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**The Overwintering Biology of
the European earwig *Forficula auricularia* Linnaeus
(Insecta: Dermaptera: Forficulinae)**

by

Stephen Goodacre

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment
of the requirements for the degree of**

Master of Science

**Department of Biology
Carleton University
Ottawa, Ontario
April 23, 1997.**

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the Faculty of Graduate Studies and Research
acceptance of the thesis,

"The Overwintering Biology of the European earwig
Forficula auricularia Linnaeus
(Insecta: Dermaptera: Forficulinae)"

submitted by

Stephen Goodacre, B.A. Hon.

in partial fulfilment of the requirements
for the degree of Master of Science

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ABSTRACT

This paper investigates the role of overwintering micro-habitat temperatures on the meso-distribution of the European earwig, *Forficula auricularia* L. Populations were examined in areas close to, and removed from, human disturbance. Micro-habitat characteristics and overwintering survival were examined. The supercooling point (SCP) and freeze tolerance of *F. auricularia* were assessed. The role of diapause in overwintering survival was examined.

Areas close to human disturbance favour large populations of *F. auricularia* due to greater egg production by females ($P = 0.0136$). Overwintering survival was low (0 to 15%) and is not affected by micro-habitat temperature. Mortality is predominantly from non-freezing damage. *F. auricularia* overwinter in a quiescent state and do not use diapause to increase cold-hardiness. *F. auricularia* is freeze intolerant, supercools to -8°C , and does not alter the SCP to avoid cold damage. Cold-hardening is rapidly induced by low temperatures, reduces non-freeze damage and is mandatory for overwintering survival.

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Introduction

Every year there are many accidental introductions of insect species to North America. These often result from importing crops and ornamental plants that contain insects (Elton, 1958; Weems and Skelley, 1989). The majority of successful colonizations are associated with areas of human activity (Elton, 1958). Orions (1986) postulated that introduced insects establish more readily in disturbed areas because there are fewer indigenous predators. These areas are also richer in non-native plants with which the introduced insects may have evolved. Other theories postulate that new species cannot invade natural habitats because they are more complex than human-altered areas and that they present a greater "ecological resistance" to alien species (Elton, 1958). This ecological resistance takes the form of resource availability, predation and inter-species and intra-species competition that the flora and fauna exert on the new species.

In addition to biotic resistances to colonization, abiotic factors are also important. Central Canada has long, cold winters (Danks, 1978). Low temperature limits insect population ranges (Krebs, 1994). Low temperature limits a species' range by affecting survival, reproductive output, development and biotic interactions of insects (Danks, 1978; Krebs, 1994). To determine if low temperature has an effect on these features, it needs to be determined (1) at what life stage the insect is exposed to low temperatures, (2) what the

tolerance of the insect is and (3) whether the micro-habitat where the insect overwinters is within the tolerance limits while micro-habitats where they are not found are outside the tolerance limits (Krebs, 1994).

Several species of earwigs (Dermaptera) have been introduced to Canada, but most are limited to coastal regions or areas along the St. Lawrence River and Great Lakes regions (Vickery and Kevan, 1985). These regions are characterized by heavy snowfall and a moderating effect on low temperatures due to the large bodies of water (Danks, 1978). Only *Forficula auricularia* L. (Dermaptera) (the European earwig) is found in all the provinces (including the very cold Prairie Provinces (Danks, 1978)) and the North West Territories (Vickery and Kevan, 1993).

F. auricularia has recently been proposed as a potential biological control agent for aphids and psyllids in pear orchards (Lenfant et al., 1994). Ideally, biological control agents should establish viable populations where they are introduced. Thus, it is important that we understand the overwintering biology of *F. auricularia*, to maximize their use as a biological control agent and for potential population control where they are perceived as pests.

This paper investigates the possible role of temperature as an agent regulating the meso-range distribution (tens of meters) of *F. auricularia* by investigating their overwintering biology. The hypothesis to be tested is if overwintering site

temperatures preclude *F. auricularia* from surviving in fields.

To answer this question, Chapter I examines the population levels in frequently disturbed areas (yards and gardens around houses) and undisturbed areas (old field habitats). Micro-habitat overwintering site characteristics (disturbance/soil quality) were examined to determine if micro-habitat characteristics can affect adult survival or reproductive output, which would affect the next year's population level. This will test the hypothesis that overwintering micro-habitat characteristics account for the difference in population size).

Chapter II examines the role of overwintering micro-habitat temperature, to test the hypothesis that heat radiated by buildings during the winter affects survival of *F. auricularia*. Temperature and snow depth of micro-habitats close to foundations were compared to sites distant from building foundations.

In Chapter III the tolerance range of *F. auricularia* is examined, to determine if they can survive temperatures experienced at overwintering sites. The ability to develop a rapid cold-hardening response is examined. The supercooling point (temperature at which ice forms spontaneously below 0°C), is measured in individuals acclimatized to summer conditions and winter conditions, as is their survival after the supercooling point is reached, to determine if they are freeze tolerant (able to survive ice formation in extra-

cellular liquids). Changes in super cooling points and survival to cold exposures above the supercooling point (long and short) for summer and winter acclimatized individuals will indicate what defensive mechanisms *F. auricularia* may employ to survive the winter, e.g., diapause, cryoprotectants, anti-freezes, etc.

To investigate the possibility that the duration of the cold period affects the range of *F. auricularia*, a metabolic study on the rate of resource use (lipids etc.) is conducted at cool ($\sim 4^{\circ}\text{C}$) and cold (0°C) temperatures. Winter acclimated (cold hardened) and summer acclimated (non cold-hardened) earwigs will be compared. A consistently lower rate of resource use by the cold hardened individuals would indicate that diapause plays a role in overwintering survival.

Chapter I

F. auricularia Population Biology

1.1 Introduction:

Although *F. auricularia* has reached pest proportions in gardens and orchards (Fulton, 1924; Lamb and Wellington, 1974; Weems and Skelly, 1989), they have not been reported to reach "pest" proportions in undisturbed areas (e.g., centres of old fields, woods). This could be for several reasons: European earwigs are poor dispersers, as they tend not to fly, though they have well-developed wings (Lamb, 1975; Weems and Skelley, 1989). They may be rarely encountered in natural habitats as they are nocturnal. *F. auricularia* displays negative geotaxis (Weyrauch, 1929 in Lamb, 1975) and preys upon aphids and psyllids (Lenfant et. al., 1994) thus they may be present, but at the tops of trees which are rarely sampled by casual observers. An alternate explanation is that they cannot survive the long central Canadian winters and are continually being reintroduced with imported plant materials into highly populated regions (Elton, 1958; Weems and Skelly, 1989).

This chapter examines the population levels of *F. auricularia* in an area close to human activity (the edge of a field) and an area removed from human disturbance (centre of a field) to test the hypothesis that there is no effect of human disturbance on the population levels of *F. auricularia*. Micro-habitat characteristics are examined to test the

hypothesis that overwintering micro-habitat characteristics do not affect population levels in disturbed vs. non-disturbed areas.

1.2 Methods:

1.2.1 Study area

The area sampled was National Capital Commission (NCC) parkland (Braemar Park) in Ottawa, Ontario. The dimensions of the field are 995 meters long by 137 meters wide. The area is composed of old field vegetation with a few widely dispersed clumps of trees (*Prunus* spp., *Acer negundo*, *Acer* spp.). The dominant vegetation is milkweed (*Asclepias* spp.), stinging nettle (*Urtica dioica* and *U. urens*) and grasses (subclass *Commelinidae*). The vegetation at the edge was cut by the NCC as a fire break, and was adjacent to the back yards of the surrounding houses. The cut area followed the perimeter of the field and was 5-7 meters wide. The centre had not been cut for at least five years. Any region at least 20m from the cut area was regarded as being in the centre of the field.

1.2.2 Population measurements

Populations were censused by trapping during the summer of 1995 and by walking transects in 1996 to determine if the population trend was unique to one area or if it was consistent on all sides of the field. Traps consisted of inverted flower pots with crumpled newspaper inserted into the pot. The trap was then placed on top of a stick, 20-60cm high. Sixteen traps were set out in a 4m x 4m grid. Three locations were assessed; one at the field edge in cut grass, one at the edge in undisturbed vegetation, and one placed 75m from the edge in undisturbed grass. Sampling took place prior to sunset at two to three day intervals.

Five transects, 50m in length and parallel to the edge of the field were taken 5m from the edge of the mowed area of the field. Five transects were taken in the centre of the field, 50-75m from the edge. The distance between replicate transects was a minimum of 100m. All milkweed plants that were within 1m of the transect were checked for the presence of earwigs, as the tops were easily sampled and appeared to be a favourite place for *F. auricularia* to hide during the day. To standardize the sampling method, the number of plants sampled was recorded, and the number of earwigs per plant then reported.

1.2.3 Nest location

From April 22 to May 8 1996, nine plots, (1m², 15 cm deep) were randomly selected at the edge of the field (within 15-20m of the houses) and searched. The mowed area of the field was selected as it would resemble a yard, regarding the level of disturbance and distance to a building foundation. When a female *F. auricularia* was located, the soil near her was closely searched for eggs. Eggs could be identified as elliptical globes 1-2mm long and pearly white to pale yellow in colour. Eggs found were collected and counted using a pair of fine tweezers. The number of females and eggs for each plot were recorded. Nine plots (1m², 15 cm deep) were also randomly sampled in a garden devoid of vegetation and 15-20m from a building.

1.3 Results:

1.3.1 Population size

F. auricularia displays a larger population at the edge of the field (Table. 1-1). *F. auricularia* is more abundant in areas with short vegetation. This trend was consistent over the summer (Fig.1-1)

Figure 1-1. The number of earwigs present in 16m² by vegetation height and location in the field.

Table 1-1. Average number of earwigs per plant in 50m transects, 5m from the edge and 50-75m from the edge.

Location	Average # of earwigs per plant $\pm$ Standard error	N	t value	P value
Edge	32.8 $\pm$ 17.2	5	11.14	P < 0.01
Centre	0.828 $\pm$ 0.6	5		

1.3.2 Nest characteristics

Only one female was found per nest and nest density was not significantly different between disturbed and undisturbed areas. Nests in both areas were of similar depth. Nests in the disturbed area had a significantly more eggs per nest (Table 1-2).

Table 1-2. Nest characteristics for *F. auricularia* in a disturbed and undisturbed area.

Area Sampled	# of plots Sampled (N)	# of Females (N)	# of Males (N)	Min # of eggs (N)	Average # of eggs* (N)	Max # of eggs (N)	Mean Depth (cm)
Garden	9	8	0	3	48	73	6.3±3.4
Field	9	4	0	0	15	35	~6**

* Significant (df=10, F= 3.05, P= 0.0136)

** represents an estimate because the destructive nature of the sampling resulted in no intact nests, but eggs and females were always found in the root mat, which did not extend below 7cm.

1.4 Discussion:

1.4.1 Population size

The population size of *F. auricularia* is significantly greater in areas close to human activity (Table 1-1), as the population was larger at the edge of the field (close to buildings). The difference in population sizes was consistent through the summer (Fig.1-1), indicating that there is some feature about the disturbed area that is beneficial to *F. auricularia*.

1.4.2 Nest location

No males were found in the nests regardless of the location sampled. Females evict the males early in spring at the beginning of oviposition (Fulton, 1924; Lamb, 1976). Nest density was not significantly different in areas with disturbed vegetation relative to non-disturbed areas. As only one female is found per nest, the population differences are not due to differences in adult survival (i. e. more females to survive, more females to lay eggs).

1.4.3 Overwintering microhabitat effect on reproductive output

Female *F. auricularia* laid significantly more eggs in highly disturbed habitats, as the area with no vegetation had significantly more eggs (Table 1-2). In both locations the

nests were of similar depth (Table 1-2), but in the highly disturbed soil (no vegetation) the female earwig does not need to penetrate the root mat formed by grass. This would result in the females expending less energy during nest establishment that could then be allocated to egg production.

The reduced fecundity caused by the energetic demands of nest establishment in undisturbed areas demonstrates that some overwintering micro-habitat features can have impacts on overwintering success. Thus overwintering micro-habitat characteristic effects on reproductive output would partly explain the larger population in the disturbed habitat at the edge of the field. Chapter II examines if micro-habitat temperature affects overwintering survival.

Chapter II

Microhabitat Characteristics

2.1 Introduction:

Chapter I determined that micro-habitat characteristics (soil disturbance) can affect overwintering success by altering the reproductive output of *F. auricularia*. This chapter examines the role of micro-habitat temperatures and its effect on the survival of adult earwigs.

Specifically, this chapter investigates overwintering micro-habitats in an environment altered by human activity. Overwintering sites close to sources of thermal energy (buildings) are compared to overwintering sites distant to buildings. Temperature limits species ranges (Danks, 1978; Krebs, 1994) and can affect overwintering survival rate (Leather et al., 1993; Tauber et al., 1986). Of particular interest is if the European earwig uses heat from buildings to moderate cold temperatures to survive the winter. The hypothesis to be tested is if heat leaked from building foundations affects survival by alteration of soil temperature. If distance from the heat source affects survival then the null hypothesis should be rejected.

2.1.1 Winter climate

The climate of Ottawa, Ontario is characteristic of the Great Lakes region with heavy snowfall (mean 215.9 cm per year) (Danks, 1978). Frost can begin as early as late

September and continue till late May. The long-term mean air temperature from November to March is -6°C (Danks, 1978). The average January air temperature is -10.9°C (Danks, 1978).

In contrast, the Prairie Provinces obtain only 112.7 cm of snow per year and the November to March mean air temperature is -12.5°C . The average January air temperature is -18.7°C (Danks, 1978).

It can be seen from the temperatures and the lack of protective snow cover (Marchand, 1987), that climate conditions lead to a harsher winter in the Prairie Provinces (Danks, 1978).

2.1.2 Overwintering behaviour

F. auricularia overwinter as a pair in a tunnel that the female digs into the soil in mid-October (Fulton, 1924; Lamb, 1976). Nest structure varies from a single tunnel to having three chambers (Lamb, 1976). The mean depth of the nest is 4.2 cm (Lamb, 1976). Nests are often associated with stones which the female uses for structural support and for temperature regulation in incubation of the eggs (Lamb, 1976). In early spring the female evicts the male and seals the single entrance for the duration of the brooding period.

2.2 Methods and Materials:

2.2.1 Micro-habitat temperature

Location;

A west facing wall of a single level brick house, in the west end of Ottawa, Ontario, was selected as the experimental site. The ground was devoid of grass for the first 1.5 m from the house. Beyond 1.5 m was a lawn of mixed grasses. The soil composition is a loamy sand mixed with clay.

Equipment;

Cages to hold the earwigs consisted of plastic vials with holes cut in the sides and bottom. A fine cloth screening covered the holes to allow free air and water movement. The top was covered with cheese cloth folded over several times, then taped to the vial.

Temperature was recorded using a 16-channel analog data logger constructed at Carleton University Science and Technology centre. All sixteen channels (the maximum available) were equipped with one thermistor probe (Model AD590 JH 8209). The data logger was powered by a 12 volt battery. The time between recordings could be set for intervals of 15 seconds (fast recording) or minutes (regular recording), with 1-16 cycles between recordings; e.g., on fast setting, recordings could be made every 15, 30, 45, 60 seconds etc. up to every four minutes. The recording period was set to the maximum time between recordings (every four hours).

Experimental protocol;

On 14 - 17 November, 30 plastic cage-vials were placed in the ground at each distance of 0m, 0.5m, 1.5m, and 6m from the building foundation. A tunnel 4cm deep was made by forcing a pencil into the soil inside the vials. A pair of earwigs was then introduced into the vial and the top was sealed with cheese cloth folded over several times and taped to the sides. Thermistor probes were placed on the surface and 4cm deep in the soil at each end of the distance treatments. One probe was suspended on a rope 1 meter in the air 1.5m away from large structures and was covered by a white paper tube to avoid heating from the sun. At no point during the study was the probe in the air allowed to become covered by snow. Readings were taken at Midnight, 4 A.M., 8 A.M., Noon, 4 P.M. and 8 P.M. Snow depth measurements were taken as required.

On 31 March to 2 April, the cages were removed from the ground and checked for survivors.

Data Analysis;

Temperature data were analyzed to determine if there was spatial variation depending upon vertical position (on the soil surface or below the surface) and distance (to determine if the temperature was cooler/warmer as distance from the foundation increased). The time of recording was also examined to determine if diurnal factors such as sunlight would have an effect on the micro-habitat temperature.

The analysis was done by fitting the temperature data to a model, then performing a 3-way ANOVA on the temperature residuals. To obtain the residuals, the overall mean for the probes on each Julian date was computed. The residual is the difference in temperature reading for that probe (at a given time and location) and the overall mean. This would account for the temporal variation that would occur naturally over a period of months.

A three-way ANOVA was conducted on the residuals to determine if any of the predictor variables (distance, vertical position and time) were significant as well if any interaction effects were significant.

2.2.2 Overwintering survival

The number of surviving males and females were counted at each distance as were the number of missing individuals. To determine if there was a difference in survival rate, a Monte Carlo Contingency test was performed on the survivors. A Monte Carlo test was also performed on the missing earwigs to determine if there was a difference in the number of migrators.

2.3 RESULTS:

2.3.1 Environmental factors

Snow cover;

The snow was consistently deeper the greater the distance from the foundation (Table 2-1). No subnivian space was found over the winter. Ice formed down to the soil surface. By April all the snow had melted. The 1.5m treatment region was flooded two times; the first in late January, the second on the 20th of February.

Table 2-1. Snow depth (cm) during the winter of 1995 at 0m, 0.5m, 1.5m, and 6m from a building foundation located in Ottawa.

Date	Distance			
	0m	0.5m	1.5m	6m
17 Jan	8.4±8.0	26±11.1	42.7±3.6	50.6±1.5
21 Feb	0±0	6.6±3.8	35.5±1.3	43.8±2.3
25 Feb	0±0	5.1±3.7	27.8±0.3	34.3±2.2
3 Mar	0±0	0±0	3.2±3.1	23.8±1.5
20 Mar	0±0	0±0	0.5±1.2	17±11.3

Temperature;

The mean air temperature decreased from December to January, then began to increase again from March until May (Table 2-2).

Micro-habitat temperature was affected by distance from the foundation, whether it was above or below ground and the time of day that the temperature reading was taken (Figs 2-1, 2-2 and 2-3, and Table 2-4). All the two-way interaction effects were significant (Table 2-4). The three-way interaction effect was not significant. As distance from the foundation increases, the mean temperature decreases (Fig. 2-1). The underground temperatures are warmer than the surface temperatures (Fig. 2-2). The time of day affected the temperature. During the late evening the temperature dropped until after 8 A.M. whereupon the temperature would rise from late morning till nightfall (Fig. 2-3). For the interaction between distance and time of day we see that the temperature readings nearest to the foundation vary more over time than the positions far from the foundation (Fig. 2-4). The nighttime air temperatures affect the 0m and 0.5m treatments in particular. The 6m treatment displays a slight time lag in the drop in temperature but the change in temperature is slight compared to the other distance treatments.

The position and time of day interaction demonstrates that the daily temperature variation is greater on the surface than in the soil (Fig. 2-5).

For the distance and position interaction there is a great difference in pattern for the 0m treatment, with the above soil temperature being very cool, while the below soil temperatures are very warm. In the other cases, the below soil temperature is warmer than the surface temperature. The 6m treatment is an exception to this case, where the surface temperature is warmer than the in soil temperature (Fig. 2-6).

Table 2-2. Mean monthly air temperature (°C) from December to April 1996 at the experimental site (Ottawa, Ontario).

Date (Month)	Air Temperature (°C)
December	-9.53
January	-11.48
February	-8.51
March	-1.74
April	3.12

Table 2-3. Mean monthly ground temperatures (°C) at 0m, 0.5m, 1.5m and 6m from a foundation.

Month	Distance			
	0m	0.5m	1.5m	6m
December	-1.9	1.07	0.36	0.54
January	-4.3	-1.04	0.36	0.75
February	-3.9	-2.84	-0.61	0.025
March	1.18	-0.01	0.097	2.03
April	5.59	4.43	4.24	3.64

The temperatures remain relatively constant over time at greater distances from the foundation.

Table 2-4. Summary table of the Type III Sum of Squares for the ANOVA of the temperature residuals from model with distance, position and hour of day as predictor variables and interaction effects.

Source	df	SS	MS	F	P
Distance	3	1153.2	384.4	50.16	0.0001
Position	2	35682.1	17841	2327.9	0.0001
Hour of day	5	3773.1	754.62	98.4	0.0001
Distance*Position	6	6562.2	1093.7	142.7	0.0001
Distance*Hour of day	15	423.6	28.24	3.7	0.0001
Position*Hour of day	10	4098.5	409.85	53.48	0.0001
Distance*Hour of day*Position	30	203.89	6.796	0.887	> 0.05
Residuals	8668	66429.6	7.664		

Dependant variable: Temperature residuals

Fig. 2-1. Temperature residuals by distance (m) from a foundation.

Fig. 2-2. Residual temperatures by position (on soil surface and 4cm down in the soil) with standard error bars.

Fig. 2-3. Residual temperatures and time of day of recording (Midnight, 4am, 8am, Noon 4pm, 8pm) with standard error bars.

Fig. 2-4. Interaction effects of distance and hour of day, on the temperature residuals. Standard error bars included.

Fig. 2-5. Interaction effects of position and hour of day, on the temperature residuals. Standard error bars included.

Fig. 2-6. Interaction effects of distance from the foundation and position, on the temperature residuals. Standard error bars included.

2.3.2 Survival

Males and females survived poorly regardless of the distance to the foundation (Table 2-5). Thus the null hypothesis that survival is not affected by distance to the foundation is kept. Survival ranged from 0% to 5% for the males and from 0% to 11.5% for the females. The distance with the lowest survival for both sexes was the 0.5m treatment where there were no survivors.

The rates of disappearance were significant however (Tables 2-6.). Females are more likely to disappear from the 0m treatment ($P = 0.0003$) than any of the other distance treatments. Males displayed an equal probability to be missing at any distance in the spring (Table 2-6). Thus for the females the null hypothesis that presence is not affected by distance is rejected.

Table 2-5. Summary table of earwigs found alive at the end of March, by overwintering distance from the foundation. P value reported from Monte Carlo analysis at a minimum of 15 000 iterations.

Sex	Distance from the Foundation					P value
	0m	0.5m	1.5m	6m		
	N=30	N=30	N=30	N=30	N total	$\alpha=0.05$
Males	0	0	0	1	120	0.454 $\pm$ 0.004
Females	2	0	1	3	120	0.258 $\pm$ 0.003

Table 2-6. Number of missing earwigs by distance from the foundation. P value calculated from Monte Carlo analysis with a minimum of 15 000 iterations.

Sex	Distance from the Foundation				P value $\alpha=0.05$
	0m	0.5m	1.5m	6m	
Male	10	6	4	7	0.327 $\pm$ 0.004
Female	12	2	1	4	0.0003 $\pm$ 0.0001

2.4 DISCUSSION:

2.4.1 *Effects of snow cover*

Snow cover and heat from the building foundation affected the micro-habitat where earwigs may overwinter (Table 2-3).

Snow can act as an insulator, with the effectiveness increasing as the depth increases, because the volume of the snow's trapped air increases and air is a poor conductor of heat (Marchand, 1987). The snow depth was lowest near the foundation (Table 2-1), due to melting from the heat generated in the house and from warming of the wall by the sun during the day. Thus the micro-habitats closer to the wall were less insulated from fluctuations in air the temperatures (Figs. 2-1 and 2-4). Adjacent to the wall, where there was no snow cover at all, we see the largest effect on temperature relative to the time of day (Fig. 2-4). This is because the diurnal heating and cooling during the day and night (Fig. 2-3) is not buffered by a layer of insulating snow. Thus sunlight can warm the ground during the day, but the ground will cool greatly at night.

The layer of snow will retain the latent heat released from the ground through the winter because heat is released when the soil freezes. This can be seen in the mean soil temperatures remaining stable over time at the 6m treatment where the snow is deepest and the larger drop over the same period in mean soil temperatures closer to the foundation

where the snow is shallow (Table 2-1 and 2-3). The fact that soil is warmer at 4cm deep, than at the surface (Fig. 2-2) suggests that the source of heat is from within the soil rather than from above ground, e.g., heating from the sun. The heat in the soil is derived from the heat of crystallization as water in the soil freezes. This would result in a warmer temperature below the soil. The blanket of snow and the water content in the soil buffers the below soil areas from the diurnal fluctuations (Fig. 2-5). This is because the snow insulates the ground, while the water in the soil has a high heat capacity, thus requiring more light energy to cause a change in temperature.

At the 6m treatment the soil surface was warmer than below ground (Fig. 2-6). One possible explanation for this is that the treatment was exposed to direct sunlight for a greater period each day. Snow acts as a perfect absorber of infrared radiation (Marchand, 1987). Water has a high heat capacity thus a large amount of energy can be stored in melt water percolating down the snow column from the snow air interface. The water would cool as it descends, releasing the energy as heat of crystallization. Evidence for this is the diurnal cycling on the soil surface, with less dramatic, cycling underground (Fig. 2-5).

2.4.2 Heat from the foundation

Heat would flow from the building foundation from the

warmest area to the coolest. Since heat is the kinetic energy of molecules, rapidly moving molecules will transfer energy to slower moving molecules. This would mean that the area adjacent to the foundation (a heat source) would be warmer than soil distant to the foundation (a heat sink). Similarly, soil temperature would rise as depth increases. This is because the surface is in contact with the cold air (Table 2-2) and snow (the heat sink), while the deep soil still retains the thermal energy gained from the sun during the summer and fall (the heat source).

Heat from the foundation affected the microhabitat by moderating the temperature. The heat from the foundation had the greatest effect under the soil surface (Fig. 2-6). The effect on the environment is reduced as the distance increases. At the 0m treatment the below soil temperature is much warmer than the soil surface. At the other distances the difference is not as great.

2.4.3 Note about the ANOVA on temperature residuals

Because it was of interest to obtain spatial and temporal information on the temperature characteristics, the statistical analysis lacks independence due to spatial and temporal auto-correlation. Due to the fact that there is a lack of independence in the temperature measurements, the validity of the F tests that yield the P values is in question. However, the general trends in the temperature

characteristics can still be examined legitimately because the cell means calculated would not change (McClave and Dietrich, 1988).

2.4.4 Overwintering survival

The overwintering survivorship of *Forficula auricularia* is low despite the distance from the foundation (Table 2-5), thus micro-habitat temperature characteristics do not affect survival. The disappearance rate differs however (Table 2-6). This could be a result of the habitat next to the foundation being unsuitable for the earwigs; thus they emigrate or die early in the winter. Also the warm daytime temperatures early in the spring allow the survivors a longer period for emigration before the vials were collected. Emigration is likely since *F. auricularia* is active at cool temperatures (personal observation and Lamb and Wellington, 1974) and two female earwigs were located on the side of the foundation outside the vials.

Since the survival rate was very low, yet the temperature at the 6m treatment never went below -0.3°C and at the other distances, the subterranean temperature never went below -4.6°C , it indicates that *F. auricularia* cannot tolerate cool temperatures for long periods.

To elucidate the cause of mortality, the tolerance of *F. auricularia* to low temperatures must be determined. Death can be caused by freeze or non-freeze damage (Turnock, 1994; Lee,

1989). If *F. auricularia* can supercool beyond -5°C , or if they can survive freezing, then the cause for the high mortality probably is non-freeze damage.

Starvation may be a problem for *F. auricularia* if they do not enter diapause and overwinter in an area where the mean temperature is high, since they are poikilothermic exotherms and their metabolic rate depends on ambient temperature. That may be one potential reason for the disappearance of *F. auricularia* at the base of the foundation: they were warmer earlier and so were metabolically active enough to leave in search of food. The supercooling point, freeze-tolerance and the role of acclimation and diapause will be examined in Chapter III.

Chapter III

Cold-Defense Strategies

3.1 Introduction:

The purpose of this chapter is to investigate the limits of *F. auricularia*'s tolerance to cold and to determine what mechanisms are used to prevent cold damage or death. The role of cold acclimation is also examined.

The tolerance range of *F. auricularia* must be known to test the hypothesis that they can survive temperatures experienced at overwintering sites. The supercooling point (temperature at which ice forms spontaneously below 0°C), is measured in individuals acclimatized to summer conditions and winter conditions, as is their survival after the supercooling point is reached, to test the hypothesis that they are freeze intolerant (cannot survive ice formation in extra-cellular liquids). Changes in super cooling points and survival to cold exposures above the supercooling point (long and short) for summer and winter acclimatized individuals will indicate what defensive mechanisms *F. auricularia* may employ to survive the winter, e.g., cryoprotectants, antifreezes.

3.1.1 Overwintering strategies

Insects can adopt one of two strategies to deal with below optimal temperatures that they experience in temperate

regions. They can be freeze avoidant by migrating away from the cold (e.g., monarch butterflies) or they can dig down into the soil to avoid extreme temperatures (Leather et al., 1993). Insects that do not avoid cold temperatures are either freeze tolerant or freeze intolerant. Freeze tolerant species can survive the formation of ice in the extra-cellular fluid (Asahina, 1969).

3.1.2 Cold survival mechanisms

Neither freeze tolerant nor intolerant species can survive ice formation within cells (Asahina, 1969; Salt, 1961). Freeze tolerant species have high supercooling points (the temperature below 0°C at which ice forms spontaneously), caused by ice nucleating agents (proteins, etc.) (Baust, 1982). Ice nucleating agents cause ice formation at warmer temperatures to avoid flash freezing which damages tissues. Since the supercooling point (SCP) is regarded as the lower limit to survival in freeze intolerant species (Lee and Denlinger, 1985), they tend to lower their supercooling points, using antifreeze agents (proteins and polyols) (Baust, 1982; Miller, 1982). Insects using both strategies can produce cryoprotectant substances to protect proteins and cellular structures (Baust, 1982). Some substances serve dual purposes such as glycerol, which acts as a cryoprotectant and an antifreeze (Salt, 1961; Danks, 1978).

Supercooling points are not set at any fixed temperature, but are subject to several factors such as the rate of cooling, the physiological state of the insect and the duration of time at the low temperature (Danks, 1978). Since ice crystal formation is a probabilistic event, the greater the volume of water, the longer the time at sub zero temperatures, the faster the rate of cooling, and the greater the concentration of ice nucleating agents (e.g., food in the gut), the greater the probability that ice will form in the hemolymph (Asahina, 1969; Salt, 1961). The supercooling point was measured in acclimatized and non-acclimatized individuals to test the hypothesis that *F. auricularia* modifies the supercooling point to increase overwintering survival.

3.1.3 Diapause

Regardless of freeze tolerance, insects must acclimatize to cool temperatures to survive the winter (Lee and Denlinger, 1985; Kostal and Simek, 1995). Diapause (a form of dormancy that is not readily broken), leads to several physiological changes that increase overwintering success, and is common in insects (Lee and Denlinger, 1985; Kostal and Simek, 1995; Leather et al., 1993). Diapause reduces water content, in some but not all species (Lee and Denlinger, 1985) and induces production of high concentrations of cryoprotectants (Leather et al., 1993; Lee and Denlinger, 1985; Kostal and Simek, 1995). Diapausing freeze intolerant species produce

antifreezes, while freeze tolerant species produce ice nucleating agents (Zachariassen, 1982; Leather et al., 1993; Baust, 1982).

Diapause is induced by short day length (photoperiod), exposure to low temperatures, and in some species to crowding and water stress (Leather et al., 1993; Tauber et al., 1986). The required temperatures and photoperiod to induce diapause varies greatly from species to species. Diapause can generally be induced by a prolonged exposure (two weeks to one or more months) to very short photoperiods (< 8 hrs) and cool temperatures (< 10-5°C).

F. auricularia enters a post-reproductive diapause (Vancassel and Quris, 1994), which must be broken before any eggs will be laid in late winter or early spring (Lamb, 1976; Lamb and Wellington, 1975). The role of diapause in earwig overwintering is examined to test the null hypothesis that it does not play a role in overwintering survival.

3.2 Methods:

3.2.1 Culture rearing methods

Earwigs were randomly assigned to three pretreatment groups: non cold-hardened, rapidly cold-hardened and long-term cold hardened. Non-cold hardened earwigs were maintained in a room held between 21-28°C with a 24-hour photoperiod for at least two weeks. The rapidly cold hardened earwigs were reared

in the same conditions as the non-hardened specimens, but exposed for 1-2 hours at 4°C before being tested for the supercooling point. The long-term cold-hardened earwigs were reared at 4°C±2°C for at least one month with an 8-hour photoperiod. This photoperiod was then reduced to complete darkness at least two weeks before the supercooling points were measured.

All earwigs were fed sliced carrots and egg yolks.

3.2.2 Supercooling point and Freeze tolerance

F. auricularia were placed in small glass vials with a copper-constantan thermocouple attached to their cuticle by a thin strip of duct tape. The glass vials were then placed in a larger plastic vial filled with cotton as insulation. Four such vials were set up at a time and the temperature monitored on an ADControl datalogger. The initial temperature was room temperature 21-25°C. The cooling rate was between 3°C/min and 5°C/min. The body temperature was continuously monitored for 1/2 to 1 1/2 hours, until a temperature of -18°C was steadily maintained. The experimental subjects were thawed at room temperature and survivors counted. The temperature curve for each individual was then plotted and the minimum temperature before the first exotherm was recorded. The first exotherm is characterized by a sudden increase in temperature as heat is released when ice forms in the extra-cellular fluid.

To determine if acclimation affects ice tolerance, the same procedure was followed as for the supercooling point determination, except that the earwigs were tested individually and removed 1 minute after the first exotherm was observed. Survivors were counted.

A literature review of supercooling points and freeze tolerance was conducted to determine the mean supercooling points (and 95% confidence interval) of freeze tolerant and freeze intolerant species to determine if the mean supercooling points for the freeze tolerant and intolerant groups are significantly different. The supercooling point of *F. auricularia* will be compared to these ranges.

Statistical analysis: An ANOVA was conducted on *F. auricularia*'s supercooling points to determine if there was a significant difference for the acclimation treatments.

A Monte Carlo test was performed on the survivors after the first exotherm was passed for 1 minute, to determine if acclimation affected survival after ice formed in the body.

To determine to which group (freeze tolerant species or freeze intolerant species), *F. auricularia* has a similar supercooling point to, an ANOVA was conducted.

3.2.3 Non-freeze mortality

Four earwigs were placed in a glass vial sealed with

parafilm and the cap replaced. The vials were then placed in a salt water ice bath at -7°C to -7.5°C . They remained there for 24 hours and the temperature at removal was measured to be between -5°C to -4°C . The earwigs were removed from the vials and allowed to warm up at room temperature. After one hour they were ranked as dead, moribund (alive but unable to walk, or alive (no apparent damage, or slight damage to appendages but walking about). They were again assessed at 24 hours after removal. Monte Carlo analyses were performed to determine if there was a significant difference in the number of surviving earwigs depending upon the rearing conditions. A minimum of 15 000 iterations were performed for each Monte Carlo analysis.

3.2.4 Long-term cold survival

Non-hardened and long-term hardened male and female earwigs were weighed and placed as pairs in test tubes filled with moist sand. The test tubes were then placed in large water baths held at 10.5°C , 4°C and 0°C . The water baths were kept in a cold room at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The 10.5°C bath was heated by aquarium heaters (Hagen Radiant Model LR-91923 75 Watt and 50 Watt heaters). The 4°C bath remained at cold-room temperature. The 0°C bath was maintained by adding ice. Every fourth day, the test tubes were randomly rearranged in the baths, and once a month the baths were rotated in the cold room.

After two months, the earwigs were removed and frozen at

-20°C for one week. They were weighed for final fresh weight, placed in a drying oven at 50°C for a minimum of 24 hours for final dry weight. Lipids were then extracted and the dry weight taken again. This would provide the % water, % lipid and % protein/other constituents of each earwig.

Lipids were removed by a chloroform-methanol wash (Folch method as described by Christie (1973)). Dried earwigs were crushed, then washed in 3ml of solvent for half an hour at room temperature, followed by centrifugation (Sorval GLC-2B general lab. centrifuge) for 15 min at 1500 rpm. The solvent was then removed with a pipette and 6ml fresh solvent added. The wash procedure was repeated again, after which the earwigs were dried and weighed.

An ANCOVA was performed on the weight lost (difference between the estimated initial dry weight and the final dry weight) to determine if temperature, sex and acclimation affected the amount of weight lost. An ANCOVA was also performed on the % fat to determine if the weight lost was due to fat metabolism. This should indicate if diapause was induced in hardened earwigs, and if *F. auricularia* possessed enough fat resources to survive the winter in eastern Canada.

Percentage of water in non cold-hardened and long-term cold-hardened earwigs were recorded to investigate if water loss occurs in cold-acclimated specimens and to estimate the initial dry weights.

3.3 Results:

3.3.1 Freeze tolerance

The supercooling point is characterized by the release of heat (the first exotherm) as ice forms in the extra-cellular fluid (Fig. 3-1). No specimens survived after the supercooling point was surpassed for a prolonged period (Table 3-1). Some (37.5% to 58.3%) *F. auricularia* survived formation of ice within their bodies for a short period of time. Cold hardening did not affect the ability to withstand ice formation in *F. auricularia* (Table 3-2).

Fig. 3-1. Cooling curve for *F. auricularia* displaying the first exotherm.

Table 3-1. Number of rapidly hardened and long-term cold hardened earwigs surviving after cooling beyond the supercooling point.

Acclimation	Number of Survivors	N
Rapid	0	13
Long term	0	18

Table 3-2. Tolerance to ice in the extra-cellular space one minute after the first exotherm for long term cold hardened and non-acclimated earwigs.

Rearing condition	Survival		
	Alive	Moribund	Dead
Long term hardening	6	5	5
Unhardened	7	0	5

P value= 0.079 ± 0.0037 at 15150 trials. We retain the null hypothesis that acclimation had no effect on freezing mortality: freezing mortality is independent of acclimation.

3.3.2 Supercooling points

The supercooling points were not affected by cold hardening (Tables 3-3 and 3-4.). The supercooling point of *F. auricularia* coincide with the literature for freeze tolerant insects, being significantly different from the supercooling points of freeze intolerant insects (Fig. 3-2 and Table 3-6). In general the supercooling points of freeze tolerant insects are higher than those for freeze intolerant species (Fig. 3-2).

Table 3-3. Mean supercooling points (SCP) and standard error (S.E.) of *F. auricularia* for non-hardened, pre-chilled (rapidly hardened) and long-term hardened earwigs.

Rearing condition	Mean SCP $\pm$ S.E. (°C)	N
Non-hardened	-8.213 $\pm$ 0.436	12
Rapid hardened	-8.041 $\pm$ 0.311	13
Long term hardened	-7.516 $\pm$ 0.226	34

Table 3-4. ANOVA summary table for the super cooling points of non-hardened, rapidly hardened and long-term hardened earwigs.

Source of Variation	df	SS	MS	F	P
Between treatments	2	5.495	2.747	1.58	0.215
Within treatments	56	97.373	1.739

Figure 3-2. Mean supercooling points and 95% confidence intervals for freeze tolerant and intolerant species found throughout Canada, and for long-term cold hardened *F. auricularia*.

Table 3-5. Number of species and order of cold tolerant and cold-intolerant species found in Canada.

<u>Order</u>	<u>Freeze Tolerance</u>	<u>Number of species</u>
Coleoptera	No	7
Diptera	No	7
Hymenoptera	No	2
Lepidoptera	No	4
Coleoptera	Yes	17
Diptera	Yes	7
Hymenoptera	Yes	1
Lepidoptera	Yes	4

Table 3-6. ANOVA results of the supercooling points of long-term cold-hardened *F. auricularia* to the means of the freeze intolerant species and freeze tolerant species found throughout Canada.

Source of Variation	df	SS	MS	F	P
Between treatments	2	4991.99	2496	20.3	6.21×10^{-8}
Within treatments	84	10302.4	122.65		

3.3.3 Non-freeze mortality

Non-freeze mortality is high but significantly reduced by prior exposure to cold temperatures (Table 3-7). The duration of the acclimation period is not significant; there was no difference in survival between rapidly hardened and long-term hardened earwigs (Table 3-8). For long-term survival at temperatures near freezing, cold hardening is mandatory (Tables 3-9 and 3-10). This trend held for both males and females.

Moribund earwigs were alive and responded to stimulation but could not walk or coordinate movement. No moribund insects at the 24 hour recovery period improved and all died within one week. Surviving earwigs groomed legs and antennae vigorously upon revival and there was a slight discoloration of the tarsal segments and joints in some individuals. Joint discoloration also occurred after exposure to cold temperatures above the supercooling point and was also seen in some specimens reared in the long term cold acclimated cultures.

Table 3-7. Non-freeze mortality of *F. auricularia* at 24 hours after an exposure to -6°C for 24 hours and resulting P value from Monte Carlo analysis (20070 trials).

	Dead/Moribund	Alive
	(N)	(N)
Non-hardened	40	4
Rapid hardened	31	13
Long-term hardening	13	11

P value=0.00204±0.00032 The null hypothesis that acclimation didn't have an effect on non-freezing mortality is rejected: non-freeze mortality is dependent on acclimation.

Table 3-8. Non-freeze mortality of Hardened and Rapid hardened earwigs to a 24 hour exposure to -6°C 24 hours after removal. Resulting P value from Monte Carlo analysis provided.

Rearing condition	Dead/Moribund (N)	Alive (N)
Rapid hardened	31	13
Long-term hardened	13	11

P value= 0.19423 ± 0.0027 at 21140 trials. The null hypothesis that the duration of acclimation has no effect is retained: non-freeze mortality is independent of the duration of acclimation.

Table 3-9. Survival of Non-hardened female earwigs after a 2 month exposure to 10.5°C, 4°C and 0°C temperatures. P value from Monte Carlo analysis provided. $\alpha=0.05$

Rearing Temperature (°C)	Dead	Alive	Moribund
10.5	0	17	0
4	0	17	0
0	9	3	5

$P=0.0 \pm 0.00$ at 14930 trials. The null hypothesis that survival is not affected by temperature is rejected: survival is dependent on temperature.

Table 3-10. Survival of Hardened female earwigs after a 2 month exposure to 10.5°C, 4°C and 0°C temperatures. P value from Monte Carlo analysis provided. $\alpha=0.05$

Rearing Temperature (°C)	Dead	Alive	Moribund
10.5	1	16	0
4	0	15	0
0	1	15	0

$P=1.0 \pm 0.000$ at 15280 trials. The null hypothesis that temperature does not affect hardened earwig survival is retained: survival is independent of temperature.

Males followed the same trend.

3.3.4 Diapause

Eggs were laid by both hardened and unhardened female earwigs over the 2-month period (Table 3-13), at the 10.5°C treatment but not at cooler temperatures (Tables 3-11 and 3-12). Weight loss was greater as temperature increased (Tables 3-14 and 3-15). Females lost slightly more weight than males, but weight loss was not affected by the pre-treatment rearing conditions (Table 3-15).

Females possess more fat than males (Tables 3-16 and 3-17). As the incubation temperature increases, the amount of fat present decreases. Females held at 0°C had the greatest amount of fat. Acclimation had no effect on the final amount of fat present.

Percentage water did not differ significantly for cold-hardened and non cold-hardened *F. auricularia* (Table 3-18).

Table 3-11. Number of long-term hardened females laying eggs during a 2 month period at 10.5°C, 4°C and 0°C.

Temperature (°C)	Number of females laying eggs	Number of females not laying eggs
10.5	6	8
4	0	15
0	0	14

$P = 0.00125 \pm 0.000287$ at 15200 iterations. The null hypothesis that oviposition is independent of temperature is rejected.

Table 3-12. Number of unhardened females laying eggs during a 2 month period at 10.5°C, 4°C and 0°C.

Temperature (°C)	Number of females laying eggs	Number of females not laying eggs
10.5	8	9
4	0	17
0	0	3

$P = 0.001785 \pm 0.000343$ at 15130 iterations. The null hypothesis that oviposition is independent of temperature is rejected.

Table 3-13. Number of hardened and unhardened females laying eggs at 10.5 °C. $\alpha=0.05$

Rearing condition	Ovipositing	Not Ovipositing
Hardened	6	8
Unhardened	8	9

$P = 1.0 \pm 0$ at 15270 iterations. The null hypothesis that oviposition is independent of acclimation (rearing condition) is retained.

Table 3-14. Mean weight loss (mg) and Standard error over 2 months for Long-term hardened and unhardened earwigs maintained at 10.5°C, 4°C and 0°C.

Temperature (°C)	Hardened		Unhardened	
	Male	Female	Male	Female
10.5	6.3±1.6	12.3±1.9	7.53±1.4	11.1±1.9
4			8.0±1.9	3.4±0.88
0	4.0±1.0	5.9±2.4	0.0*	3.8±2.1

* only one male survived to the end of the experiment.

Table 3-15. Summary table of type III sums of squares for a 3-way ANCOVA results for weight loss (mg) over time with acclimation, temperature and sex as predictor variables.

Source	df	SS	MS	F	P
Acclimation	1	1.9×10^{-6}	1.9×10^{-6}	0.066	0.7985
Temperature	1	6×10^{-4}	6×10^{-4}	20.29	0.0001
Sex	1	1×10^{-4}	1×10^{-4}	3.239	0.0746
Acclimation*Temperature	1	5.7×10^{-7}	5.7×10^{-7}	0.019	0.8893
Acclimation*Sex	1	1×10^{-4}	1×10^{-4}	2.765	0.0991
Temperature*Sex	1	3×10^{-4}	3×10^{-4}	8.781	0.0037
Temp*Sex*Acclimation	1	4.1×10^{-6}	4.1×10^{-6}	0.141	0.7072
Residual	113	3.3×10^{-3}	2.9×10^{-5}		

Dependant variable: Mass lost (g)

Table 3-16. Mean % fat content and Standard error in *F. auricularia* after 2 months in 10.5°C, 4°C and 0°C baths for long term hardened and unhardened earwigs.

Temperature (°C)	Hardened		Unhardened	
	Male	Female	Male	Female
10.5	23.13±1.99	22.37±1.43	10.13±1.77	16.6±1.59
4			6.75±3.03	22.42±2.03
0	18.09±2.26	31.4±1.4	27.45*	48.26±9.49

* only one male survived to the end of the experiment.

Table 3-17. Summary table of type III sums of squares for a 3-way ANCOVA results for % fat composition, with acclimation, temperature and sex as predictor variables.

Source	df	SS	MS	F	P
Acclimation	1	72.343	72.343	0.469	0.4946
Temperature	1	1382.73	1382.73	8.967	0.0033
Sex	1	2897.56	2897.56	18.79	0.0001
Acclimation*Temperature	1	314.431	314.431	2.039	0.1557
Acclimation*Sex	1	139.562	139.562	0.905	0.3432
Temperature*Sex	1	1351.94	1351.94	8.768	0.0037
Temp*Sex*Acclimation	1	68.494	68.494	0.444	0.5063
Residual	129	19891.7	154.199		

Dependant variable: % Fat

Table 3-18. Mean % water in long term hardened earwigs and non-hardened earwigs prior to treatment assignment for the 2 month incubation period. t result and P value reported.

Non-hardened (% water)	Hardened (% water)	t	t _{crit}	P _{value}
47.946±2.03	49.579±2.56	0.587	1.99	P > 0.2

3.4 DISCUSSION:

3.4.1 Freeze tolerance

According to the criteria set out by Asahina (1969), *F. auricularia* is freeze intolerant because individuals could not survive for prolonged periods when the temperature was lowered below the supercooling point. The supercooling point is regarded as the absolute lower limit for survival in freeze intolerant species (Salt, 1961; Block, 1982). Ring and Tesar (1980) demonstrated that the freeze tolerant beetle *Pytho americanus* can surpass its supercooling point by 34°C for 16 days without ill effects. To date no known exopterygote has been shown to be freeze tolerant (Tauber et al., 1986).

3.4.2 Supercooling point

The supercooling point was not altered by cold hardening. This indicates that antifreeze agents such as glycerol and trehalose were not produced in large quantities, (Duman et al., 1982) nor were ice nucleators masked (adsorbed to a cryoprotectant so as to not cause ice crystal formation), as in some freeze intolerant species. Nor were ice nucleators produced as in some freeze tolerant species (Zachariassen, 1982). Thermal hysteresis proteins (antifreeze proteins) were apparently not produced either, as they would cause the supercooling point to decrease (Duman et al., 1982). Water content was not reduced in long-term cold hardened earwigs,

thus dehydration is not used as a protection against freezing.

As indicated (Fig. 3-2) by the difference in the supercooling ranges of a majority of species, a rule of thumb can be applied that freeze intolerant species supercool to lower temperatures. However, *F. auricularia*'s supercooling point is high for a freeze intolerant insect and is within the range seen normally in freeze tolerant insects (Table 3-4 and Fig. 3-2). *F. auricularia* is by no means an exception to this general trend. Ring (1982) demonstrated that several freeze tolerant species have extremely low supercooling points. Thus, Kostal and Simek's (1995) and Turnock's (1993) statements that the supercooling point alone should not be used to determine a species tolerance to freezing is supported.

3.4.3 Non-freeze mortality

Survival to short-term cold exposure did increase with prior exposure to low temperatures, but the duration of the cold hardening process (long-term or rapid) increased survival to the same extent. This indicates that *F. auricularia* possesses a rapid cold hardening response similar to that seen in *Danaus plexipus* (Larsen and Lee, 1994). Glycerol is rapidly produced by the up-regulation of glycogen phosphorylase which is more active at low temperatures (Lee et al., 1987). Glycerol acts as a cryoprotectant, by stabilizing membranes at low temperatures (membranes become less fluid and interrupt membrane transport, or even collapse (Lee, 1989; Lee et al.,

1987; Danks, 1978). Glycerol also acts to stabilize proteins against denaturation by coating them and preventing a tertiary structure change as solute concentrations increase (Lee, 1989; Kostal and Simek, 1995). Glycerol blunts ice crystals during formation and slows their growth (Salt, 1961). Glycerol can perform these cryoprotectant functions at low concentrations that would not substantially change the supercooling point (Lee, 1989). This would account for the increase in survival with cold hardening, but no change in supercooling point in *F. auricularia*.

Most of the mortality that *F. auricularia* experiences is due to non-freeze chilling damage. This is indicated by the 54%-91% mortality at -6°C exposure for 24 hours for all earwigs, and the low survival of non-hardened earwigs in long-term cold exposures (Table 3-7). Because these temperatures were above the supercooling point, death would not have resulted from ice in the intra-cellular space (Asahina, 1969). Non-freeze mortality can be caused by biochemical pathway uncoupling resulting in a build up of metabolites to toxic levels, enzyme denaturation, membrane transport interruptions and membrane failure (Danks, 1978; Duman et al., 1982; Turnock, 1993; Lee, 1989; Kostal and Simek, 1995).

In terms of exposure to cold temperatures for long periods, it is clear from Tables 3-8 and 3-9, that long-term cold hardening is mandatory for winter survival. Since the supercooling point was not altered, antifreeze substances and

thermal hysteresis proteins (antifreeze proteins) would be ruled out as possible cold defense mechanisms. Biochemical changes in membrane lipids allowing for greater fluidity may play a role as a defense against chill damage (Turnock, 1993; Asahina, 1969). This is the most reasonable explanation for the survival of long-term cold hardened individuals, since no change in supercooling points were observed and changing the membrane lipid constituents to less hydrogenated fatty acids would require longer than a few hours to produce. Changing the membrane fluidity affects the membrane permeability and may reduce non-freeze damage by allowing efficient membrane transport that would be otherwise inhibited by cooler temperatures (Asahina, 1969).

Increasing membrane permeability also reduces the resistance against water moving out of the cells as ice crystals form in the extra-cellular space, preventing osmotic shock associated with the increase of extra-cellular solutes that occur when ice forms in the blood (Asahina, 1969). It would also reduce the mechanical damage that ice would cause by allowing greater deformation of cells as ice crystals form in the extra-cellular space (Salt, 1961). Evidence that this may have occurred is the fact that long-term cold hardened earwigs are marginally ($P=0.079$) more tolerant to extra-cellular ice for short periods.

3.4.4 Diapause

F. auricularia undergoes a post-reproductive diapause period that must be broken before the female will lay eggs (Vancassel and Quris, 1994). There are several explanations as to why acclimation was not significant for the amount of weight loss and % fat composition. The first is that diapause developed in the non-hardened earwigs during the metabolic study, due to exposure to cool temperatures and short photoperiod. The above explanation implies that diapause is induced by cool temperatures and a short photoperiod and that it be broken by cues such as temperature or photoperiod. The first explanation can be discounted since there should not have been a difference in survival if cold-hardiness developed along with diapause as would be expected (Leather et al., 1993; Tauber et al., 1986; Lee et al., 1985).

An alternate explanation is that *F. auricularia* pass the winter (or in this case a 2-month incubation period) in a post-diapause quiescent state (Tauber et al., 1986). Thus both culture treatments were at the same metabolic state (diapause) before and quiescent during the incubation period. Vancassel and Quris (1994) report that a short photoperiod and cool temperatures for a few weeks are required for females to break diapause and oviposit. Being in a post-diapause state would mean that earwigs are quiescent rather than dormant and would respond metabolically to environmental factors such as temperature (Tauber et al., 1986). This was observed where

weight loss was greatest and fat was reduced at warm temperatures for both sexes. Females were more metabolically active than males since they lost slightly more weight than males, as would be expected during post-diapause egg development. Females also possessed more fat than males, to deploy towards egg production. Quiescence would also explain why both cold-hardened and non-hardened females oviposited at warm temperatures. Quiescence would allow females to resume their life cycle early in spring and lengthen their brooding periods to include two broods rather than one (Lamb and Wellington, 1975; Vancassel and Quris, 1994).

This implies however that diapause is not the cause of metabolic changes responsible for the long term survival of cold-hardened earwigs. In fact cold-hardening would only occur at the end of diapause or after diapause was broken. This is unusual as diapause will usually increase cold hardness in insects (Lee et al., 1985; Leather et al., 1993; Tauber et al., 1986).

F. auricularia may sacrifice adult survival to increase the potential reproductive output of overwintering survivors, leading to a selective advantage to individuals resistant to non-freeze damage. Particularly for those individuals who have two successful broods as 50% of overwintering adults come from the second brood (Vancassel and Quris, 1994). This reproductive strategy also leads to greater population increases (Leather et al., 1993).

GENERAL DISCUSSION

4.1 *F. auricularia* as an introduced species

F. auricularia is typical of introduced species: *F. auricularia* was accidentally introduced in imported in crop plants (Weems and Skelly, 1989). They are associated with human disturbance, as the population levels are lower in the centre of old field habitats relative to areas of frequent human disturbance e.g., lawns and gardens. Microhabitat characteristics such as root density affect *F. auricularia*'s ability to establish by increasing the energy required for nest establishment, reducing the resources available for egg production.

Most colonizing organisms display an r-selected reproductive strategy (many offspring with minimum investment per young and rapid growth). *F. auricularia*'s reproductive strategy resembles that of a k-selected species (few eggs are produced and a large maternal investment in care of the young). In addition to the large investment in their young, they maximize the potential output of young by sacrificing adult overwintering success for a longer rearing season and greater number of broods. This results in greater population growth and would increase the probability of a viable population in the face of adverse conditions. The population dynamics of *F. auricularia* also follow the trend for most introduced species: populations grow to large levels, then

level off (Lamb and Wellington, 1975; Weems and Skelly, 1989).

4.2 Temperature and colonization

Temperature can limit the distribution of introduced species (Krebs, 1994; Danks, 1978) and can affect overwintering survival (Leather et al., 1993). The large scale distribution of *F. auricularia* is affected by temperature as they are restricted to overwintering in well protected areas with deep snow cover. While this does not preclude earwigs from overwintering in fields, it is important for their large scale distribution. It would limit them to coastal areas and the Great Lakes region as these areas experience warmer temperatures and greater snow fall (Danks, 1978). The continental distribution of *F. auricularia* reported by Vickery and Kevan (1993) supports this assumption by displaying a distribution of earwigs along the Great Lakes and coastal regions. *F. auricularia* depends on humans in the sub-arctic and do not persist if humans are not present (Danks, 1981). This indicates that *F. auricularia* reaches its northern limit for permanent populations in Ontario.

Overwintering microhabitat temperature is not a factor regarding the differences in population size of *F. auricularia* at the meso-scale (e.g., in disturbed and undisturbed areas), as survival is consistently low (85-100% mortality) despite the difference in mean monthly temperatures, or variation in those temperatures as a result of the proximity to building

foundations. High mortality rates are not unique to *F. auricularia*. Several species have been reported to have overwintering mortality rates above 80% (Leather et al., 1993). Despite the high adult mortality, enough young are produced by overwintering survivors to maintain the population, or there is substantial yearly immigration by new individuals to maintain the populations in eastern Ontario. It is possible that in northern communities, populations of *F. auricularia* (and possibly other introduced species) persist only through repeated introductions. Overwintering behaviours and tolerance ranges would have to be assessed for each introduced species before any conclusions could be drawn, as little is known about the overwintering ecology of most insects (Danks, 1981). This is an area in need of further research (Leather et al., 1993; Danks, 1981).

4.3 Disturbance and colonization

In chapter I it was shown that areas with disturbed soil are favorable to *F. auricularia* as the energetic costs of nest establishment are reduced. This is evident by the greater number of eggs produced by females in disturbed microhabitats. As urban areas are rich in habitats at various levels of disturbance (Rebele, 1994), urban areas are favorable for earwig establishment. This is particularly true as *F. auricularia* is imported to urban areas on a regular basis in plant materials (Weems and Skelley, 1989). Thus *F.*

auricularia not only is likely to find a suitable habitat but also has a high probability of being introduced into these areas. These factors, in addition to the large maternal investment in their young (Lamb, 1976) and because they sacrifice adult overwintering survival for a longer rearing period in the next season, partly explain why *F. auricularia* is effective at establishing in new areas.

In terms of *F. auricularia*'s potential as a biological control agent in orchards, it may be beneficial to generate areas of disturbance in the early fall by tilling soil close to trees, to increase the number of offspring in the next season. Particularly if a pest outbreak is predicted, or for areas with heavy aphid infestations.

4.4 Overwintering strategies

F. auricularia is a freeze intolerant species that cold-hardens by methods other than altering the supercooling point. The result of cold-hardening is that they are more resistant to non-freeze damage. *F. auricularia* can rapidly cold-harden, likely by producing glycerol as this is common in insects (Lee et al., 1987).

F. auricularia does not overwinter in a typical fashion to most temperate insects. Most insects that are freeze intolerant reduce their supercooling points and produce cryoprotectants (Baust, 1982; Miller, 1982; Tauber et al., 1986). Many species enter diapause after an exposure to a

shortened photoperiod and cool temperatures (Leather et al., 1993). Diapause causes physiological changes that induce mechanisms that will reduce freezing and non-freezing damage (Tauber et al., 1986; Leather et al., 1993).

F. auricularia displays an overwintering strategy similar to tropical insects: They enter a period of diapause that is short and involves few or subtle behavioral changes (Tauber et al., 1986). *F. auricularia* does not enter diapause in the fall but instead breaks a post-reproductive diapause and enters a quiescent state (Vancassel and Quris, 1994). This would allow for rapid development as soon as temperatures are favourable, where as diapausing insects have to build up a threshold limit of stimuli (degree hours) before breaking diapause and resuming development (Leather et al., 1993). This would lengthen the active period for *F. auricularia* allowing it to extend its population range northwards, since the short active period is limiting to many insects (Danks, 1981).

Given that *F. auricularia* overwinter in the soil where the temperature rarely reaches their supercooling point, they would not benefit by lowering the supercooling point. However, they would be restricted to overwintering in well protected areas with deep snow cover. Overwintering in the soil is common among insects and is one of the most important overwintering sites for arctic species (Danks, 1981).

4.5 Further research

It is curious to note that *F. auricularia* is highly susceptible to cool temperatures as are several species of aphids (Leather et al., 1993). They are also effective predators of aphids (Lenfant et al., 1994). They display a strong negative geotaxic response (Weyrauch, 1929 in Lamb, 1975), which would indicate that they may be adapted to an arboreal lifestyle. This may in part, explain why earwigs are located around dwellings rather than in the centre of old fields. Trees and shrubs are often used for ornamental purposes, shade, etc. near dwellings, while the centre of old fields are depauperate in trees. It would be worthwhile to conduct a correlation with tree density and earwig population size. It would also be worthwhile to determine if *F. auricularia* is established in wooded areas both frequented and isolated from human disturbances.

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