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Winter survival of nuisance fly parasitoids (Hymenoptera: Pteromalidae) in Canada and Denmark

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Abstract

Independent studies were performed in Canada and in Denmark to assess the survival of parasitic wasps (Hymenoptera: Pteromalidae) wintering in puparia of house fly, *Musca domestica* Linnaeus (Diptera: Muscidae). Data in Canada were collected for *Muscidifurax raptor* Girault & Saunders, *M. raptorellus* Kogan & Legner, *M. zaraptor* Kogan & Legner, *Nasonia vitripennis* (Walker), *Spalangia cameroni* Perkins, *Trichomalopsis sarcophagae* (Gahan) and *Urolepis rufipes* (Ashmead) in three microsites at an outdoor cattle facility in southern Alberta. Survival was highest for *N. vitripennis*, *T. sarcophagae* and *U. rufipes*, ranging from near zero to *c*. 7%. No survival was observed for *S. cameroni*. Daily mean values for ambient air temperature (DMAT) averaged about −3.5°C during exposure periods. Data for Denmark were collected for *M. raptor*, *S. cameroni* and *U. rufipes* in a dairy barn and in a swine barn. Survival of *M. raptor* and *U. rufipes* was higher than that of *S. cameroni* in the dairy barn (DMAT = 8.6°C), with the three species having similar survival in the swine barn (DMAT = 15.4°C). In both studies, parasitoids in egg stages were least likely to survive. These results identify the potential for *T. sarcophagae* and *U. rufipes* to be commercialized for use in northern climates as biocontrol agents for nuisance flies, compare directly the cold-hardiness of commercialized species (i.e. all of the above species excluding *T. sarcophagae* and *U. rufipes*), and document the importance of microsite on winter survival.

Introduction

Several species of pupal parasitoids (Hymenoptera: Pteromalidae) have been commercialized as biocontrol agents of the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae), and stable fly, *Stomoxys calcitrans* (Linnaeus) (Diptera: Muscidae) for use in livestock confinements (Legner, 1995). Purchasers receive parasitized house fly pupae to be scattered at fly-breeding sites. The adult parasitoids that develop from these pupae subsequently locate and parasitize naturally-occurring fly pupae. Releases generally are recommended every second or fourth week throughout the fly season at varying rates, e.g. 1100 parasitoids per 100 m^2 or 250–500 parasitoids per cow (Cranshaw *et al*., 1996). Hence, thousands to millions of parasitoids may be released in relatively small areas during the course of the year. These inundative releases must be repeated annually, mainly because the parasitoids are regarded as having low winter survival. Greater knowledge

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of the inherent biological and physical factors affecting winter survival of these parasitoids may identify pathways to reduce the need for massive releases early in the fly season.

Parasitoids overwinter primarily as immature stages inside fly puparia, i.e. the hardened cuticle of the third-instar fly larva that surrounds the fly pupa. The proportion of immature parasitoids that survive a winter period is primarily dependent on differences in the duration of exposure, ambient temperatures, parasitoid species and life stage. *Muscidifurax zaraptor* Kogan & Legner (Pteromalidae) is described as being more cold-tolerant than *Spalangia cameroni* Perkins (Pteromalidae) and may survive for at least four months at 10°C with survival of both species generally highest for late larval and early pupal stages (Guzman & Petersen, 1986a,b). Survival can be six to seven months for *Spalangia endius* Walker (Pteromalidae) at 10°C (Legner, 1976) and 16.5°C (Shibles, 1969), and exceeds four months for *S. cameroni* at 15°C (Mourier, 1971). Acclimatization also affects winter mortality. Survival of immature *M. zaraptor* and *S. cameroni* is enhanced at 0°C by prior exposure to 10°C for periods of up to 30 days (Guzman & Petersen, 1986a,b). The microsite also may influence the winter survival of a given parasitoid species. In one of the few such studies, survival in open silage of *S. cameroni* was lower than that of *M. zaraptor* and *Urolepis rufipes* (Ashmead) (Pteromalidae) with depth in silage as the key factor affecting overwintering success (Guzman & Petersen, 1986b). Results of the above studies suggest that it may be possible to enhance the success of biocontrol programmes either by selecting parasitoid species with high winter survival or by tailoring the timing and location of late season releases to increase winter survival. However, relatively little information has been published on the winter survival of these parasitoids, particularly under field conditions.

The current paper presents the results of two studies that ask the question 'What is the winter survival for parasitoid species under conditions of use common to the local area?'. Both studies were performed concurrently, but independently, to address the lack of information on the winter survival of nuisance fly parasitoids. The study by K. Floate was performed at an outdoor facility in Alberta, Canada, where parasitoids are used for fly control in cattle feedlots. The study by H. Skovgård was performed indoors, in livestock facilities in Denmark, where parasitoids are used to control flies in swine and dairy barns. Both studies examined the winter survival of *Muscidifurax raptor* Girault & Saunders (Pteromalidae), *S. cameroni* and *U. rufipes* with four additional species included in the Canadian study. Subsequently realizing the similarity of their studies, the authors present here their joint results to facilitate comparisons.

Materials and methods

Canada

Experiments were performed over two years using parasitoids from cultures maintained on house fly pupae at the Lethbridge Research Centre, Lethbridge, Alberta, Canada (49°42'N, 112°49'W). *Muscidifurax zaraptor*, *Trichomalopsis sarcophagae* (Gahan) (Pteromalidae) and *U. rufipes* were cultured in 1996–1997 with stock recovered from cattle feedlots in southern Alberta (Floate *et al.*, 1999). *Muscidifurax raptor* and *Nasonia vitripennis* (Walker) (Pteromalidae) were cultured in 2001 with stock from these same feedlots. *Muscidifurax raptorellus* Kogan & Legner (Pteromalidae) was cultured in 1995 with stock originally recovered from feedlots in eastern Nebraska in 1990 (Petersen & Currey, 1996). *Spalangia cameroni* was cultured in 1999 with stock obtained from Kunafin (Quemado, Texas), a commercial insectary in the United States of America. Unless otherwise stated, rearing conditions were 25°C, 60–70% RH, and a photoperiod of 12L:12D.

Year 1 (2000–2001)

To test the effect of developmental stage on winter survival, house fly pupae (24–48 h) were placed in cages of *M. raptorellus*, *M. zaraptor*, *T. sarcophagae*, *S. cameroni* or *U. rufipes*. After 48 h of exposure, pupae were removed and held for four days. Pupae that did not produce flies by this time were assumed to be parasitized. These parasitized pupae were divided into samples of about 100 pupae and placed in fibreglass screen bags with a mesh size of 1.7 mm. Bags subsequently were placed in the field at each of three microsites (see description below) on dates corresponding to first instar, late instar (i.e. second/third instar), or newly formed pupal stages of parasitoid development. Developmental stage was determined by dissecting a subset of 50–93 pupae per parasitoid species at time of placement.

For all parasitoid species, first- and late-instar stages were placed in the field on 6 and 10 November, respectively. Due to differences in developmental times, pupal stages were placed in the field on November 20 for *T. sarcophagae* and *U. rufipes*, November 23 for *M. raptorellus*, November 25 for *M. zaraptor*, and December 1 for *S. cameroni*. At monthly intervals, one bag for each combination of developmental stage, species, and microsite ($3 \times 5 \times 3 = 45$ bags per month) was returned to the laboratory and held for 6–8 weeks for parasitoid emergence. May 10, 2001 (i.e. month 6) was the last date of field collection. A total of 67 bags were recovered in this last month due to the initial placement of extra bags as a precaution against losses in the field. For use as controls, parasitoid emergence was monitored for subsets of pupae held indoors with individual pupa placed in wells of two, 96-well immunoassay plates, i.e. $n = 192$ pupae per subset.

Year 2 (2001–2002)

The experiment was repeated in a second year with three modifications. Firstly, winter survival was considered only for parasitoids placed in the field as newly formed pupae. This was because results from year 1 showed winter survival of larval stages to be virtually zero. Secondly, establishment in 2001 of laboratory colonies allowed for inclusion of *M. raptor* and *N. vitripennis* as additional test species. Thirdly, exposure of fly pupae to parasitism in colony cages was staggered to synchronize developmental stage such that all parasitoid species were placed in the field on the same date, i.e. November 9. This latter modification allowed each species to be exposed in the field for the same period of time.

At monthly intervals for seven months, one bag for each combination of microsite and species ($3 \times 7 = 21$ bags per month) was returned to the laboratory and held for parasitoid emergence. June 10, 2002 was the last date of field collection. Extending the experiment for a seventh month was intended to assess the effect of a late spring on winter

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survival. For use as controls, similar procedures were followed as per year 1.

Microsites

The same microsites were used in each year of the study and were selected to represent extremes in representative overwintering sites for parasitoids. The sites were located 2–3 m apart arranged around an approximately 50 cm high pile of barley silage enclosed on north, south and west sides by three bales of hay. This arrangement was set up specifically for the study. The 'north' microsite was located at the base of the north bale on its north side. This microsite was expected to be most buffered by variation in air temperature, because it was protected from direct exposure to sunlight. The 'south' microsite was located at the base of the south bale on its south side. This location was expected to be most affected by variation in air temperature, because it was directly exposed to sunlight. Fly pupae placed at north and south sites were covered by 2–3 cm of barley silage to mimic natural conditions of the field site. The 'centre' microsite was centred on top of the pile and was not covered by silage. It was predicted to provide the harshest conditions for overwintering parasitoid immatures. Pupae at the three sites were placed between two layers of hardware cloth (1 cm mesh) to protect against mice and bird predation.

Temperature

Temperature probes attached to a CR10 Datalogger (Campbell Scientific (Canada) Corp., Edmonton, Alberta) recorded ambient air temperature 1.5 m above the silage pile and at each microsite immediately adjacent to pupae, e.g. below the silage covering pupae at north and south sites and beside the uncovered pupae at the centre site. Temperatures were recorded every 5 min by two probes at each site. These temperatures were then averaged to obtain an hourly value per probe. Hourly values in turn were averaged across probes to obtain a site value for each hour.

Denmark

The experiment was performed over one year (1998–1999) using parasitoids obtained from cultures maintained on house fly pupae at the Danish Pest Infestation Laboratory, Lyngby, Denmark. All cultures were established with stock from swine and dairy farms in Denmark. *Spalangia cameroni* and *M. raptor* were cultured in 1996–1997 with wild material added each year. *Urolepis rufipes*, a newly described species for Denmark (Gibson, 2000; Stenseng *et al*., 2003), was cultured in 1996. Unless otherwise stated, rearing conditions were 25°C, 60–70% RH, and a photoperiod of 12L:12D.

To test the effect of developmental stage on winter survival, house fly pupae (12–36 h) were placed in cages of *M. raptor*, *S. cameroni* and *U. rufipes*. After 48 h of exposure, pupae were removed and held until the parasitoids within had reached egg/first instar, second instar, or third instar/newly formed pupal stages of development. *Muscidifurax raptor* and *U. rufipes* reached these stages after 0, 6, and 12 days post-parasitism, respectively. *Spalangia cameroni*, with its longer developmental period, reached these stages after 0, 12 and 18 days post-parasitism, respectively. Colony-exposed pupae (n = 200 for each combination of species and developmental stage) were used to obtain control values for percentage parasitism as emergence of adult parasitoids (n = 100 pupae) and to validate developmental stage by dissection at time of field placement (n = 100 pupae).

Exposure of fly pupae in colony cages was staggered to allow for the placement of the three developmental stages for each parasitoid species in the field on October 28, 1998. Field placement was at a swine farm (55°43′N, 11°44′E) and a dairy cattle farm (55°40′N, 11°42′E) about 60–70 km west of Copenhagen. Pupae at each farm were placed in barns on the floor near sites of natural occurrence for fly pupae, but outside of pens to avoid trampling by livestock. Fly pupae were protected from mice and beetle predation by placement in screened plastic vials (20 ml capacity, 20 pupae per vial).

At monthly intervals for six months beginning December 3, 1998, six samples for each combination of developmental stage and species were returned to the laboratory for each farm ($6 \times 3 \times 3 = 54$ samples per month per farm). Five of these samples were held for parasitoid emergence. Puparia without parasitoid emergence after 42 days were dissected to detect dead but fully developed parasitoids. The sixth sample was held at −18°C for 24 h and then stored in 70% ethanol until dissection to determine the developmental stage of parasitoids at the time they were recovered from the field.

Temperature

Tinyview data loggers (Model 9906–0050, Chichester, West Sussex, UK) were used to record ambient temperature at ground level in the indoor facilities adjacent to where samples of pupae were located. Temperatures were recorded every third hour at both swine and dairy farms. Outdoors, daily average temperature at 2 m height were obtained from a weather station of the Danish Meteorological Institute located at Roskilde 20 km and 40 km from the two dairy and swine farms, respectively.

Statistics

Survival for both studies was measured as the number of emergent parasitoids per sample of fly pupae (Canadian study: 1 sample = a bag of 100 pupae; Danish study: 1 sample = a vial of 20 pupae) expressed as a percentage of the controls. Hence, emergence of 20 parasitoids from a sample exposed in the field of the Canadian study or 12 parasitoids in the Danish study was expressed as 67% survival if 30 and 18 parasitoids, respectively, emerged from the controls. Analyses of variance (ANOVA) tests were performed using percentage survival as the dependent variable. Depending upon the particular experiment, parasitoid species, month of exposure, microsite, farm, and development stage were used as independent variables. When effects of independent variables on percentage survival were detected ($P < 0.05$), Duncan post-hoc tests with Bonferroni adjustments were performed to assess the effect of specific treatments.

Values of percentage parasitism were heteroscedastic and exhibited non-normal distribution. Hence, data were rank-transformed (Conover & Iman, 1984) in the Canadian study and arcsine ($p^{\frac{1}{2}}$) transformed in the Danish study prior to analyses. The need for different transformations reflected much higher levels of mortality, and hence heteroscedasticity, in the Canadian study. Analyses were performed using Systat 8.0 (SPSS, 1998) and SAS (SAS Institute, 1999) for Canadian and Danish studies, respectively. Percentage survival is reported throughout the text as mean ± SE.

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Results

Canada

Year 1 (2000–2001)

Parasitoids exposed as first-instar larvae and *S. cameroni* did not survive even one month of exposure in the field. Hence, these data were excluded from analyses. A four-way ANOVA (species, developmental stage, month, microsite) performed on the remaining data detected a significant effect on survival of species ($F = 31.48$; $df_{3, 144}$; $P < 0.001$), month ($F = 21.25$; $df_{5, 144}$; $P < 0.001$) and microsite ($F = 12.40$; $df_{2, 144}$; $P < 0.001$). No difference was detected in the overall effect between developmental stages, i.e. late-instar larvae versus pupae ($F = 1.40$; $df_{1, 144}$; $P = 0.24$).

Each of the species exhibited significantly different levels of survival. For data combined across months, microsites and developmental stages (i.e. late-instar larvae + pupae), average percentage survival was: *U. rufipes* (6.9 ± 1.5, $n = 36$ replicates of *c*. 100 fly pupae), *M. zaraptor* (4.1 ± 2.0, $n = 40$), *T. sarcophagae* (2.3 ± 1.0, $n = 41$), and *M. raptorellus* (0.2 ± 0.1, $n = 39$). Only *T. sarcophagae* and *U. rufipes* emerged from puparia exposed in the field for six months, by which time their percentage survival had declined to 0.4 ± 0.2 ($n = 11$) and 2.1 ± 1.5 ($n = 6$), respectively. No difference in survival was detected between the two species at this time ($F = 0.41$; $df_{1, 15}$; $P = 0.531$). The greatest decline in survival for parasitoid species occurred during the first two months of field exposure. From a control value of 100% at month 0, average percentage survival of parasitoids for data combined across species and microsites was 14.9 ± 3.6 ($n = 24$) in month 1 and 2.7 ± 1.0 ($n = 24$) in month 2. Patterns of monthly survival for individual species are provided in fig. 1a.

Microsite differences reflected higher survival at south versus central and north microsites (fig. 2a). For data combined across species, months and developmental stages, average percentage survival was: south (5.4 ± 1.7, $n = 53$), central (2.2 ± 0.8, $n = 54$) and north (2.3 ± 0.9, $n = 49$). When data only for month 6 were considered, no parasitoids survived at the central microsite and no difference was detected between north (0.1 ± 0.1, $n = 9$) and south (1.2 ± 0.8, $n = 13$) microsites ($F = 2.06$; $df_{1, 20}$; $P = 0.167$).

Year 2 (2001–2002)

As was observed in year 1, *S. cameroni* did not survive even one month of exposure in the field. Hence, data for this species was excluded from analyses. A three-way ANOVA (species, month, microsite) performed on the remaining data resulted in a significant effect on survival, of species ($F = 28.39$; $df_{5, 112}$; $P < 0.001$), month ($F = 47.90$; $df_{6, 112}$; $P < 0.001$) and microsite ($F = 45.44$; $df_{2, 112}$; $P < 0.001$).

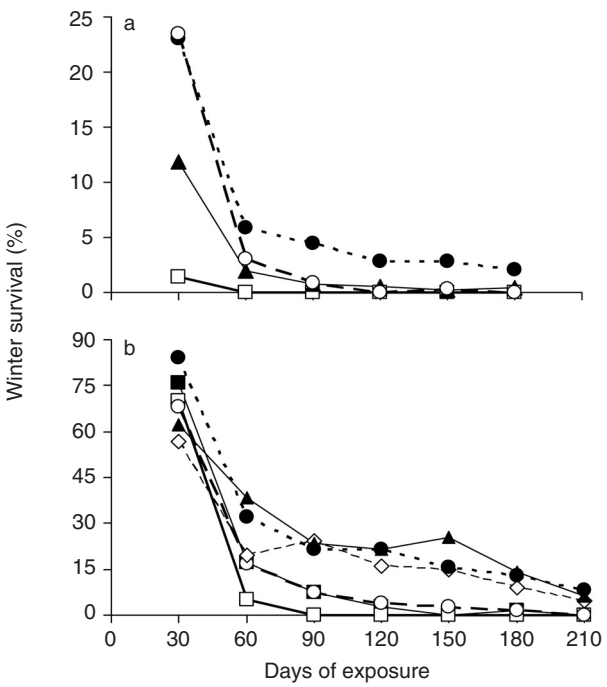

Fig. 1. Species differences in winter survival for parasitoids developing inside house fly puparia in Canada. a) 2000–2001: late instar larval and pupal stages of *Muscidifurax raptorellus* (□), *M. zaraptor* (○), *Trichomalopsis sarcophagae* (▲) and *Urolepis rufipes* (●) placed in the field on 10–25 November and sub-sampled at monthly intervals. b) 2001–2002: pupal stages of *M. raptor* (■), *M. raptorellus* (□), *M. zaraptor* (○), *Nasonia viripennis* (◇), *T sarcophagae* (▲) and *U. rufipes* (●) placed in the field on 9 November and sub-sampled at monthly intervals. Data not shown for *Spalangia cameroni*, which did not survive to 30 days.

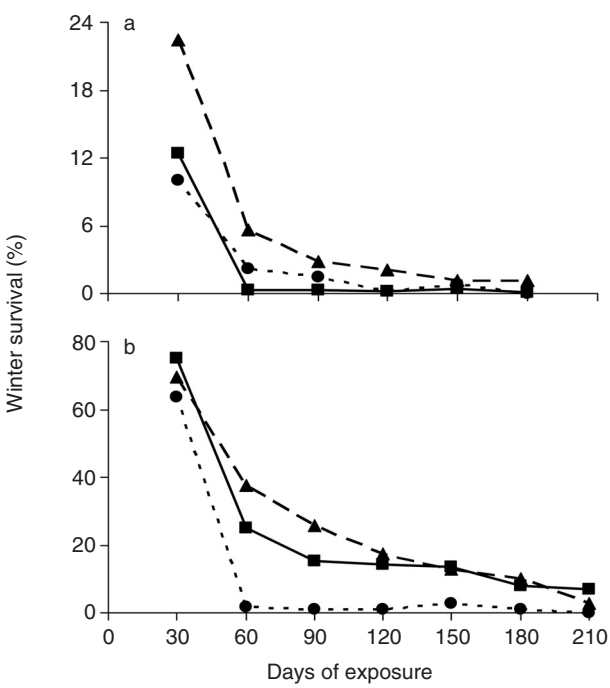

Fig. 2. Microsite differences (–■–, north; --▲--, south; --●--, centre) in winter survival for parasitoids developing inside house fly puparia in Canada. Data combined across species. a) 2000–2001: late instar larval and pupal stages of *Muscidifurax raptorellus*, *M. zaraptor*, *Trichomalopsis sarcophagae* and *Urolepis rufipes* placed in the field on 10–25 November and sub-sampled at monthly intervals. b) 2001–2002: pupal stages of *M. raptor*, *M. raptorellus*, *M. zaraptor*, *Nasonia vitripennis*, *T. sarcophagae* and *U. rufipes* placed in the field on 9 November and sub-sampled at monthly intervals.

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Species differences reflected a division between two groups. For data combined across month and microsite, average percentage survival for species in the first group was: *N. vitripennis* (20.7 ± 4.1, n = 21 replicates of *c*. 100 fly pupae for each species), *T. sarcophagae* (27.3 ± 5.2), and *U. rufipes* (27.9 ± 6.0). No differences in survival were detected among these species, but each had higher survival than species in the second group. For species in the second group, average percentage survival was: *M. raptor* (15.1 ± 5.9), *M. raptorellus* (10.7 ± 5.5), *M. zaraptor* (14.4 ± 5.3). Significant differences were detected between the latter two species, but no differences were detected between *M. raptor* vs. *M. raptorellus* or *M. zaraptor*. *Muscidifurax raptorellus* did not survive six months of exposure. By this time, percentage survival for species in the first group averaged 11.9 ± 2.7 (n = 9) vs. 1.6 ± 1.0 (n = 6) for the remaining species in the second group. Only species in the first group survived seven months of exposure with an average percentage survival of 6.5 ± 2.2 (n = 9) with no difference detected between species (F = 0.046; df $_{2, 6}$; P = 0.955).

Similar to year 1, the greatest decline in survival occurred during the first two months of field exposure. From a control value of 100% at month 0, average percentage survival of parasitoids for data combined across species and microsites was 69.5 ± 3.4 (n = 18) in month 1 and 21.5 ± 4.8 (n = 18) in month 2. Patterns of monthly survival for individual species are provided in fig. 1b.

Microsite differences reflected higher survival at south and north vs. central microsites (fig. 2b). For data combined across species and months, average percentage survival was: south (25.2 ± 3.7, n = 42), north (22.7 ± 4.0, n = 42), central (10.3 ± 3.6, n = 42). Emergence in spring reflected this general pattern. For data combined across months 6 and 7, average survival at south and north microsites was 6.5 ± 2.0% (n = 12) and 7.5 ± 2.5% (n = 12), respectively, vs. 0.6 ± 0.6% (n = 12) at the centre microsite (F = 4.153; df $_{2, 33}$; P = 0.025).

Temperature

Greater variation in daily mean values was the main difference separating ambient air temperature 1.5 m above the microsites from temperatures within microsites (table 1). Daily means for ambient air temperature varied by 39°C from early November to mid-April in year 1 and again in year 2. During these same periods, daily means for the centre site varied by 28°C. Variation in daily mean temperatures for south and north sites was similar in year 1 at about 22°C. However, minimum daily mean temperatures were about 4°C lower at north vs. south sites in year 2. This latter result was attributed to the insulative properties of snow, which accumulated to greater depths on the south vs. north site due to wind.

Denmark

A four-way ANOVA (farm, species, developmental stage, month) resulted in a significant effect on survival, of farm (F = 28.34; df $_{1, 514}$; P < 0.0001), species (F = 73.13; df $_{2, 514}$; P < 0.0001), developmental stage (F = 251.68; df $_{2, 514}$; P < 0.0001), and month (F = 77.87; df $_{1, 514}$; P < 0.0001). Significant interactions were observed between farm and species (F = 26.72; df $_{2, 514}$; P < 0.0001), farm and developmental stage (F = 7.01; df $_{2, 514}$; P < 0.001) and between farm, species and stage (F = 60.05; df $_{8, 514}$; P < 0.0001). The difference between farms was reflected in a higher overall percentage survival of parasitoids in the swine barn (46.5 ± 2.4, n = 245) vs. the dairy barn (36.1 ± 2.4, n = 269). Among species, overall percentage survival was highest for *M. raptor* (54.4 ± 2.5, n = 169), intermediate for *U. rufipes* (43.5 ± 3.3, n = 174) and lowest for *S. cameroni* (25.4 ± 2.3, n = 171). Among the developmental stages, percentage survival for pupae, larvae and eggs was 64.5 ± 3.2 (n = 170), 44.7 ± 2.5 (n = 169) and 14.8 ± 1.5 (n = 175), respectively. Survival across months showed an overall negative slope for the survival of each species with time of exposure in the field (figs 3 and 4).

Most of the above differences in survival could be attributed to lower survival in the dairy, particularly of *S. cameroni*. At this site *S. cameroni* exposed as eggs and larvae did not survive more than 30 and 90 days, respectively (fig. 3). For the three developmental stages combined, survival of *S. cameroni* after six months in the dairy barn was <1 vs. 40% at the swine barn. Survival of *M. raptor* exposed as larvae in the dairy barn was 68.8 ± 4.4% (n = 5) after six months, but zero when exposed as eggs, and 3.8 ± 2.5% (n = 5) in the pupal stage. In contrast, the percentage survival for *M. raptor* after six months in the swine barn was 26.7 ± 5.6 (n = 5), 76.6 ± 5.9 (n = 4), and 56.3 ± 13.3 (n = 4), when exposed as eggs, larvae and pupae, respectively (fig. 4). Survival of *U. rufipes* was similar in dairy and swine barns. When exposed as pupae in the latter facility, survival stayed close to 100% throughout the period of exposure. In the swine barn, survival of pupae remained near 100% through 150 days of exposure, but then declined to 60.0 ± 24.5% (n = 5) at the end of the study period. Survival of *U. rufipes* exposed as egg and

Table 1. Mean daily values (°C) for ambient air temperature 1.5 m above microsites and for temperatures within the three microsites used to study winter survival of parasitoids at Lethbridge, Canada.

Time period	Air	Centre	South	North
6 November 2000 to 17 April 2001				
Number of days[1]	163	163	163	163
Minimum daily mean temperature	−29.1	−18.1	−16.4	−16.2
Maximum daily mean temperature	9.9	10.4	6.4	5.1
Average daily mean temperature	−3.4 ± 0.6	−3.3 ± 0.5	−2.5 ± 0.4	−3.7 ± 0.4
10 November 2001 to 17 April 2002				
Number of days	158	158	158	158
Minimum daily mean temperature	−25.5	−18.3	−12.9	−17.2
Maximum daily mean temperature	12.7	10.5	8.7	8.2
Average daily mean temperature	−3.8 ± 0.7	−3.1 ± 0.5	−1.8 ± 0.3	−3.1 ± 0.4

[1] Excluding days with missing temperature records.

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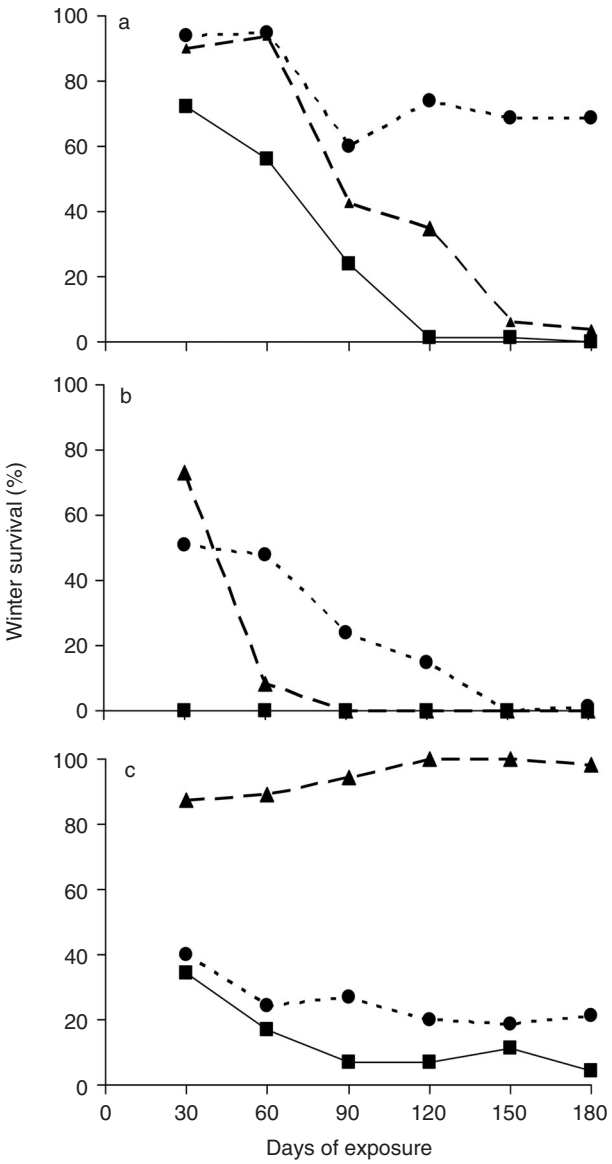

Fig. 3. Developmental stage differences in winter survival for *Muscidifurax raptor* (a), *Spalangia cameroni* (b) and *Urolepis rufipes* (c) developing inside house fly puparia in Denmark. Parasitoids in egg/first larval (–■–) instar, second instar (--●--), or third instar/pupal (--▲--) stages exposed in a dairy barn from late October through early April, 1998–1999.

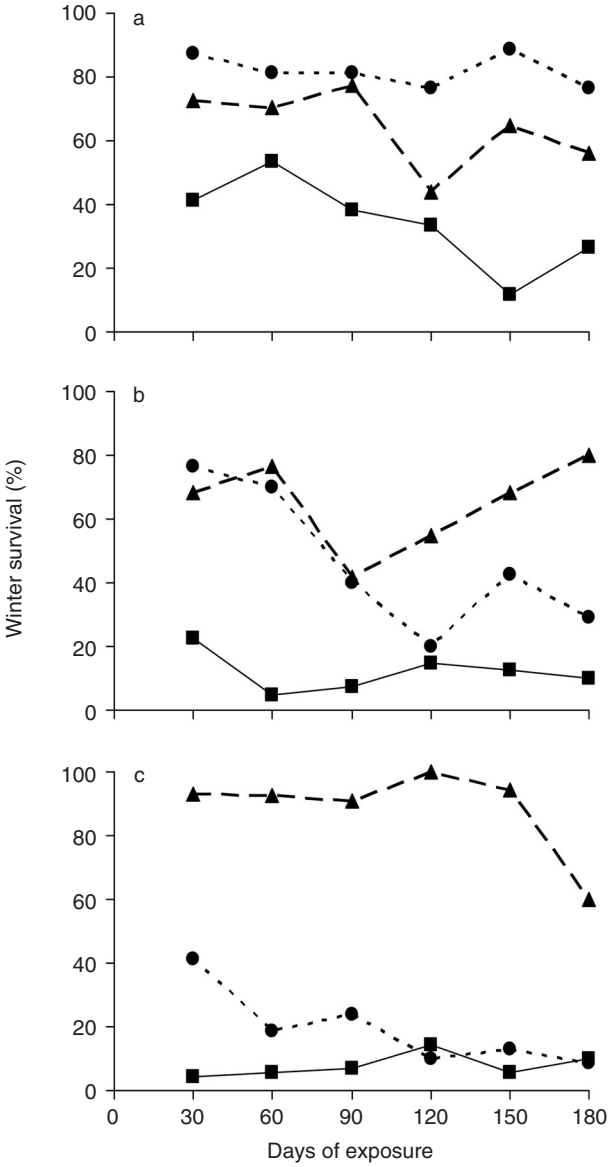

Fig. 4. Developmental stage differences in winter survival for *Muscidifurax raptor* (a), *Spalangia cameroni* (b) and *Urolepis rufipes* (c) developing inside house fly puparia in Denmark. Parasitoids in egg/first larval instar (–■–), second instar (--●--), or third instar/pupal (--▲--) stages exposed in a swine barn from late October through early April, 1998–1999.

larval stages declined steadily until 60 days of exposure in the dairy barn where it remained at a relatively constant level towards the end of the period, i.e. 10.0 ± 6.2% (n = 5) for eggs and 8.6 ± 4.2% (n = 5) for larvae, respectively. For the swine barn, survival remained below 10% after day 90 of exposure and throughout the period (fig. 4).

Dissection of fly puparia

Dissection of fly puparia provided no evidence of parasitoid development after placement in the field (table 2). Subsamples of puparia containing egg/first instar, second instar, or late larval instar/pupal stages of parasitoids at time of field placement, still predominately contained the same developmental stages after six months of exposure. Dissections, however, did recover higher numbers of parasitoids from puparia than might have been expected from the emergence of adult parasitoids. For *M. raptor* exposed in the dairy barn as third larval instar/pupae, adult emergence was 3.8% after 180 days whereas dissection of puparia produced parasitoids from 61.1% for subsamples of these same puparia. The results for *S. cameroni* were similar

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Table 2. Developmental stage of parasitoids at time of placement (control) on 28 October 1998, and after 180 days in dairy and swine barns in Denmark.

Species Developmental stage	Control (0 days)	Dairy (180 days)	Swine (180 days)
Muscidifurax raptor			
Egg/first instar	90.3	18.7	38.3
Second instar	90	73.6	80
Third instar/pupal	80	61.1	72.2
Spalangia cameroni			
Egg/first instar	82.5	18.7	42.1
Second instar	83.2	6.2	84.1
Third instar/pupal	89.1	64.7	70.6
Urolepis rufipes			
Egg/first instar	94.4	73.6	68.2
Second instar	90.8	27.7	18.8
Third instar/pupal	95.9	94.4	94.4

Values are expressed as percentages, based on dissections of 100 parasitized house fly puparia for 0 day values, and on dissections of 20 puparia for 180 day values.

to those for *M. raptor*, whereas adult emergence of *U. rufipes* more closely resembled levels of parasitism for second instar larvae and third instar larvae/pupae as determined by dissection (fig. 3, table 2). For the swine barn, adult emergence of *M. raptor*, *S. cameroni* and *U. rufipes* was more similar to the numbers found when dissected. Because pupae were freeze-killed prior to dissection, the proportion of parasitoids alive in these pupae at the time of field collection could not be determined.

Temperature

In the swine barn, temperature was significantly higher than in the dairy barn during the course of the study (F = 337.1; df $_{1,54}$; $P < 0.0001$) (fig. 5). Daily mean temperature in the swine barn averaged 15.4°C ± 0.03 with daily minimum and maximum values averaging 13.5 and 16.8°C, respectively. Temperatures in the dairy barn fluctuated more with daily mean values at the start of the study (late October) initially averaging about 10°C then declining to 5–7°C from December and into March whereafter it slowly increased again. Daily mean temperature in the dairy barn during the study averaged 8.6°C ± 0.1 with an average daily minimum and maximum value of 5.8 and 12.4°C, respectively. Daily mean air temperatures outside of the swine and dairy barns during the experimental period averaged 2.9°C ± 0.5 (fig. 5).

Discussion

Results of the current study are attributed to differences in cold hardiness (quiescence) between species, rather than to differences in diapause. Both factors influence winter survival, but involve different mechanisms. Cold hardiness is a physical and metabolic adjustment by the organism in response to sudden environmental change. This adjustment allows survival at freezing temperatures either by increasing the tolerance of the organism to freezing, or by preventing freezing through the accumulation of cryoprotectant chemicals (Lee & Denlinger, 1991). Diapause is an endocrine-mediated dormancy. This dormancy generally is restricted to

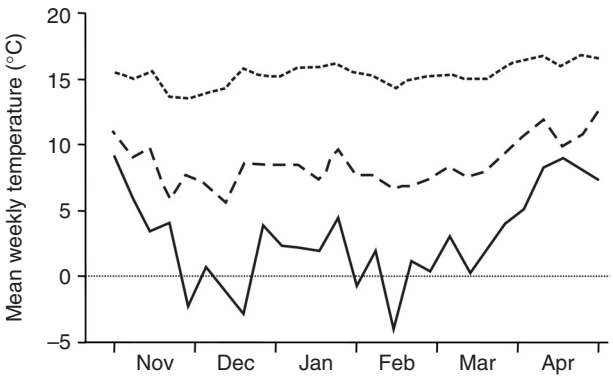

Fig. 5. Weekly mean temperatures of ambient air (——), in dairy barn (–––), and swine barn (-----) from 28 October, 1998 to 28 April 1999 in Denmark.

a developmental stage characteristic for the given species and typically is triggered by 'token stimuli' that are not adverse *per se*, but which typically precede unfavourable conditions (Saunders, 1982). Nuisance fly parasitoids have been reported to exhibit both cold hardiness (Petersen & Meyer, 1983; Guzman & Petersen, 1986a,b) and diapause (Simmonds, 1946; Legner & Gerling, 1967; DeLoof *et al.*, 1979). As is common practice when used in fly control programmes, parasitoids in the current study were reared indoors at room temperature and then placed in the field without a period of acclimation. Hence, it is assumed that they would have had little opportunity to implement endocrine changes necessary for diapause. This was further supported by the Danish study where no diapausing individuals were observed when subsamples of field-exposed puparia were dissected.

Categories of cold hardiness

The observed cold hardiness of the species examined was consistent between countries, agrees with previous observations, and provides new information on the winter survival of nuisance fly parasitoids. Previous studies showed *M. zaraptor* and *S. cameroni* to best survive winter conditions as late larval or early pupal stages inside host puparia with survival of even these stages extremely low at temperatures of 0–10°C (Guzman & Petersen, 1986a,b). For the outdoor study in a cattle feedlot in Canada, none of the four species of parasitoids tested in year 1 survived even one month of field conditions when exposed as early instar larvae, although survival was observed for late larval and pupal stages. For the indoor study in swine and dairy barns in Denmark, data combined across months, species and farms showed survival of pupae to be 1.5-fold greater than that for second instar larvae, which in turn was 3-fold greater than that for egg/first instar stages. Combined across the two studies, the similar results for *M. raptor*, *M. raptorellus*, *M. zaraptor*, *S. cameroni*, *T. sarcophagae* and *U. rufipes* identify poorest survival in the egg/first instar stage as a general phenomenon for this guild of parasitoids. To our knowledge, there have been no previous studies on cold hardiness of *M. raptorellus* or *T. sarcophagae*.

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Species differences in winter survival, consistent between the two studies, suggest three categories of parasitoids based upon survival when ambient air temperatures are near or about 0°C. Species in the first category, exemplified by *S. cameroni*, have extremely low survival under such conditions with most individuals likely to perish early in the winter regardless of developmental stage. No individuals of this species survived even one month of field exposure in the Canadian study with the same result obtained in each of two years. Even in the dairy barn in Denmark, where temperatures ranged from 5.8 to 12.4°C, survival of *S. cameroni* was lowest among the three tested species. Guzman & Petersen (1986a) previously showed survival at 10°C for *S. cameroni* in any stage of development to be consistently lower than that for *M. zaraptor* and to be virtually zero for egg and late pupal stages after 60 days. They subsequently showed survival of *S. cameroni* to be much lower than that for either *M. zaraptor* or *U. rufipes* exposed to winter conditions in outdoor piles of silage in Nebraska (Guzman & Petersen, 1986b). The low cold tolerance of *S. cameroni* may partially explain the virtual absence of *Spalangia* in surveys of nuisance fly parasitoids in southern Alberta (Lysyk, 1995; Floate *et al*., 1999, 2000) and near Winnipeg, Manitoba, Canada (McKay & Galloway, 1999) where low snow cover and cold temperatures, respectively, provide particularly harsh winter conditions.

The second category is comprised of species likely to survive several months of winter conditions when ambient air temperatures are near or about 0°C, but which may not survive to emerge in the spring. This category would include *M. raptor, M. raptorellus* and *M. zaraptor*. In year 1 of the Canadian study, individual *M. raptorellus* and *M. zaraptor* survived exposure to one month of field conditions with individuals of the latter also surviving five months of exposure. *Muscidifurax raptor* was not examined in year 1. In year 2, individuals of *M. raptorellus* survived two months of field exposure, whereas individuals of *M. raptor* and *M. zaraptor* survived six, but not seven, months of exposure. Greater winter survival of *M. raptor* and *M. zaraptor* explains the much greater recovery of these species vs. *S. cameroni* in western Canada (Lysyk, 1995; Floate *et al*., 1999; McKay & Galloway, 1999; Floate *et al*., 2000). Although the current study shows *M. raptorellus* to be least likely of the three *Muscidifurax* species to overwinter in western Canada, overwintering of this species in the region has been reported at least once (Floate *et al*., 2000).

The third category is comprised of species for which a portion are likely to survive to emerge in the spring after experiencing winter conditions with ambient air temperatures near or about 0°C. This final category includes *N. vitripennis*, *T. sarcophagae* and *U. rufipes*. In year 1 of the Canadian study, *T. sarcophagae* and *U. rufipes* were the only two species to survive six months of winter exposure. In year 2, these species and *N. vitripennis* were the only species to survive seven months of exposure. *Nasonia vitripennis* was not tested in year 1. In contrast to the findings of the current study, winter survival of *M. zaraptor* and *U. rufipes* in Nebraska did not differ when host pupae were placed in barley silage at a depth of 0–3 cm (Guzman & Petersen, 1986b), which suggests that they should be members of the same category for cold hardiness. However, this discrepancy may reflect more mild temperatures during the Nebraska study. Daily mean temperatures for Lincoln, Nebraska from 1971 to 2000 (Anon., 2003), with corresponding values for Lethbridge, Alberta in brackets, averaged 3.4 (−1.2), −3.1 (−5.9), −5.3 (−7.8), −2.1 (−4.1) and 4.1 (−0.3)°C for November, December, January, February and March, respectively. This conclusion is consistent with results of the current study from Denmark. The greater cold hardiness of *U. rufipes* versus *M. raptor* was apparent only in the colder environment of the dairy barn. In contrast, comparisons of these species in the warmer environment of the swine barn seem to indicate the reverse pattern. These collective results reiterate that this tentative classification of parasitoids into categories of cold hardiness is based upon survival when ambient air temperature is near or about 0°C.

Microsite differences

Results of the Canadian study emphasize the importance of microsite on winter survival (table 1). Average daily mean values in 2000–2001 appear to identify similar temperature conditions of ambient air and the three microsites. Corresponding values for 2001–2002 showed larger differences, but even the greatest difference was only 2°C. However, comparison of average daily mean values masks large microsite differences in diurnal patterns of temperature variation. During periods of relative warmth, temperatures of air and the centre site attained the highest values and exhibited the highest levels of variation during 24-h cycles (fig. 6a). At such times, temperatures at the centre site frequently exceeded that of ambient air temperature during daytime hours. This phenomenon presumably reflected the additional heating of air near the surface of the centre site, which was unprotected by barley silage. Less pronounced variation in diurnal patterns at south and north sites was attributed to the insulative properties of the 2–3 cm of barley silage that covered these sites.

During periods of relative cold, ambient air temperatures consistently were colder than temperatures at the three microsites, occasionally by +20°C (fig. 6b). On the morning of March 8, 2002, recorded air temperature was −31.4°C, whereas temperatures recorded for the centre, south and north microsites were −12.6, −5.2 and −7.3°C, which were covered at that time by an estimated 10, 20 and 30 cm of snow, respectively. Much colder temperatures were observed for microsites when snow cover was absent. On the morning of December 31, 2001, recorded air temperature was −23.5°C, whereas temperatures recorded for centre, south and north sites were −24.1, −15.6 and −17.3°C, respectively. In contrast to observations during periods of relative warmth, temperatures for the centre site during periods of relative cold always were lower than that observed for south and north sites. Given temperature differences, it is perhaps not surprising that winter survival of parasitoids was greatest at north and south microsites, and zero (year 1) or near zero (year 2) at the centre site.

Further study is needed to determine the relative importance on winter survival of average daily mean temperature versus average variation in diurnal temperature. All else being equal, survival may be lower at a relatively warm site with more variable temperatures, than at a much colder site with less variable temperatures.

Consideration of microsite explains the presence of *S. cameroni* in more northerly climates. Despite its low tolerance to cold temperatures, this species and its congeners comprise a significant portion of the parasitoid fauna in some regions of Canada (Gibson & Floate, 2004). This

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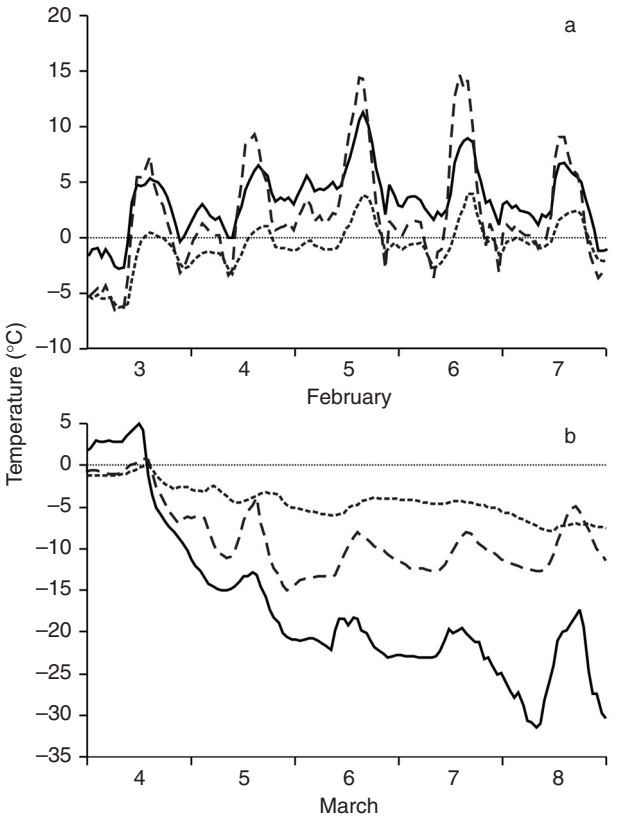

Fig. 6. Temperature profiles for ambient air (——), and for centre (– – –) and north (-----) microsites in Canada. Profile of south microsite, similar to that of north microsite, is excluded for clarity. Temperatures at the north microsite were: a) most variable during periods of relative warmth, and b) lowest during periods of prolonged cold.

apparent discrepancy may reflect deeper penetration into substrate by species of *Spalangia* searching for host pupae. Laboratory studies show *Spalangia* spp. to penetrate deeper into various substrates to parasitize hosts than do other parasitoid species (Legner, 1977; King, 1997). Field studies suggest that species of *Spalangia* are responsible for most parasitism of nuisance fly pupae below a depth of 3 cm (Legner, 1978; Rueda & Axtell, 1985; Neves & de Faria, 1988). Parasitizing host pupae more deeply buried in substrate provides greater protection to *S. cameroni* during winter months from both variation in diurnal temperatures and from cold ambient air temperatures. Smith & Rutz (1991) hypothesized that *Spalangia* species may have evolved the behaviour of parasitizing deeply buried pupae as a mechanism to avoid competition for hosts from *Muscidifurax* species, which tend to parasitize more exposed pupae. Alternatively, this behaviour may have been selected as a mechanism to increase overwintering survival.

To summarize, independent studies in Canada and in Denmark show relatively few parasitoids survive a six-month overwintering period in northerly climates with survival highest for parasitoids overwintering as pupae. Of the species tested in Canada, *N. vitripennis*, *T. sarcophagae* and *U. rufipes* exhibited the highest levels of overwintering survival. Previous studies indicate that *N. vitripennis* is generally ineffective as a biocontrol agent (Legner, 1967; Kaufman *et al*., 2001). Hence, the latter two species may be of greatest value as biocontrol agents. Although neither has been commercialized, field trials of *T. sarcophagae* have been performed in outdoor cattle confinements (Floate, 2003). Of the species tested in Denmark, *M. raptor* and *U. rufipes* exhibited higher winter survival than *S. cameroni* in dairy barns, whereas the three species exhibited similar winter survival in swine barns. Because *U. rufipes* normally occur outside of dairy or swine barns (Skovgård & Jespersen, 1999), *M. raptor* and *S. cameroni* may be the most appropriate species for use as biocontrol agents in these facilities.

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