated 25 times and results were averaged for each year and parameter value before analysis. The number of days after snow melt and parasitoid age producing the highest overall correlation were subsequently used as model parameters in Scenario 1 and 2, respectively.

The threshold of critical day length for entry into diapause ($D_C$), the lethal temperature for individuals not in diapause ($T_L$), and survival from the temperature-independent culling ($S_C$) were determined by testing their influence on the annual *T. rostrale* population growth rate $R_A$ (number of individuals entering diapause at the end of the growth season / initial number of individuals). To this end, a common optimum of $R_A$ was searched for in three locations where *T. rostrale* is known to occur (Armagh, Epaule, and Fredericton) by running the model under Scenario 1 for the
period 2008-2015, gradually increasing values of $D_C$ and $T_L$. The values that produced the maximum overall $R_A$ were then used to calibrate $S_C$ so as to obtain an average $R_A$ near 1 (indicative of long-term persistence) for the three locations combined. Each simulation was replicated 25 times and results were averaged for each parameter value before analysis.

**Voltinism, and Population Growth Rate**

To assess *T. rostrale*’s voltinism and potential population growth rate in the study sites as well as over its probable habitat in North America (specifically over the range of spruce budworm distribution), we ran the model under Diapause Scenario 1 with the values of $D_S$, $D_C$, $T_L$ and $S_C$ obtained above. The model was run for 10,000 random points between 41°N and 69°N across the range of spruce budworm in North America. Each run was replicated 30 times using daily minimum and maximum air temperature data generated from 1981-2010 monthly normals based on the four Environment Canada or NCDC weather stations nearest to each simulation point, following compensation for differences in latitude, longitude and elevation by the BioSIM system (Régnière & Saint-Amant 2008).

To compare the phenologies of *T. rostrale* and spruce budworm, the *T. rostrale* model and the Spruce Budworm Phenology Model (Régnière et al. 2012b) were run based on Armagh and Epaule for the period 1991-2015 under the same observed temperature regimes (25 replications per site and year).

**Results**

**Overwintering Strategy and Comparison of Observed and Simulated Development**

The best correlation between the predicted and observed attack rates of spruce budworm larvae by *T. rostrale* in Armagh and Epaule was obtained with a delay in development summation of 15 days after snowmelt in Diapause Scenario 1 (Fig. 6.2a). Under Scenario 2, where the parasitoid is assumed to overwinter in-host, the age for entry into diapause ($a_D$) producing the highest correlation between predicted and observed attack is approximately $a_D = 0.1$, or 10% through in-host development (Fig. 6.2b). Under the optimal parameter values, both scenarios yielded very similar correlations with the observed attack rates.

The remaining results focus on Diapause Scenario 1, however, they are equally applicable under Diapause Scenario 2 because of equal statistical plausibility. The critical day length for entry into diapause resulting in optimal annual population growth rates $R_A$ in the three study sites (Armagh,
Epaule, and Fredericton) was 13.5 h, which occurs on 24-25 August in those locations (Fig. 6.3a). With this critical day length, the model predicts that the average number of generations per year (defined here as the average generation to which overwintering individuals belong) is 2 in Epaule, 3 in Armagh, and 3.8 in Fredericton (Fig. 6.3b).

Changes in $T_L$ had a marked influence on $R_A$ in the three sites only when $T_L > -5^\circ C$ for non-diapausing individuals (Fig. 6.4a). Thus, $T_L = -5^\circ C$ is used in the model. Finally, values of $R_A$ averaging 1 over the 3 sites where obtained with $S_C=0.19$ (Fig. 6.4b). Using these parameters, the correlation between predicted and observed attacks of spruce budworm larvae over the 8-year period was 0.73 and 0.79 in Armagh and 0.49 and 0.42 in Epaule, for Diapause Scenarios 1 and 2, respectively (see Fig. 6.5 for fit under Diapause Scenario 1).

**Phenology**

The predicted phenology of *T. rostrale*’s life stages is similar in both study sites but all stages occur about 2 weeks later in Epaule than in Armagh (Fig. 6.6). First adults emerge in mid-May in Armagh and early June in Epaule when 4\textsuperscript{th}-instar spruce budworm larvae are starting to appear and most larvae are in the 3\textsuperscript{rd} instar in the field. The peak of adult parasitoid activity is in early- and late June in Armagh and Epaule, respectively. Oviposition peaks about 10-12 d later in both study sites and coincides with the peak occurrence of 4\textsuperscript{th}- and 5\textsuperscript{th}-instar spruce budworm larvae. *Tranosema rostrale* pupae of the first generation occur from late June to beginning of July in Armagh and in from July to the beginning of August in Epaule, with the second generation of adults egressing about 20 d later.

**Voltinism**

The mean number of predicted generations generally increases from north to south over northern North America, and increases within the area of spruce budworm distribution from one generation in central Quebec, the Labrador mainland, and the northern Rocky Mountains to four generations in southern Ontario and the northeastern USA (Fig. 6.7).

**Population Growth Rate**

The predicted annual population growth rate ($R_A$) of *T. rostrale* is generally low in northern North America, and forms a band of $R_A \geq 1$ around the 50° N latitude, decreasing thereafter to the south (Fig. 6.8). Particularly high $R_A$ values are predicted for coastal regions in the west around Vancouver Island (where spruce budworm is absent) and in the east north of Anticosti Island
(Quebec), the island of Newfoundland, and Prince Edward Island. Within the area of spruce budworm distribution, \( R_A \geq 1 \) except in its northwestern habitat, central Quebec, and parts of Labrador mainland.

**Discussion**

The IBM presented in this paper conclusively shows how ecological modeling approaches can be efficiently used to gain insights into the spatiotemporal biology of parasitoids. By taking the influence of temperature on key life history traits of the parasitoid *T. rostrale* into account, we were able to simulate its seasonal pattern of attack, potential voltinism, and population growth rate in North America, and formulate hypotheses about other unknown facts of its seasonal biology that can help fill the current gap in knowledge about this system.

The model accurately predicts the observed pattern of seasonal parasitism of spruce budworm larvae. Field data suggest that parasitism of 2\(^{nd}\)-instar spruce budworm larvae occurs only when this stage overlaps with 3\(^{rd}\)-instar larvae (Chapter 3). The onset of parasitoid attack predicted by the model is precisely during this overlap in 2\(^{nd}\) and 3\(^{rd}\)-instar larvae, confirming field observations. In addition, the observed increase in parasitism from 3\(^{rd}\)- to 4\(^{th}\)-instar that peaks during 4\(^{th}\)- and 5\(^{th}\)-instar and decreases in later instars (Chapter 3) is predicted by our model. The increase in late-season attack frequency observed over many years in Epaule (*e.g.*, 2010 and 2012) is also predicted to be due to a second generation of eggs being laid (Fig. 6.5). These results confirm the hypothesis that the parasitoid’s phenological response to temperature is mainly responsible for the observed seasonal pattern in spruce budworm parasitism, and that other explanations such as host instar preference or competition with other parasitoid species such as *Elachertus cacoeciae* (Howard) (Hymenoptera: Eulophidae) (Chapter 3) are only playing a minor role, if any. However, some of the variation in the observed pattern of seasonal parasitism of spruce budworm larvae by *T. rostrale* cannot be explained by the model. The model only takes temperature into account, but other factors such as humidity, rain, and wind (*e.g.*, Weisser et al. 1997; Kalyebi et al. 2005), or undefined behavioural responses (*e.g.*, functional response) may influence parasitism rates locally, leading to a deviation from the predicted values.

The model predicts 1–4 generations of *T. rostrale* per year within the area of spruce budworm distribution. Through rearing in an outdoor insectary, it was determined that this parasitoid can undergo three generations in Quebec City (Cusson et al. 1998a), which is in accordance with our findings. However, the effort to find late season hosts that *T. rostrale* attacks after spruce budworm
larvae so far has been unsuccessful (Cusson et al. 1998a). With our model, this search for hosts can now be focused more efficiently because we can now predict voltinism and the timing of parasitism in the field. One potential source of error in this prediction may be due to the fact that some parasitoids have variable temperature-dependent development times in different host species (e.g., Bazzocchi et al. 2003). Our model is based on development rates recorded from spruce budworm, however, development times for different host species seem to be uncommon or marginal. More often, differences in parasitoid survival or other traits related to fitness may be observed (Carton & Kitano 1981, Janssen 1989, Brodeur et al. 1998, Harvey et al. 1999).

The host and its population dynamics are not explicitly modeled here, and thus, potential differences in fitness of the parasitoid developing in different host species cannot be accounted for, and temperature-independent mortality of the parasitoid is imposed by a constant ($S_C$). This means that the predicted values of $R_A$ are overly simplified and strictly theoretical, and can only be used to compare the parasitoid’s temperature-dependent overall fitness within the modelled habitat, as estimated by the modeled life history traits. Within most of the spruce budworm’s area of distribution $R_A \geq 1$, suggesting that the parasitoid’s thermal requirements for optimal fitness are well suited across this habitat. Figure 6.8 reveals a trade-off between an increased development rate and detrimental effects on other life history traits at high temperatures. As mean temperatures increase towards the south, $T$. rostrale will undergo more generations each year due to a higher development rate, but mortality will rise simultaneously, and longevity as well as realized fecundity (Chapter 4) will be lowered, restricting $R_A$ in the southern latitudes. High $R_A$ values on the east and west coasts are probably the result of moderate temperatures that allow for an additional generation while also favouring other life history traits.

Increasing voltinism of the parasitoid implies increasing dependence on alternate hosts. Increasing the number of alternate hosts required to complete a year’s life history probably leads to asynchrony and unpredictable host abundances. As a result, $R_A$ values in regions with a greater number of generations may be overestimated. For this reason, Fig. 6.8 cannot provide complete information on spatial differences in the efficacy of $T$. rostrale attacking and developing in spruce budworm larvae. Interestingly though, when calibrating the model for optimal $T_L$, $S_C$, and $D_C$ values, $R_A$ was always lower in Fredericton than in the two other Quebec sites (Fig. 6.3a and 6.4). At optimum $D_C$, 2, 3, and 3.8 generations are predicted for Epaule, Armagh, and Fredericton, respectively. Because the 4th generation cannot be fully completed in Fredericton before $D_C$, the
probability of the parasitoid’s mortality from $T_L$ might increase in this region. These results therefore could offer possible explanations for lower spruce budworm parasitism by *T. rostrale* in Fredericton (Eveleigh et al. 1994, 1997, 2007) when compared to Armagh and Epaule (Cusson et al. 1998a, Seehausen et al. 2013, 2014).

Many tortricids, including spruce budworm, overwinter as small larvae in bark crevices or similar shelters exposed to ambient air temperatures (Nealis 2015), and this makes it biologically possible that the parasitoid uses an overwintering strategy similar to that considered by Diapause Scenario 2. Early studies proposed that *T. rostrale* could overwinter in 2\textsuperscript{nd}-instar spruce budworm larvae (Coppel 1947, Wilkes et al. 1948, Miller 1963), however this was later dismissed after extensive sampling of overwintering larvae in Quebec and Ontario (Cusson et al. 1998a). The fact that the parasitoid is active early in the spring also suggests a different overwintering strategy. *Tranosema rostrale* is a koinobiont parasitoid, for which development is dependent on continued host development, at least in small hosts. For many insects, diapause is terminated after only a fraction of their dormant phase in winter, and the onset of development depends on temperatures above a lower thermal threshold for development (Tauber & Tauber 1976, Koštál 2006). Therefore, most potential host larvae cannot continue their development until foliage is available in the spring, and this introduces a delay in parasitoid development that would be too long for *T. rostrale* to reach adulthood in time to attack 3\textsuperscript{rd}- and 4\textsuperscript{th}-instar spruce budworm larvae. Thus, it is more likely that the parasitoid overwinters in the late larval, prepupal, or pupal stage under the snow (Scenario 1) than in overwintering host larvae.

Based on the results of our model, we hypothesize that *T. rostrale* develops in an alternate host until diapause is induced by a critical day length of 13.5 h during its late larval, pre-pupal or pupal stage. Parasitoids that do not reach this developmental stage when day length is below this threshold and temperatures are -5°C or below are killed by frost. Parasitoids successfully entering diapause will be covered by snow on the ground where they can overwinter until next spring. After snow melt, a soil warmup period is required for the onset of parasitoid development from the pupal stage (a 15-day delay was used here for this). It may be that this is the time required for soil temperatures to reach air temperature (Régnière & Griffiths 1992), or that diapause actually takes place in the late larval or pre-pupal stage. This hypothesis remains to be validated through field observations, and these can be facilitated using the model’s predictions for voltinism and timing of attack before the predicted induction of diapause.
The next step will be to run the model described here under climate change projections to determine the influence of increasing temperature on the overall performance of this important spruce budworm parasitoid. IBMs are easily expandable and can be applied to other species because of their mathematical simplicity and object-oriented nature (Régnière et al. 2015). Thus, in the future, the model described here may be combined with the existing Spruce Budworm Seasonality Model (Régnière et al. 2012b) to gain further insight into the influence of changing temperatures on this parasitoid-host interaction. Such a combined model may not only help to understand important processes in the population dynamics of these two species, but can also be used to study the impact of climate change on their interaction. As descriptions of thermal responses exist for many species, the model may also be used as a framework to fill knowledge gaps in the spatiotemporal biology of other parasitoid species or poikilothermic organisms.
Fig. 6.1: Flowchart of the individual-based model showing stage-specific temperature-dependent development, survival, and fecundity for the parasitoid *Tranosema rostrale* in-host (egg and larva), pupa, and adult under two diapause scenarios, (1) in the pupal stage, and (2) in-host. Processes that are dependent on air temperature are bordered by dotted lines.
Fig. 6.2: Correlation between observed and simulated attack rates of spruce budworm larvae by *Tranosema rostrale* at changing values of (a) start-date shift of parasitoid development after snow melt under Diapause Scenario 1, and (b) parasitoid age of entry into diapause under Diapause Scenario 2, overall and in the two sites Armagh and Epaule, Quebec (2008 to 2015).
Fig. 6.3: Sensitivity analysis of the impact of varying times of critical day length past summer solstice for entry of *Tranosema rostrale* into diapause on (a) the predicted annual population growth rate and (b) the number of generations per year in three sites from eastern Canada: Armagh, Epaule (Quebec), and Fredericton (New Brunswick).
Fig. 6.4: Sensitivity analysis of changing values of (a) lethal lower temperature threshold for non-diapausing parasitoids, and (b) survival from temperature-independent mortality (culling) on the predicted annual population growth rate in three sites: Armagh, Epaule (Quebec), and Fredericton (New Brunswick).
Fig. 6.5: Simulated (pointed line) and observed (black dots with solid line) attack rates of spruce budworm larvae by *Tranosema rostrale* and subsequent simulated attack rates of further generations (dashed lines) from May to September between 2008 and 2015 in (left panels) Armagh and (right panels) Epaule, Quebec, Canada.
Fig. 6.6: Frequency distribution of (upper panels) spruce budworm life stages from feeding second instars to pupae and (lower panels) *Tranosema rostrale* life stages from pupa of the first generation to adults of the second generation over the average season from May to July in (left panels) Armagh and (right panels) Epaule, Quebec, Canada.
Fig 6.7: Spatial distribution of the simulated mean number of generations (pupa to pupa) of *Tranosema rostrale* in (highlighted) the area of spruce budworm distribution and (greyed out) the surrounding habitat in northern North America.
Fig. 6.8: Spatial distribution of the simulated annual population growth rate ($R_A$; number of individuals entering diapause at the end of the growth season / initial number of individuals) of *Tranosema rostrale* in (highlighted) the area of spruce budworm distribution and (greyed out) the surrounding habitat in northern North America.
Chapter 7
General Conclusions and Future Research

The goal of this thesis was to investigate factors influencing the efficacy of *T. rostrale* as one of the most important mortality factors in low-density spruce budworm populations (Cusson et al. 1998a; Seehausen et al. 2013; 2014). Specifically, the dissertation is aimed at filling knowledge gaps about the biology and ecology of *T. rostrale* outlined in Chapter 1 and investigating the effect of temperature on key life-history traits. Results presented in Chapter 2 clearly show that key traits of *T. rostrale*’s reproductive biology and behaviour contribute to its high rate of success in terms of parasitizing the spruce budworm. Seasonal parasitism by *T. rostrale* under field conditions was partially affected by multiparasitism of spruce budworm larvae, but not by any preference for a specific larval instar (Chapter 3). Temperature influenced key life history traits of *T. rostrale* (Chapter 4 and 5), and this ultimately allowed a simulation model to be developed that accurately predicted parasitism (Chapter 6). This last data chapter also provides new insight into the seasonal biology of this parasitoid and its potential performance as a mortality factor in regulating spruce budworm dynamics under the scenario of changing temperatures across North America.

Chapter 2 addressed knowledge gaps about *T. rostrale*’s reproductive biology. A number of life history traits related to its reproduction contributed to the success of *T. rostrale* when attacking low-density spruce budworm populations, namely: 1) its lack of a pre-mating or preoviposition period; 2) the relatively rapid maturity of its eggs soon after emergence, despite being synovigenic; and 3) its efficacy in host searching and oviposition that appear to successfully circumvent basic host defenses. Results from Chapter 2 also provide advanced understanding for rearing *T. rostrale* in future laboratory studies. It appears that optimal mating success increases with the number of males present in a cage and adult parasitoids live longest when sugar water is provided. As well, no pre-mating or preoviposition period seems to be necessary as mating and oviposition can take place immediately after emergence. Combined, these results greatly facilitate parasitoid rearing and were critical for completing the many experiments developed in this thesis.

Chapter 3 investigated life history traits of *T. rostrale* that might possibly influence its pattern of parasitism over the season. Results showed that parasitism on spruce budworm larvae increased during 3rd and 4th instars and then decreased during 5th to 6th instars. Multiparasitism by both *T. rostrale* and the ectoparasitoid *E. cacoeciae* occurred in spruce budworm larvae, and their pattern
of seasonal parasitism was negatively correlated. It is known that when *E. cacoeciae* is given a choice, it prefers to attack 5th- and 6th-instar spruce budworm larvae (Fidgen et al. 2000), however my results from the manipulative choice experiments in the field suggest that *T. rostrale* has no host instar preference and that it can successfully develop in all 3rd- to 6th-larval instars without any significant impact on the parasitoid’s overall performance. Thus, host instar preference does not appear to be a factor in the observed seasonal pattern of parasitism by *T. rostrale*, and competition with *E. cacoeciae* is more likely an explanation since the latter species is thought to outcompete *T. rostrale* when multiparasitism occurs. Based on my observations however, the incidence of multiparasitism in the field is likely to be too rare and sporadic, and thus multiparasitism is unlikely to be the only factor reducing spruce budworm parasitism by *T. rostrale* during 5th to 6th instar. My work suggests that the observed phenological responses in *T. rostrale* that determine its seasonal patterns are most likely to be explained by the seasonal changes in temperature. Thus, this chapter shed light on unknown facts about the parasitoid’s seasonal biology and multiparasitism as outlined in Chapter 1.

The examination of *T. rostrale*’s developmental, survival, and reproductive response to temperature (Chapter 4) showed that its key life-history traits are strongly dependent on air temperature. Parameter estimates derived from non-linear maximum likelihood modeling were used to describe changes in these key life history traits at each temperature and to build a seasonal biology model to predict its phenology in the field. While the optimal temperature for the immature development of *T. rostrale* was 25°C, temperatures above 20°C had a negative effect on its survival, longevity, and fecundity. This sharp decrease in survival with increasing temperature was unexpected and led to further experiments to investigate those factors that might result in such low survival at high temperatures.

During the successful development of *T. rostrale* in its host, its ichnovirus (TrIV) abrogates the host’s immune system to enable the parasitoid to avoid encapsulation and melanisation (Doucet & Cusson 1996b; Cusson et al. 1998b). When parasitized larvae exposed to high temperatures were dissected here, it was clear that low parasitoid survival was due to encapsulation and melanisation. This raises two hypotheses as to how high temperatures might lead to low parasitoid survival, namely: 1) they reduce the performance of TrIV through a depression of viral gene expression that limits TrIV’s effectiveness in abrogating the host immune response, and 2) they enhance the budworm’s immune system by enabling more effective encapsulation and melanisation resulting
from a rise in the expression of immunity-related genes. These two hypotheses were tested in Chapter 5 with molecular and physiological experiments. The results support both hypotheses, showing that both the expression of several TrIV genes is reduced at high temperatures, and the host immune system is enhanced. However, temperature only had a minor effect on both the spruce budworm’s immune response and the transcription of genes related to melanisation, but not the melanisation process itself was enhanced in spruce budworm larvae exposed to high temperatures. My work suggests that the influence of temperature on TrIV gene transcription is the key factor responsible for the negative correlation between parasitoid survival and temperature observed here.

Remaining knowledge gaps about the seasonal biology of *T. rostrale* were then addressed by the individual-based model developed in Chapter 6. The model gave insight into the spatiotemporal biology of *T. rostrale* in response to changing temperatures, and predicted spruce budworm parasitism in any given year or region with a relatively high degree of accuracy. By comparing patterns in the field with the predicted occurrence of spruce budworm instars from the Spruce Budworm Seasonality Model (Régnière et al. 2012b), it is clear that the seasonal pattern of parasitism presented in Chapter 3 can be explained by the temperature-dependent phenology of *T. rostrale*, and that multiparasitism with *E. cacoeciae* plays only a minor role in this, if at all. The model also accurately predicted *T. rostrale* voltinism in the study area; i.e., 2-3 generations per year in Epaule and 3-4 in Armagh, Quebec. Moving from north to south in Canada and through the northern USA, the model forecasts a general increase in 1 to 4 parasitoid generations per year across the budworm’s geographic distribution. This result supports the hypothesis raised in Chapter 1 that *T. rostrale*’s population dynamics, in some areas, is highly dependent on alternate hosts, which may lead to an observed decrease in apparent parasitism rates with increasing spruce budworm populations. The annual growth rate of *T. rostrale* populations is predicted to form a band of values ≥1 around 50° N latitude, and this falls within the majority of the budworm’s distribution, suggesting that it is well adapted to the region. The model also predicts the highest annual population growth rates around coastal areas within this latitude, and a gradual decrease in growth rates moving north and south of this latitude. These results suggest that there is a trade-off between development rate and other life history traits for this parasitoid at increasing temperatures. High temperatures result in a greater number of generations per year moving southwards, but this is accompanied by a decrease in overall fitness (as represented here by the annual population growth rate) due to the negative effects on immature survival, adult longevity, and female
Finally, the model also provides two plausible hypotheses for the overwintering strategy of *Tranosema rostrale*. While no field observations on overwintering are yet available for *T. rostrale*, results from the model suggest that it likely spends the winter as a pupa under the snow.

Together, the chapters in this dissertation constitute a comprehensive study of one important spruce budworm parasitoid, including its basic behaviour, ecology, physiology, molecular biology, and diverse modeling approaches. Chapter 6 combines all of results from the previous chapters, either directly (Chapter 4) or indirectly (Chapters 2, 3, and 5), and provides additional insights into the biology of *T. rostrale*, which would not have been available without the findings from the previous chapters.

**Research Contributions and Implications**

The studies presented in this dissertation substantially increase our knowledge about factors influencing parasitism in low-density spruce budworm populations, especially by *T. rostrale*. Despite the importance of parasitoids in keeping this major defoliator’s populations at low levels over many years, little information exists as to the actual mechanisms. Besides *T. rostrale*, the only other parasitoid species attacking low-density spruce budworm populations for which life history traits have been studied are *E. cacoeciae* (Fidgen & Eveleigh 1998, Fidgen et al. 2000) and *A. interrupta* (Cusson et al. 2002). My results now provide key life history traits for another important parasitoid, *T. rostrale*, well beyond the basic biology that was previously described for this species (Cusson et al. 1998a). New information about the reproductive biology and behaviour of this parasitoid (Chapter 2), as well as its pattern of seasonal parasitism in relation to other parasitoids attacking spruce budworm larvae (Chapter 3) add significantly to our understanding about this parasitoid’s efficacy as a mortality factor of low-density spruce budworm populations. It is clear that investigating parasitoid life history traits in depth can facilitate further work on temperature effects that drive parasitoid development, survival, and fecundity. To date, developmental responses to temperature were only available for two larval parasitoids of rising or high-density spruce budworm populations, *A. fumiferanae* (Nealis & Fraser 1988) and *Smidtia fumiferanae* (Diptera: Tachinidae) (Hebert & Cloutier 1990b). *Tranosema rostrale* regulates low-density populations and thus, the current work in terms of its response to temperature (Chapters 4-6) is an important contribution to our understanding of this little known, and yet important parasitoid-host system.
Parasitism is the main factor contributing to the predator Allee effect, that together with a mate-finding Allee effect, explains how mortality can keep spruce budworm populations low over long periods of time (Régnière et al. 2013). Unfortunately, it is still unclear what actually causes such populations to escape from these low levels to form outbreaks; current speculation rests on either a reduction in parasitism/predation mortality or some improvement in mate finding. Migrating female moths are thought to overcome the mate-finding problem (Régnière et al. 2013), and both theoretical and empirical studies are now being conducted to examine whether long-distance migration helps form spruce budworm outbreaks (e.g., Sturtevant et al. 2013). Alternatively, it has been suggested that spruce budworm densities may increase as a result of a reduction in parasitism due to natural events. Results here show that high temperatures do negatively affect the efficacy of *T. rostrale* as a factor in the mortality of spruce budworm, and this supports the hypothesis that high temperatures may increase spruce budworm survival, especially during hot summers, thereby allowing populations to reach outbreak levels. Although the influence of temperature on the dynamics of spruce budworm is beyond the scope of this dissertation, the model developed here (Chapter 6) provides a strong basis on which to examine the implications of high temperatures on spruce budworm survival.

Climate change is predicted to result in higher temperatures and more extreme weather events over time (IPCC 2013), however its actual effects on insects are difficult to predict because of the complex ecological interactions that take place over multiple trophic levels (Fleming & Volney 1995; Harrington et al. 2001; Dukes et al. 2009). It has been suggested that for at least some insect species, changes in outbreak frequency, extent, and severity will occur with higher temperatures (Fleming & Volney 1995; Fleming 1996; Dale et al. 2001; Logan et al. 2003; Jepsen et al. 2007; Dukes et al. 2009; Haynes et al. 2014). For the spruce budworm, climate is known to affect the severity and duration of outbreaks (Gray 2008), as well as their synchrony at a landscape level (Williams et al. 2000; Royama 2005). Modeling approaches applied to data on temperature-dependent performance of the spruce budworm predict a shift in its range towards the north as the climate changes (Williams & Liebhold 1997; Candau & Fleming 2011; Régnière et al. 2012b). Climate warming is predicted to also have severe impacts on parasitoids and their interactions with hosts (Hance et al. 2007; Jeffs & Lewis 2013). Some authors suggest that a ‘temporal refuge’ for spruce budworm might develop under a scenario of climate warming due to the differential optima needed for development of the spruce budworm versus its parasitoids. Such phenological mismatches have the potential to lower overall parasitism, thereby allowing spruce budworm
populations to escape and develop outbreaks (Fleming & Volney 1995). This theory of phenological mismatch is based on work done with only one spruce budworm parasitoid, *A. fumiferanae* (Nealis & Fraser 1988). More information is needed on the response of other parasitoids to reliably predict the influence of climate change on parasitism in this system. Here, the response of an additional important spruce budworm parasitoid to changing temperature is studied in detail and thus, the data presented contribute significantly to our scientific understanding about mechanisms that might drive changes in spruce budworm mortality with climate warming.

Despite the suggestion that high temperature has a negative effect on the efficacy of polydnaviruses (PDVs) to abrogate their host’s immune response (Khafagi & Hegazi 2004), to date, no information is available on its influence with respect to PDV gene expression. TrIV is a well-studied PDV (Doucet & Cusson 1996a; 1996b; Béliveau et al. 2000; 2003; Doucet et al. 2008; Rasoolizadeh et al. 2009a; 2009b; Djoumad et al. 2013), and therefore provides a good model to examine the effect of temperature on gene expression. There are many other parasitoids that are associated with PDVs and temperature may have a similar effect on them. Thus, findings in Chapter 5 add to our understanding about some mechanisms that might reduce parasitoid performance under increasing temperature, and this will help predict the impact of climate change on natural enemies in other pest insect systems.

Numerous models of parasitoid-host interactions have been developed in the past, most exploring population control by natural enemies (*e.g.*, Mills & Getz 1996; Hassell 2000) or specific aspects of parasitoid life histories (*e.g.*, Jervis & Kidd 1986; Ellers et al. 2000). Very few simulation models exist that investigate aspects of the seasonal biology of a parasitoid taking into account temperature (for exceptions see Drummond et al. 1985; Régnière & Griffiths 1992; Mols & Diederik 1995; de Souza et al. 2009). The model developed here is the first individual-based model giving insights into the seasonal biology of a spruce budworm parasitoid that predicts its spatiotemporal phenology under changing temperatures. The object-oriented individual-based structure of the model provides it with the flexibility to be updated by incorporating new aspects and details as needed (Régnière et al. 2015). Not only can it be developed further to give important insights into the population dynamics of spruce budworm, but it can also be used to study the influence of increasing temperatures and extreme weather events on the performance of this parasitoid, and as well, its effect on spruce budworm population dynamics under a scenario of
climate change. In addition, the model can also provide a framework for analyzing the influence of temperature on other parasitoids.

Future Research
While this dissertation presents new findings about the biology of T. rostrale, many aspects remain unknown and warrant further investigation. Chapter 2 shows that we are just beginning to understand the behaviour of T. rostrale, e.g., its host searching behaviour. Rigorous tests, such as choice tests in an olfactometer or with an antennogram, could help to identify specific compounds that are needed for T. rostrale to find its hosts. Also, the reason for the strong male bias in the laboratory rearing remains a mystery. Further behavioural and physiological studies (e.g., sperm count; Cusson et al. 1998a) are needed to unravel what causes this bias. Results from such experiments may help to improve rearing this parasitoid and to eventually establishing a laboratory colony for further experimental work.

One potentially important factor in the population dynamics of T. rostrale is the outcome of multiparasitism of spruce budworm larvae by T. rostrale and E. cacoeciae (see Chapter 3). Multiparasitism by these two parasitoids under controlled conditions in the laboratory would help clarify whether the ectoparasitoid E. cacoeciae actually outcompetes T. rostrale, and if so, if this contributes to the pattern of observed seasonal phenology and successful parasitism by T. rostrale. The importance of hyperparasitism in the population dynamics of T. rostrale also remains unclear. The current methods to study parasitism in low-density spruce budworm populations do not allow for a thorough investigation of hyperparasitism because parasitized larvae are either collected after only one week of exposure (sentinels) or as soon as the parasitoid egresses (cohorts). Only in rare cases have suspected hyperparasitoids of T. rostrale been reared this way, e.g., M. sylvarum (J.R., unpublished data), and no quantitative analysis of hyperparasitism and its effect on T. rostrale population dynamics exists. This knowledge gap is important to fill because hyperparasitism can play a crucial role in the efficacy of a primary parasitoid in controlling its host (Weseloh 1978; Bourchier & Nealis 1992; Sullivan & Völkl 1999; Brodeur 2000). The use of molecular tools to identify hyperparasitism might also be very useful in this context, e.g., DNA barcoding (Smith et al. 2011).

Chapter 4 represents an example of the successful use of transfer treatments at extreme temperatures and non-linear maximum likelihood modeling approaches to study an insect’s developmental response to temperature. It has been long known that the developmental response
of insects to temperature is non-linear (Damos & Savopoulou-Soultani 2012; Régnière et al. 2012a), nevertheless, researchers still use degree-day approaches, which are based on linear temperature responses. This results in imprecise predictions of development, especially at extreme temperatures. With the available strong statistical tools, computational power, and instructing literature (e.g., Régnière et al. 2012a), non-linear modeling approaches should be used for future research, especially in cases where precision at extreme temperatures is crucial, i.e., in forensic entomology or in climate change studies. A new method to model parasitoid fecundity is described in Chapter 4 that takes temperature-dependent egg resorption into account. While this approach may only represent a prototype model, it accurately describes T. rostrale fecundity under changing temperatures and can be used in future studies to predict the fecundity of other parasitoids with a similar reproductive strategy (non-host feeding synovigenic parasitoids).

There remain many unknown facts about PDVs warranting further investigation, in particular, the role of transcribed genes as they impact the host’s immune system and benefit parasitoid development. With the rapid development of new molecular tools and methods, genetic studies will be facilitated in the future, and this would allow for a better understanding of PDVs and the role of gene transcription in the host. As shown in Chapter 5, temperature has a marked influence on TrIV gene transcription, which seems to impair successful development of T. rostrale. It is possible that temperature has a similar effect on other parasitoids because many species are associated with PDVs. However, only the application of molecular experiments (as described in Chapter 5) to other PDV-parasitoid-host systems can answer the question as to whether the results from the current work can be generalized to other parasitoid systems.

The individual-based model described in Chapter 6 helped to identify knowledge gaps in the seasonal biology of T. rostrale, but as well, has provided information critical to formulating hypotheses directing future research. As a first step, key predictions from the model derived here need to be validated. The voltinism of T. rostrale predicted in the two study sites, Armagh and Epaule, Quebec, as well as in other regions across North America, could be investigated through continuous host implantation or trapping that extend beyond the spruce budworm larval season. Adult T. rostrale were never captured in ground Malaise traps over several seasons even though parasitism of budworm larvae was confirmed in the field at these same sites (M.L.S., unpublished data). Therefore, other trapping or sampling methods are required to validate predicted voltinism of T. rostrale in the field. To identify alternate hosts of T. rostrale, the predicted voltinism and
time windows of parasitism could help to efficiently time fieldwork for the collection of potential hosts. In addition, the two diapause scenarios presented in Chapter 6 now provide hypotheses that can be tested in a range of laboratory and field experiments.

In the future, the individual-based *T. rostrale* model may be coupled with the existing Spruce Budworm Seasonality Model (Régnière et al. 2012b) to gain greater insight into the implications of changing climatic conditions on the interaction of *T. rostrale* with the spruce budworm. The coupled model may help explain the role of temperature in this system, and more importantly, facilitate investigations that address whether a rise in temperature (e.g., during very hot summers) can trigger a release of low-density spruce budworm populations from natural *T. rostrale*-caused mortality or not. The *T. rostrale* model (alone or coupled with the spruce budworm model) may be used further to predict the performance of this parasitoid and its rates of parasitism under different climate change scenarios, and in this way, help to predict whether there is the possibility for a phenological mismatch or another temperature effect that can drive the escape of spruce budworm to outbreak levels.

Finally, in terms of managing spruce budworm outbreaks, work presented in this thesis can help identify future situations in which human intervention will be necessary in order to prevent forest stand damage and loss. The model described in Chapter 6 can help managers to identify where and when low-density spruce budworm populations are likely to escape from high natural mortality through parasitism due to changes in temperature. In this way, information derived here can be incorporated into a management decision tool that can coordinate early interventions aimed at keeping spruce budworm population levels low enough to be under the control of natural enemies such as *T. rostrale*. 


Coccidae) and its effect on parasitoid size, sex, and quality. Environmental Entomology 25: 283-294.


