COLD HARDINESS AND OVERWINTERING SURVIVAL OF THE GRAIN APHID *SITOBION AVENAE* IN NORTHERN ENGLAND

A thesis submitted to the University of Leeds
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Doctor of Philosophy

by

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Cold hardiness and overwintering survival of the grain aphid Sitobion avenae in Northern England.

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Laboratory maintained Sitobion avenae of all developmental stages had a mean inherent supercooling ability below -20°C. Acclimation alone, or in conjunction with starvation had no significant effect on supercooling. Surface moisture on the aphid cuticle during cooling resulted in a significant loss in supercooling. Repeat coolings to temperatures markedly above the mean supercooling point resulted in increasing levels of mortality.

Field collected S. avenae showed a seasonal variation in supercooling with a higher mean supercooling point in the winter than in the remainder of the year. Field populations showed a dramatic decline in December when environmental temperatures were well above mean and individual supercooling points. It was concluded that the aphids were dying before they froze and that the supercooling point was not a reliable indicator of the lower lethal temperature.

The development and reproduction of S. avenae in the field over winter were directly and positively related to temperature whilst mortality increased with decreasing temperature.

The development of a multi-channel micro-bead thermistor unit allowed the study of a previously unrecorded thermal event (exotherm) during the cooling of aphids. The cause of the exotherm is unknown and warrants further investigation. Laboratory experiments on S. avenae and the lupin aphid, Macrosiphum albifrons revealed that the adults of the two species had lower lethal temperatures (LT50) of approximately -7.5°C and -7.0°C respectively. Nymphs of S. avenae were significantly more cold hardy than adults in terms of LT50 values. Acclimation prior to experimentation significantly improved the cold hardiness (LT50) of S. avenae.

It is concluded that in the case of S. avenae and other aphids supercooling points are ecologically irrelevant if aphids are already dead before they freeze. An experimental protocol is suggested for future experiments on insect cold hardiness.

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CHAPTER ONE

GENERAL INTRODUCTION

INTRODUCTION

Approximately 4000 species of aphid have been described worldwide (Dixon, 1985) and about 1500 of these are found in Britain of which around 30 are pest species (Taylor, 1977). Aphids can cause economically important crop losses. These losses occur when high numbers of aphids feed on a plant and remove large amounts of sap or, when the feeding aphid is able to transmit plant pathogenic viruses which can severely weaken or kill the plant.

Seven species of aphid are found on cereals and grasses in Europe (Vickerman & Wratten, 1979) but only three of these commonly infest cereals in Britain (Carter et al, 1980). The least prevalent of the three species on crops is Rhopalosiphum padi (L.) (Taylor & French, 1972; Plumb, 1974, 1976) whilst Metopolophium dirhodum (Walker) and Sitobion avenae (Fabricius) are the most frequently found and serious aphid pests of cereals (George, 1974; Kolbe, 1969, 1970, 1973; Rabbinge & Mantel, 1981).

It is only relatively recently that cereal aphids have reached pest status. Outbreaks were observed as early as the 18th century (Marsham, 1798; Curtis, 1845) but these were not regarded as being of any economic importance. It was not until the 1950's, when the transmission of barley yellow dwarf virus (BYDV) in Californian cereal crops was attributed to cereal aphids (Oswald & Houston, 1951, 1953), that their importance as crop pests was realized. This discovery prompted research in Britain on both the effects of BYDV on cereal yields and also the role and importance

of aphids in the transmission of this disease (Watson, 1959; Watson & Mulligan, 1957, 1960). In Europe cereal aphids were not regarded as serious pests until the severe outbreak of the grain aphid (S. avenae) in 1968 (Anglade, 1969; Fletcher & Bardner, 1969; Jacob-Haupt, 1969; Kolbe, 1969; Leclant, 1969).

The reasons for the change in status of cereal aphids from minor to major pests is not known. It has been suggested that changes in agricultural practice are important (Baronyovits, 1973; Kolbe, 1973; Potts, 1977; Potts & Vickerman, 1974; Way, 1978), but there is little evidence to support this theory in Britain (Vickerman & Wratten, 1979).

The damage caused by aphids to the cereal crop is therefore twofold, firstly direct (feeding) damage and secondly transmission of the virus BYDV. It has been demonstrated that S. avenae can reduce the yield of commercial crops of wheat, oats and barley by feeding damage alone (George, 1974, 1975; Kolbe & Linke, 1974; Vereiken 1979). Reductions in yield may be as high as 42% (Kolbe, 1969) but this is probably exceptional and a level of 9 to 14% (Kolbe, 1970) would be more typical for most years. The level of damage is dependant on the number of aphids on each plant and the length (of time) of the infestation.

The virus BYDV causes most damage when a plant is infected at an early stage of growth. For example, inoculations of wheat, barley and oats with BYDV at growth stage (G.S.) 2 led to mean reductions in yield of 36, 35

and 67% respectively. Inoculations at later stages (G.S. 9), reduced yield by 31, 19 and 29% for the three types of cereals (Doodsen & Saunders, 1970). In most years the annual losses associated with BYDV in Britain are 3-10% (Saunders & Doodsen, 1969). The extent and severity of any crop damage is dependant on the variety of the crop and the strain of the virus. Severe strains of BYDV are normally found in the south and south-west of Britain (Plumb, 1978) although S. avenae has recently been reported as transmitting the severe strain in the north of the country (McGrath et al, 1987).

The ancestors of modern aphids originally lived on woody hosts throughout the year; aphids which live on only one type of host (woody or herbaceous) are called monoecious or autoecious. Initially, in the Mesozoic era aphids lived on conifers and the most ancient of the angiosperms such as Salicaceae. When other trees became available in the later Cretaceous period alate aphids were able to spread and colonize these new hosts. However woody tree hosts become unfavourable for aphids after the initial flush of spring growth and do not provide an adequate source of food. For this reason some aphids go into a resting condition in mid-summer with no development or reproduction until autumn when activity restarts as the tree produces a more favourable food supply from its senescing leaves.

From the Cretaceous period onward flowering herbaceous plants became more numerous and as woody hosts became unfavourable in late spring, alate aphids through random

dispersal found and colonized these herbaceous hosts. There is however a problem associated with this behaviour because in the autumn, the sexual forms are produced which have to lay their eggs on the woody host. Therefore selection favours those species which are able to return to the primary host in the autumn. This process has successfully occurred many times to provide the variety of heteroecious (host alternating) species of today.

Evolution has continued and we now find species that live all the year round on herbaceous hosts of which S. avenae is an example. Clearly a lifecycle of this type is only possible if (i) herbaceous hosts are available all year round and (ii) aphids which are sexual (holocyclic) are able to reproduce on the secondary herbaceous hosts. This strategy does have the advantage that the migratory stages moving to and from the woody host in the autumn and spring are eliminated and although migratations still occur at these times the aphids are searching for cereal crops and grasses which are more abundant than the woody hosts of heteroecious species, so mortality at this time may be reduced. Both the monoecious and heteroecious types of aphid described here reproduce sexually and are holocyclic.

Both monoecious and heteroecious species respond to the stimuli of decreasing photoperiod and temperature for the production of sexual forms; however in both types of life cycle clones have evolved as a stable mutation which do not respond to photoperiod but retain the ability to reproduce anholocyclically (asexually) throughout the year as long as food plants and climate remain favourable. A

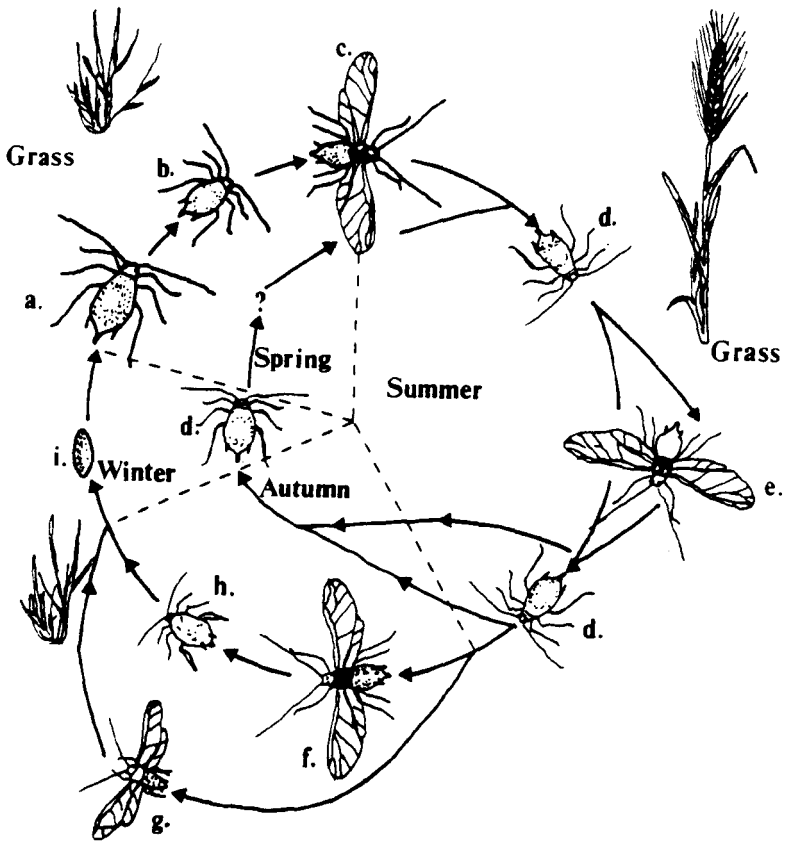
fuller account of the evolution of aphid lifecycles is given by Kennedy and Stroyan (1959).

S. avenae is monoecious and exhibits both the holocyclic and anholocyclic lifecycles (Figure 1.1). In the autumn females of holocyclic clones produce oviparae and males which mate to produce overwintering eggs. The eggs of cereal aphids are very cold hardy and well adapted to survive the low temperatures of winter. Eggs of R. padi supercool to -37°C (Sømme, 1969) and those of R. insertum to at least -35°C, but even with this ability, a proportion of the eggs when at temperatures of only -5°C failed to hatch (James and Luff, 1982). The eggs hatch in spring giving rise to the fundatrices which produce, asexually, nymphs which after a few generations may be alatiform. These aphids then reproduce parthenogenetically throughout the rest of the year until the next autumn when the cycle repeats itself. The anholocyclic lifecycle involves the overwintering of virginoparae which do not respond to the cues of decreasing daylength and temperature and so do not produce any sexual morphs. The ability of these virginoparae to feed, develop and reproduce in winter is dependent on the prevailing climate. At temperatures below -4°C S. avenae is totally immobile (Smith, 1981).

S. avenae appears to overwinter mainly as anholocyclic virginoparae (Adams and Drew, 1964; Forbes, 1962; George, 1974; Greene, 1966; Philips, 1916) since eggs have been rarely found (Hille ris Lambers, 1939; Dean, 1978). Although eggs of S. avenae have been discovered by Hand (1980) he did not find any oviparae or fundatrices.

Figure 1.1.

Life cycle of the grain aphid Sitobion avenae (a, fundatrix; b, apterous fundatrigenia; c, emigrant; d, apterous exule; f, gynopara; g, male; h, ovipara and i, egg; e, alate virginopara).


Grass
b.
c.
d.
a.
?
Spring
Summer
Grass
d.
i.
Winter
Autumn
e.
h.
d.
f.
g.

Occasionally some of the overwintering virginoparae produce a number of males and these can mate with oviparae produced by holocyclic clones. Thus in severe winters, the genetic information for anholocyclic overwintering will not be lost even though all the anholocyclic aphids may have perished.

The anholocyclic lifecycle of S. avenae can be advantageous in the post-winter period since reproduction resumes as soon as temperatures increase and this gives rise to the migratory alate population much earlier in spring than alates derived from overwintering eggs. With holocyclic overwintering at least one or two generations are required before any alates are produced. Consequently BYDV is much more likely to to be introduced into a spring sown cereal, or spread within winter sown cereals, by the progeny of overwintering virginoparous aphids than by those derived from overwinting eggs (Plumb, 1983). The majority of progeny born on virus infected plants will become viruliferous and hence vectors of BYDV whether apterous or alate adults. The ability of S. avenae to overwinter viviparously, even if only in sheltered positions (Dean, 1974a,b) makes the overwintering biology of this species of great interest becase of its increasing pest status in British agriculture.

The pest status of S. avenae and cereal aphids in general has resulted in attempts to predict both the timing and size of the aphid migration in order to forecast possible outbreaks and the need for control. Providing a reliable forecast can be developed, the need for insecticide sprays can be reduced and consequently wasteful

'insurance treatments' which also increase the probability of pesticide resistance occurring in these aphids, as it has done in Myzus persicae, can be avoided. This will be cost-effective for farmers, increase the useful life of present pesticides and reduce environmental pollution.

A number of aphid forecasting systems have been developed; one of the most successful has been that for Aphis fabae the black bean aphid (Way and Cammel, 1973). This species is almost entirely holocyclic on its primary host, spindle (Euonymous europaeus). This makes egg counts easy in autumn and also the spring assessment of survival. The migration date is estimated and a recommendation to spray is issued when the number of plants infected at the end of the spring migration exceeds 5%.

The grain aphid has presented more problems since it has a number of overwintering hosts and both holocyclic and anholocyclic clones; however some forecasts for the timing and probability of outbreaks have been made (e.g. Turl, 1980; Dewar and Carter, 1984; Walters and Dewar, 1986). Most forecasts are based on the negative relationship between winter temperature and the timing of first catches in suction traps. The suction traps cover most of Britain and some of Europe and are part of the Rothamsted Insect Survey which regards predictive forecasting as its main objective (Taylor, 1977). Even though the relationships have been derived it is surprising that no direct assessment of aphid cold hardiness has been attempted, making this the weakest link in our knowledge of aphid biology.

Low temperature is not the only aspect of climate that is important to overwintering survival; leaf surface wetness and rainfall have both been correlated with population change (Harrington and Cheng, 1984; Taylor, 1977). The main factor regulating the winter survival of temperate insects is winter climate (Danks, 1978); high mortality occurring in severe winters when temperatures are lower than normal.

Insects exhibit two distinct strategies to survive at sub-zero temperatures. Freezing tolerance describes the ability to survive the formation of extra-cellular (and possibly intra-cellular) ice. The majority of species are freezing intolerant i.e. unable to tolerate the formation of ice in the body tissues and fluids, and avoid ice formation by the process of supercooling (Salt, 1961). In the majority of reviews and research papers the terms cold hardiness and cold tolerance refer only to mechanisms associated with the survival or avoidance of freezing and furthermore, cold hardiness and supercooling ability are virtually synonymous when applied to freezing intolerant species.

Freezing tolerant insects often contain ice nucleating agents, mainly proteins, which promote freezing, extra-cellularly at a few degrees below the insects true freezing point (Zachariassen and Hammel, 1976; Zachariassen, 1980, 1982; Duman, 1980). Intra-cellular freezing was thought to be fatal (Asahina, 1969) but there is now some doubt that this is universally true amongst insects (Baust and Rojas, 1985). This category of insects

which are found in the more extreme climates (Block, 1982) also contain polyhydroxy alcohols which function to limit freeze damage (Duman and Horwath, 1983).

Freezing intolerant insects avoid freezing by supercooling, a process in which the body tissues and fluids are maintained unfrozen below their equilibrium feezing point (Salt, 1963). Insects are able to supercool to temperatures below -20°C without significant levels of polyols (Zachariassen, 1985) but seaonal increases in the concentration of one or more polyols extends the inherent ability to supercool (Baust, 1981; Somme, 1982). In addition to the polyols, antifreeze proteins which lower the freezing point of the haemolymph relative to its melting point may act to stabilise the supercooled state (Zachariassen and Husby, 1982) thus improving their ability to overwinter.

Supercooling points are widely used as a convenient laboratory method to assess and compare the cold hardiness of freezing intolerant species (Sømme, 1982) and because of the ease by which they can be determined have been widely used as an index of cold hardiness for freezing intolerant species. However for some species, other injurious effects of cold may be a more important threat to life (Bale, 1987). Whilst the supercooling point of a species is not fixed but influenced by a number of inherent and environmental factors, these changes may be entirely irrelevant in ecological terms if the insect dies before reaching the limit of its supercooling. The main purpose of the work described in this thesis was to evaluate in

broad terms the cold hardiness of the grain aphid S. avenae and evaluate the use of this type of data as part of a forecasting system for aphid migrations and outbreaks. Earlier studies on aphid cold hardiness have desribed all species as freezing intolerant (Powell, 1974; Sømme, 1982; O'Doherty, 1984; Williams, 1984) and thus reliant on supercooling for survival. However Williams (1980, 1984) recorded substantial mortality at temperatures much above the supercooling point of cereal aphids and thus emphasised the need for an integrated study of the overwintering ability of S. avenae combining both field and laboratory observations.

The second chapter of this thesis describes the methods used to culture the aphids and the method for determining supercooling points. Chapter 3 gives an account of the freezing status, supercooling ability and the effects of acclimation, starvation and surface moisture. The study of S.avenae field populations and the supercooling capacity of individuals from them are outlined in chapter 4 whilst chapter 5 is concerned with the investigation of the development, reproduction and survival of individual aphids overwintering in the field. Chapter 6 describes the development of the apparatus required to detect very small changes in thermal energy that occur in aphids when they are cooled, the results of which are described in chapter 7.

CHAPTER TWO

MAINTENANCE OF *SITOBION AVENAE* CLONES AND MODIFICATION OF SUPERCOOLING SYSTEM

SUMMARY

A culture of a single clone of S. avenae was maintained in the laboratory on cereal plants at 20° ±2°C in a 18L:6D photoperiod. The aphids were reared in a variety of ways using whole plants or leaf strips depending on the conditions under which the experiments were taking place.

Maintenance of the clone under a short photoperiod (8L:16D) showed that it was of the anholocyclic type. Mean values for development to adult, longevity, and nymphal production at 20 ±2°C were 10.0 days, 31.7 days and 45 nymphs per adult respectively.

The method used in this study for the determination of supercooling points was developed by Bale, O'Doherty, Atkinson & Stevenson (1984). In the course of the research the computer program which monitored and displayed the cooling profile was modified to provide clearer resolution of supercooling points on the graphical display and to reduce the effects of fluctuations in the electrical supply which on occassion, generated spurious supercooling points.

INTRODUCTION

This chapter outlines the methods of aphid culture used in the study and the general method used for the determination of aphid supercooling points (SCPs). Methods that relate to specific areas of study are discussed in the relevant chapters.

The development of standardised methods of culture to reduce variation within and between experiments was important because different cereal cultivars and different ages of plants can affect the fecundity of S. avenae (Lowe, 1978). It is possible that these factors may also affect the development and other physiological characteristics of the aphids.

Insects are described as freezing tolerant or intolerant depending on their ability to survive the formation of ice in the body tissues and fluids. The only protection for freezing intolerant insects is the ability to supercool, in which the body tissues and fluids are maintained in the liquid state below the equilibrium freezing point. The supercooling point is the lowest temperature reached before freezing occurs and is detected as the heat emmission associated with the spontaneous transformation of water to ice. The supercooling point can therefore be regarded as the instantaneous low temperature death point (Salt, 1958) and is widely used as a comparative index of cold hardiness for freezing intolerant species (Sømme, 1982).

The method employed for the determination of

supercooling points of S.avenae was that of Bale, O'Doherty, Atkinson & Stevenson (1984) with modifications to the computer program. During the course of this study it was evident that the very small amounts of energy produced by first instar nymphs on freezing were close to the limits of sensitivity of the thermocouples and that other physiological changes with lower heat emmissions would not be detected. A system capable of detecting such small energy changes was developed and is described fully in Chapter 6.

MATERIALS AND METHODS

Culture and Maintenance.

Aphids used in all experiments were descended from a single apterous, viviparous adult of S. avenae. The aphid was collected from a winter wheat plant (variety unknown) at Pocklington, nr. York, North Yorkshire. A single clone was established to limit biological variation within and between experiments. In order to reduce any such variation still further the culture methods were standardised. The regulation of environmental conditions such as temperature, daylength, light intensity and also food quality ensured that any intraclonal variation was kept to a minimum.

The rearing and maintenance of the clone was conducted in a room in which the temperature was controlled at 20°C±2°. The relative humidity, which was not controlled, ranged between 60-80%. The cultures were kept under banks

of 4 or 5 fluorescent tubes with a photoperiod of 18L:6D. The photon flux density at the bench surface was 40μmol m^{-1} sec^{-1}.

Plants.

All of the plant material used in the experiments was either winter wheat (var. Avalon) or winter barley (var. Igri). The seed was sown in John Innes number 3 compost in 125mm plastic pots and subsequently kept in a greenhouse at approximately 20°C. The plants were used when they were about 15cm high (G.S. 11-12, Zadoks, Chang & Konzak, 1974)

Rearing methods.

Method I Whole plant caged.

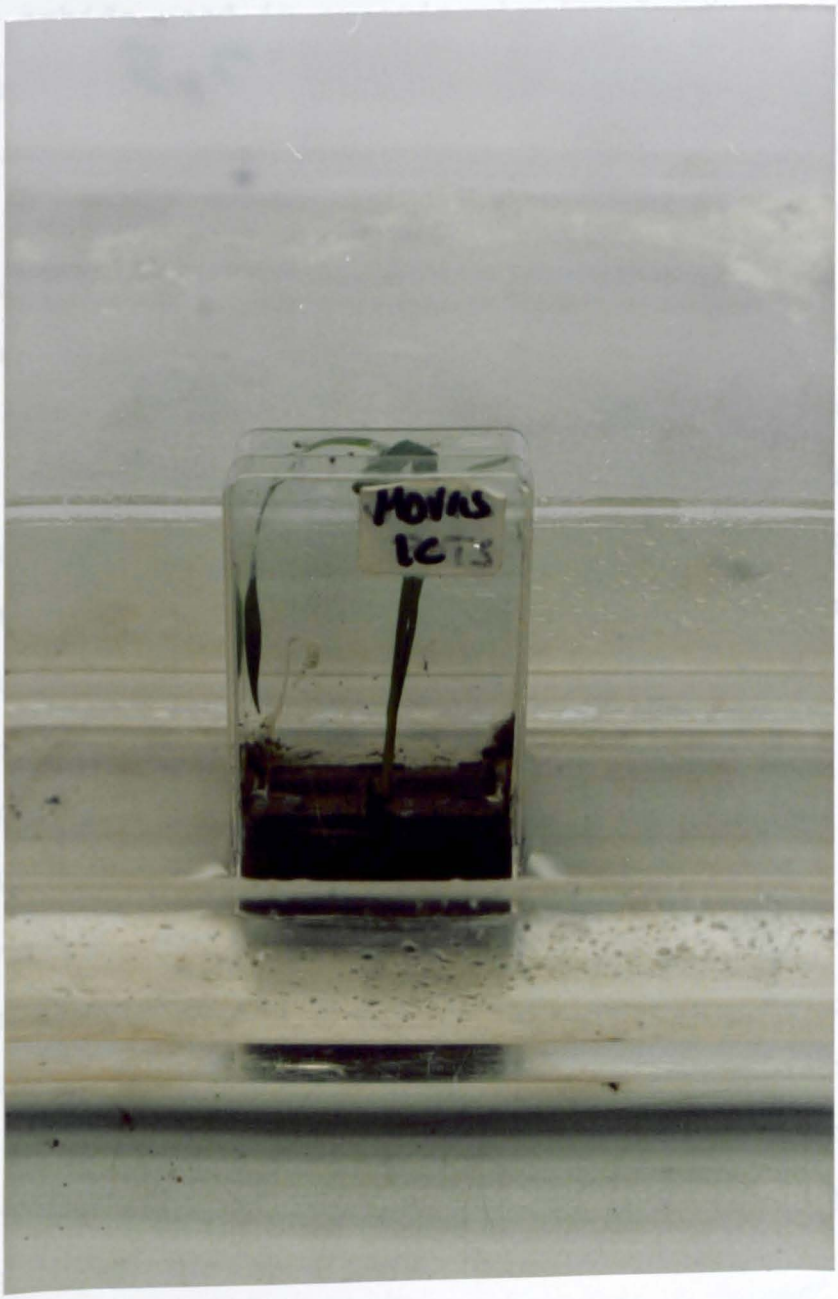
A colony of S.avenae was kept on whole plants in a cylindrical insect cage (Watkins & Doncaster, Hawkhurst, Kent) in the controlled environment room (Plate 1). It was from this colony that aphids were taken to obtain individuals for experimental purposes. The plants were renewed every two weeks when they became too large for the cages.

Method II Blackman boxes.

A small number (2-3) of S. avenae were maintained in transparent plastic boxes ('Blackman boxes'), described by Blackman (1971), as a reservoir clone in the event that the main colony died out from disease, parasitism or other factors (Plate 2). The clones were maintained by collecting the progeny of the previous adult generation and placing them on the new plants. The plants used within the boxes were only 4 or 5cm high (G.S. 9-10), grown in water throughout, and replaced every 2-3 weeks.

Plate 1. Rearing method I, whole plants caged.

Plate 2. Rearing method II, Blackman boxes.


HOVAS
1C

Method III Leaf strips.

The aphids used in experiments involving acclimation were maintained on leaf strips 2.5cm long and cut from wheat plants at growth stage 11-12. These strips were placed, lower surface uppermost, on moist tissue paper which was stretched over a glass or perspex block. The tissue and the block were in a tray of tap water which could be covered with a lid to reduce evaporation (Plate 3). The excess tissue at either end of the block acted as a wick to keep the surface moist. The trays were placed in illuminated cooled incubators running at the required temperature and with a photoperiod of 18L:6D. The leaf strips were replaced every 2-3 days to prevent any significant effects from senescence.

Method IV Whole plant propagator.

When large numbers of adults and nymphs were required they were reared on whole plants covered with perspex propagator lids. The top of the lid was removed and replaced with nylon mesh to increase ventilation and so reduce the incidence of cereal disease such as moulds and mildew (Plate 4).

In the following chapters the rearing method used in each experiment is indicated by the relevent title and roman numeral. Aphids were handled using a damp camel hair paintbrush, and lifted from the head end to reduce damage to the aphid and handling variability (Adams & van Emden, 1972). The different rearing methods used provided a reliable and reproduceable way of obtaining aphids for experimentation. The adults were all of similar size and

Plate 3. Rearing method III, leaf strips.

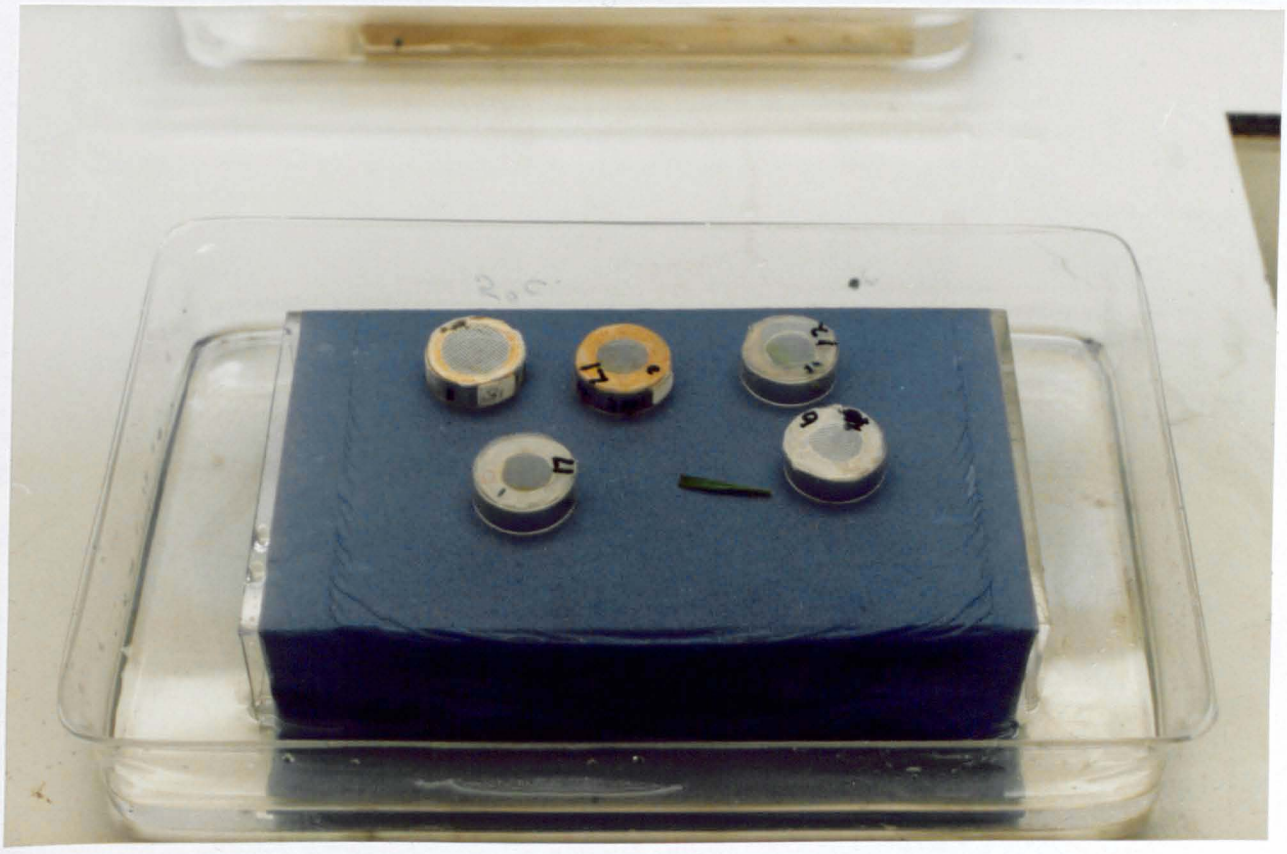


Plate 4. Rearing method IV, Whole plants in propagator.

there was little variation in the length of time to develop from first instars to the adult stage (1 day); thus there was little variation between or within the different rearing methods.

Therefore, any differences occurring between experimental results can safely be attributed to changes in experimental factors rather than differences inherent in the aphids prior to the experiment.

Biological Characteristics.

Life cycle.

The life cycle of the clone of S.avenae was investigated by exposing some individuals to a short photoperiod (8L:16D) and lower temperatures (18°C ±2°). These were left to reproduce for several generations to induce production of sexual forms which would occur if the clone was holocyclic. Over a period of 4 or 5 months only viviparae, (no gynoparae, males or oviparae), were produced. The clone was therefore assumed to be anholocyclic and typical of the aphids that would overwinter in the fields in this region.

Development, reproduction and longevity.

In order that the experimental work could be carefully planned it was neccessary to know the time required for this clone of S. avenae to develop from nymph to adult. This information was also of use in comparing the time taken for development of these aphids with those that were reared in the field (Chapter 5).

The aphids were maintained on leaf strips (method III) in an illuminated cooled incubator at 20°C±1°. Newly moulted adult viviparae were placed on strips of wheat leaf and allowed to to reproduce for 48 hours in the incubator. They were then transferred to fresh leaf strips and allowed to reproduce for 8 hours. The resulting progeny were placed individually on leaf strips, and returned to the incubator. The nymphs were observed twice daily at 0900 hrs and 2100 hrs, and any moults were noted at these times. When the nymphs were mature and had commenced reproduction their progeny were removed and counted at daily intervals.

RESULTS

The results for the development, reproduction and longevity of the clone of S. avenae used in all experiments are given in tables 2.1, 2.2 and 2.3. The aphids required 11.1±0.3 days at 20°C to develop from first instars to reproductive adult and had an adult lifespan of 20.7±2.4 days. Of the original 20 aphids 12 became apterae and 8 alatae and they had an average lifespan of 31.8±2.4 days. During the reproductive phase the mean number of nymphs produced per adult was 45±4.5.

Supercooling point method.

The supercooling point of an insect can be measured by the rise in temperature associated with the liberation of heat when the body fluids of the insect freeze. The aphid to be supercooled was attached to the thermocouple with a spot of petroleum jelly and then placed in a chamber of an

Table 2.1. Duration in days of the instars and pre-reproductive period of the experimental clone of S.avenae at 20°C (mean ± S.E. N=20).

	1st instar	2nd instar	3rd instar	4th instar	Pre-reproductive period	Total
Time (Days)	2.1 ± 0.1	2.8 ± 0.1	2.2 ± 0.1	2.9 ± 0.1	1.1 ± 0.2	11.1±0.3

Table 2.2. Longevity in days of adult S.avenae at 20°C (mean ± S.E. N=20).

	Reproductive period	Post-reproductive period	Total
Longevity (Days)	16.7 ± 1.6	4.0 ± 1.3	20.7 ± 2.4

Table 2.3. Reproductive ability (number of nymphs) of S.avenae at 20°C (mean ± S.E. N=20).

	Total	Per day
Number of offspring produced	45 ± 4.5	2.4 ± 0.1

aluminium block mounted on a Peltier device. The thermocouple was interfaced to a microcomputer via a thermocouple converter for the sensing and recording of supercooling points. The Peltier device had an automatic temperature controller for the maintenance of a constant cooling rate of 1 deg per minute (Plate 5). The experimental details are entered into the computer program along with the required duration of the recording period. The program provides a digital readout on the monitor of the temperature of each thermocouple, (i.e. the temperatures of the four specimens), the cooling block and the cooling fluid for the thermoelectric module. There is also a graphical display which is described later in the modifications to the system. On completion of the experiment, i.e. when all specimens have passed their supercooling point, the results can be output to a dot matrix printer and a hard copy of the experimental details and the supercooling points of the four specimens obtained. The graphical display can also be output to the printer or saved on disk (Figure 2.1). The system can then be rapidly reset and the next run started.

Determination of Supercooling points.

The apparatus used for the determination of the supercooling points was developed by Bale, O'Doherty, Atkinson and Stevenson (1984). During the course of early experiments it was found that fluctuations in the mains electrical supply could trigger the recording of spurious supercooling points. In the original program supercooling points were recognized by the computer as an increase in

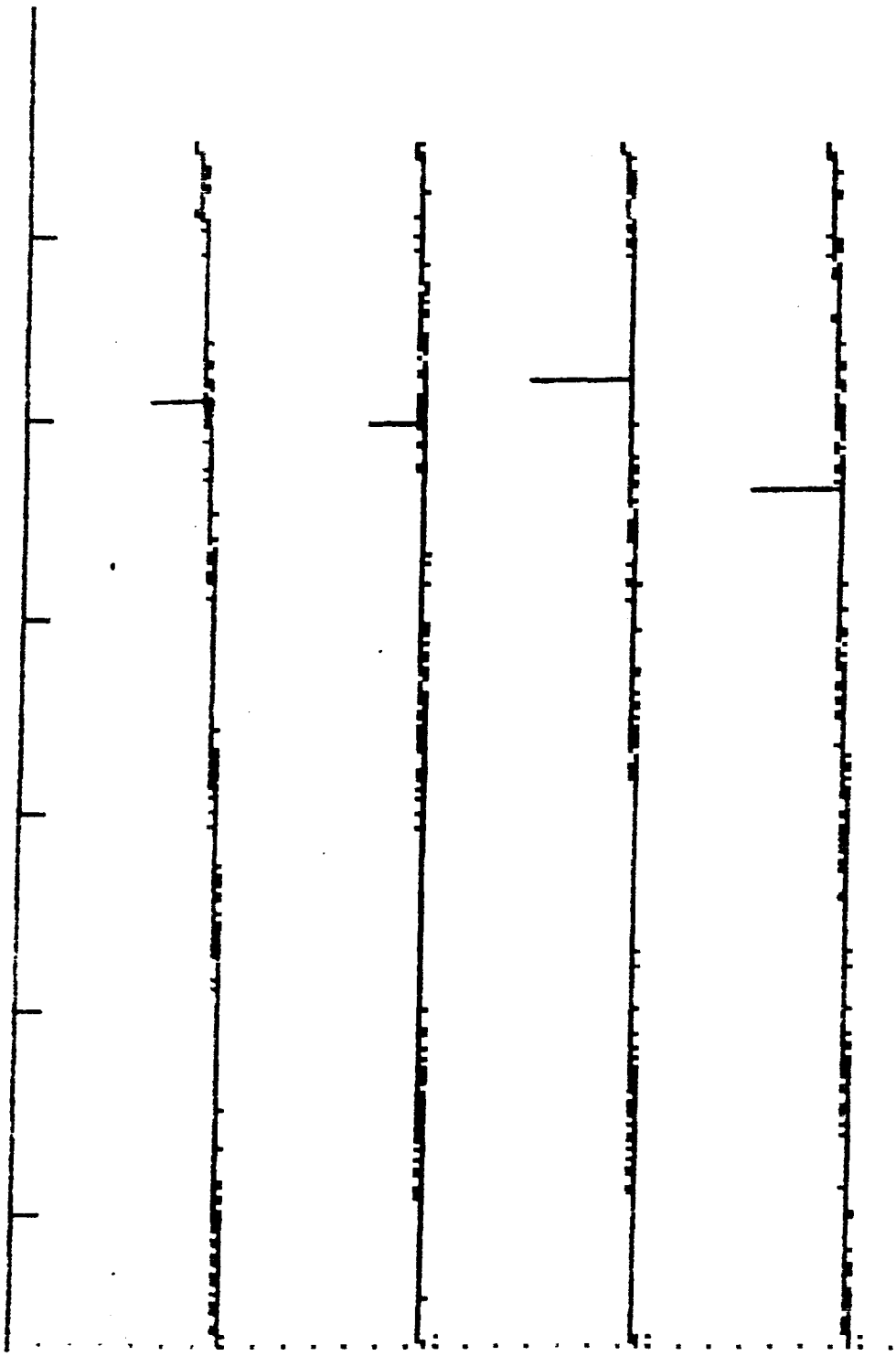
Plate 5. System used for the determination of supercooling points showing individual components.

1. Grant circulator and flow cooler.
2. Automatic temperature controller.
3. Bipolar controller.
4. Cooling unit (thermoelectric module).
5. Microcomputer.


1
2
3
4
5
Grant

Figure 2.1.

Output of dot matrix printer showing graphical display and supercooling points of specimens.

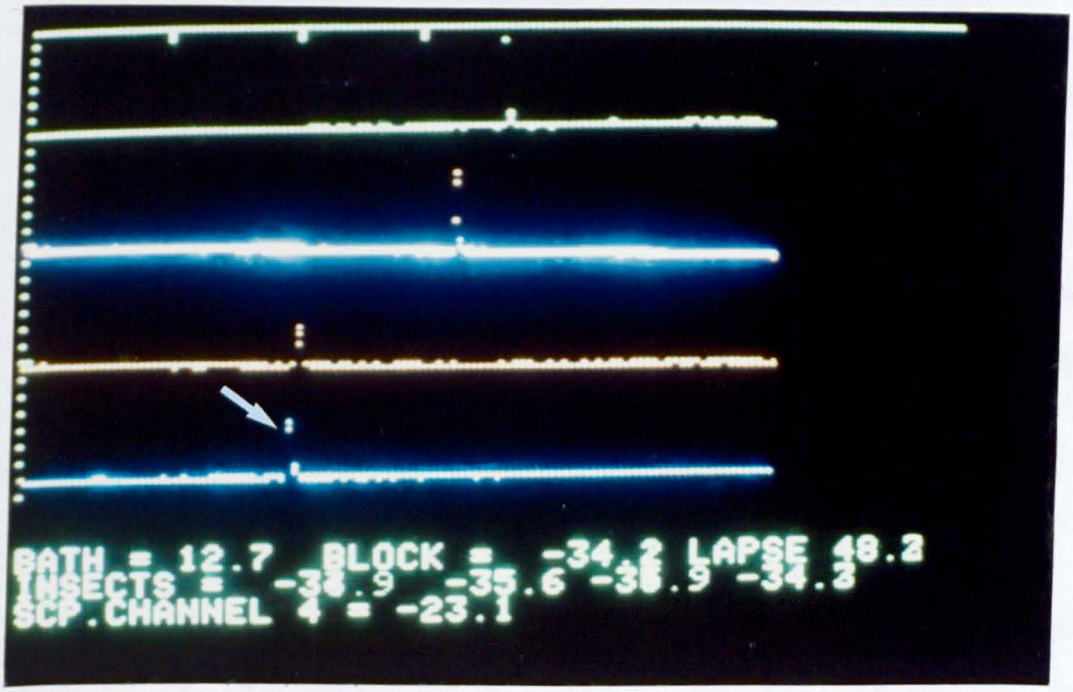


temperature in excess of a preset value above the previous reading for a particular thermocouple. The preset value could be altered to suit the size of the insect so that large insects could have a high threshold e.g. 0.5 deg, which was much greater than any electrical interference. Experiments using smaller specimens required a lower threshold e.g. 0.15-0.2 deg, and such changes could be caused by electrical interference. The electrical interference caused an increased temperature reading on all channels which was subsequently recorded as a supercooling point value. To negate this effect the BASIC program was modified. In the modified program a supercooling point was only recognized when the differential temperature between a reference thermocouple and that carrying a specimen exceeded the preset value. Any external influence had an equal effect on both thermocouples thus ensuring that only true supercooling points were recorded. At the same time the program was altered to plot the graphical representation of the temperature decrease during cooling as a horizontal line from left to right insted of the more traditional diagonal temperature v. time plot. This enabled supercooling points to be more easily distinguished and simplified the cross checking of graphical and digital results (Plate 6).

DISCUSSION

The results obtained for the rates of development, fecundity, and longevity of S.avenae in this study are

Plate 6. Graphical and digital display as observed on the computer monitor. Supercooling point on channel 4 is arrowed.


BATH = 12.7 BLOCK = -34.2 LAPSE 48.2
INSECTS = -38.9 -35.6 -36.9 -34.3
SCP.CHANNEL 4 = -23.1

comparable with those of other workers. Markkula and Pulliainen (1965) recorded a pre-reproductive period of 9 days, a reproductive period of 28-35 days, a longevity of 42-49 days and a mean fecundity of 40 nymphs for S. avenae reared at 20°C. Equivalent values from Dean (1974b) are 8.8 days, 17 days, 30.8 days and 61 nymphs respectively. Lykouressis (1985) recorded the total time from birth to larviposition at 20°C as 8.7 days for apterae and 9.2 days for alatae. The results obtained from the aphids used in this study show that they are comparable to other laboratory clones and are probably representative of S. avenae that may be found in the field.

The supercooling method used allows rapid and accurate detection of the supercooling points of the insects. The system provides constant and repeatable cooling rates and is unaffected by changes in ambient temperature. The modifications to the computer program have increased the sensitivity of the unit and eliminated spurious results whilst retaining all the virtues of the original system.

CHAPTER THREE

SUPERCOOLING ABILITY OF THE GRAIN APHID AS AFFECTED BY ACCLIMATION, STARVATION AND SURFACE MOISTURE

SUMMARY

The grain aphid S. avenae was found to be freezing intolerant. All developmental stages reared at 20°C supercooled to below -20°C. Acclimation at 5°C and 0°C caused no biologically significant changes in supercooling ability but some nymphs died during the period of acclimation at 0°C after 14 days. Starvation at the same temperatures resulted in a slight reduction in supercooling associated with an increase in time of exposure. Nymphs showed mortality at 5°C and 0°C after only 7 days of starvation.

Exposure of the cuticle to moisture during cooling resulted in a significant loss of supercooling in a proportion of both the adults and nymphs although the mean values did not rise above -20°C. Mortality increased when aphids with or without moisture on the cuticle were cooled repeatedly.

INTRODUCTION

Insects of the temperate and polar regions of the earth are commonly faced with the problem of surviving temperatures below 0°C. These insects can be divided into two main groups with respect to cold-hardiness. Freezing tolerant insects are, as their name implies, able to tolerate the formation of ice in the body fluids and members of this group often exhibit poor supercooling ability associated with the action of ice nucleating proteins which ensure extracellular freezing at high sub-zero temperatures to prevent damage to the tissues (Ring, 1980; Sømme, 1982). Freezing intolerant insects cannot tolerate the formation of ice in the body fluids and have developed mechanisms allowing extensive supercooling. This group constitutes the largest proportion of overwintering insects (Block, 1982). Some insects have been found to change from freezing tolerant to freezing intolerant in successive winters (Duman, 1984).

Supercooling in insects can be defined as the ability to maintain the body fluids in a liquid state below the equilibrium freezing point (Ring, 1980). The supercooling point of a freezing intolerant insect is therefore widely regarded as the instantaneous low temperature death point and a convenient basis on which to assess and compare the cold-hardiness of different species (Sømme, 1982).

The comparison of supercooling abilities is only valid if the method of measurement is standardised. Salt (1961) proposed a standard cooling rate of 1 deg per minute which

has been widely adopted by insect cryobiologists. Many investigations have been made on the supercooling ability of overwintering insects and these have been reviewed by Sømme (1982).

Whilst starvation has been shown to increase the supercooling ability of some freezing intolerant insects by the removal or masking of gut nucleators, increased supercooling is normally associated with the winter accumulation of cryoprotective substances such as polyhydric alcohols (polyols) (Danks, 1978; Ring, 1980; Duman, 1982).

Previous studies on the cold-hardiness of aphids have shown them all to be freezing intolerant and able to supercool to temperatures around -20°C (Powell, 1974, 1976; Parry, 1978; Williams, 1984; O'Doherty and Bale, 1985).

In the field during winter S. avenae is exposed to the elements and therefore subject to the full effects of precipitation, wind and low temperatures, unlike some insects which seek overwintering sites that provide some protection (Danks, 1978).

This chapter determines the supercooling responses of S. avenae to acclimation, starvation and surface moisture under laboratory conditions. The supercooling capacity of aphids reared at 20°C was used as a baseline with which to compare the other results. Studies on the seasonal variation in supercooling under field conditions are reported in chapter four.

MATERIALS AND METHODS

The method used for the determination of the supercooling points was as described in chapter 2. Adult apterous virginoparous aphids from the stock culture maintained on young plants (Method IV) at 20°C ± 2°C and in a photoperiod of 18L:6D, were placed on leaf strips (Method III) overnight in an illuminated incubator at 20°C ± 1°C 18L:6D. The transferred aphids had already produced some nymphs so the atypical biological responses sometimes observed in the first born offspring (Murdie, 1969) did not arise. The adults were removed from the leaf strips the following morning and the nymphs arranged at a density of one per strip. The supercoolng ability of 20 of these first instars was assessed immediately and the rest were allowed to develop at 20°C ± 1°C 18L:6D to the appropriate instar at which time their supercooling points were determined.

To investigate acclimation responses first instar nymphs or newly moulted adults, produced as in the first part of this method, were maintained on leaf strips (Method III) in illuminated incubators at 5°C ± 1°C or 0°C ± 1°C, 18L:6D for periods of 2, 7, 14, 21 or 28 days. These temperatures were known to be the optima for the induction of cryoprotectant production in other species but were still above the threshold temperature for activity (Smith, 1981) and hence normal physiological function in aphids. Lower, sub-zero, temperatures may have induced chill coma or injury (Salt, 1961; Ring, 1980) in the aphids and they

would then have been unable to synthesise any cryoprotectants. Supercooling points were measured at the end of the acclimation periods.

In order to assess the effects of starvation on the supercooling ability of the aphids the preceeding experiment was repeated but the aphids were kept on the moist tissue paper without any leaf strips.

Finally batches of first instar nymphs and newly moulted adults were cooled in contact with water to see if inoculative freezing occurred above the supercooling point. The aphids were attached to the thermocouples as normal but were moistened with a paintbrush wetted with rainwater prior to cooling. A further experiment was conducted on the effects of repeat coolings on aphids. Two batches of 32 adult aphids, one wetted with rainwater, were cooled to -8°C and then warmed to room temperature. After 1 hour the survivors were counted, re-wetted as necessary and then cooled to -8°C again. This procedure was repeated until all the aphids had died.

Results from all the experiments were expressed as the mean ($\bar{x}$) and its standard error (S.E.). Further analysis was carried out using a proprietry statistics package (SPSSX, Nie, 1983), on an Amdahl 470/V7 mainframe computer, using oneway analysis of variance followed by Student-Newman-Keuls tests (SNK) where appropraiate.

RESULTS

No aphids survived below their supercooling point and *S. avenae* was concluded to be a freezing intolerant species. The first experiment showed that all developmental stages had a mean supercooling point below -20°C with little intra-stage variation. A progressive loss of supercooling was exhibited with increasing age; thus first instars had a significantly lower mean supercooling point than the fourth instars or adult morphs ($F=29.800$, 5,114 d.f., $P<0.001$) (Table 3.1), although the differences were small.

In the acclimation experiments in general, supercooling was unaltered, and the few significant differences that were found were so small as to be of no biological importance in the field (Tables 3.2 & 3.4). Nymphs with access to food died at 0°C when exposed for more than 14 days but mortality occured after 7 days when the aphids were starved at the same temperature (Table 3.2a & 3.3a). At 5°C starved nymphs also began to die after only 7 days (Table 3.3b).

Nymphs in general showed no changes in supercooling ability in these experiments, whereas adults showed a loss with increased exposure to the combined effects of starvation and the low temperatures of acclimation; the mean supercooling point after 21 days at 0°C is significantly less than that after 2 days but it improves again by 28 days ($F=6.141$, 4,95, d.f., $P<0.001$) (Table 3.5a). Starvation at 5°C resulted in a significant loss of

TABLE 3.1. Supercooling ability ($\bar{x}$ ±S.E. and range °C N=20) of developmental stages of *Sitobion avenae* maintained at 20°C

(Means followed by the same letter do not differ significantly at 5% level)

First instar	Second instar	Third instar	Fourth instar	Apterous adult	Alate adult
-27.0 ±0.2	-25.9 ±0.2	-25.2 ±0.2	-24.4 ±0.3	-24.2 ±0.3	-23.2 ±0.3
-25.6/-28.6	-23.8/-26.8	-23.0/-26.4	-18.8/-25.8	-20.2/-25.9	-20.5/-26.6
			a	a	

TABLE 3.2. Supercooling ability ($\bar{x}$ ±S.E. and range °C N=20) of first instar Sitobion avenae after acclimation at (a) 0°C or (b) 5°C.
(Means followed by the same letter do not differ significantly at the 5% level).

Temperature	Days of acclimation 2	7	14	21	28
(a)					
0°C	-25.8 ±0.2	-26.0 ±0.1	-26.1 ±0.1	All dead	All dead
	-23.5/-26.9	-25.1/-26.8	-25.2/-27.8		
	a	a	a		
(b)					
5°C	-26.2 ±0.2	-26.4 ±0.1	-26.2 ±0.2	-25.6 ±0.3	-26.1 ±0.2
	-24.4/-26.9	-24.6/-27.1	-24.1/-27.8	-22.0/-27.2	-24.6/-27.1
	ab	a	ab	b	ab

TABLE 3.3. Supercooling ability ($\bar{x}$ ±S.E. and range °C N=20) of first instar Sitobion avenae after combined starvation and acclimation at (a) 0°C and (b) 5°C.
(Means followed by the same letter do not differ significantly at the 5% level).

Temperature	Days of acclimation and starvation 2	7	14	21	28
(a)					
0°C	-26.0 ±0.1 -24.2/-27.2	-25.9 ±0.2 -24.8/-27.6	All dead	All dead	All dead
(b)					
5°C	-25.4 ±0.2 -23.3/-26.4	-25.4 ±0.2 -24.2/-26.5	All dead	All dead	All dead

TABLE 3.4. Supercooling ability ($\bar{x}$ ±S.E. and range °C N=20) of adult apterous Sitobion avenae after acclimation at (a) 0°C or (b) 5°C.
(Means followed by same letter do not differ significantly at 5% level).

Temperature	Days of acclimation 2	7	14	21	28
(a)					
0°C	-23.1 ±0.5	-24.1 ±0.5	-24.5 ±0.3	-22.2 ±0.3	All Dead
	-17.9/-25.0	-16.4/-25.7	-21.0/-26.1	-20.1/-24.5	
	ab	a	a	b	
(b)					
5°C	-24.0 ±0.3	-25.0 ±0.2	-24.4 ±0.3	-23.9 ±0.2	-24.4 ±0.2
	-20.6/-25.3	-21.7/-26.3	-21.1/-26.3	-20.8/-25.7	-21.3/-25.6
	b	a	ab	b	ab

TABLE 3.5. Supercooling ability ($\bar{x}$ ±S.E. and range °C N=20) of adult apterous Sitobion avenae after combined starvation and acclimation at (a) 0°C and (b) 5°C.
(Means followed by same letter do not differ significantly at 5% level).

Temperature	Days of acclimation and starvation 2	7	14	21	28
(a)					
0°C	-24.2 ±0.2	-23.5 ±0.3	-24.2 ±0.1	-21.7 ±0.7	-22.3 ±0.6
	-21.4/-25.8	-19.8/-25.1	-22.6/-25.0	-14.9/-25.2	-16.7/-25.0
	a	ab	ab	b	b
(b)					
5°C	-23.8 ±0.2	-23.2 ±0.6	-23.3 ±0.4	-22.1 ±0.3	All dead
	-21.2/-25.2	-14.9/-25.3	-19.1/-25.0	-18.8/-24.4	
	a	ab	ab	b	

cold hardiness in the adults after 21 days compared to that at 2 days ($F=3.436$, 3,76 d.f., $P<0.05$) (Table 3.5b).

Surface moisture caused a significant loss of supercooling ability in a proportion of both adult apterae and first instar nymphs ($t=5.955$, 30 d.f., $P<0.001$ and $t=3.7022$, 23 d.f., $P<0.01$ respectively) although in both cases the mean supercooling point did not rise above -20°C. (Fig. 3.1). The repeat coolings resulted in a high cumulative mortality at temperatures far above the mean supercooling point of adult aphids (Fig. 3.2). This research has been published as Knight & Bale (1986).

DISCUSSION

Supercooling points indicate the variation in the instantaneous low temperature death point of a population and are widely used as a convenient laboratory method to assess and compare the cold-hardiness of freezing intolerant species (Sømme, 1982). These experiments show that S.avenae has a supercooling ability below -20°C at all stages of development. This is comparable with values obtained for a variety of polar and temperate species of invertebrates (Sømme, 1981).

Freezing intolerant insects share a number of common features based on supercooling point data; (i) a seasonal pattern of supercooling, which is at a maximum in winter, first noted in Bracon cephi (Salt, 1957); (ii) an acclimation response triggered by laboratory exposure to low temperatures (e.g. Young & Block, 1980; Bale & Smith,

Figure 3.1.

Supercooling point distribution histograms of first instar nymphs and adults of Sitobion avenae when dry and after wetting with water.

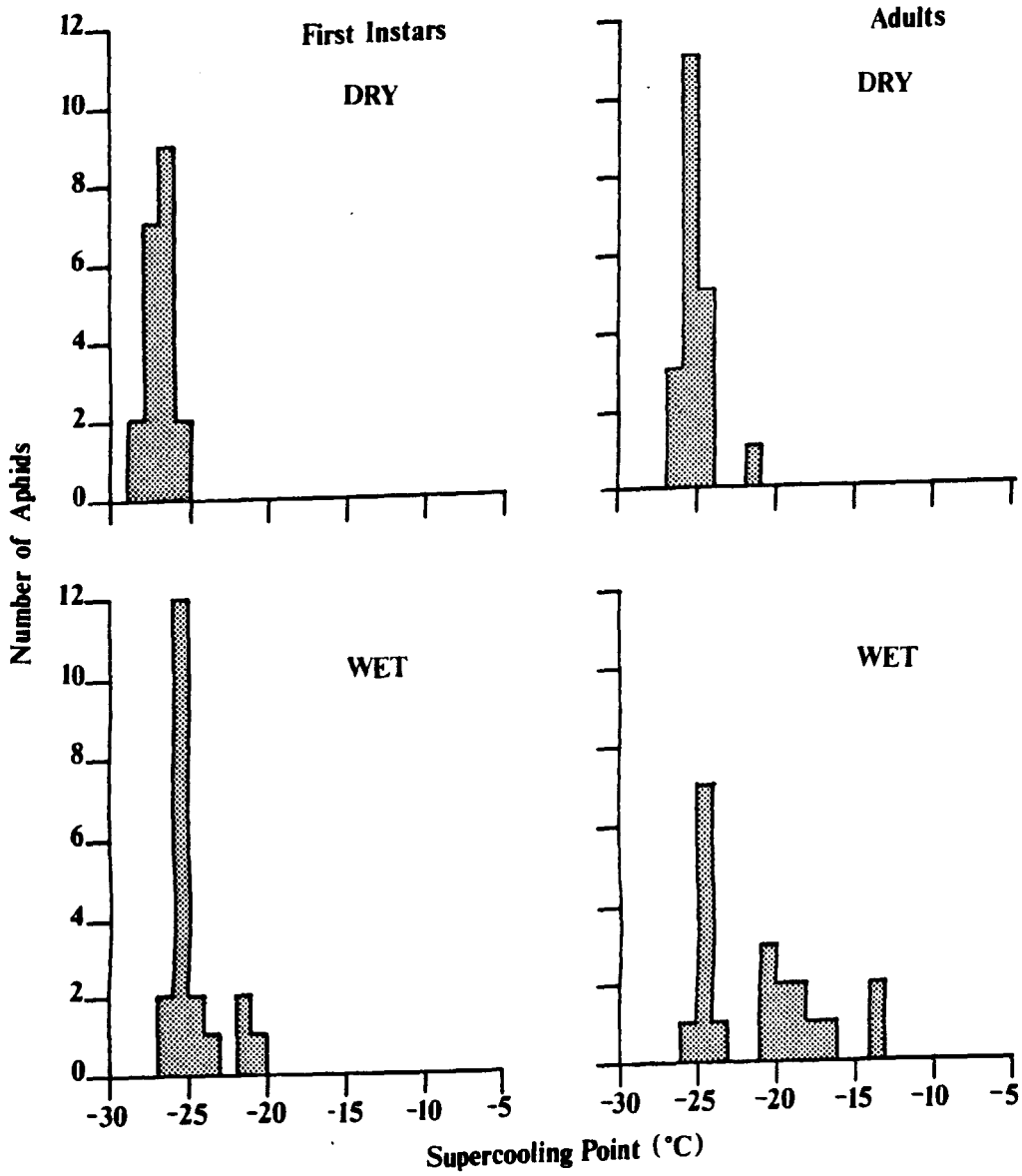

First Instars
DRY
Adults
DRY
WET
WET
Number of Aphids
0
2
4
6
8
10
12
-30
-25
-20
-15
-10
-5
Supercooling Point (°C)

Figure 3.2.

Result of repeat coolings on mortality of wetted and dry adult Sitobion avenae.

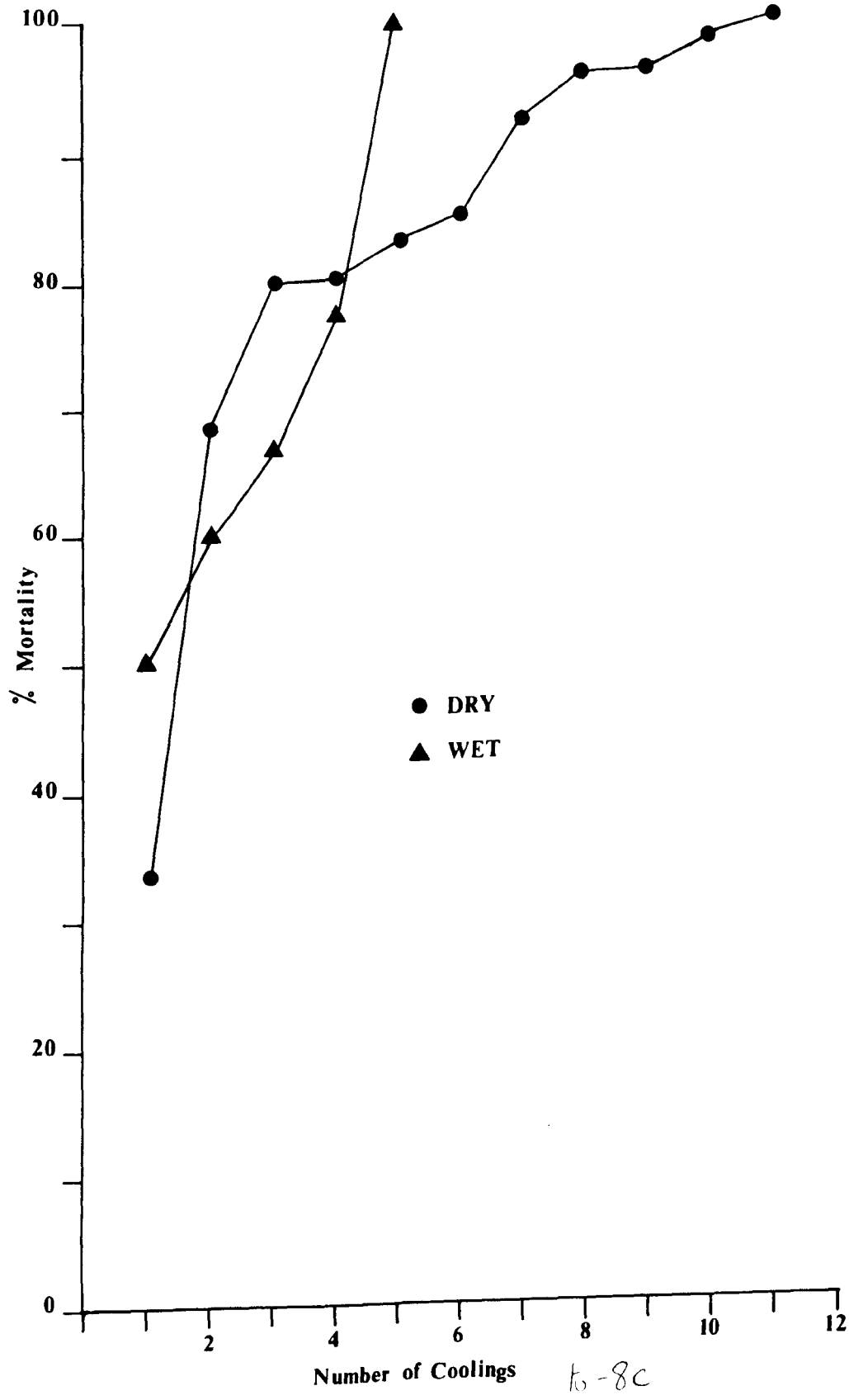

100
80
60
40
20
0
% Mortality
DRY
WET
2
4
6
8
10
12
Number of Coolings

Fig. 8c

1981); (iii) inoculative freezing above the supercooling point induced by moisture on the body surface (Salt, 1963); (iv) reduced supercooling when feeding associated with nucleators in the gut (Sømme, 1982).

The results from these experiments show that S. avenae is atypical in its supercooling characteristics compared to other freezing intolerant insects in the last three of the features described above. The fourth, a seasonal variation in supercooling ability is investigated in chapter four. Thus the aphids showed little or no acclimation response, a low incidence of inoculative freezing above the inherent supercooling point when wet, and no improvement in supercooling ability when starved. Similar results have been obtained for the aphid Myzus persicae (Sulz.) (O'Doherty and Bale, 1985) and indicate that aphids may be an atypical group of freezing intolerant insects. Therefore, any conclusions drawn from work on other freezing intolerant insects may not be applicable to aphids.

Aphids that fed at 20°C showed the greatest supercooling ability whilst those that were deprived of food had, in general, a lesser ability to supercool; a similar result was found with M. persicae adults starved for 7 days at 5°C where supercooling points ranged from -12.6°C to -25.8°C (O'Doherty and Bale, 1985). Thus it is possible that aphids have to feed in order to maintain their supercooling ability or at least to survive. The method of feeding used by aphids is highly specialised and the diet obtained from phloem sap is high in some of the

carbohydrates which are known to be cryoprotectants in other freezing intolerant insects (Danks, 1978); these substances may provide protection against freezing in aphids. In such circumstances the aphid would not need to synthesise any cryoprotectants and would not necessarily show any responses to acclimation and none of any biological significance were observed in these experiments.

The food of aphids is very different from that of most other insects and the combination of a fluid diet containing few nucleators and a high concentration of carbohydrates may account for the extensive supercooling ability exhibited by the Aphididae.

Starvation had no significant effect on the supercooling ability of the aphids which is consistent with the theory that the diet contains very few nucleators. Starvation is unlikely to modify the supercooling of aphids since, unlike many other insects, gut contents are retained during starvation rather than excreted (Auclair, 1963). The supercooling ability of Elatobium abietinum remained unaffected during enforced starvation (Powell, 1974) as did that of M. persicae first instar nymphs, although adults showed some changes (O'Doherty and Bale, 1985).

The overwintering site of S. avenae is, in general, fairly exposed and affords little protection from wind, rain or low temperatures. Thus the aphids can easily become wetted either from precipitation or dew and may, if the temperature falls sufficiently low, be exposed to the dangers of inoculative freezing. Many freezing intolerant insects die when water freezes on the body surface at

temperatures considerably above their 'dry' supercooling points, for example 'wetted' Rhynchaenus faqi (L.) supercool to only -9°C compared to -23.6°C when dry (Bale, 1980). Insects overwintering in sheltered positions, e.g. in cocoons or hibernacula, reduce the probabilty of contact moisture inoculation and so are less likely to be killed (Danks, 1978). S. avenae exposed to surface moisture on cooling showed a reduced supercooling ability compared to dry individuals. A proportion of the aphids died at temperatures above their supercooling point due to inoculative freezing but the mean did not rise above -20°C for either adults or nymphs in a single cooling. Griffiths and Wratten (1979) showed that low-temperature survival ability (LT50) of S. avenae and Rhopalosiphum padi (L.) was not influenced by contact moisture but it did reduce that of Metopolophium dirhodum (Wlk.) from -11.7°C to -7.5°C. It has been suggested that aphids with more cuticular wax have a greater resistance to inoculative freezing than those with a lesser amount (Bevan and Carter, 1980).

The laboratory experiments on the effects of surface moisture and inoculative freezing only exposed the aphids to one cooling; in the field they may experience extended periods of sub-zero temperatures and also be subject to the effects of repeated exposures. Thus unless the aphids can maintain their resistance to inoculative freezing a considerable cumulative mortality may occur. The experiment on repeat coolings to only -8°C caused 100% mortality of wetted and dry aphids after four and eleven coolings respectively. The aphids that had died did not

all appear to have frozen as they did not show the change in colouration that is normally associated with this event nor were any supercooling points detected. The conclusion from this experiment was that the aphids were dying from the effects of cold above their mean supercooling point and hence not from the effects of freezing. This phenomenon was investigated further and is described in chapter seven.

During the same winter as the laboratory experiments on the effects of starvation, acclimation and surface moisture on supercooling ability, a field population was monitored for seasonal changes in supercooling ability and also changes in population density. This work is the subject of chapter four.

CHAPTER FOUR

CHANGES IN THE SUPERCOOLING ABILITY AND DENSITY OF FIELD POPULATIONS OF *SITOBION AVENAE* DURING THE WINTERS 1983-4 and 1984-5

SUMMARY

Seasonal variation in the supercooling ability of a field population of S. avenae was investigated for two consecutive years, 1983-4 and 1984-5, and the changes in population density for one year, 1983-4. Both adults and nymphs of S. avenae showed a consistent level of supercooling from late spring until late winter at which time it decreased markedly. This persisted until the end of spring when supercooling potential was regained.

The field population showed a rapid decline in December when environmental temperatures were well above mean and individual supercooling points. It was concluded that the aphids were dying before they froze and that the supercooling point was not a reliable indicatior of the lower lethal temperature. No correlation could be found between mortality and any other metereological factor.

INTRODUCTION

The successful overwintering of *S. avenae* as active stages in the field will be dependent on its ability to develop, reproduce, and survive, under unfavourable conditions. The mortality occurring in a population may be due to predation, parasitism and disease, or the effects of the weather. Dean (1974a) found that *S. avenae* could survive the winter in sheltered positions but not in exposed situations. Periods of snow and low temperatures caused a reduction in the number of cereal aphids on perennial ryegrass (Hand, 1980). Low temperatures below about -4°C were necessary to kill *S. avenae* and *M. dirhodum* overwintering on winter wheat (Williams, 1980). For *S. avenae* to survive the low temperatures of winter it must be able to supercool to temperatures lower than those it will experience since it is a freezing intolerant insect.

Many freezing intolerant insects show a seasonal variation in supercooling (Sømme, 1982) which is associated with decreasing temperatures experienced in autumn (Lee and Baust, 1981). This seasonal change has been recorded in a number of freezing intolerant insects from temperate and colder regions (Sømme, 1981, 1982; Block, 1982). Supercooling reaches a maximum in the winter and is at a minimum in the summer months. The lowering of supercooling points can be caused by an increase in the level of cryoprotectants, a lowering in the level, or inactivation of nucleating agents in the body (Zachariassen, 1985), or starvation and the associated evacuation of gut contents.

This chapter investigates the possible correlation between metereological conditions and supercooling capacity of S. avenae throughout two winters and also changes in the population density in one of the years.

MATERIALS AND METHODS

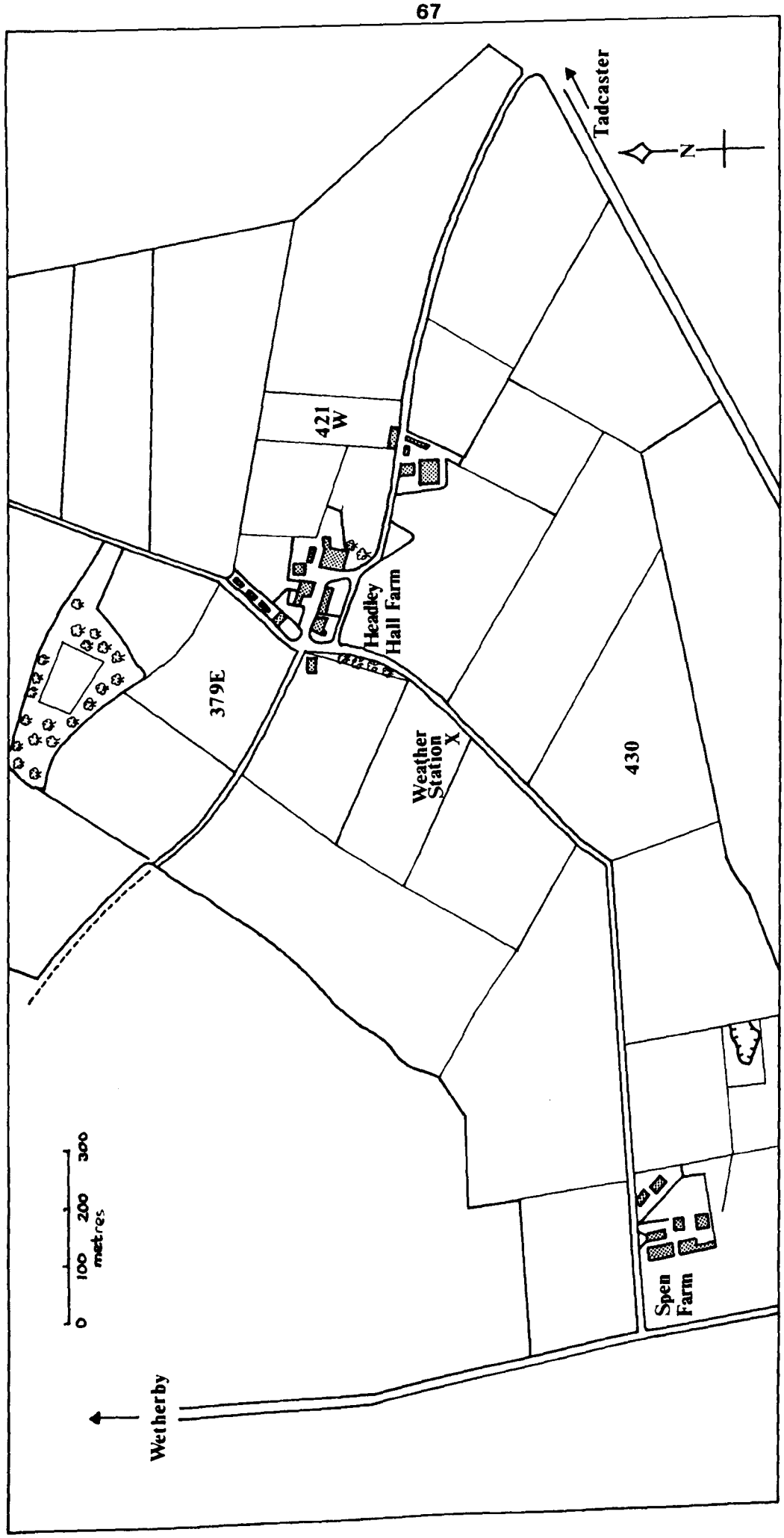
Winter 1983-4.

This field experiment monitored (i) the density of a natural population of S. avenae and (ii) the seasonal variation in the supercooling ability of first and second instar nymphs and adults drawn from the population. The experiment was conducted at the University of Leeds field station near Tadcaster North Yorkshire (Grid Ref. 445415) from November 1983 to July 1984. The sites were in fields of winter barley (var. Igri) sown on 13 September 1983 (fields 379E and 421W, Figure 4.1). The crop was sprayed with a pyrethroid insecticide ('Ambush' ICI) on 29th November 1983 for the control of cereal aphids carrying barley yellow dwarf virus. Unsprayed strips were left in the experimental fields to enable sampling of the natural populations as described. The unsprayed strips were 12m wide by approximately 120m long.

The population density was estimated at fortnightly intervals by examining 10 groups of 20 tillers, selected at random, and recording the number of aphids present. The selection of tillers was randomised by throwing a marker into the crop and examining the the nearest 20 tillers from the row in which the marker had landed.

Figure 4.1.

Map of the University field station showing the location of the experimental plots and weather station.


Tadcaster
N
421
W
Headley
Hall Farm
379E
Weather
Station
X
430
Spen
Farm
0
100
200
300
metres
Wetherby

The supercooling points of the aphids were determined according to the method described in chapter 2. The aphids used in the supercooling point determinations were collected by hand at monthly intervals; a target sample size of 40 adults and 40 nymphs was set. Due to the reduction in the population density that occurred as the experiment progressed actual sample sizes were sometimes smaller than this. Aphids for supercooling point determination were collected from a different area to that used for the population estimates. During March and April the population density became so low that hand collection was no longer practicable and consequently a vacuum insect sampler (Burkhard) was used to obtain sufficient numbers of aphids. The use of this machine coincided with a marked reduction in supercooling so a controlled experiment was set up to assess the effect of such mechanical sampling on supercooling ability. Laboratory reared aphids were vacuum sampled from plants and their mean supercooling point compared with that of aphids from the same plants that had been collected by hand. Metereological data were collected at the University farm weather station approximately 500m from the experimental sites.

Winter 1984-5.

The field experiment in this year was conducted at the University farm in field 430 (Figure 4.1). The crop of winter barley (var. Igri) was sown on the 18 September 1984. The experiment began in September 1984 and continued until June 1985. Samples of aphids were taken at approximately fortnightly intervals in the autumn and early

winter and then at monthly intervals as the population density decreased in late winter and spring. The vacuum sampler was used from January until March. During April and May the population density was so low that insufficient numbers of aphids could be collected to obtain a meaningful measure of their supercooling ability. Metereological data were recorded as in the previous year.

RESULTS

The changes in population density and the mean monthly grass minimum temperature with time for 1983-4 are shown in figures 4.2a and 4.2b respectively. The results for the changes in the supercooling points for both 1983-4 and 1984-5 are shown in figure 4.3. In both periods adults and nymphs collected from the field showed a marked seasonal variation in their mean supercooling point. In the first winter the adult mean supercooling points remained below -20°C in all samples with the exception of -19.8°C and -15.8°C for those in February and March; the mean supercooling points of the nymphs were always lower than those of the adults and only rose above -20°C in the March sample (-18.8°C). No nymphs could be found in the field during April. In the second winter, adult mean supercooling points remained below -20°C in all samples except those from the end of January until the middle of March (-16.8°C and -19.0°C respectively). Mean supercooling points of nymphs remained below -20°C for all periods except the sample at the end of January (-18.3°C).

Figure 4.2a.

Mean monthly grass minimum temperature recorded at the University field station, September 1983 to July 1984.

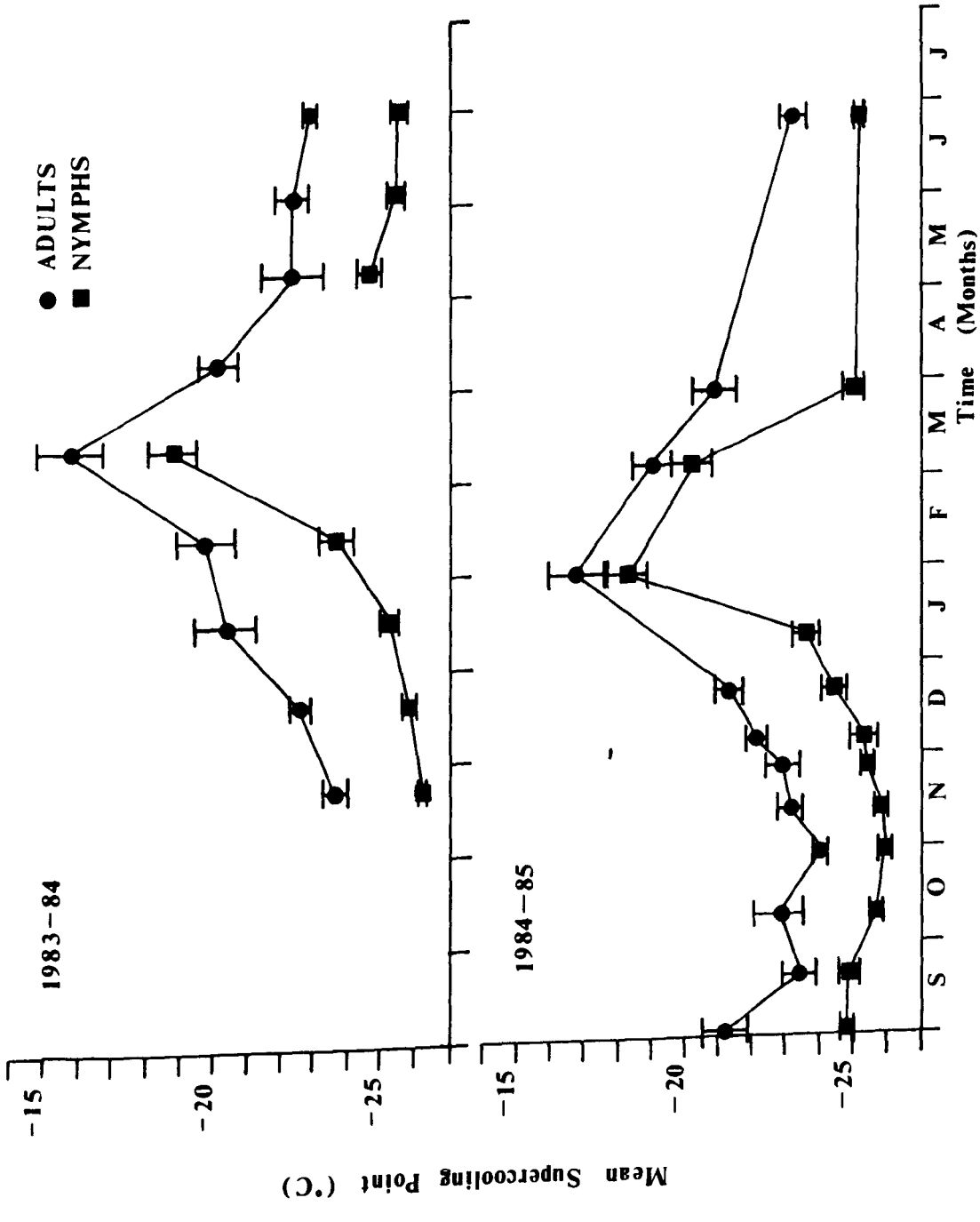
Figure 4.2b.

Number of Sitobion avenae found per 100 tillers on each sampling occasion.

A
B
10
5
0
-5
Mean Monthly
Temperature (°C)
30
20
10
Aphids / 100 Tillers
S
O
N
D
J
F
M
A
M
J
J
Time (Months)

Figure 4.3.

Mean supercooling point (±S.E.) of adult apterae and nymphs of *Sitobion avenae* collected from the field in 1983-4 and 1984-5.


● ADULTS
■ NYMPHS
1983–84
1984–85
-15
-20
-25
S
O
N
D
J
F
M
A
M
J
J
Time (Months)
Mean Supercooling Point (°C)

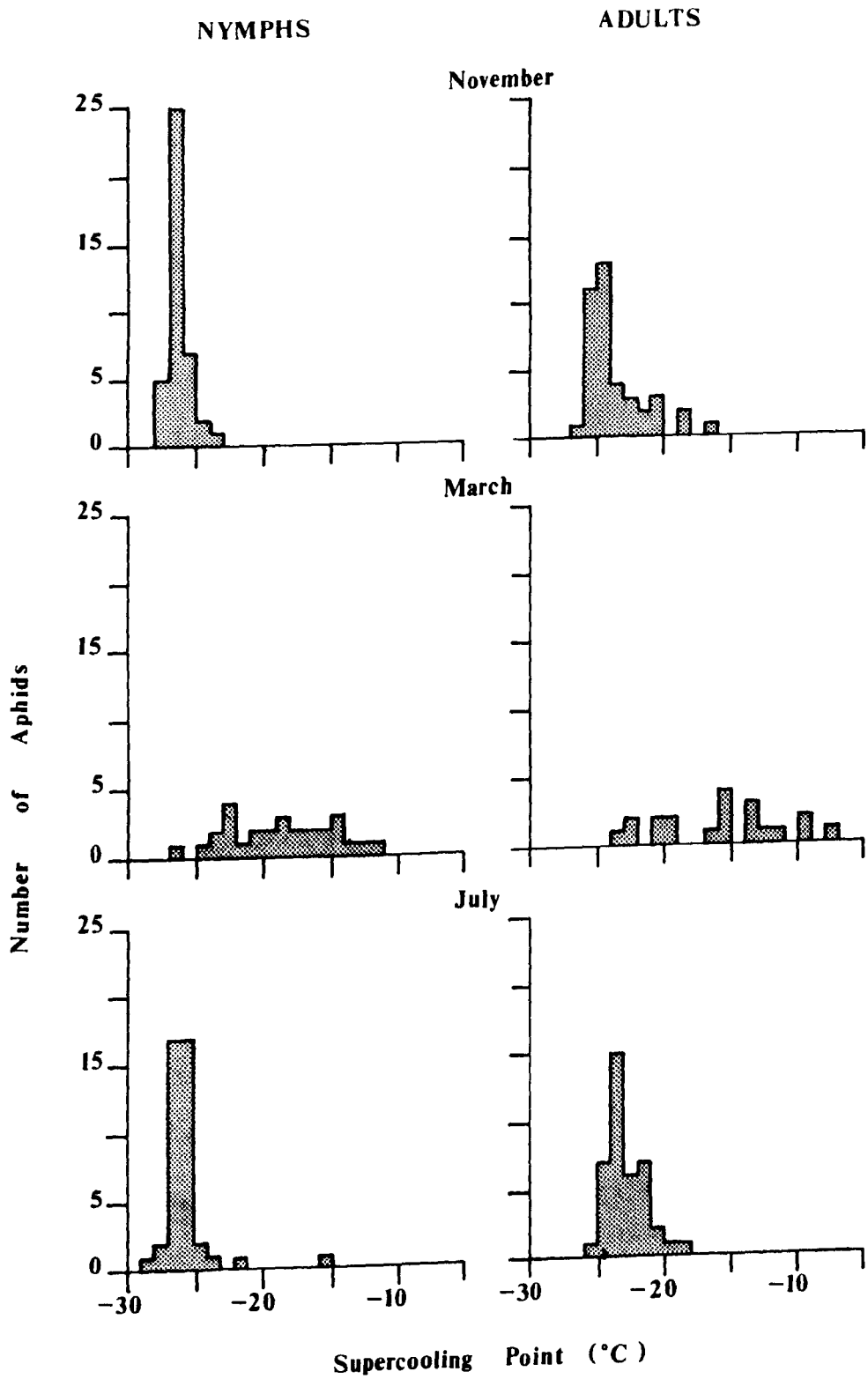
The nymphs again supercooled more extensively than the adults at all times. No adults could be found in mid-January and neither nymphs nor adults in April or May. The pattern of supercooling was therefore similar in most respects in both years. Frequency histograms of the supercooling points of monthly samples in winter and early spring were skewed but there were no significant deviations from normal (figure 4.4).

Both the adult mean monthly supercooling point and the population density were positively and linearly related to the mean monthly grass minimum temperature occurring two months previously ($r = 0.93$, 3df., $P < 0.05$ and $r = 0.92$, 3df., $p < 0.05$ respectively) for the first five months of the experiment in 1983-4. In 1984-5 the adult mean monthly supercooling points were positively and linearly related to the mean monthly grass minimum temperature occuring one month previously ($r = 0.84$, 5df., $P < 0.02$). No such relationships could be found with the nymph mean monthly supercooling points. There were also no other relationships between any of the aphid parameters measured and any other metereological data.

The control experiment showed that there was no significant difference between the mean supercooling points of laboratory aphids collected by hand and those that were collected using the vacuum sampler ($t = 1.85$, 32df.).

Figure 4.4.

Supercooling point distribution histograms of Sitobion avenae, nymphs and adult apterae, showing seasonal changes.


NYMPHS
ADULTS
November
March
July
25
15
5
0
Number of Aphids
−30
−20
−10
Supercooling Point (°C)

DISCUSSION

The supercooling of S. avenae shows a seasonal variation but the change is counterintuitive; instead of supercooling capacity increasing in winter it decreases. S. avenae is therefore atypical compared to other freezing intolerant insects. S. avenae nymphs always had lower mean supercooling points than the adults but this may simply be a result of the different volumes of the two stages. Small volumes of water supercool to lower temperatures than do larger ones as the probability of nucleating agents being present is reduced.

The field population showed a rapid decline during the early winter period when the mean supercooling points for both adults and nymphs were below -20°C; the temperatures in the field at this time had only fallen to a minimum of -11°C. The supercooling points of individual aphids were all below this temperature and therefore there should have been little or no mortality due to freezing deaths. In fact the population decreased by 84% during the month of December which implies that the mortality was not due to freezing. Since the decline in the population could not be correlated with rainfall or windspeed it appeared that low temperature was the main factor responsible for the reduction in population level. However the reduction could have been partly due to the effects of predation, parasitism, or disease, although none of these factors were evident during the monitoring of the aphid population.

The strong correlation between the mean monthly grass

minimum temperature and both the mean adult supercooling points and changes in population density occuring two months later may be due to the low temperatures affecting feeding and consequently the maintenance of normal physiological processes. In such circumstances a lag relationship may be expected between temperature and its influence on metabolic related processes such as supercooling ability.

It is possible that aphids can accumulate a 'cold dose' leading to a freezing death (Salt, 1950) but the laboratory experiments of the previous chapter indicated that aphids die after being exposed for just a few moments at temperatures of only -8°C when wet and not from freezing. There is no doubt that aphid populations decline markedly in winter and some mortality, albeit a very small proportion, may occur through freezing as a result of inadequate supercooling ability. The non-freezing mortality occuring above the supercooling point, also described by Williams (1984) in S. avenae and other cereal aphids, is more likely to be due to 'cold injury' (Ring, 1980). This cold injury make take the form of membrane disruption, through changes in lipid fluidity or protein denaturation, changes in enzyme kinetics, starvation or desiccation. In contrast to the active stages, the overwintering eggs of aphids appear to be very cold hardy with supercooling points as low as -43°C and with an ability to acclimate (James and Luff, 1982). These eggs should ensure the successful overwintering of the species in severe winters although the adults and nymphs will

perish. There have however been reports of quite high levels of mortality in overwintering eggs of R.padi on bird cherry trees which are due to factors other than predation and parasitism (Leather, 1981). The cause of the mortality of S. avenae during the winter could not safely be attributed to the effects of low temperatures alone so an experiment to monitor individual aphids over the winter was designed and this is the subject of chapter 5.

CHAPTER FIVE

THE DEVELOPMENT, REPRODUCTION AND SURVIVAL OF OVERWINTERING *SITOBION AVENAE* ON WINTER BARLEY IN THE FIELD

SUMMARY

The development, reproduction and mortality of adult and first instars of *Sitobion avenae* in clip cages in a field of winter barley were studied throughout the winter of 1984-85. Development and reproduction were directly and positively related to temperature whilst mortality increased with decreasing temperature with adults showing high levels of mortality below -8°C. Some individuals survived down to -11.8°C indicating the potential for survival in anholocyclic clones. Aphids which survive and are active in winter can spread virus diseases such as BYDV and also form the nucleus of a new population in the spring. Populations of overwintering anholocyclic virginoparae give rise to alates which migrate earlier in spring than aphids derived from eggs and are more likely to be viruliferous when alighting in new sown crops. The high mortality of *S. avenae* recorded during the winter occurred at temperatures well above the supercooling points of individuals sampled from the same field.

INTRODUCTION

Studies on the cold hardiness of insects have generally concentrated on supercooling and modifying influences such as feeding and surface moisture. Most experiments have been conducted at a cooling rate of 1 deg per min to facilitate comparisons between species and experimentors. In nature however cooling rates are not consistent and rarely at the same rate as that used in the laboratory; in addition climatic factors may act in combination in the field. The relevance of such laboratory data to the natural environment can be investigated by comparing the results obtained with those from field populations and individuals exposed to natural fluctuations in climatic factors such as temperature, precipitation and windspeed. If mortality in the field occurs at the same temperature as the supercooling point measured in the laboratory, then supercooling data can be used as a reliable index of insect cold hardiness, at least in response to short term exposures.

Much work has been done on the effects of summer temperatures on the rate of development, reproduction and survival of cereal aphids (Dean, 1974b; Campbell et al, 1974; Cannon, 1984; Lykouresis, 1985) but less is known about the effects of the low temperatures that are experienced over the winter period. Other studies on aphids (Messenger, 1964; Harrison and Barlow, 1972; Wood and Starks, 1972) have suggested that development at low fluctuating temperatures cannot be predicted by

extrapolation from results obtained at relatively high constant temperatures, the object of the experiments discussed in this chapter was to obtain such data for S. avenae from a population on winter barley in the north of England. Data on the rate of development, reproduction and lifespan of S. avenae on winter wheat in the south of England has recently become available (Williams and Wratten, 1987; Williams, 1987) and allows useful and interesting comparisons.

Winter cold which would be expected to cause a slowing of development, could cause an increase in mortality and consequently a reduction in population density. Similarly a reduction in the rate of reproduction due to low temperatures would reduce recruitment to the population again affecting the overall density. Low densities of aphids during the summer of 1978 have been attributed to the low level of spring immigration into the crop (Carter et al, 1980) implying that the number of aphids or eggs that overwinter successfully can determine the population density the following year. This emphasises the need for a better understanding of the winter biology of cereal aphids.

The prediction of years when aphid outbreaks are likely due to high overwintering survival, requires a detailed knowledge, relating climate to mortality and the subsequent performance of the surviving population and this can only be gained from a combination of field and laboratory experiments.

MATERIALS AND METHODS

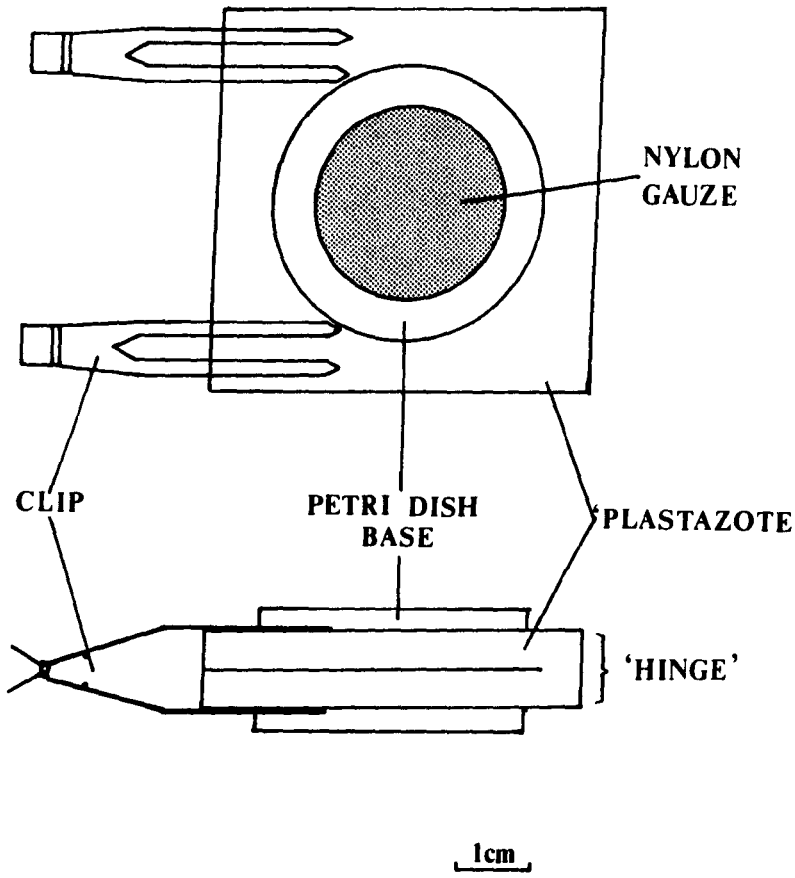
This experiment was carried out during the winter of 1984-5 at the University field station. The experimental site was in an exposed position in field 430 and occupied an area of 24 m by 190 m. The crop was winter barley (var. Igri) sown on 18th September. The aphids used in the experiment were taken from the clonal culture reared on whole plants (method I) at 20°C±2°C and 18L:6D. First instars used in the experiment were collected over a 24 hour period from apterous adults which had already commenced reproduction so that no atypical first born nymphs were used (Murdie, 1969). The adults that were used in the experiment were raised from nymphs produced as above but were then reared to adults at 20°C±2°C on whole plants (method IV). The aphids that moulted to adults over a 24 hour period were used in the experiment. All the aphids were acclimated at 10°C±1°C for 2 days prior to being placed in the field.

Three batches of aphids each consisiting of 100 newly moulted adults and 100 first instar nymphs were placed in the field. The first group was put out on October 26th, the second on December 10th, and the third and final group on February 20th. The adults in the first batch consisted of 50 alatae and 50 apterae as both morphs were present in the wild populations at this time; the later two groups of adults were entirely apterous.

To remove the effects of predation and parasitism each aphid was placed in a separate, individually numbered, clip

Figure 5.1.

Illustration of clip cage used in field experiments.


NYLON
GAUZE
CLIP
PETRI DISH
BASE
'PLASTAZOTE'
'HINGE'
1cm

cage (figure 5.1) which contained a barley leaf attached to a growing plant (plate 7). The aphids were inspected at weekly intervals (weather permitting) and any moults, deaths or offspring recorded. Nymphs were removed from the cages containing reproducing adults to prevent overcrowding and simplify the counting of new offspring. The cages were moved to new leaves every few weeks to prevent any effects from senescing or diseased leaves. The positions of the cages in the field were marked with canes (Plate 8). Weather data was recorded as in chapter 4. The temperatures were converted to accumulated temperature (day degrees) to investigate the relationship between this and rates of development and reproduction.

The day degree values were calculated from the following formulae using a threshold for development of 3°C. The threshold of 3°C was chosen on the basis of previous studies with the same species conducted by Williams and Wratten (1987). The values were summed for each sampling period.

Calculation of day degree values (Anon, 1943).

D=Developmental threshold. Min=Grass minimum temperature. Max=Screen maximum temperature. DD=Day degrees.

If $Min > D$ then $DD=(Max+Min)-D$

If $Max > D > Min$ and $Max-D > D-Min$ then $DD=\frac{Max-D}{2} - \frac{D-Min}{4}$

If $Max > D > Min$ and $Max-D < D-Min$ then $DD=\frac{Max-D}{4}$

If $Max < D$ then $DD=0$

Plate 7. Close-up of clip cage used in the field experiments, showing position of leaf (top part of cage removed).

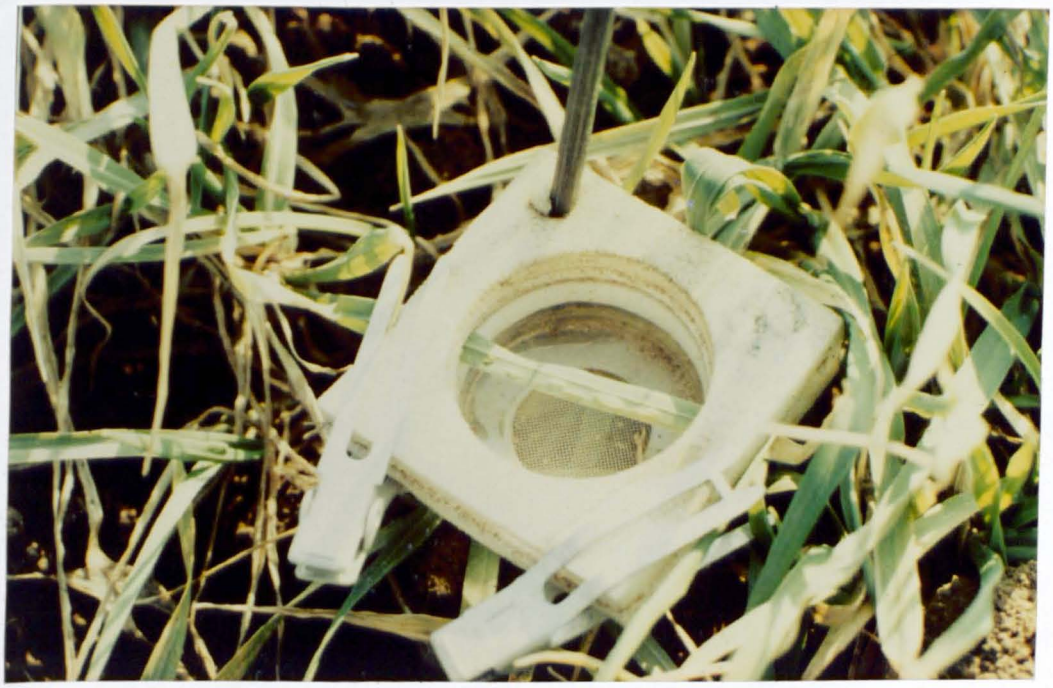


Plate 8. Site of overwintering experiments, canes mark the position of clip cages.

The temperature inside the clip cage was monitored with a Grant Recorder using DS type probes and compared with the temperature recorded outside the cage with a similar probe.

RESULTS

In this field study aphids were inspected at intervals of approximately 7 days. The exact dates of birth, moults or death were not known but for the benefit of the analysis and presentation of data were assumed to have occurred at the mid point between sampling occasions. Since development and reproduction were relatively slow under the winter field conditions these approximations represent very small errors. The temperatures recorded inside the clipcages varied by a maximum of ±0.5°C from the temperature recorded on the outside and was considered to be insignificant.

The rate of development was seen to be directly and positively related to temperature. In the first batch some nymphs had developed to adults in 4 weeks, whereas in the second and third batches the times required were 11 and 5 weeks respectively (Fig. 5.2). The rate of development expressed as the number of moults per aphid per sample period was linearly and positively correlated with the sum of day degrees above 3°C (DD$>$3°C) ($r = 0.60$ 17 d.f. $P < 0.01$; Fig. 5.3). The mean thermal time requirement for the aphids to develop from nymph to adult was 142.6 DD $>$3°C (Table 5.1). This figure was obtained by summing the

Figure 5.2.

Breakdown of development of the three batches of Sitobion avenae placed in the field and the mean grass minimum temperature over the same period.

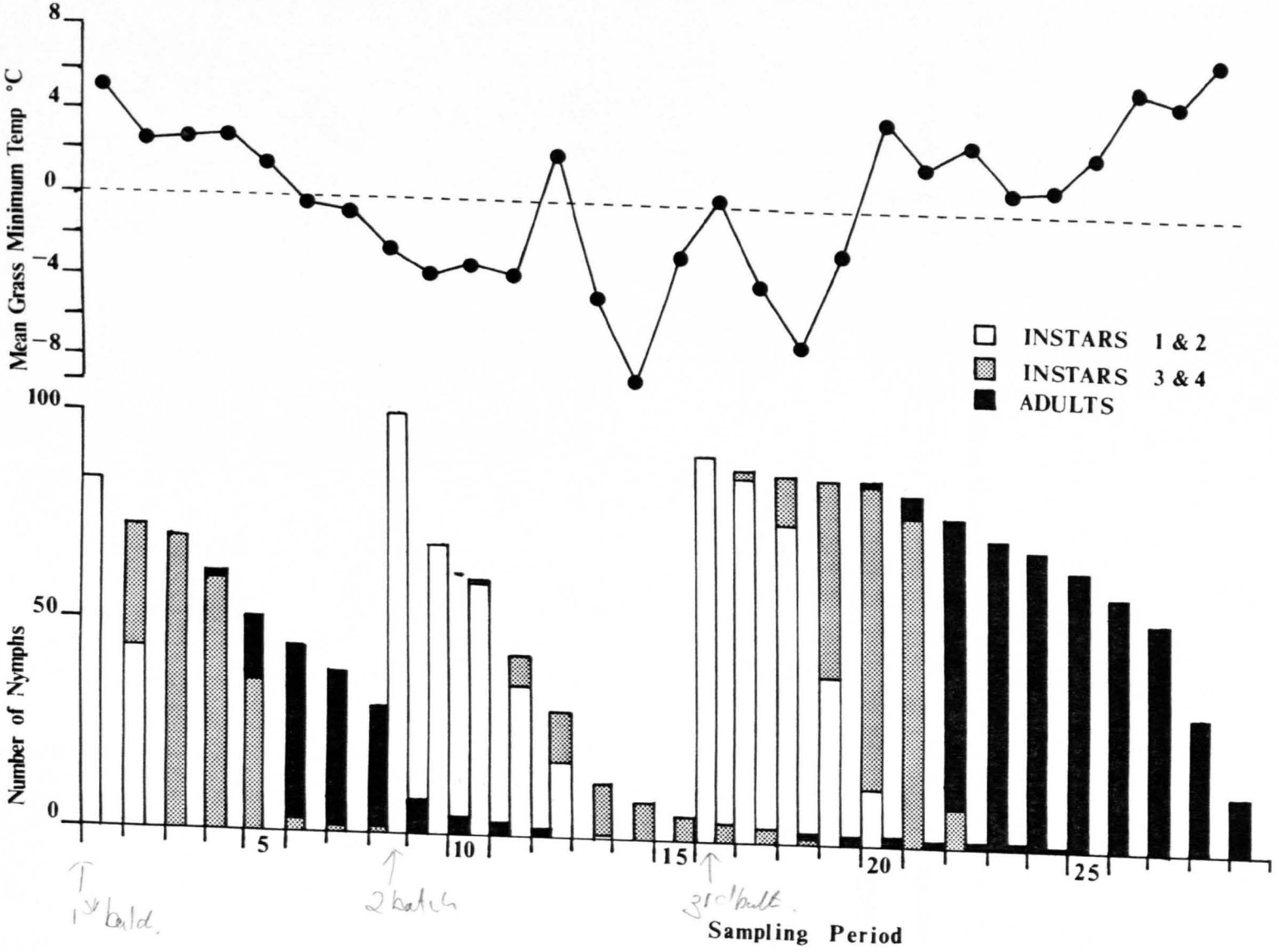

Mean Grass Minimum Temp °C
8
4
0
−4
−8
INSTARS 1 & 2
INSTARS 3 & 4
ADULTS
100
50
0
Number of Nymphs
5
10
15
20
25
1st bald.
2 batch
3rd/adult.
Sampling Period

Figure 5.3.

The number of moults per Sitobion avenae nymph during each sample period plotted against the number of day degrees above 3°C for the same period.

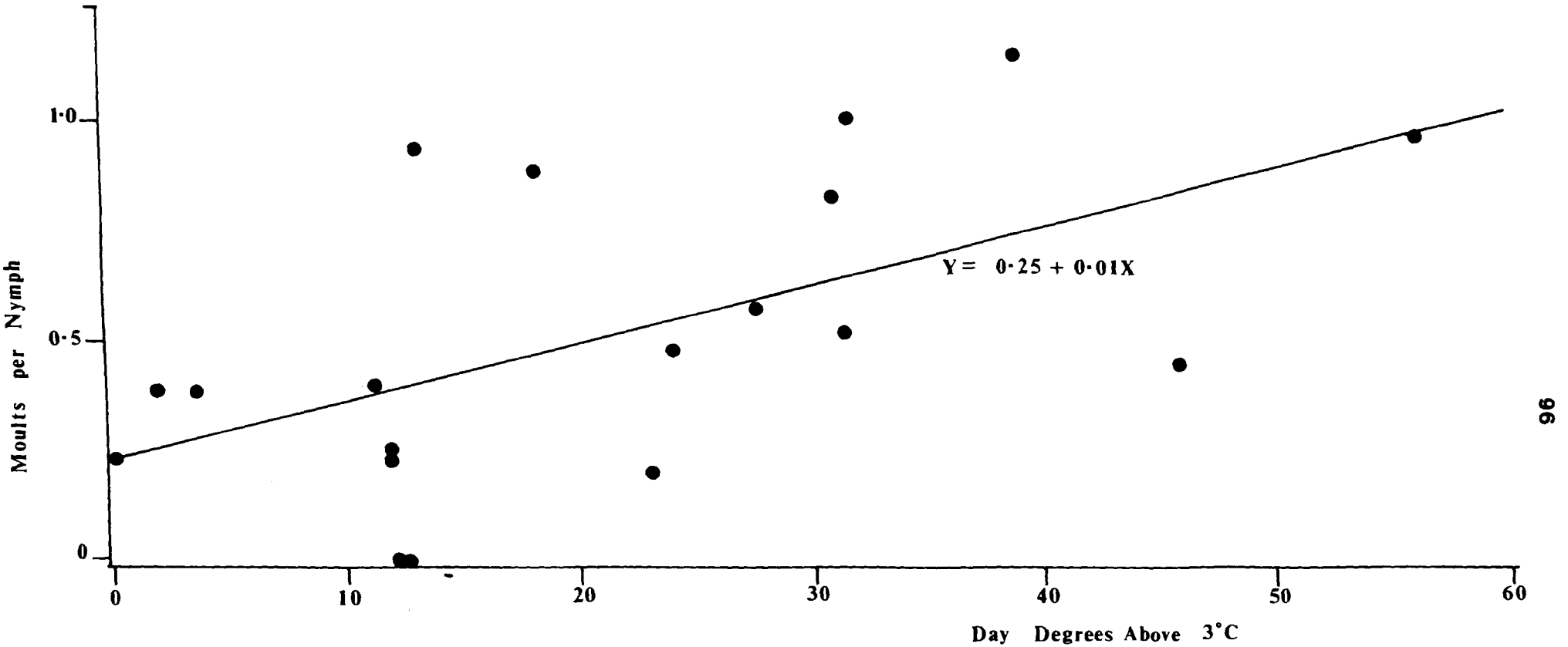

1·0
0·5
0
Moults per Nymph
Y = 0·25 + 0·01X
0
10
20
30
40
50
60
Day Degrees Above 3°C

TABLE 5.1. Thermal time requirement for the development of Sitobion avenae first instar to adult (x ±S.E. N=120).

Day degrees above 3°C in field	111.6 ±3.4
Day degrees above 3°C in laboratory	31.0
Total day degrees above 3°C	142.6 ±3.4

TABLE 5.2. Mean reproductive rate of Sitobion avenae in field experiment 1984-85 ($\bar{x}$ ±S.E.).

	Nymphs/period/ day degree above 3°C
Aphids placed as adults (N=1548)	0.113 ±0.007
Aphids placed as nymphs (N=499)	0.106 ±0.006

number of DD >3°C for the time the aphid was in the field developing to adult and adding a further 31 DD >3°C for the initial period spent at 20°C and 10°C in the laboratory.

Reproductive rate was directly and positively related to temperature. Mean reproduction measured as the number of births per adult per sample period was linearly and positively correlated with the sum of DD >3°C for the sample period ($r = 0.62$ 23 d.f. $P < 0.01$; Fig. 5.4). The mean reproductive rate of aphids placed in the field as adults, expressed as nymphs per DD >3°C was calculated as 0.113, and for the aphids that were placed in the field as nymphs, the value was 0.106 (Table 5.2). The total number of nymphs produced per aphid varied between 4.3 and 23.8 for the three batches (Table 5.2).

The relationship between mortality and grass minimum temperature is shown in Figure 5.5 (curve fitted by eye). The rate of change in aphid numbers is calculated from the following formula (Harrington and Cheng, 1984);

$$\frac{\log N_2 - \log N_1}{t} = \text{Rate of change}$$

where N_1 = number of aphids in first sample

N_2 = number of aphids in second sample

t = time (in days) between samples

The results indicate that mortality is inversely related to temperature and increases rapidly at temperatures below about -8°C (Fig. 5.5). When the sampling periods were grouped according to minimum

Figure 5.4.

The number of births per *Sitobion avenae* adult during each sample period plotted against the number of day degrees above 3°C for the same period.

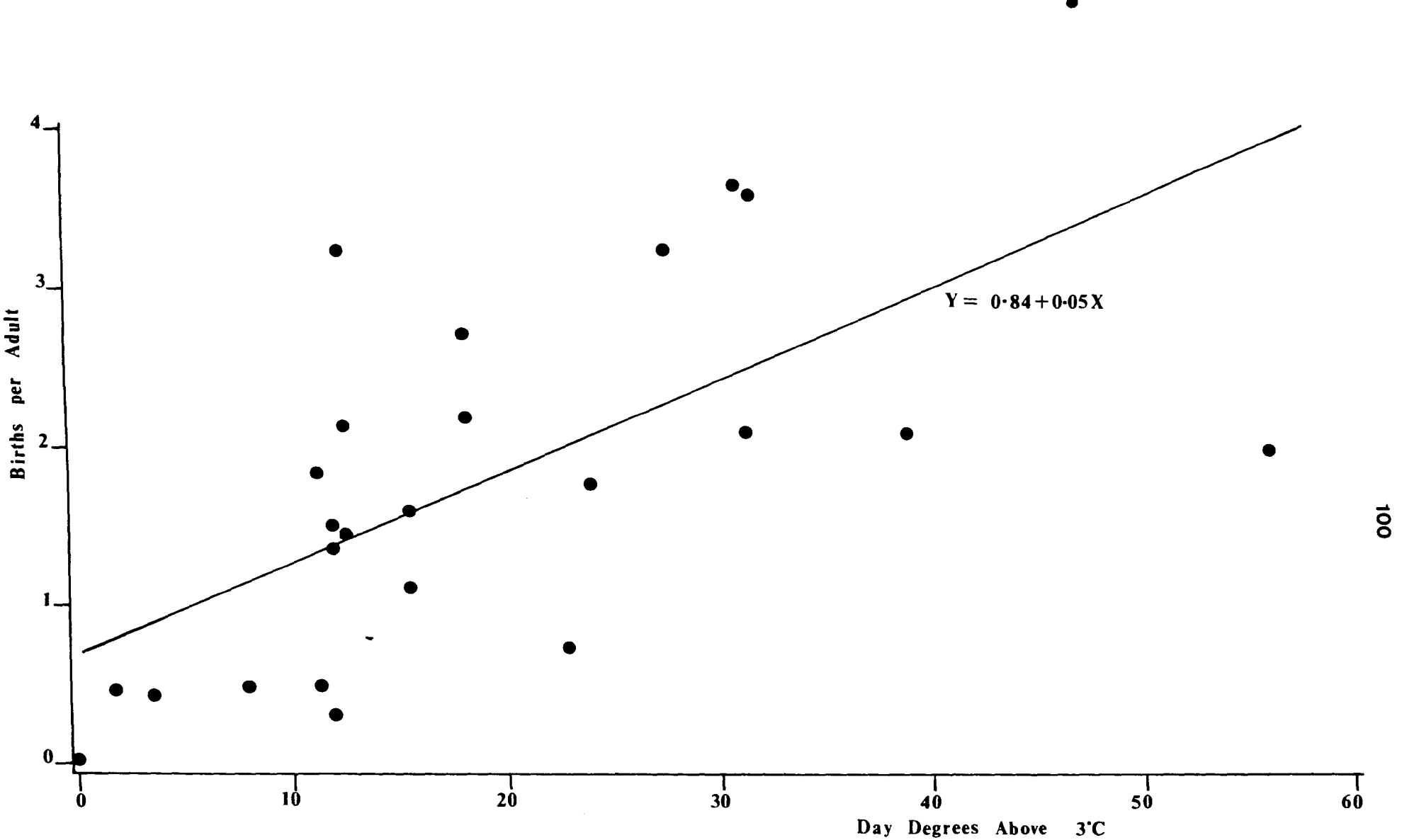
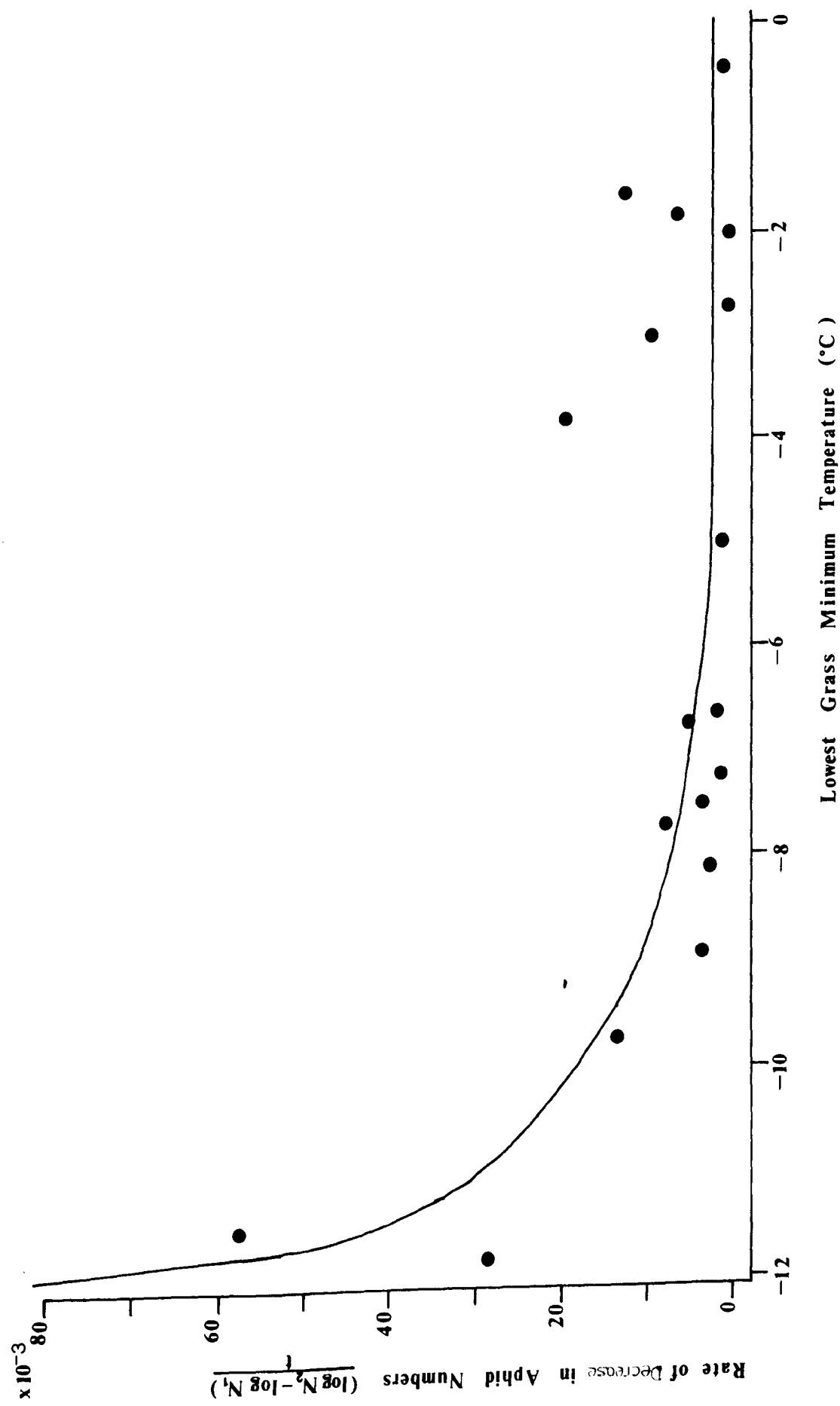

Births per Adult
4
3
2
1
0
Y = 0·84 + 0·05X
0
10
20
30
40
50
60
Day Degrees Above 3°C

TABLE 5.3. Total nymph production per adult ($\bar{x}$ ±S.E. and maximum and minimum)

		Adults		Nymphs
Batch 1	Apterae N=50	8.4 ±0.9	N=41	5.2 ±0.5
		(0/27)		(0/14)
	Alatae N=43	4.4 ±0.3		
Batch 2	Apterae N=97	5.2 ±0.3	N=2	7.5 ±1.5
		(0/17)		(5/9)
Batch 3	Apterae N=87	23.8 ±1.2	N=75	15.7 ±0.8
		(0/46)		(0/41)

FIGURE 5.5.

Rate of change in numbers of Sitobion avenae against lowest grass minimum temperature.


Rate of Decrease in Aphid Numbers $\frac{(\log N_2 - \log N_1)}{t}$
$\times 10^{-3}$
80
60
40
20
0
−12
−10
−8
−6
−4
−2
0
Lowest Grass Minimum Temperature (°C)

temperature and calculations made on the the rate of change of aphid numbers, there was a significant correlation between mortality of adults and grass minimum temperature (Spearmans rank correlation coefficient = 0.42, n=19 $P < 0.05$) but not for nymphal mortality.

DISCUSSION

The results of this chapter show that S. avenae can develop, reproduce and survive in all but the most severe winter conditions. Whilst it is not unexpected to find that development and reproduction are directly related to temperature it is perhaps surprising to find that the relationship is linear. It is widely accepted that the relationship between insect development rate and temperature is a sigmoid curve approximating to a straight line only at intermediate temperatures (e.g. Wigglesworth, 1965; Chapman, 1971; Gilbert et al, 1976). The results recorded here are in accordance with the findings of Williams and Wratten (1987) that the rate-temperature relationships for development and reproduction were well described by straight lines when temperatures were measured above 3°C and postulated that this occurs because the error zone, where the development-temperature relationship is curved, is narrow relative to the normal fluctuations in day and night temperatures in winter. This means that aphids will only experience error zone temperatures for a small proportion of their development. During periods of low temperature their development is effectively arrested;

at higher temperatures the relationship is close to linear. The thermal time requirement for development from nymph to adult calculated from this study also agrees with the result of Williams and Wratten (1987); this indicates that rates of development of S. avenae in winter on both wheat and barley are similar, simplifying any forecasting system. The reproductive rate and total nymph production was much lower than in studies on S. avenae conducted at higher temperatures. At temperatures between 10°C and 20°C the total number of nymphs produced is between about 33 and 60 (Markkula and Myllymäki, 1963; Dean, 1973, 1974 a and b; Wratten, 1977). When aphids were exposed to lower temperatures (Dean, 1974a; Williams and Wratten, 1987) conflicting results have been obtained; in the first study, nymph production was reduced, while in the second the reproduction was not markedly different to that at higher temperatures. This study shows a much reduced level of reproduction during the winter period similar to Dean (1974a) and the difference from the results of Williams and Wratten (1987) could be due to number of factors. Firstly, the experiment was carried out on barley and not wheat. Secondly, adult aphids were acclimated only briefly before being placed in the field and the rapid change in conditions may have arrested embryo development, although it is worth noting that nymphs which were raised in the field also showed much lower levels of reproduction. Thirdly, the temperatures experienced by the aphids in this study were substantially lower than in that of Williams and Wratten (1987). Furthermore few of the aphids showed any

post-reproductive period indicating that they may have perished before reaching their full reproductive potential.

The mortality of S.avenae in the field appears to be due mainly to the effects of low temperatures. It should perhaps be noted that clip cages may give some protection from wind, rain and natural enemies. Minimum temperature is the only weather parameter with a statistically significant effect on the mortality of S. avenae (Williams, 1980) whilst rainfall and temperature have the greatest effect on mortality of Myzus persicae in the field (Harrington and Cheng, 1984). This study and others (Hand,1980; Williams, 1980; Williams and Wratten, 1987) have revealed that temperatures much above those required to cause death by freezing i.e. the supercooling points measured in chapter 3, have produced high levels of mortality in field populations of S. avenae. Wind and rain may have an indirect effect on mortality by dislodging aphids from plants and then low temperatures could prevent the aphid regaining a feeding position on the plant. Smith (1981) has stated that temperatures of -1°C prevented the movement of first instar S. avenae whilst at -4°C no aphids were able to move. Therefore during prolonged periods of low temperature aphids may die of starvation or be eaten by predators that are able to move at these low temperatures. At extreme low temperatures a direct effect on mortality can be expected and this was observed in February 1985 when aphids were found hanging from leaves with stylets still inserted. Because of their position these aphids would almost certainly have been killed by the

effects of cold and not wind and rain.

The results indicate that aphids can respond quickly to short periods of higher temperatures and resume reproduction. This ability results in an irregular production of nymphs in short periods of warmer weather, but this may be very important to the overall success of anholocyclic overwintering of S. avenae, particularly because nymphs are more cold hardy than adults. The second batch of nymphs placed in the field in December showed very poor survival but even if only 1% survives to adult and produces some nymphs in late winter and early spring, then their offspring have a much greater chance of survival judged by the performance of the third batch of aphids put out in February, and will produce many offspring in the warm weather of late spring and thus lead to an early build up in numbers and a likely pest outbreak.

The mortality of adults appeared to occur around a threshold of about -8°C whilst nymphs did not show high levels of mortality until the temperature fell below -11.8°C, the lowest recorded temperature during the experiment. This would indicate that although nymphs are more cold hardy than adults, for both age groups, mortality occurs at temperatures much above their supercooling points.

It was concluded that experiments were necessary to determine the lower lethal temperatures of different age groups of S. avenae and this research is described in chapter 7. The research described in this chapter has been published in part as Knight and Bale (1987).

CHAPTER SIX

DEVELOPMENT OF A SYSTEM FOR THE DETECTION OF LOW LEVEL THERMAL ENERGY CHANGES IN APHIDS

SUMMARY

An instrument was developed in conjunction with R.A Stevenson of Stonegate Instruments for the detection of very small changes in thermal energy during the study of the effects of low temperatures on aphids.

The instrument comprises 6 thermistors to monitor the temperature of the aphids (or any other specimens); each thermistor can be individually calibrated and the signal is then amplified before it goes to the output. When used in this mode the instrument can measure supercooling points of very small specimens. In the differential mode the unit can measure extremely small changes in temperature. The instrument is suitable for use with any cooling system and the output can be recorded directly on a chart recorder or, via an analog-digital converter, on a micro-computer using a suitable program. The instrument is inexpensive to construct, has proved to be reliable and has the advantage of being able to handle relatively large specimens compared to a differential scanning calorimeter. It is hoped that this instrument will be of use in many areas of cryobiology.

INTRODUCTION

The measurement of supercooling points has been of interest since Salt (1961) first proposed a standardised method for their determination. This allowed comparison between results from different experiments and hence the cold-hardiness of one species could be compared with that of another.

A range of methods are used to cool specimens for supercooling point measurements but most systems employ thermocouples as the temperature sensors because of their small size, low price and robust nature (e.g. Sømme 1964; Block & Young 1979; Ring 1980; Bale et al. 1984). There is, however, a limit to their sensitivity and during the course of this study it became neccessary to be able to detect very small thermal energy changes that occurred in the aphids at temperatures above their supercooling points. Initial experiments were carried out on a Differential Scanning Calorimeter (DSC) in collaboration with Professor Felix Franks of the Botany Department at Cambridge University. A suitable instrument with the appropriate sub-ambient attachment was not available at Leeds University. It was because of this that a system capable of detecting such low-level energy changes was developed. The cooling system employed was the same as that used in the determination of supercooling points but the thermocouple temperature sensors were replaced by a system employing micro-bead thermistors capable of detecting much smaller temperature changes.

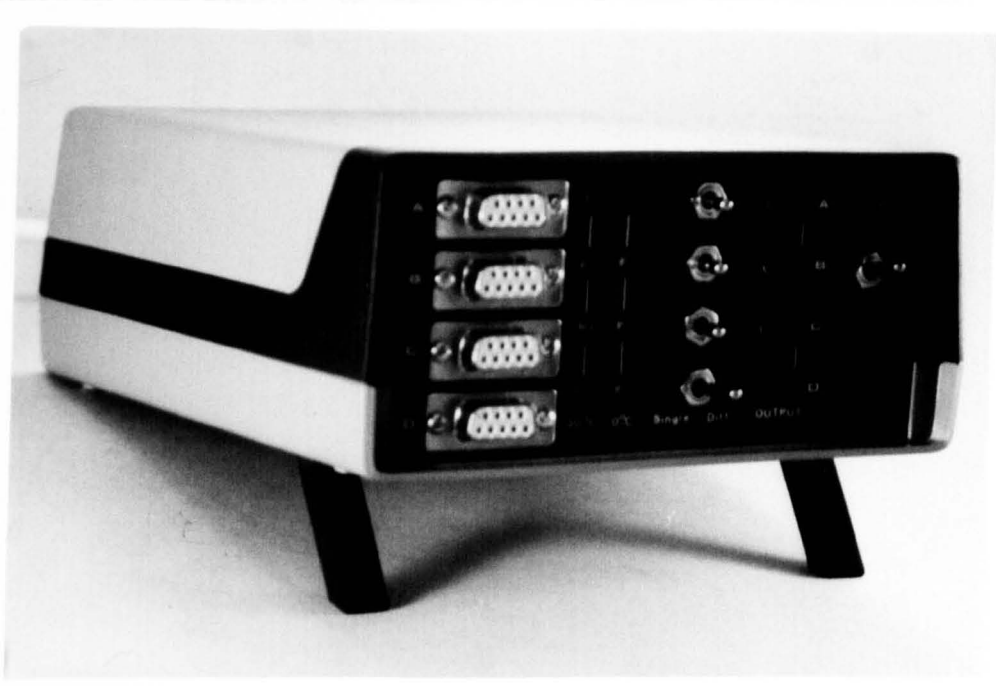
MATERIALS AND METHODS

The thermistors used in the system are type U53 US (Standard Telephone and Cable manufacturing code number, R.S. Components Ltd. thermistor equivalent to RA53 type stock number 151-114) with a resistance of 5Kohm at 20°C ±20%, a maximum power dissipation of less than 20μW and a self-heating effect of less than 0.07°C.

RA53 thermistors were selected because the fine bead wires are welded to stronger support/connecting wires which pass through a glass envelope around the bead. The envelope was scored with a diamond pen approximately 5mm from the base and broken off to expose the bead. The thermistors were connected to the amplifier by a 15 way D connector on the back panel or via an extension cable to a 12 way terminal block.

The amplifier can operate at temperatures between 10°C and 35°C, has a linearity of ±1.25% of full scale deflection (f.s.d.) and a power consumption of 3VA at 240V A.C.. The unit comprises six amplifier channels and a stabilised power supply (Plate 9). Each amplifier channel has two overlapping temperature ranges, +20°C to -20°C and 0°C to -40°C. The amplifier output voltage is zero for the low temperature of each range (-20°C and -40°C) and is adjustable between 0.7V and 1.3V for the high temperature. This enables the maximum output voltage to be set to 0.8V for potentiometric recorders (1.0V range), giving 0.5°C per division on 100 division chart paper, or to 1.0V for a computer interface to gain maximum resolution from the

Plate 9. Micro-bead thermometer.



computer analog to digital converter. Computer software is then used to scale the output in degrees Celsius. The output is available on a 15 way D connector for a computer interface and pairs of 4mm sockets for potentiometric recorders. The zero volt terminals are all ground referenced i.e. connected to supply earth. The wide tolerance of the thermistors necessitates the matching of each to a particular channel. If the thermistor is changed then the channel must be recalibrated.

Calibration was carried out by placing the themistor in a thermoelectric cooling module, monitoring the temperature and adjusting the amplifier channel accordingly. The calibration procedure was as follows;

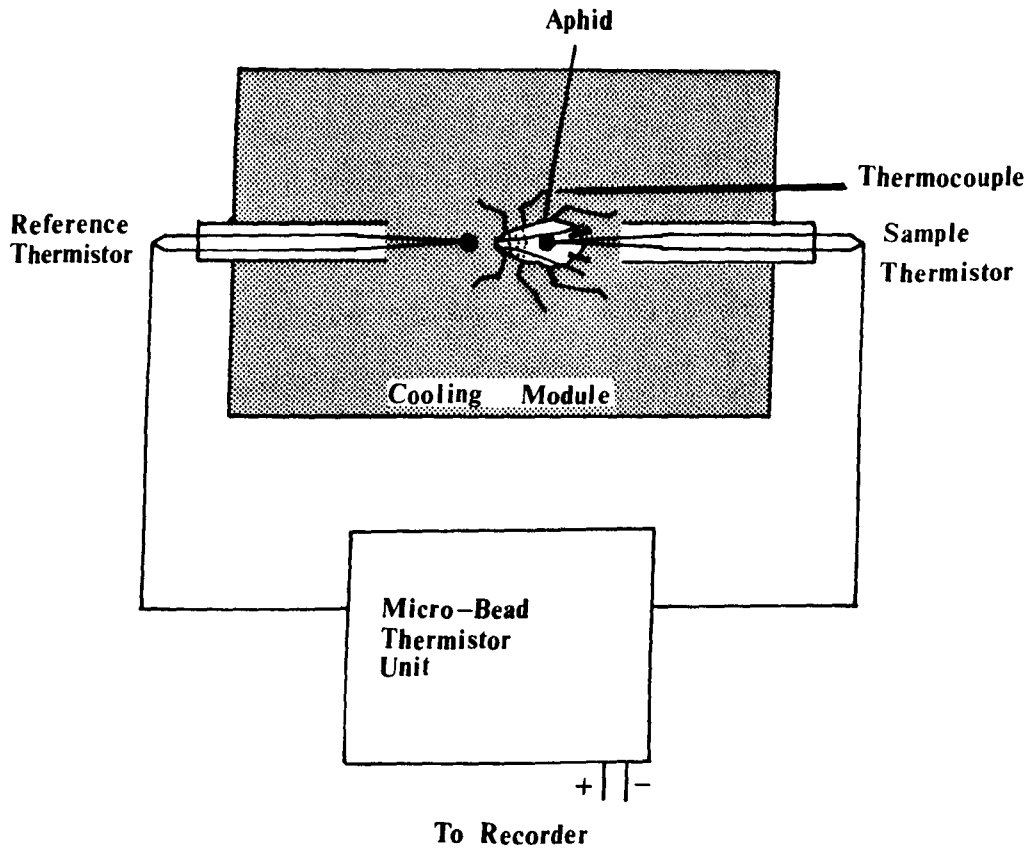
1. The 0°C to -40°C range was selected, the thermistors cooled to -40°C and the -40°C presets adjusted to give 0V at the outputs (measured on a Thurlby 1503 digital multimeter).
2. The 20°C to -20°C range was selected, the temperature raised to -20°C and the appropriate presets adjusted to read 0V at the outputs.
3. The 0°C to -40°C range was reselected, the temperature raised to 0°C and the 0°C presets adjusted to give 1V at the outputs.
4. Finally the -20°C to 20°C range was reselected, the temperature raised to 20°C and the presets for this temperature set to give 1V at the outputs.

Pre-freeze thermal events.

To study these low-level thermal changes the output was recorded on a potentiometric chart recorder in

Figure 6.1.

Schematic diagram showing the arrangements of thermistors, thermocouple and specimen relative to the cooling block. (Not to scale).

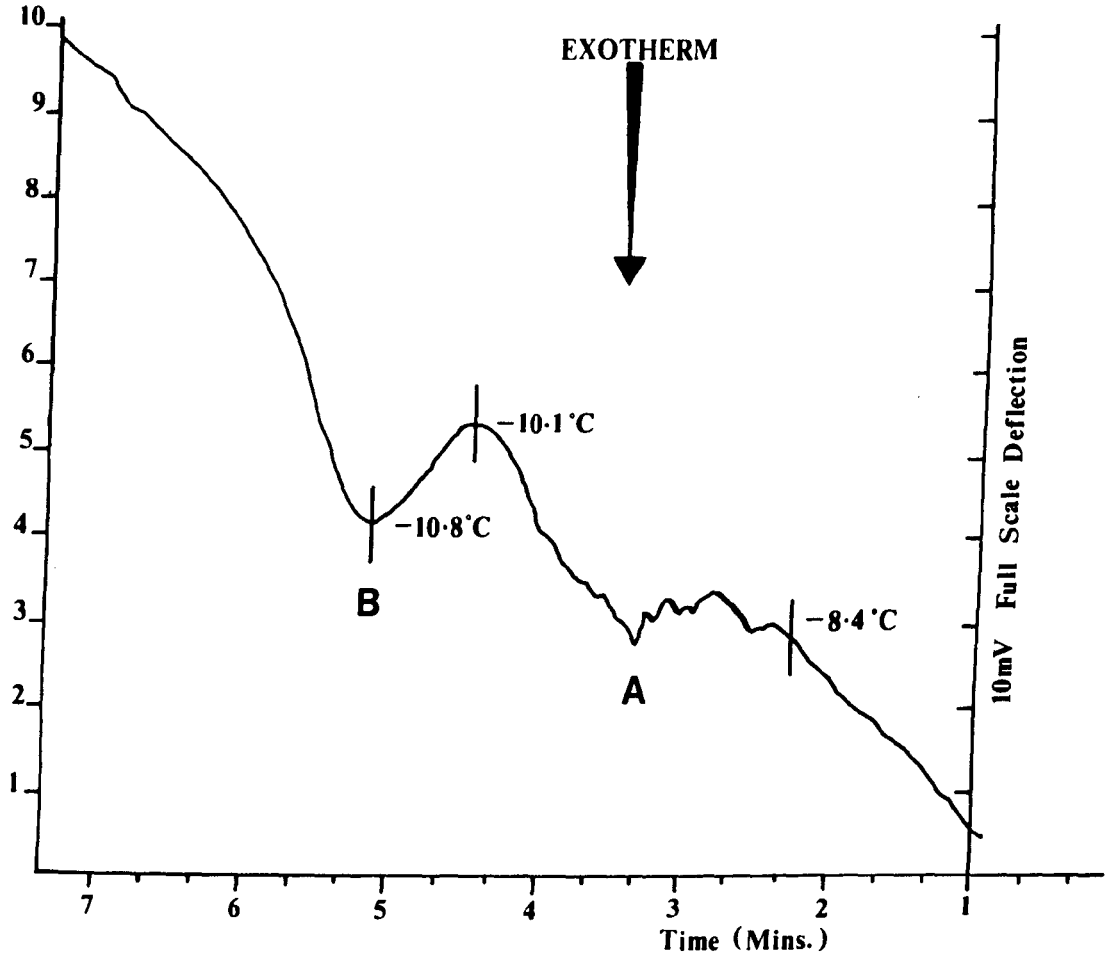

Aphid
Thermocouple
Reference
Thermistor
Sample
Thermistor
Cooling Module
Micro-Bead
Thermistor
Unit
+
−
To Recorder

preference to a computer to allow greater resolution. The temperature was measured using a thermocouple in close proximity to the thermistors in the cooling chamber which was connected to a micro-computer via a thermocouple converter. The arrangement of the thermistors and thermocouple relative to the cooling stage is shown in figure 6.1. The positive terminals of the thermistors were connected to the two input sockets of the chart recorder to give a positive voltage and so gave the differential voltage between the two thermistors. Any abrupt changes in the readings were therefore due to heat coming from the specimen. The chart recorder (Servoscribe 544.2) had a variable offset (0-400%) and was used at a sensitivity of 10mV f.s.d. The use of matched pairs of thermistors provides a reasonably stable baseline with the minimum of drift. The system has been used extensively with aphids and the pre-freeze exotherm detected by the DSC in the scanning ranges of 0.1-0.2 mcal sec is clearly resolved by the differential thermistor when operated as described above. A typical trace from an experimental run is shown in figure 6.2.

The micro-bead thermistor unit has also been used successfully in the detection of supercooling points of very small organisms such as plant parasitic nematodes, e.g. eggs and juveniles of the potato cyst nematode Globodera, with a fresh weight of only 0.1µg. In this application the thermistors are a straight replacement for thermocouples, the specimens being placed directly onto the thermistors and the recording of results is achieved by the

Figure 6.2.

Typical chart trace from an experimental run of the micro-bead thermometer in differential mode showing the pre-freeze exotherm (peaks A and B) produced by an aphid cooled at 1 deg per min.


EXOTHERM
−10·1°C
−10·8°C
B
A
−8·4°C
10mV Full Scale Deflection
Time (Mins.)
10
9
8
7
6
5
4
3
2
1
7
6
5
4
3
2
1

computer based system. The micro-bead thermistor unit is connected to the computer via an analog-digital converter (Interactive Structures, AI13).

DISCUSSION

When used to detect supercooling points the micro-bead thermometer provides a useful increase in sensitivity compared to thermocouples thus facilitating studies on a wider spectrum of invertebrate groups. The thermistors are not as robust as thermocouples, but a coating of varnish on the fine connecting wires reduces the risk of breakage; for larger specimens thermocouples remain the preferred sensors for supercooling point experiments.

The option to use thermistors in the differential scanning mode provides a sensitive and inexpensive system that has produced comparable results to those obtained from modern DSCs. Clearly the DSC has distinct advantages; the thermal events can be quantified as an aid to identifying the nature of the change. Additionally the temperature is recorded automatically at 1 deg intervals as part of the trace on the chart recorder and the baseline drift can be adjusted. For many invertebrate studies however it will be sufficient to record the thermal events without the need for quantification . There is also a limit to the size of specimens that can be studied in a DSC dictated by the dimensions of the sample pans which have a maximum diameter of less than 1cm. For these reasons of sensitivity, adaptability to different cooling systems and low cost, the

micro-bead thermistor unit will be a useful aquisition for research in many areas of cryobiology.

The original system has since been developed further and now has four amplifier channels and only requires calibration at -30°C and 0°C. Output is now via pairs of 2mm sockets only. Development of this system was carried out in collaboration with R. A. Stevenson of Stonegate Instruments Ltd. details of which have been published as Knight, Bale, Gleave, and Stevenson (1986).

CHAPTER SEVEN

INVESTIGATION OF PRE-FREEZE MORTALITY IN THE APHIDS *MACROSIPHUM ALBIFRONS* AND *SITOBION AVENAE*

SUMMARY

Adults of the grain aphid *S. avenae* and the lupin aphid *Macrosiphum albifrons* had lower lethal temperatures (LT50) of approximately -7.5°C and -7.0°C respectively. Nymphs of *S. avenae* were significantly more cold hardy than adults in terms of LT50 values. Acclimation at 10°C prior to experimentation significantly improved the cold hardiness (LT50) of *S. avenae*. Differential scanning calorimetry of single aphids revealed a previously unreported exotherm, at approximately the LT50 temperature of unacclimated aphids, corresponding to less than 1% of the energy liberated at the freezing point, followed by normal supercooling points. The cause of the exotherm is unknown but it is not an artefact. It is concluded that supercooling points are ecologically irrelevant if insects are already effectively dead before they freeze, consequently any study of insect cold hardiness should include experiments to determine the lower lethal temperature as well as the supercooling point.

INTRODUCTION

The supercooling point, measured as the lowest temperature reached before spontaneous freezing occurs, is widely used as a comparative index of cold hardiness for freezing intolerant insects (Sømme, 1982) representing the instantaneous low temperature death point (Salt, 1958). It has been widely assumed, since the pioneering studies of Salt in 1930 and throughout the modern literature, that during a supercooling experiment, at the standard cooling rate of 1 deg per minute, the specimen remains alive until nucleation occurs and the ice front advances through the body. This assumption has rarely been tested.

The results from the previous chapters have shown that S. avenae dies from the effects of cold at temperatures well above the supercooling point and therefore the death cannot be due to freezing. Additionally mortality of S.avenae at temperatures above the supercooling point has been reported in other field experiments (Dean, 1974a; Hand, 1980; Williams, 1984). The purpose of this chapter was to investigate at what temperature mortality actually occurred and therefore provide an estimate of the lower lethal temperature of this species.

MATERIALS AND METHODS

Determination of lower lethal temperatures. (LT50)

Initial experiments were carried out on adult lupin aphids Macrosiphum albifrons which were maintained and

reared on whole lupin plants (Russel strain) using method IV. These were reared at 20 ±1°C with a photoperiod of 16L:8D. The aphids were used shortly after they had moulted to adults. Lupin aphids were selected for their larger size compared to S. avenae which made subsequent investigations easier.

The S. avenae used in the experiment were reared under similar conditions on young barley plants (var. Igri). Experiments were carried out with adults and nymphs reared at 20 ±1°C and also at 10 ±1°C to test for any acclimation effects. The LT50 (the temperature which causes 50% mortality in a sample of individuals) was determined by cooling 5 replicates of 10 individuals in the same apparatus as that used for the supercooling point determinations and at the same rate of 1 deg per minute. The aphids were cooled to a pre-set lower limit and held at this temperature for 1 minute. The minimum temperatures ranged from -5°C to -20°C. The aphids were then warmed to room temperature and placed in petri dishes containing moist filter paper and strips of barley or lupin leaves. A control group of aphids was also monitored for mortality over the 96 hours in order to correct the experimental results. The temperature was monitored throughout the experiment using the thermocouples of the supercooling system. Mortality was recorded after 24, 48, 72 and 96 hours. Probit analysis was used to determine the LT50 value for each of the four groups (Finney, 1971).

Differential scanning calorimetry (DSC) studies were performed on single adult aphids of S.avenae (average adult

Table 7.1. LT50 and 95% confidence limits (°C) for adult *Macrosiphum albifrons* after cooling at 1 deg per min to minimum temperatures.

LT50 and 95% confidence limits (°C) at intervals (hours) after cooling at 1 deg per min.	
24	48
-7.8	-6.5
-7.4/-8.2	-5.7/-7.2

weight 0.42mg) and M. albifrons (average adult weight 4.5mg) placed into sealed DSC sample pans. They were cooled from 17°C at 1.25 deg per min (closest available rate to that used in previuos supercooling point and LT50 experiments) in a Perkin-Elmer DSC-2, fitted with a sub-ambient temperature accessory and a scanning auto-zero device. The instrument was operated at its maximum sensitivity. Subsequently both species were investigated using the system described in the previous chapter (chapter 6).

RESULTS

Determination of LT50

Within 1 - 2 hours of exposure grain aphids could be classified as dead, comatose (twitching of appendages) or fully mobile. Invariably aphids which were unable to walk normally within 2 hours of exposure died during the next 24 - 96 hours. Mortality was assessed 24, 48, 72 and 96 hours after exposure and LT50 values calculated for each time interval after the results had been corrected by the Abbotts correction factor derived from the controls.

In the case of the lupin aphids the mortality was assessed after only 24 and 48 hours since longer periods caused high mortality in the control samples. Adult M. albifrons had a LT50 of about -7.0°C (Table 7.1).

S. avenae nymphs were consistently more cold hardy than adults when reared at both 20°C and 10°C based on LT50 values. (Tables 7.2 and 7.3). The LT50 values of S.

Table 7.2. LT50 and 95% confidence limits (°C) for Sitobion avenae adults and first instar nymphs reared at 20°C after cooling at 1 deg per min to minimum temperatures.

Age group	LT50 and 95% confidence limits (°C) at intervals (hours) after cooling at 1 deg per min.		
	24	48	72
Adults	-8.1	-7.7	-7.0
	-7.6/-8.6	-7.1/-8.2	-6.6/-7.4
Nymphs	-11.2	-10.8	-10.8
	-10.3/-12.2	-9.7/-12.1	-9.6/-12.0

avenae differed by a maximum of 1.9 deg between 24 and 96 hours for both adults and nymphs indicating that most fatally injured aphids would die within 24 - 48 hours of exposure. No development (moulting) or reproduction was observed before death in aphids which died up to 96 hours after exposure.

The aphids reared at 10°C showed a significant increase in cold hardiness over those reared at 20°C indicating that acclimation occurs (Table 7.3).

DSC experiments.

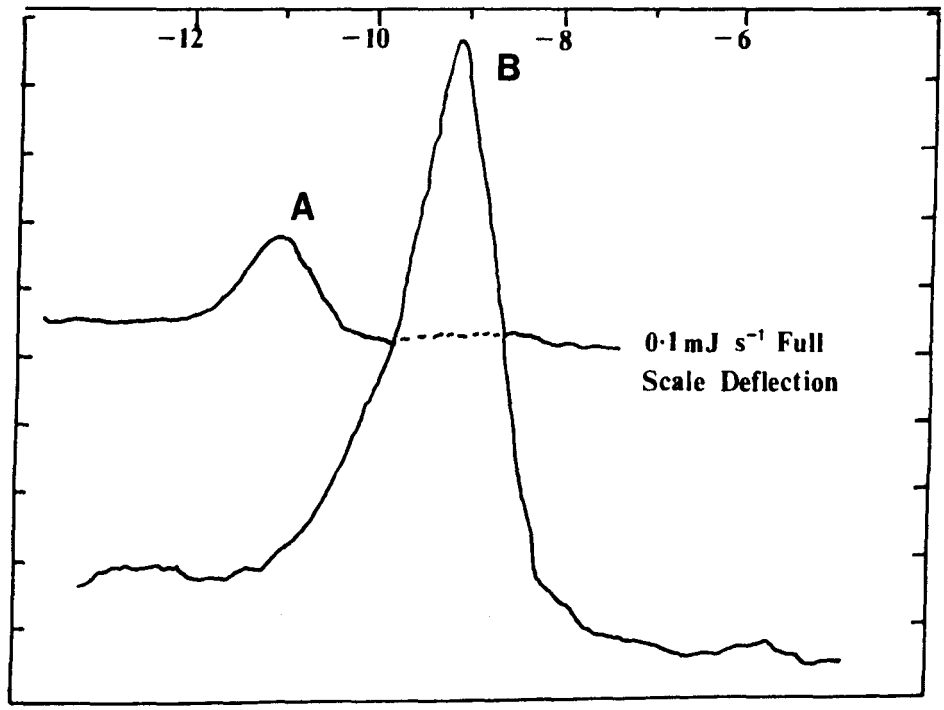
An exotherm was invariably observed above the supercooling point in both species and figure 7.1 illustrates typical results for adults of S. avenae and M. albifrons. With both species, although the amplitudes of the exotherms were variable, the half-widths were remarkably constant (0.8 deg for S. avenae and 1.25 deg for M. albifrons). The onset temperature varied by 1 deg within each species (-9°C for M. albifrons and -10°C for S. avenae). A shoulder on the low temperature side suggested the presence of two partly superimposed processes. On rewarming the sample from -13°C no endotherm could be observed, indicating the irreversibility of the thermal event. Furthermore, the absence of an endotherm at 0°C is strong evidence that the exotherm is of direct biological origin rather than an artefact associated with condensation in the system or evaporative water loss from the specimen; additionally no exotherm was present in aphids killed prior to cooling. A second cooling scan revealed no further thermal event except the supercooling point below -13°C for

Table 7.3. LT50 and 95% confidence limits (°C) for *Sitobion avenae* adults and first instar nymphs reared at 10°C after cooling at 1 deg per min to minimum temperatures.

Age group	LT50 and 95% confidence limits (°C) at intervals (hours) after cooling at 1 deg per min.		
	24	48	72
Adults	-9.7	-9.4	-9.3
	-9.4/-9.9	-9.1/-9.7	-8.9/-9.6
Nymphs	-19.2	-20.6	-20.2
	-17.4/-21.3	-18.1/-26.3	-18.2/-24.4

Figure 7.1.

Power - time curve obtained on cooling a single Sitobion avenae (0.7 mg) (A) and Macrosiphum albifrons (2.5 mg) (B) at 1.25 deg per min.


−12
−10
−8
−6
A
B
0·1 mJ s⁻¹ Full
Scale Deflection

M. albifrons and -20°C for S. avenae.

When studied by the differential scanning thermistor system (which had been calibrated against the thermocouples used for temperature sensing in the supercooling point and LT50 experiments) the exotherm for S. avenae had an onset temperature of -7.5 ±0.4°C and a mid-peak value of -8.3 ±0.4°C (Mean ±S.E. n=10). Two peaks less than 1.5 deg apart were frequently detected by the thermistor system, in which the second peak was markedly and consistently larger. With M. albifrons the onset temperature was -6.9 ±0.2°C and mid-peak value -8.1 ±0.2°C (n=10). These values for S. avenae and M. albifrons confirm the narrow range of onset temperatures for the exotherm observed with the DSC. There was a consistent difference of about 2 deg between values obtained from the DSC and the thermistor system, attributable to the calibration procedures in the different laboratories; however when the exotherm and LT50 values are derived from temperature sensors calibrated against a common reference source (automatic thermoelectric supercooling point cooling system with thermocouples and the differential scanning thermistors) there is a good correlation between the exotherm and LT50 with values of -8.3 ±0.4°C (mid-peak) and -8.1 ±0.5°C respectively. Adult S. avenae reared at 10°C produced an exotherm at -8.4 ±0.2°C (start point) which was significantly lower than the start point value for adults reared at 20°C (t=3.22, N=10, $P<0.01$).

DISCUSSION

During its 50 year history, the study of insect cold hardiness has concentrated almost exclusively on the mechanisms by which insects survive or avoid freezing, and the factors which influence this ability. The supercooling point is the most widely used index of cold hardiness and the adoption of a standard cooling rate has facilitated comparative investigations. The convenience of this method (rapid experimental times, small sample sizes) allied to a diversity of modifying influences to study (acclimation, surface moisture, feeding status,production of cryoprotectants) may have discouraged any critical appraisal of the method, particularly its relevance as an indicator of survival or death under natural conditions.

The mean supercooling point of laboratory populations of S. avenae and of field samples collected throughout the year is consistently below the lowest minimum temperature recorded in the field (Chapters 3 and 4). Whilst some individuals do lose supercooling ability this has little effect on the mean value. If considered in isolation, this information might suggest that S. avenae is adequately protected from the deleterious effects of cold throughout most British winters, since temperatures close to -20°C are extremely rare.

The studies detailed in Chapters 4 and 5 indicate however that (i) environmental temperatures are continuously above the mean supercooling point but (ii)

populations decline markedly and abruptly in winter at temperatures of about -8°C, much above the mean supercooling point.

Overwintering insects are subject to an interaction of ecological and physiological factors, which are difficult to model in the laboratory, but the close agreement of the LT50 value for adult S. avenae (-8.1°C) to the field temperatures at which significant increases in mortality occur, suggests that for S. avenae, cold is the single most important winter mortality factor and the rate of cooling to the lethal temperature may have little effect on the level of mortality. Clearly for some freezing-intolerant insects with extensive supercooling ability, predictions of winter survival based solely on supercooling point data can be highly misleading and emphasises the need to quantify cold death above the supercooling point and assess population changes in winter in relation to known environmental temperatures.

The LT50 values for S. avenae adults in this study compare favourably with the value of -8.17°C obtained by Williams (1984) with S. avenae from the south of England reared on wheat and also those of Griffiths and Wratten (1979) of -8.8°C.

The fact that S. avenae acclimates when reared at low temperatures indicates that they can respond to decreases in temperature and therefore improve their chances of survival through periods of low temperature. However the laboratory experiments conducted may over- or under-estimate the extent of the change that would occur

under normal field conditions. Williams (1984) also recorded similar results with acclimation causing a significant increase in cold hardiness, although Griffiths and Wratten (1979) state acclimation at temperatures from 5°C to 28°C had little effect on LT50 values. Interestingly the nymphs are significantly more cold hardy than the adults implying that it will be the development, survival and reproduction of these individuals that will determine the size and timing of any outbreak in the spring.

It is recognized that aphids are not typical cold hardy freezing-intolerant insects; there is no seasonal acclimation and no response to laboratory acclimation regimes with respect to supercooling ability (Knight and Bale 1986). In previous studies on the most cold hardy freezing-intolerant species which accumulate polyols in winter and show thermal hysteresis effects, experiments have (apparently) never assessed the viability of test specimens in supercooling point experiments above the supercooling point. Rarely have such studies quantified winter mortality in the subject species or measured accurately the microclimate of overwintering sites. For these reasons, it is possible that the inconsistencies between supercooling points, environmental temperatures and population decline, so readily apparent in S. avenae, may also apply to other 'more typical' freezing intolerant species. Future studies on such species should at least confirm that the insects remain alive in supercooling point experiments until they freeze, and recover normal behaviour

if not frozen.

The thermal event described in this chapter is a novel discovery but its physiological significance or cause at the present time is unknown. Whilst the temperatures at which it occurs are very close to the LT50 values of aphids reared at 20°C it has been shown that individuals that have been cooled to the point where an exotherm is produced and then rewarmed, survive and go on to reproduce normally; therefore the event cannot be assumed to be indicative of the process which is responsible for the aphids death. (Clough, Bale and Harrington, 1987).

Further research could be done to investigate if the reproductive performance of these individuals differs from individuals that have not been cooled. The actual cause of the exotherm is not known but possibilities include a thermotropic membrane phase transition, a cold inactivation of protein(s) (Franks and Hatley, 1985), or some perturbation of normal metabolic processes, giving rise to an unsuccessful attempt by the organism to repair the damage.

The results of this chapter may shed light on another established principle of insect cryobiology. Whilst it has been recognised that insects may die during prolonged exposure to sub-zero temperatures above the supercooling point, this has traditionally been explained in terms of the increasing probability of ice nucleation under such regimes (Salt, 1966). Freezing has been presumed but very rarely proven to be the cause of death in these conditions, even in laboratory experiments. These studies would

suggest that for some species non-freezing cold death is the more likely explanation. It is concluded that the physiologically or biochemically based abilities of insects to avoid lethal freezing by supercooling to -20°C and below is ecologically irrelevant when non-freezing cold death occurs at higher temperatures. Furthermore, the classification of such species as 'freezing intolerant' may not be appropriate (except in the strictest sense) since the organisms are invariably dead or fatally injured before they freeze. A reappraisal of the ecological worth of cold hardiness studies based primarily on supercooling points would seem timely since the accepted dogma on the influence of cooling rate on the freezing status (tolerant or intolerant) of species and the identification of sites of nucleation is also under review. (Baust and Rojas, 1985). The research described in this chapter has been published in part as Knight et al (1986).

CHAPTER EIGHT

GENERAL DISCUSSION

GENERAL DISCUSSION

The likelihood of death for an individual insect from the effects of cold depends on (i) the cold hardiness of the specimen and (ii) the temperature and periods of exposure experienced in the overwintering site (Bale, 1987). It is therefore the interaction between these two factors that determines the proportion of a population that survives in winter.

Historically the study of cold hardiness has concentrated on the physiology and biochemical mechanisms of avoiding or surviving freezing, while for the most part disregarding the possibility that for some species, other injurious effects of cold may be a more important threat to survival. Indeed the physiological and biochemical have been stressed, and the ecological aspects such as behaviour and winter conditions in micro-habitats, largely overlooked (Danks, 1978). The aim of this thesis was to conduct a combined ecophysiological study on one species of aphid, Sitobion avenae.

Supercooling experiments provide a convenient method by which to assess the 'cold hardiness' of freezing intolerant insects and allow comparisons to be made between different species, experimental groups, or strains and populations within the same species, provided a standard technique is used (Ring, 1980). In this study the recommended standard rate of cooling of 1 deg per min (Salt,1966) was adopted and conditions in the experiments were standardised as far as possible so that only the direct effects of low

temperature could be studied. Obviously this does not apply to the field studies where many factors varied, but this has the advantage of providing information on the relevance of climatic factors which can be compared to the isolated effects of low temperature from the laboratory experiments. It should also be noted that the terms cold hardiness and supercooling are often used synonymously but supercooling points are only indicative of levels of cold hardiness in insects which survive down to the temperature at which they freeze.

The general features of freezing intolerant arthropods, as reviewed by Sømme (1982)have thus been expressed largely in terms of supercooling and include (i) a seasonal variation in supercooling which is at a maximum in winter, (ii) increased supercooling induced in the laboratory by exposure to low temperatures e.g. acclimation, (iii) inoculative ice formation through the cuticle above the inherent supercooling point due to contact with surface moisture and (iv) reduced supercooling when feeding associated with the action of ice nucleators in the gut. The findings of this work on the supercooling of S. avenae show that the aphid is atypical in all of these four features. Thus there was seasonal variation in supercooling, no response to acclimation, very few cases of inoculative freezing and no changes in supercooling associated with feeding or starvation.

The supercooling ability of S. avenae maintained under favourable conditions was comparable to that of some polar species (Sømme, 1981, 1982). This result considered in

isolation and without the knowledge of the pre-freeze mortality was unexpected since S. avenae is generally thought only to overwinter successfully anholocyclically in sheltered conditions (Dean, 1974a), or in average rather than 'severe' winters (Dewar and Carter, 1984). Whilst these results are atypical to other freezing intolerant insects they are almost identical to the pattern of supercooling characteristics observed in M. persicae (O'Doherty and Bale, 1985).

The work discussed in this thesis has provided unequivocal evidence for the widespread occurence of pre-freeze mortality in aphids which renders the extensive supercooling in these insects to be of little ecological relevance, since in a regime of decreasing temperature aphids die before they freeze. Nevertheless the supercooling features of aphids contrast markedly with those of many other insects which are genuinely freeze intolerant and the nature of these differences is worthy of discussion.

The lack of seasonality and extent of supercooling in S. avenae may perhaps be explained by the feeding method and specialised diet of aphids. Aphids feed on the phloem sap of plants, (which is high in some of the carbohydrates which are known to be cryoprotectants in other freezing intolerant insects (Danks, 1978)), may provide additional supercooling capacity in aphids which are in any case extremely efficient vessels for supercooling as judged by the supercooling points of new born nymphs (O'Doherty and Bale, 1985). The extensive supercooling of S. avenae is

therefore a natural ability and not an adaptation for survival at low temperatures. The lack of response in acclimation experiments indicates that aphids do not produce any cryo-protectants that improve their supercooling. The phloem sap that the aphids feed on is very 'clean' and unlikely to provide any catalysts for the nucleation of water. This is indicated by the lack of any increase in supercooling in S. avenae when the aphids are starved, a similar result to that observed in M. persicae (O'Doherty and Bale, 1985) and Elatobium abietinum (Powell, 1974).

In some freezing intolerant insects the action of body surface moisture reduces inherent supercooling ability by inoculation of an ice front through appertures in the cuticle to the internal tissues and fluids. This effect can cause a dramatic reduction in supercooling as with the beech weevil, Rhyncaenus fagi (Bale, 1980). A few individuals of S. avenae did die at temperatures above their supercooling point due to inoculative freezing from surface moisture and it is possible that with the repeated frosts of winter a substantial number of individuals would die from this effect. The feeding habit of aphids may also be a source of inoculative freezing from fluids within the plant. It has been proposed that mortality during winter in E. abietinum could occur through inoculative nucleation via the mouthparts when ice is formed in freezing spruce needles (Powell, 1974; Parry and Powell, 1977). It is possible that this may also occur in S. avenae feeding on grasses such that the aphid would freeze

and die when the plant froze. Since most plants are freezing tolerant, they normally only supercool a few degrees below their true freezing point before freezing extracellularly, so avoiding lethal intracellular ice formation (Levitt, 1980; Li and Saki, 1982). Thus aphid mortality would be significantly increased at higher temperatures around the freezing point of the host plant and substantially above their own ability to supercool. The laboratory research on supercooling point data assesses the ability of a species to avoid a freezing death and inevitably isolates factors which in nature act together. A high level of mortality in samples of S. avenae was recorded in experiments where aphids were cooled repeatedly to only -8°C and also in field situations where the lowest temperature recorded was only -12.1°C, much above the mean supercooling ability of the population at that time. These results suggest that mortality was occurring at temperatures above the inherent freezing point of the aphids. Possible explanations for this are: (i) Inoculative freezing from the effects of surface moisture on the cuticle, which has already been discounted since it caused only a small number of premature freezings in individual aphids; (ii) Inoculative freezing from ice formation in the host plant passing into the aphid via its mouthparts. Since the last explanation is the most tenable, the established view that the supercooling point is a direct measurement of the lower lethal limit (instantaneous death point) (Sømme, 1982) of a freezing intolerant species has no validity in the case of aphids.

The use of supercooling data for this purpose is only valid when a species (or individual) dies from the effects of freezing but remains alive (and capable of recovery) down to the temperature at which freezing occurs (Bale, 1987). Thus for S.avenae, only those individuals (and there may be none) which die from freezing at the limit of supercooling are freezing intolerant and the species could be more appropriately described as cold intolerant. Williams (1984) stated that cereal aphids could not be described as entirely freezing intolerant since he also observed mortality above the supercooling point and suggested that 'chill susceptible' may be a more appropriate description.

A proportion of the field mortality may have been due to starvation of the aphids since at temperatures below -4°C S. avenae becomes totally immobile (Smith, 1971) and therefore probably unable to feed. Furthermore if aphids fall or are dislodged from plants at low temperatures they may starve to death before they are able regain a feeding position on the host plant (Harrington and Cheng, 1984).

Low temperatures do not only pose a threat of death to insects but can also cause a general lowering of the metabolism. These changes can in turn influence a number of processes (Block, 1980) that require regulation or acclimatisation at the metabolic level (Bullock, 1955; Block and Young, 1978). Reduced metabolic activity may lead to reduced rates of development, reproduction and overall population performance. The results of this study suggest that aphids overwintering in the field do show

reductions in rate of development and reproduction. Interestingly the relationship between the number of day degrees above the developmental threshold of 3°C and the rates of development and reproduction was linear rather than curvilinear. This confirms the findings of Williams and Wratten (1987) for S. avenae on winter wheat that rate-temperature relationships for development and reproduction are well described by straight lines. The thermal time requirements for development from nymph to adult are reasonably close in this study to that of Williams and Wratten (1987), (142.6 DD>3 and 150.1 DD>3 respectively), implying that similar conclusions can be drawn from the results for both barley and wheat. However Williams (1987) has shown that development and reproduction of S. avenae on perennial ryegrass is markedly different to that on wheat (e.g. development time on wheat and ryegrass 146.9 DD>3 and 188.8 DD>3 respectively) and therefore care should be taken in comparing results obtained on different host plants.

In order to overwinter successfully anholocyclic aphids must not only be able to survive low tempera tures but also continue to develop and reproduce in order to maintain recruitment to the population and compensate for mortality caused by disease, predation, parasitism and 'old age'. If a species is unable to do this then the population will decline and eventually die out. In the field experiments described in chapter 5 only two individuals survived to become adults from the second inoculation of nymphs placed in the field; although this

represents only 2% of the original sample, given the high reproductive potential of aphids these survivors could soon give rise to an increasing population under warmer conditions. The results of these experiments may underestimate the ability of S. avenae to survive overwinter since the aphids placed in the field had undergone only a limited period of acclimation, whereas in the natural environment there would be sufficient time for a complete acclimatisation and therefore an increased chance of survival. Conversely the fact that the aphids were enclosed in clip cages may have protected them from the effects of wind, rain and hoar-frost and also excluded predators, thus giving an overestimate of their longevity.

Mortality due to low temperatures was only really significant at temperatures below about -8°C for adults and about -10°C to -11°C for nymphs, implying that in winters where temperatures did not fall below this level mortality would be much reduced. Laboratory results of the LT50 experiments confirm that high levels of mortality do occur at these temperatures and it is proposed that these values may be of use in predicting the overwintering success of the aphid by comparing mortality expected at particular temperatures to grass minimum temperatures recorded throughout the winter. This would give an early indication on the likely level of survival of anholocyclic aphids. This may also give some information on the likelyhood of cereal aphid outbreaks, although the holocyclic population must also be considered. The timing of the spring migration of S. avenae has been closely correlated with

the mean temperatures in the months of January and February (Walters and Dewar, 1986) the same relationship has also been found for M. persicae (Bale, Harrington, Knight and Clough, 1987) thus relatively high temperatures during these months will lead to a greater proportion of a population surviving the winter, subsequently building up and leading to overcrowding which would in turn lead to an early migration.

All aphid eggs, like those of most freezing intolerant arthropods, are capable of extensive supercooling (James and Luff, 1982; Sømme, 1982); holocyclic aphid species, or clones within a species, which overwinter as eggs can therefore be considered to be cold hardy and well adapted for winter survival (Dixon, 1985).

Whilst it is now clear that aphids die before they freeze, the cause(s) of this death is (are) not known. The thermal event described in chapter 7 is not related to supercooling and freezing because no corresponding endotherm can be observed in the neighbourhood of 0°C on rewarming. Possible causes could include a thermotropic membrane phase transition, a cold inactivation of proteins (Franks and Hatley, 1985) or the decoupling of the normal metabolic processes giving rise to an attempt, successful or otherwise, to repair the damage. The coincidence of this event at temperatures similar to those obtained for LT50 values suggested that this may be related to the process that causes the lethal injury. However Clough, Bale and Harrington (1987) have shown that there is no connection between the two, since aphids which have 'passed

through´ their exotherm are able to moult and reproduce. This still leaves the question as to why the aphids do die at low temperatures since for the majority it is not attributable to starvation or freezing. This is therefore an area that warrants further study.

Two main conclusions can be drawn from this study, the first concerning the general area of insect cryobiology and the second, the overwintering biology of S. avenae.

If this particular study had been confined to a laboratory investigation of supercooling in S. avenae then the general conclusion would have been that the aphid was freezing intolerant but able to withstand temperatures down to about -20°C. This would clearly be untrue but unfortunately supercooling is often the only aspect of the cold hardiness of an insect that is investigated (Bale, 1987). ´Field studies´ in insect cryobiology have tended to be limited to the collection of individuals for subsequent supercooling or cryoprotectant analysis.

For research on insect cryobiology to be meaningful it must include studies on the biology and ecology of the specimen as well as its physiology and biochemistry. Conversely many studies by population ecologists have identified low temperature as a regulating factor on insect populations but failed to investigate the freezing tolerant or intolerant nature of the species.

It has been proposed that insect cryobiologists should adopt a standard protocol in which all aspects of the low temperature biology of a species are investigated in an integrated study. In making this recommendation Bale

(1987) cites the work on S. avenae described in this thesis and recommends the following. (i) Firstly the freezing status of the insect should be investigated i.e. is it freezing tolerant or intolerant? This is relatively easy to do and does not require much time or many specimens. (ii) Secondly the dynamics of a field population overwinter should be determined. The microclimatic details of the insects habitat should be monitored and any correlations between these and mortality, development or reproduction investigated. If high levels of mortality are correlated with low temperatures then this should be compared to the measured supercooling ability of freezing intolerant species. If the mortality is higher than can be accounted for by freezing deaths then the LT50 of the insect should be determined. This can be done by varying cooling rate, minimum temperature of exposure and prior acclimation in laboratory experiments enabling freezing deaths, both instantaneous and time dependant to be separated from pre-freeze mortality.

Whilst it is recognized that these methods will require a large number of individuals it is essential that experiments of this type are included in the study. A comparison of laboratory and field survival at low temperatures will also give an indication of the magnitude of the mortality attributable to other factors in the field. Furthermore by obtaining this information at an early stage it can indicate if there is any justification for investigating levels or spectra of cryoprotectants.

Some recent studies on insect cold hardiness have

combined physiological and biochemical methods in the laboratory with field ecology. The goldenrod gall moth Epiblema scudderiana overwinters as mature larvae in stem galls. The mean supercooling point decreased from -13.9°C in early autumn and stabilised between -35 to -40°C in the winter; the lowest winter temperature was -26°C. In an overwintering field population 90% pupated successfully indicating that supercooling which increases in winter accompanied by an increasing glycerol content allows the insects to survive (Kelleher, Rickards and Storey, 1987).

In contrast the temperate Collembola Tomocerus minor and Orchesella cincta have mean winter supercooling points of -11°C and -14°C respectively. However the LT50 of winter samples is only -6.5°C and -8.7°C (van der Woude and Verhoef, 1986). The differences between these two values have been attributed to the time dependent nature of freezing in the supercooled state (Salt, 1950) but no evidence is given to show that freezing was the cause of death. It is possible that pre-freeze processes acting at higher temperatures are responsible for the mortality. However when field mortality was studied it did not exceed 65% since the overwintering site provided good protection from the extreme effects of low temperatures. These two examples and the work described in this thesis highlight the benefits of a combined ecophysiological approach rather than selecting isolated processes (e.g. supercooling) for study. There now seems little doubt that some individuals of anholocyclic colonies of S.avenae virginoparae are able to survive most winters

in many areas of Britain as shown by the results of this study and those of Williams and Wratten, (1987), Dean, (1974a), and Dewar and Carter (1984). However the importance or success of holocyclic overwintering of S. avenae is still relatively unknown (Dewar and Carter, 1984) although information on the survival of eggs of Rhopalosiphum padi (Leather, 1981) and R. insertum (James and Luff, 1982) indicates that levels of mortality are quite high.

A number of forecasting systems for the timing of the first spring migration have been proposed on the basis of the correlation between the date of first catch of an alate and temperatures recorded in the winter months (Turl, 1980; Dewar and Carter, 1984; Walters and Dewar, 1986). In general terms, the milder the winter the earlier is the spring migration since aphid survival is higher in mild winters and this leads to a rapid build up in numbers on the winter hosts, followed by crowding and then the alate migration (Walters, 1982). The ability to forecast the timing of spring migration is useful since an early infestation on cereals by spring migrants can lead to an aphid problem in the following summer (Sparrow, 1974; Walters, Watson and Dixon, 1983; Bale et al, 1987); With prior knowledge of potential outbreaks appropriate treatments can be recommended. Whilst the timing of the first migration is important, so also is the size of the migration and this has also been linked to the level of winter survival. A number of theories have been put forward to explain the circumstances that lead to outbreaks

of S. avenae. Vickerman (1977) associated high populations of the the grain aphid in summer with cold spring weather, on the grounds that in years with a warm spring, populations of the grass aphid Rhopalosiphum insertum on grassland are high, predators and parasites emerge early and are able to increase rapidly, exploiting the abundance of their prey. When cereal crops are subsequently invaded by the grain aphid in May and June the relatively high predator and parasite populations are able to regulate the number of aphids. By contrast in cold springs, there are fewer grass aphids and hence fewer predators and parasites and the number of grain aphids increases in the absence of any effective control. Similarly Suter and Keller (1977a and b) found that when aphid populations on grassland or primary hosts in northern Switzerland were low in the spring, the number of parasites did not build up and this led to outbreaks of grass aphids in June and July. In contrast when the number of aphids was high in spring there was a rapid build up in the populations of beneficial insects, and there were no summer outbreaks.

The size of the spring migration of S. avenae at Norwich has been negatively correlated with the number of day degrees below 0°C from October to April inclusive (Watson and Carter, 1983) i.e. the size of the spring migration was large following a cold winter and spring.

It is proposed that the results of this thesis go some way to explaining the relationships between winter temperatures and aphid numbers. Low temperatures can

effect the size of overwintering populations in more than one way. The most direct effect is that of mortality caused by low temperatures through pre-freeze or freezing death. Populations will also be reduced by starvation caused by chill coma of individuals. However the largest effect will probably be due to low temperature affects on the fecundity and rate of developmentof surviving aphids. Lewontin (1965) states that developmental time has 10 times the effect on the intrinsic rate of increase as changes in fecundity, and more than any other parameter of population growth.

During severe winters populations will decrease rapidly and the rates of development and reproduction will be much reduced. Consequently spring populations will be low and slow to build up and the eventual alate population will be relatively low and migrate later than normal. The results of this study also shows that nymphs are more cold hardy than adults and are therefore more likely to survive the winter. Their smaller size may also make them less obvious to predators and more able to find sheltered locations such that they would be less likely to be dislodged from host plants. However if the late winter population consisted only of nymphs then population growth would not increase until early spring when the nymphs became adult and this would also delay migration. In the most severe winters virtually all anholocyclic aphids would perish and the resulting spring migration would be derived entirely from holocyclic or androcyclic clones. In mild winters a higher survival of anholocyclic adults and nymphs

would be expected and the spring populations would increase rapidly leading to crowding, production of alates and an early migration. Walters and Dewar (1986) have recorded a proportion of anholocyclic individuals in southern populations of S. avenae in winter and proposed that northern populations have a higher proportion of holocyclic individuals. If this is the case then the forecasting of the timing of migration will be made more difficult; furthermore if the severity of winter conditions determines the ratio of anholocyclic:holocyclic individuals surviving overwinter then the numbers of aphids will vary locally from field to field due to local differences in aspect and shelter from trees and hedges. These factors make the formulation of a forecasting system for the whole country very difficult and it shows the need for a number of regional studies to be undertaken over a number of years. Since predators and parasites also have an effect on aphid populations, a study of their overwintering biology would be useful in providing information on the effects that severe winters may have on their populations and hence their ability to control aphids in the following spring and summer.

It is hoped that the information in this thesis and that from other past and future studies will enable a reliable forecasting system to be developed.

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APPENDIX

Abbreviations

AC	alternating current
BYDV	Barley yellow dwarf virus
cal	calorie
°C	degree centigrade
cm	centimetre
deg	degree
DD	day degree
df	degree of freedom
DSC	differential scanning calorimeter
fsd	full scale deflection
g	gramme
GS	growth stage
HMSO	Her Majestys Stationary Office
ICI	Imperial Chemical Industries
J	Joule
L:D	light:dark
LT50	lower lethal temperature (50% of sample)
m	metre
mg	milligramme
µg	microgramme
min	minute
mm	millimetre
P	probability

SNK	Student Newman Keuls
SE	standard error (of the mean)
sec	second
Temp	temperature
V	Volt
VA	Volt-Ampere
W	Watt
$\bar{x}$	mean

PRINCIPLES OF THE COLD HARDINESS AND OVERWINTERING SURVIVAL OF APHIDS

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Summary. Cold weather is potentially a major cause of mortality in overwintering anholocyclic aphids. Temperature is also a dominant influence on the timing and size of the spring migration. Previous research has described aphids as freezing intolerant (species which do not survive below their supercooling point - SCP) although the features of their cold hardiness when expressed in terms of an ability to supercool, are atypical compared with many insects in the freezing intolerant category. Most aphid species supercool (avoid ice formation) as adults and nymphs to below -20°C throughout the year. The number of frost days and frequency of very low temperatures (below -15°C) varies from year to year but temperatures close to the mean SCP of overwintering aphids are extremely rare. In field and laboratory studies aphids die in large numbers at temperatures much above their SCP. In aphids extensive supercooling is insufficient protection against winter cold and pre-freeze processes account for the majority of low temperature deaths.

INTRODUCTION

Several aphid species of agricultural importance in Britain have both holocyclic and anholocyclic clones and are thus able to overwinter as eggs and active stages. The economic importance of these species whether in causing direct feeding damage, or as virus vectors, is often determined by the level of survival of the overwintering anholocyclic clones and the timing of the migration of these aphids to new sown crops in spring. The earlier spring migration associated with the anholocyclic life-cycle (compared to populations derived from eggs) provides increased potential for virus epidemics on various crops (Watson et al, 1975; Howell, 1977).

It is widely acknowledged that winter climate, particularly low temperature is a major influence on the ecology and overwintering biology of aphids, governing both the extent of mortality in winter and the timing and size of the spring migration (Taylor, 1977; Turl, 1978; 1983; Harrington and

Cheng, 1984). There have been two main approaches to the study of aphid overwintering. Field studies have examined the relationship between winter survival and meteorological factors and laboratory work has investigated aphid cold hardiness. Previously however the two approaches have not been coherently combined.

This review describes recent research at Leeds University and Rothamsted Experimental Station which has investigated the cold hardiness of different aphid species by various methods, and related this information to field population studies on aphids in winter.

STRATEGIES OF INSECT COLD HARDINESS

With the exception of tropical insects and similar species which die at temperatures above 0°C because they are unable to adjust their metabolism to enter a dormant state, other insects have been classified as freezing tolerant or intolerant depending on their ability to survive the formation of extracellular ice in the body tissues and fluids. The principles of the cold hardiness of these two categories of insects were discussed by Salt (1961) and have formed the basis for many reviews of the subject.

Freezing tolerant species can survive body freezing and often contain ice nucleating agents (proteins) which are normally only present in winter and ensure protective extracellular freezing at high sub-zero temperatures; in these species cryoprotective polyhydroxy alcohols (polyols) function to limit freeze damage. By contrast, freezing is lethal to freezing intolerant insects and this event is avoided by the process of supercooling in which the body tissues and fluids are maintained in the liquid state below their equilibrium freezing point. In these insects there are normally seasonal increases in the concentration of one or more polyols (e.g. glycerol) which extend the inherent ability of the insect to supercool. Additionally some freezing intolerant species contain thermal hysteresis agents in winter which lower the freezing point of the haemolymph relative to its melting point and may act to stabilize the supercooled state. A full account of the physiology of cold tolerance in insects is given by Zachariassen (1985).

FREEZING INTOLERANT INSECTS

The majority of insects are freezing intolerant and apparently depend on supercooling as their only protection against low temperature. Freezing occurs at the limit of supercooling and therefore the supercooling point (SCP) has been regarded as a measure of the low temperature death point and widely used as a comparative index of cold hardiness. For such species, the terms cold hardiness and supercooling ability have been virtually synonymous. The general characteristics of these insects have been reviewed by Sømme (1982) and can be described in these terms:

1. Cold hardiness (supercooling) increases seasonally to a maximal level in winter.
2. Increased cold hardiness (supercooling) is related to the accumulation of cryoprotective substances.

3. Production of cryoprotectant antifreezes may be induced by low temperature.
4. Surface moisture can reduce supercooling by inoculative freezing through the cuticle.
5. Feeding can reduce supercooling by the action of gut nucleators.

FREEZING STATUS OF APHIDS

In the supercooling experiments aphids were cooled at 1°C min^{-1} to their supercooling point in a thermoelectric system described by Bale, O'Doherty, Atkinson and Stevenson (1984).

Age

The mean SCPs of the first instar nymph and adult stage of a range of aphid species of economic importance collected from the field between spring and autumn or reared in the laboratory at 20°C is shown in Table 1.

Table 1

Mean ± S.E. supercooling points (°C) of aphid species

Species	First Instar	Adult
Myzus persicae	-27.4 ± 0.2	-26.0 ± 0.2
Aphis fabae	-26.3 ± 0.6	-25.3 ± 0.2
Sitobion avenae	-26.9 ± 0.2	-25.5 ± 0.2
Brevicoryne brassicae	-26.9 ± 0.3	-23.0 ± 0.3

There is little variation in supercooling between or within age groups or species. No specimen survived below its supercooling point indicating that aphids are probably freezing intolerant insects.

Acclimation

For many freezing intolerant insects 5°C is the optimum temperature for the induction of acclimation responses. The mean SCPs of first instar and adult Myzus persicae (Bale, Harrington and Clough, in press) and Sitobion avenae (Knight and Bale, 1986) maintained at 20°C and 5°C are shown in Table 2.

Table 2

Mean ± S.E. supercooling points (°C) of Myzus persicae and Sitobion avenae maintained at 20°C and 5°C

Species	Mean SCP ± S.E. °C	
	20°C	5°C
M. persicae		
adult	-25.0 ± 0.1	-24.5 ± 0.3
nymph	-26.6 ± 0.2	-26.3 ± 0.2
S. avenae		
adult	-24.2 ± 0.3	-24.4 ± 0.2
nymph	-27.0 ± 0.2	-26.1 ± 0.2

There was no distinct increase or decrease in supercooling in aphids maintained at 5°C compared to populations reared at 20°C.

Surface Moisture

Artificial wetting of aphids prior to supercooling caused a marked reduction in the supercooling ability of a small proportion of a sample of M. persicae in a single cooling (O'Doherty and Bale, 1985). A similar result was observed with S. avenae although mortality increased progressively in repeat coolings of wet aphids to -10°C, (Knight and Bale, 1986).

Seasonal variation

Field samples of M. persicae and S. avenae showed a consistent pattern of supercooling from spring to mid-winter with mean SCPs of adults and nymphs always below -20°C. In late winter (January - March) there was an obvious loss of supercooling in 5 - 20% of the population with SCPs in the region of -5 to -15°C (O'Doherty and Bale, 1985; Knight and Bale, 1986).

Feeding

It has been suggested that the loss of supercooling in aphids in late winter may be associated with starvation if feeding occurs on poor quality hosts or is prevented by low temperature effects on either aphids (chill coma) or plants (frozen sap). Starvation of M. persicae at 5°C can produce a SCP distribution similar to that observed in late winter field samples, but the response is inconsistent since other populations starved under similar conditions retain supercooling levels in all individuals to below -20°C, (O'Doherty and Bale, 1985). Starvation of S. avenae at 0° and 5°C reduced supercooling in a small number of individuals; however the mean SCP of these populations was always below -20°C and many aphids died, apparently from the direct effects of starvation or low temperature without any influence on their supercooling capacity (Knight and Bale, 1986). Feeding is essential for aphid survival but starvation appears to have little effect on supercooling.

Thus the cold hardiness of aphids assessed in terms of supercooling ability largely contrasts the following features of other freezing intolerant insects:

1. There is no seasonal increase in supercooling
2. There is no acclimation response at low temperature
3. Surface moisture affects a variable proportion of aphids
4. Feeding (on sap) does not affect supercooling.

Most importantly the supercooling studies show that the majority of anholocyclic populations of M. persicae and S. avenae can avoid freezing to below -20°C in the majority of a population throughout the year.

WINTER CLIMATE

The physiological stress of low temperature on aphids in winter may be related to the frequency of cold exposure or the minimum temperatures experienced on particularly cold days; both factors vary greatly from year to year. For instance in a study of winter frosts in nine successive years (1975 to 1984) at Leeds University Field Station, the number of air frosts per winter varied from 50 to 100 (mean 67 $\pm$ 6) and ground level frosts (grass minimum temperatures) from 110 to 160 (mean 140 $\pm$ 5). In one winter the temperature did not fall below -10°C whereas in other winters, temperatures

below -15°C were not uncommon, (Bale, Harrington and Clough, in press). Significantly, throughout the nine year period no temperature was recorded below the mean SCP of overwintering M. persicae and S. avenae.

Overwintering aphids often occupy exposed sites which are close to the soil surface e.g. M. persicae on oil seed rape and S. avenae on seedling wheat and barley. Microclimate temperatures close to the soil surface can be considerably colder than the air above; the vertical distribution of overwintering sites and the insulating properties of winter host plants are therefore important considerations when assessing the level of cold experienced by aphids in winter.

OVERWINTERING SURVIVAL

The change in numbers of M. persicae overwintering on spring cabbage in successive winters (1981-82 and 1982-83) has been studied by Harrington and Cheng (1984). In both years winter population density peaked in mid-November (with over 100 aphids per plant in 1981-82) and declined progressively to less than 3 aphids per plant (and only 0.5 aphids per plant in 1981-82) by mid-January or February. Mortality levels overwinter in the two years were 99.9 and 98% respectively. Rate of change in numbers was correlated with temperature and leaf surface wetness, although in both winters air and grass minimum temperatures were consistently above the mean SCP of overwintering populations of M. persicae.

A study of the grain aphid S. avenae has been conducted by McGrath and Bale (unpublished data in Knight et al, 1986). The peak density equivalent to 1,000,000 aphids ha^{-1} in late December declined to less than 30,000 ha^{-1} (97% kill) over a two week period when the lowest grass minimum temperature was -8.1°C in the first week (approximately 50% kill) and -9.7°C in the second week. The mean SCP of aphids drawn from the same field population was below -20°C.

The results from field studies on M. persicae and S. avenae provide strong evidence that in nature, most aphids die before they freeze. In a wider context extensive supercooling is not necessarily an indicator of a cold hardy insect.

PRE-FREEZE MORTALITY

In the light of field data suggesting extensive pre-freeze mortality in aphids, large samples (n=50) of M. persicae and S. avenae nymphs and adults taken from laboratory cultures, were cooled at $1°C\ min^{-1}$ to a range of sub-zero temperatures above the known SCPs. After cooling, aphids were returned to normal culture conditions at 20°C and mortality recorded at daily intervals thereafter and expressed as LT_{50} values as indicated in Table 3. (LT_{50} refers to the temperature required to kill 50% of a population as determined by probit analysis).

For both these species, experiments indicated that (i) there was 100% mortality above the SCP, (ii) most aphids suffered cryoinjury rather than instantaneous death but died progressively within 24h of exposure, (iii) nymphs were consistently more cold hardy than adults and (iv) there was no moulting or reproduction before death.

Table 3

LT_{50} ± 95% confidence limits (°C) of adult and first instar Myzus persicae and Sitobion avenae after cooling at 1°C min^{-1} to minimum temperatures

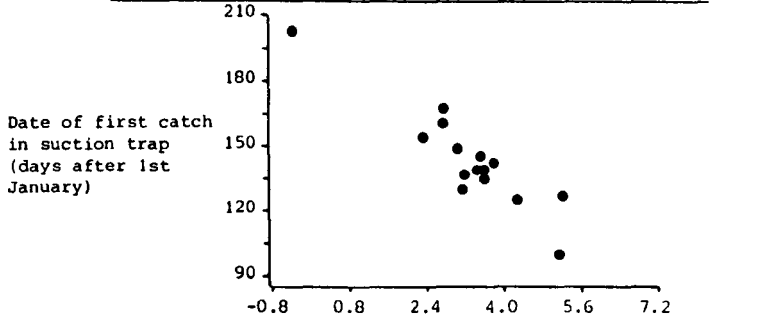
Species and age group	LT_{50} ± 95% confidence limits at intervals (h) after cooling at 1 deg min^{-1}		
	24	48	72
M. persicae			
nymph	-8.2 ± 0.6	-8.0 ± 1.0	-8.1 ± 1.0
adult	-7.4 ± 1.0	-6.9 ± 1.1	-6.9 ± 0.9
S. avenae			
nymph	-11.2 ± 0.9	-10.8 ± 1.1	-10.8 ± 1.2
adult	-8.1 ± 0.5	-7.7 ± 0.6	-7.0 ± 0.4

SPRING MIGRATION

The date on which the first *M. persicae* is caught in the Rothamsted Insect Survey suction trap at Rothamsted is better correlated with the mean temperature in January and February than with that in any other single or combination of calendar months (Fig. 1). The date of the first catch is an indication that the flying population has reached a minimum threshold size for detection and is hence a reflection of the success of overwintering. The fit of first flight to mean temperature is better than to any other measure of low temperature tested (e.g. day degrees below thresholds of 0°C, -5°C, -10°C, unpublished data), again suggesting that temperature events well above those implicated in freezing are related to winter survival.

Figure 1

Time of first catch of Myzus persicae in Rothamsted suction trap in relation to mean temperature in January and February



DISCUSSION

The classification of temperate and colder climate insects as freezing tolerant or intolerant is imperfect. There is at least one other category of cold hardiness which includes species such as aphids that are able to survive at temperatures which induce chill coma and also involves a level of supercooling, but die from the effects of cold before they freeze. Laboratory experiments in which cooling was the only stress applied to aphids have confirmed the importance of this pre-freeze mortality (Knight et al, 1986; Bale et al, in press). For these insects the supercooling point is an ecologically irrelevant index of cold hardiness. The causes of pre-freeze cold death are as yet unknown but could include phase changes in membranes, protein denaturation or the decoupling of normal metabolic processes.

Feeding is essential for the survival of overwintering aphids and prolonged cold which reduces feeding or prevents movement to new feeding sites may interact with low temperature to increase aphid mortality (Harrington and Cheng, 1984).

The cooling rate used in laboratory experiments to determine supercooling points and pre mortality ($1°C\ min^{-1}$) is fast compared to daily changes in temperature pre-freeze experienced by overwintering aphids. Interestingly cereal aphid populations on winter barley declined by about 50% when the temperature fell to -8°C and the LT_{50} of S. avenae cooled at $1°C\ min^{-1}$ was -8.1°C, suggesting that the mortality effect of low temperature occurs independently of the cooling rate.

The discovery of extensive pre-freeze mortality in aphids explains how the winter survival of these insects can vary greatly in relation to the occurrence and frequency of minimum temperatures between -10° (mild) and -18°C (severe) in different winters. Moreover it is the cumulative effect of January and February temperatures which determines the timing of the first flight of the spring migration which can vary by more than 14 weeks. Winter cold is clearly a dominant influence on the population dynamics of aphids and the combination of ecological and physiological research described in this review exemplifies the benefit of this integrated approach.

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A MULTI-CHANNEL MICRO-BEAD THERMOMETER FOR THE DETECTION OF LOW LEVEL THERMAL ENERGY CHANGES IN SMALL INVERTEBRATES

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SUMMARY

A device using thermistors as temperature sensors and capable of detecting low-level energy changes is described. It is able to detect supercooling points of small organisms weighing approximately 0.1μg and also pre-freeze thermal events recently discovered in some insects. It can be used with any cooling system, and linked to a chart recorder or computer, via a suitable analog-digital converter, for the recording of results.

KEYWORDS

Pre-freeze thermal events, supercooling point, thermistors, differential scanning calorimeter.

INTRODUCTION

Insects are described as freezing tolerant or intolerant depending on their ability to survive the formation of ice in the body tissues and fluids. The only protection for freezing intolerant insects is the ability to supercool, in which the body tissues and fluids are maintained in the liquid state below the equilibrium freezing point. The supercooling point (SCP) is the lowest temperature reached before freezing occurs (the instantaneous low temperature death point[1]) and is widely used as a comparative index of cold hardiness for freezing intolerant species[2].

A range of methods are used to cool specimens for SCP measurements but most systems employ thermocouples as the temperature sensors because of their small size, low price and robust nature[3,4,5,6]. However there is a limit to the sensitivity of thermocouples in some circumstances due to their large mass in relation to the size of the specimen. As the mass of a specimen decreases there is a corresponding reduction in the quantity of heat that is released on freezing, which for small invertebrates may be below the detection limits of the thermocouples. This limitation is manifest in two ways: (i) the SCP of some invertebrate groups e.g. soil nematodes, cannot be measured, and (ii) low level thermal energy changes occurring above the SCP[7] are not detected.

This paper discusses the development of an experimental multi-channel thermistor system which is compatible with all methods of cooling and can measure the SCP of very small invertebrates, or be operated in a differential mode with a resolution comparable to the most sensitive range of a modern Differential Scanning Calorimeter (DSC).

MATERIALS AND METHODS

The thermistors used in the system are type U53 (Standard Telephone and Cables manufacturing code number R.S. Components Ltd. bead thermistor equivalent to RA53 type, Stock No. 151-114) with a resistance of 5Kohm at 20°C ±20%, a maximum power dissipation of less than 5μW and a self-heating effect of less than 0.02°C. These thermistors were selected because the fine bead wires are welded to stronger support/connecting wires which pass through a glass envelope around the bead. The envelope was scored with a diamond pen approximately 5mm from the base and broken off to expose the bead. The thermistors were connected to the amplifier via twin screened cable connected to 9-pin D-connectors.

The amplifier (Plate 1) can operate at ambient temperatures between 10°C and 35°C, has a linearity of ±1.25% of full scale deflection (f.s.d.) and a power consumption of 3VA at 240V A.C. The unit comprises four amplifier channels and a stabilised power supply. Each amplifier channel has a temperature range of +10°C to -30°C . The amplifier is calibrated to give an output voltage of zero at 0°C, and the full scale reading at -30°C is adjustable between -300 and 750 mV to give a suitable scale on the output device. For example, to give a scale of +10°C to -30°C (40°C span) on a

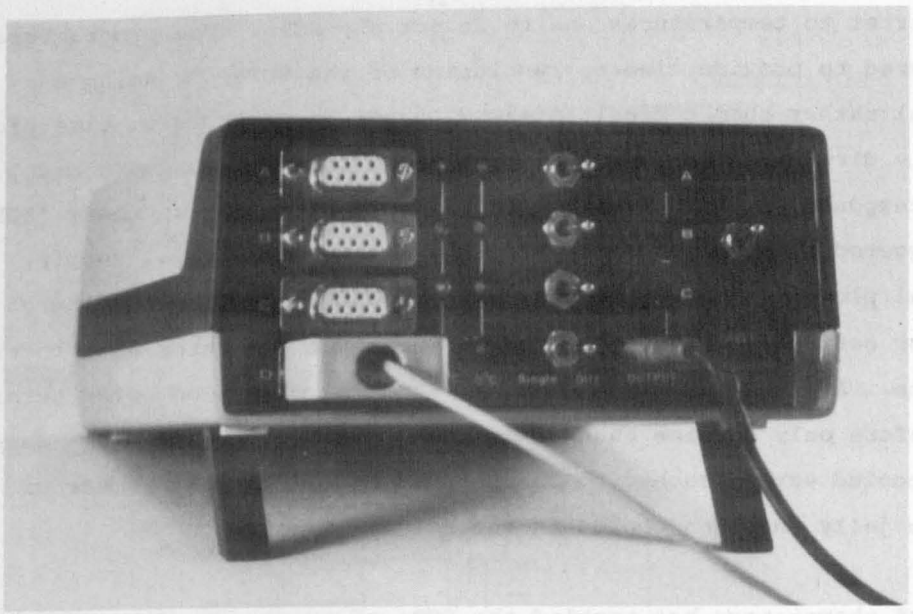

Plate 1. Micro-bead thermometer.

recorder with a 1 V full scale deflection, the amplifier can be calibrated to 25 mV per degree.

The output connection is via pairs of 2mm sockets; the negative sockets are all ground referenced (i.e. connected to supply earth). The wide tolerance of the thermistors necessitates the matching of each to a particular channel. If the thermistor is changed then the channel must be recalibrated.

Calibration was carried out by placing the thermistor in a thermoelectric cooling module, monitoring the temperature and adjusting the amplifier channel accordingly. The calibration procedure was as follows: 1. The thermistor was cooled to 0°C, and the 0°C preset adjusted to give 0 V at the output (measured on a Thurlby 1503 digital multimeter). 2. The thermistor temperature was lowered to -30°C and the -30°C preset adjusted to give the required full scale reading.

Supercooling measurements

When used to detect SCPs the thermistor unit was connected to an Apple IIe micro-computer (which records the SCPs) via an analog to digital converter (AI 13, Interactive Structures Inc.). A program in BASIC language was adapted from that used by Bale et al[3] to convert the digital values from

the converter to temperatures and to detect any SCPs. The program graphics were altered to provide clearer resolution of the SCPs, by using a horizontal rather than a traditional, diagonal temperature v. time plot. Previously direct temperature measurements of the specimen were made, but at the low response threshold required for small specimens, spurious 'SCPs' were triggered by minor interference in the mains electrical supply. The horizontal plot overcame these problems by subtracting the reading of the thermistor carrying the specimen from a reference themistor and plotting the difference. Any external influence has an equal effect on both thermistors and therefore only genuine changes in the specimen are recorded. Samples to be supercooled were attached to the thermistor beads with a smear of petroleum jelly and then cooled in the normal manner.

The use of thermistors has enabled the SCPs of organisms with a fresh weight of only 0.1μg, e.g. eggs and juveniles of Globodera, the potato cyst nematode, to be clearly resolved.

Pre-freeze thermal events

Recently a pre-freeze exotherm has been detected at temperatures between -7 and -11°C in aphids by the use of differential scanning calorimetry (Perkin-Elmer DSC-2)[7]; the exotherm corresponds to less than 1% of the thermal energy released at the SCP of the aphid and has not been recorded in previous supercooling studies on aphids[8,9] or any other invertebrate. To study these low-level changes the output was recorded on a potentiometric chart recorder in preference to a computer to allow greater resolution. The temperature was measured using a thermocouple in close proximity to the thermistors in the cooling chamber connected to a micro-computer via a thermocouple converter. The arrangement of the thermistors and thermocouple relative to the cooling stage are shown in Figure 1. The positive terminals of the thermistors were connected to the two input sockets of the chart recorder to give a positive voltage. The chart recorder (Servoscribe 544.2) had a variable offset (0-400%) and was used at a sensitivity of 10mV f.s.d. The use of matched pairs of thermistors provides a reasonably stable baseline with the minimum of drift. The system has been used extensively with aphids and the pre-freeze exotherm, consisting of either one large or two smaller distinct peaks (Figure 2), detected by the DSC in the scanning ranges of 0.1-0.2 mcal sec^{-1} is clearly resolved by the differential thermistors when operated as described above.

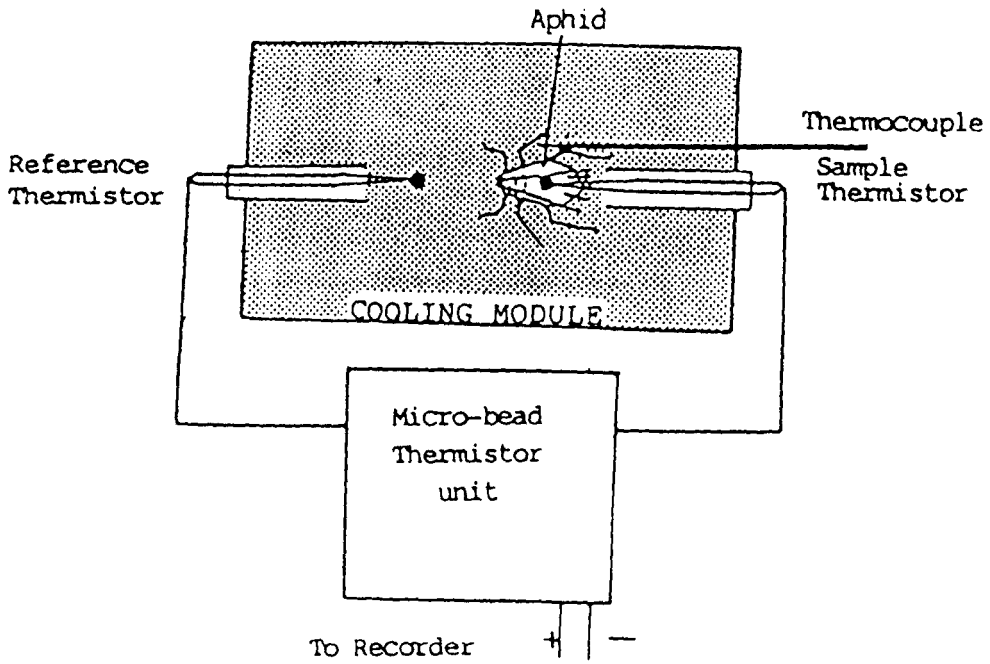

Figure 1. Schematic diagram showing the arrangement of thermistors, thermocouple and specimen relative to the cooling block. (Not to scale).

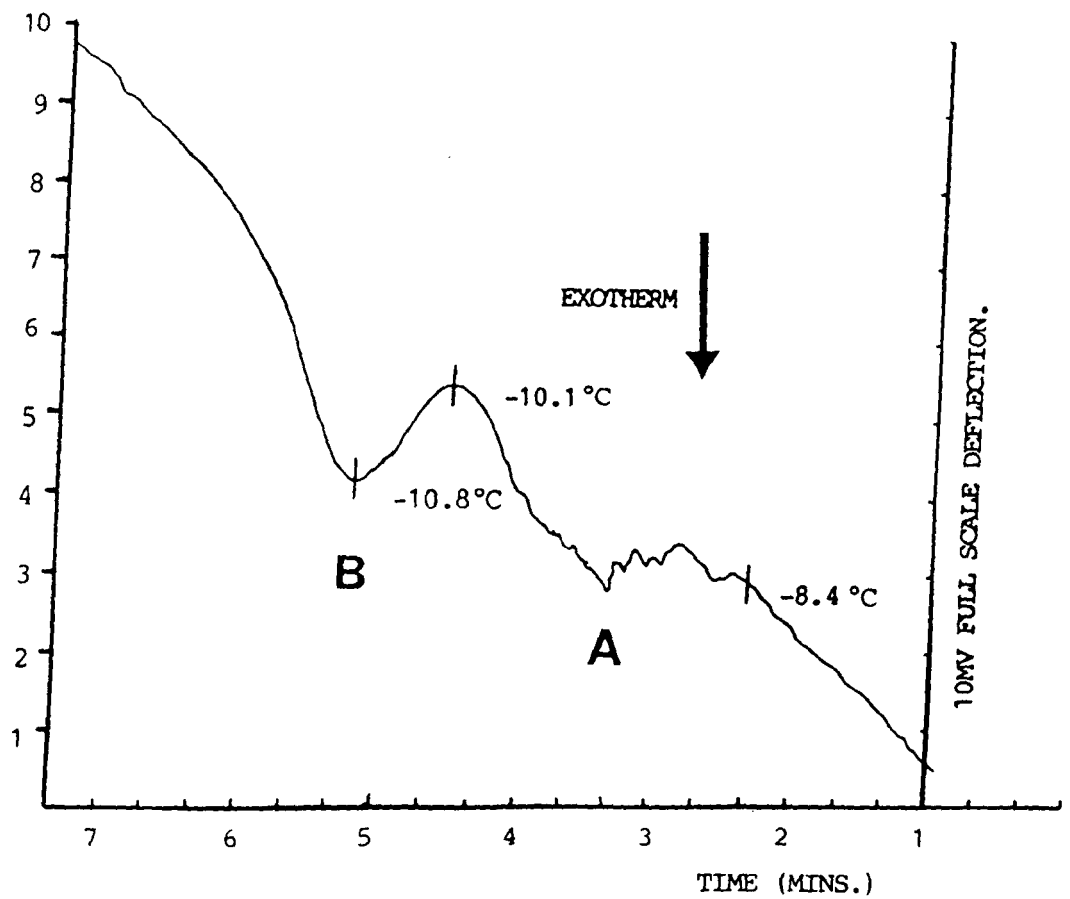

Figure 2. Typical chart trace from an experimental run with the micro-bead thermometer in differential mode showing the pre-freeze exotherm (peaks A and B) produced by an aphid cooled at 1 deg min^{-1}

DISCUSSION

When used to detect SCPs the micro-bead thermometer provides a useful increase in sensitivity compared to thermocouples thus facilitating studies on a wider spectrum of invertebrate groups. The thermistors are not as robust as thermocouples, but a coating of varnish on the fine connecting wires reduces the risk of breakage; for larger specimens thermocouples remain the preferred sensors for SCP experiments.

The option to use thermistors in the differential scanning mode provides a sensitive and inexpensive system that has produced comparative results to those obtained from modern DSCs. Clearly the DSC has distinct advantages: energies can be quantified as an aid to identifying the nature of the change, the temperature is recorded automatically as part of the trace on the chart recorder, and the baseline drift can be adjusted. For many invertebrate studies however it will be sufficient to record the thermal events without the need for quantification. There is also a limit to the size of specimens that can be studied in a DSC dictated by the dimensions of the sample pans. For these reasons of sensitivity, adaptability to different cooling systems and low cost, the micro-bead thermistor unit will be a useful aquisition for research in many areas of cryobiology. The unit has been found to be very reliable and is now in commerical production.

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INSECT COLD HARDINESS: SUPERCOOLING POINTS AND PRE-FREEZE MORTALITY

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SUMMARY

The grain aphid Sitobion avenae is a freezing intolerant insect with a mean supercooling point (SCP) of -24.2°C in the adult stage. During cooling at 1 deg min^{-1}, mortality (lethal injury) commences at -5°, the LT_{50} is -8.1° and there are no survivors at -15°C. Differential scanning calorimetry (DSC) of single aphids revealed a previously unreported pre-freeze exotherm at the LT_{50} temperature corresponding to less than 1% of the energy liberated at the freezing point, followed by normal supercooling points. Supercooling points are ecologically irrelevant if insects are already dead before they freeze.

KEYWORDS

Supercooling points, ecological relevance, pre-freeze exotherm and mortality.

INTRODUCTION

The majority of insects are freezing intolerant and die when ice forms in the body. The only protection for such species against lethal ice formation is the ability to supercool, the process by which body tissues and fluids are maintained in the liquid state below their equilibrium freezing point. The supercooling point (SCP), measured as the lowest temperature before freezing occurs, is widely used as a comparative index of cold hardiness for freezing intolerant species[1], representing the instantaneous low temperature death point[2]. Since the early studies of Salt in 1930 and throughout the modern literature of insect cryobiology, it has been assumed that during a supercooling experiment at the standard cooling rate of 1 deg min^{-1}, the specimen remains alive until nucleation occurs and the ice front advances through the body. This assumption has never been tested. Thus the aim of the research described in this paper was to quantify mortality occurring above the SCP during supercooling experiments in a freezing-intolerant species. Further preliminary experiments were carried out to investigate the nature of such pre-freeze mortality.

MATERIALS AND METHODS

Grain aphids (Sitobion avenae) used in all experiments were reared on 2 week old wheat seedlings (cv. Avalon) at 20 $\pm$ 1°C in a 16:8 LD photoperiod. Lupin aphids (Macrosiphum albifrons) were maintained under similar conditions on young lupin plants (Russell strain). SCP were determined at a cooling rate of 1 deg min^{-1} by an automatic

thermoelectric cooling system with computer-based recording of temperature changes via a multichannel thermocouple convertor[3].

To determine the LT_{50} of adult and nymphal S. avenae separate samples of 50 adult and nymphal aphids (5 replicates of 10 individuals) were cooled at 1 deg min^{-1} to a series of set minimum temperatures at intervals of 2.5 deg under identical conditions as described above.

Differential scanning calorimetry (DSC) studies were performed on single aphids of S. avenae (average adult weight 0.42 mg) and M. albifrons (average adult weight 4.5 mg) placed into sealed DSC sample pans. They were cooled from 17°C at 1.25 deg min^{-1} (closest available rate to that used in SCP and LT_{50} experiments) in a Perkin Elmer DSC-2, fitted with a sub-ambient temperature accessory and a scanning auto zero device. The instrument was operated at its maximum sensitivity. Both species were also investigated by a prototype differential scanning thermistor system (to be described elsewhere) capable of detecting the low level energy (heat) changes sensed by the DSC.

RESULTS

Supercooling experiments

S. avenae supercools to below -20°C in all nymphal instars and the adult stage with little variation between or within age groups (Table 1)[4]

Age group	Mean SCP ± S.E. °C
Nymphal instar 1	-27.0 ± 0.2
2	-25.9 ± 0.2
3	-25.2 ± 0.2
4	-24.4 ± 0.3
Apterous adult	-24.2 ± 0.3
Alate adult	-23.3 ± 0.3

Table 1. Mean ± S.E. supercooling points (°C) of Sitobion avenae cooled at 1 deg min^{-1}.

Determination of LT_{50}

Within 1-2h of exposure grain aphids could be classified as dead, comatose (twitching of appendages) or fully mobile. Invariably, aphids which were unable to walk normally within 2h of exposure died during the next 24-96h. Mortality was assessed 24,48,72 and 96h after exposure and LT_{50} (lethal temperature) values calculated for each time interval as summarised in Table 2.

Age Group	$LT_{50} \pm$ 95% confidence limit (°C) at intervals (h) after cooling at 1 deg min^{-1}			
	24	48	72	96
Nymphs	-11.2 ± 0.9	-10.8 ± 1.1	-10.8 ± 1.2	-10.9 ± 1.2
Adults	-8.1 ± 0.5	-7.7 ± 0.6	-7.0 ± 0.4	-6.2 ± 0.4

Table 2. $LT_{50} \pm$ 95% confidence limit (°C) for adult and first instar nymphal Sitobion avenae after cooling at 1 deg min^{-1} to minimum temperatures.

Nymphs were consistently more cold hardy than adults (based on LT_{50} values) by 3 to 4.7 deg. LT_{50} values differed by a maximum of 1.9 deg between 24 and 96h for both adults and nymphs indicating that most fatally injured aphids would die within a day of exposure. No development (moulting) or reproduction was observed before death in aphids which died up to 96h after exposure.

DSC experiments

An exotherm was invariably observed above the SCP in both species and figure 1 illustrates typical results for S. avenae and M. albifrons.

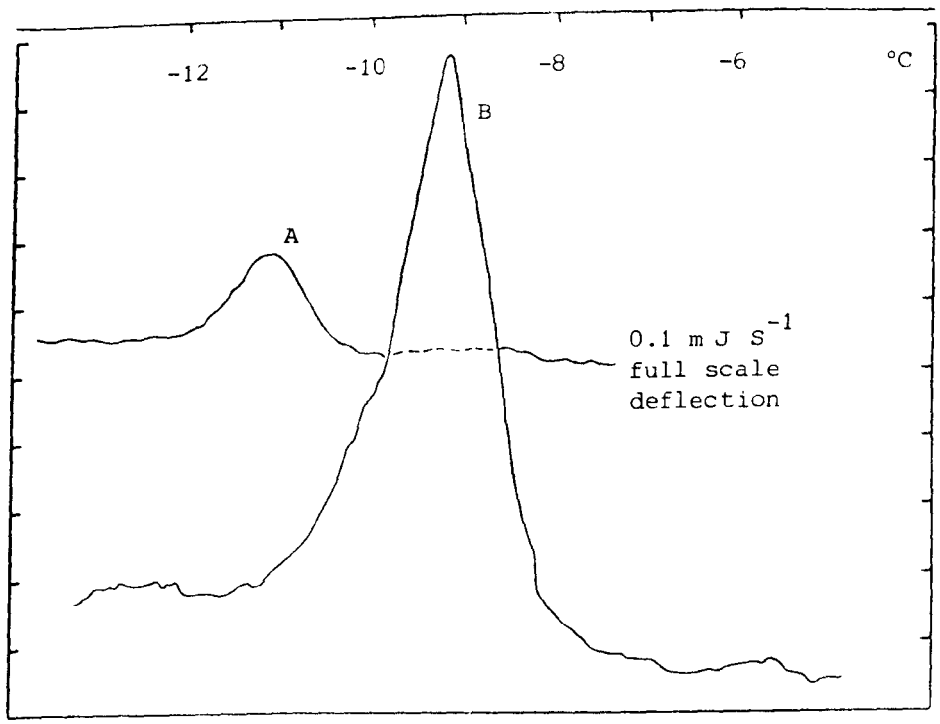

Fig. 1 Power-time curve obtained on cooling a single Sitobion avenae (0.7mg) (A) and Macrosiphum albifrons (2.5mg) (B) at 1.25 deg min^{-1}

With both species, although the amplitudes of the exotherms were variable, the half-widths were remarkably constant (0.8 for S. avenae and 1.25 deg for M. albifrons); the onset temperatures varied by 1 deg within each species, (-9° for M. albifrons and -10°C for S. avenae). A shoulder on the low temperature side suggested the presence of two partly superimposed processes. On rewarming the sample from -13°C no endotherm could be observed, indicating the irreversibility of the thermal process. Futhermore, the absence of an endotherm at 0°C is strong evidence that the exotherm is of direct biological origin rather than an artifact associated with condensation in the system or evaporative water loss from the specimen; additionally no exotherm was present in aphids killed prior to cooling. A second cooling scan revealed no further thermal event except the SCP at <-13°C for M. albifrons and <-20°C for S. avenae.

When studied by the differential scanning thermistor system (which had been calibrated against the thermocouples used for temperature sensing in the SCP and LT_{50} experiments) the exotherm for S. avenae had an onset temperature of -7.5 ± 0.4 and a mid-peak value of -8.3 ± 0.4°C (n = 10). Two peaks less than 1.5 deg apart were frequently detected by the thermistor system, in which the second peak was markedly and consistently larger. With M. albifrons the onset temperature was -6.9 ± 0.2° and mid-peak value -8.1 ± 0.2°C (n = 10). These values for S. avenae and M. albifrons confirm the narrow range of onset temperatures for the exotherm observed with the DSC. There was a consistent difference of about 2 deg between values obtained from the DSC and the thermistor system, attributable to the calibration procedures in different laboratories; however, when the exotherm and LT_{50} values are derived from temperature sensors calibrated against a common reference source (automatic thermoelectric SCP cooling system with thermocouples and the differential scanning thermistors) there is a good correlation between the exotherm and LT_{50} with values of -8.3 ± 0.4° (mid-peak) and -8.1 ± 0.5°C respectively.

DISCUSSION

In its 50 year history the study of insect cold hardiness has concentrated almost exlusively on the mechanisms by which insects survive or avoid freezing, and the factors which influence this ability, thus providing an increasing detailed literature on freezing tolerance aided by ice nucleating proteins[5], and supercooling ability extended by cryoprotectants[6] and stabilised by thermal hysteresis proteins[7]. The efficacy of these strategies and their relevance to natural environments is unknown.

The SCP is the most widely used index of cold hardiness and the adoption of a standard cooling rate (1 deg min^{-1}) has facilitated comparative investigations. The method offers rapid experimental times, requires relatively small sample sizes and can utilize a range of cooling and recording systems. These features of convenience allied to a diversity of modifying influences to study (acclimation, synthesis of cryoprotectants, surface moisture, feeding status) may have discouraged any critical

appraisal of the method, particularly its value as an indicator of survival or death in the natural environment.

The mean SCP of laboratory populations of S. avenae and field samples throughout the year is consistently below -20°C. Atypically for a freezing intolerant species, a small number of individuals lose this ability to supercool in late winter but this has little influence on the mean value[4]. If considered in isolation, this information might suggest that S. avenae is adequately protected from the deleterious effects of cold throughout most British winters, since temperatures close to -20°C are extremely rare. Comprehensive studies on the grain aphid indicate however that (i) there is 100% mortality above the mean SCP at the standard cooling rate of 1 deg min^{-1}, (ii) environmental temperatures are continuously above the mean SCP but (iii) populations decline markedly and abruptly in winter at temperatures much above the mean SCP (Figure 2, McGrath and Bale, unpublished data).

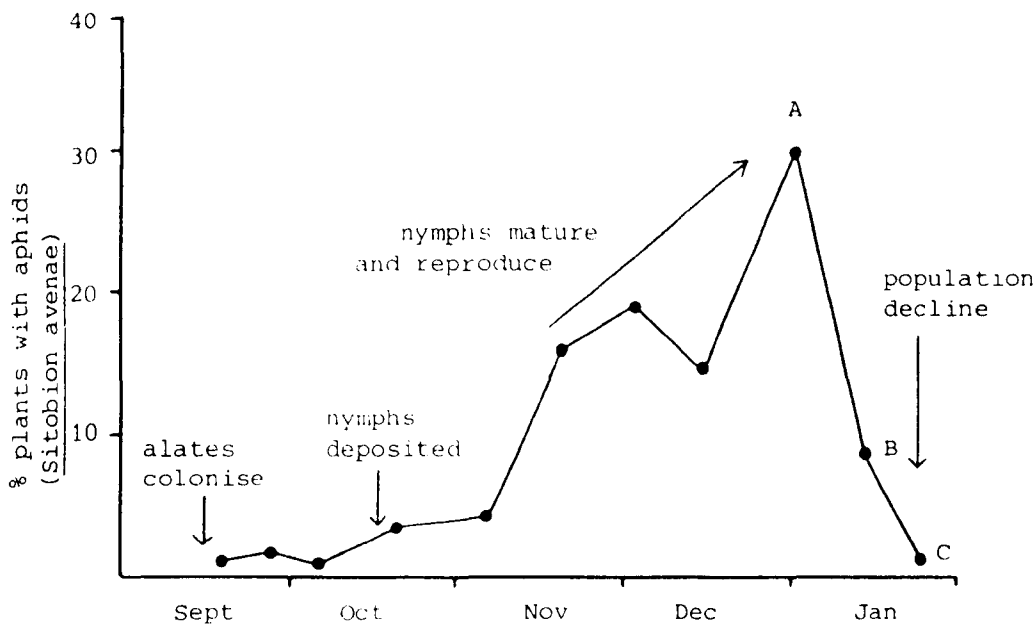

Fig. 2 Population sequence of Sitobion avenae on winter barley in winter 1984-85. Aphid density declined from 30% to 1% of plants infested (1m to 35,000 ha^{-1}) in 3 weeks when the lowest grass minimum temperature was -8.1°C between A and B and -9.7°C between B and C. The mean SCP was <-20°C.

Studies on another population of S. avenae in a different field also indicated a distinct increase in mortality when the grass minimum temperature fell below -8°C for the first time in the winter (Knight and Bale, unpublished data). Overwintering insects are subject to an interaction of ecological and physiological factors, which are difficult to model in the laboratory, but the close agreement of the LT_{50} value (-8.1°C) to the field temperatures at which significant increases in mortality occur, suggests that for S. avenae, cold is the single most important winter mortality factor and that the rate of cooling to the lethal temperature may have little effect on the level of mortality. Clearly for some freezing-intolerant insects with extensive supercooling ability, predictions of winter survival based solely on SCP data can be highly misleading and emphasises the need to quantify cold death above the SCP and assess population changes in winter in relation to known environmental temperatures.

It is recognised that aphids are not typical cold-hardy freezing intolerant insects; there is no seasonal acclimatisation and no response to laboratory acclimation regimes[4]. In previous studies on the most cold-hardy freezing intolerant species which accumulate polyols in winter and show thermal hysteresis effects, experimentors have (apparently) never assessed the viability of test specimens in SCP experiments above the SCP. Rarely have such studies quantified winter mortality in the subject species or measured accurately the microclimate temperatures of overwintering sites. For these reasons, it is possible that the inconsistencies between supercooling points, environmental temperatures and population decline, so readily apparent in S. avenae, may also apply to other 'more typical' freezing intolerant species. Future studies on such species should at least confirm that the insects remain alive in SCP experiments until they freeze and recover normal behaviour if not frozen.

The novel discoveries with S. avenae may also shed light on another established principle of insect cryobiology. While it has been recognised that insects may die during prolonged exposure to sub-zero temperatures above the supercooling point, this has traditionally been explained in

terms of the increasing probability of ice nucleation under such regimes[8]. Freezing has been presumed but never proven as the cause of death in these conditions, even in laboratory experiments, and our studies would suggest that for some species non-freezing cold death is the more likely explanation. We conclude that the physiologically or biochemically based ability of insects to avoid lethal freezing by supercooling to -20°C and below is ecologically irrelevant when non-freezing cold death occurs at higher temperatures. Furthermore, the classification of such species as 'freezing intolerant' may not be appropriate (except in the strictest sense) since the organisms are invariably dead or fatally injured before they freeze. A reappraisal of the ecological worth of cold hardiness studies based primarily on supercooling points is timely since the accepted dogma on the influence of cooling rate on the freezing status (tolerant or intolerant) of species and the identification of sites of nucleation is also under review[9].

The causes of non-freezing cold death may be variable between species and be dependent on the minimum temperature experienced and period of exposure. These lethal processes are likely to be more subtle and difficult to interpret than our simplistic view of the physiological disruption which follows internal ice formation, so clearly indicated by the SCP. The thermal event shown in Fig. 1 is not related to supercooling and freezing because no corresponding endotherm can be observed in the neighbourhood of 0°C on rewarming. Instead, the exotherm appears to be related to the process that causes lethal injury. Possible causes could include a thermotropic membrane phase transition, a cold inactivation of protein(s)[10] or the decoupling of the normal metabolic processes, giving rise to an unsuccessful attempt by the organism to repair the damage. A critical review of the research methodologies of insect cold hardiness will be required if these phenomena are common in freezing intolerant insects. In the wider context the ability to predict mortality levels in relation to known temperatures is relevant to current developments in ecological modelling, forecasting of pest outbreaks, and the storage of biological materials at low temperature.

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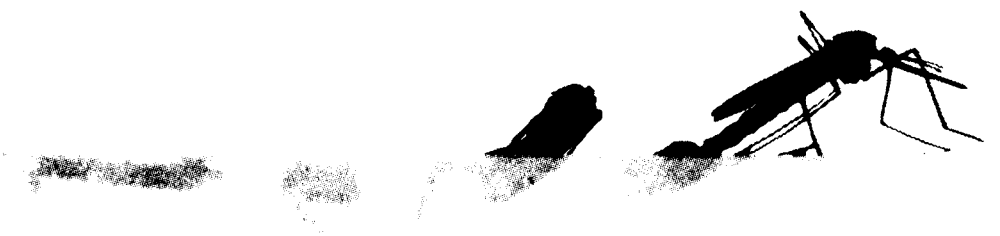

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THE EFFECTS OF LOW TEMPERATURE ON THE DEVELOPMENT, REPRODUCTION AND SURVIVAL OF THE GRAIN APHID SITOBION AVENAE

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Introduction

The grain aphid Sitobion avenae is presumed to overwinter predominantly as anholocyclic (asexual) adults and nymphs although an unknown proportion overwinters holocyclically (sexually produced eggs) (Dewar and Carter, 1984). The conditions experienced by the anholocyclic aphids over the winter will affect their survival and therefore the timing and size of the spring migration. This study was undertaken to examine the effects of metereological conditions on development, reproduction and mortality of S. avenae.

Materials and Methods

The work was carried out during the winter of 1984-85 at the University of Leeds field station in North Yorkshire, England. The experimental site was in a field of winter barley (var. Igri) in an exposed position. The aphids used in the experiment were derived from a single clone and maintained on whole barley plants (var. Igri) at 20°C ± 1°C. First instars used in the experiment were collected over a 24 hour period from alate mothers which had already commenced reproduction. The adults used in the experiment were reared from nymphs born over a 24 hour period and collected after the final moult. All aphids were acclimated at 10°C for 2 days prior to being placed in the field.

Three batches of aphids, each consisting of 100 newly moulted adults and 100 first instar nymphs were placed in the field. The first group went out on October 26th, the second on December 10th and the final group on February 20th. The adults in the first batch consisted of 50 alatae and 50 apterae, in the final two batches 100 apterae were used.

Each aphid was placed in a separate clip cage containing a leaf of a barley plant. The cages were numbered for ease of identification and inspected at weekly intervals (weather permitting) and any moults, offspring, or deaths recorded. The cages were moved to new leaves every two to three weeks to prevent any effects from senescing leaves. Weather data for the experimental period was recorded at the Field Station weather station situated about 200m from the experimental site.

Results

Mean reproduction, measured as the number of births per adult per sample period, was linearly and positively correlated with the sum of day degrees above 3°C for the same sample period. ($P<0.01$) (Figure 1). The threshold of 3°C was chosen on the basis of previous studies with the same species (Williams, 1984). The method used for the calculation of day degree values was that of Walters (1982).

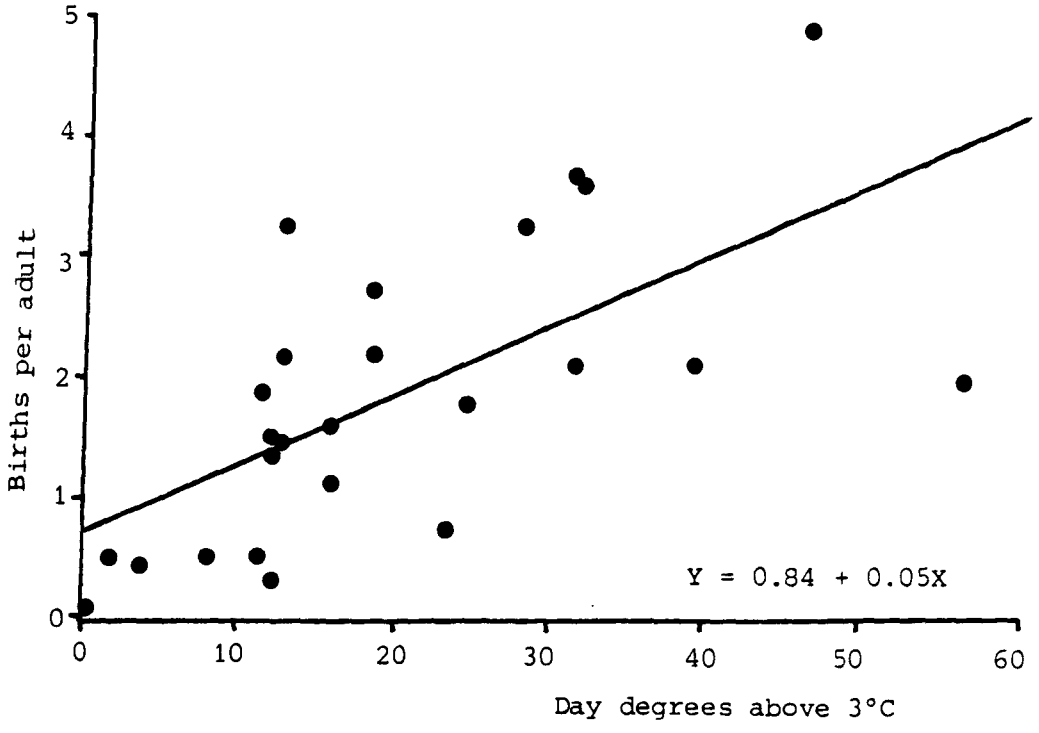

Figure 1. The number of S. avenae births per adult during each sample period plotted against the number of day degrees above 3°C for the same period.

The rate of development was inversely proportional to temperature and the mean rate of development as measured by the number of moults per aphid per sample period was linearly and positively correlated with the sum of day degrees above 3°C for the sample period. ($P<0.01$) (Figure 2).

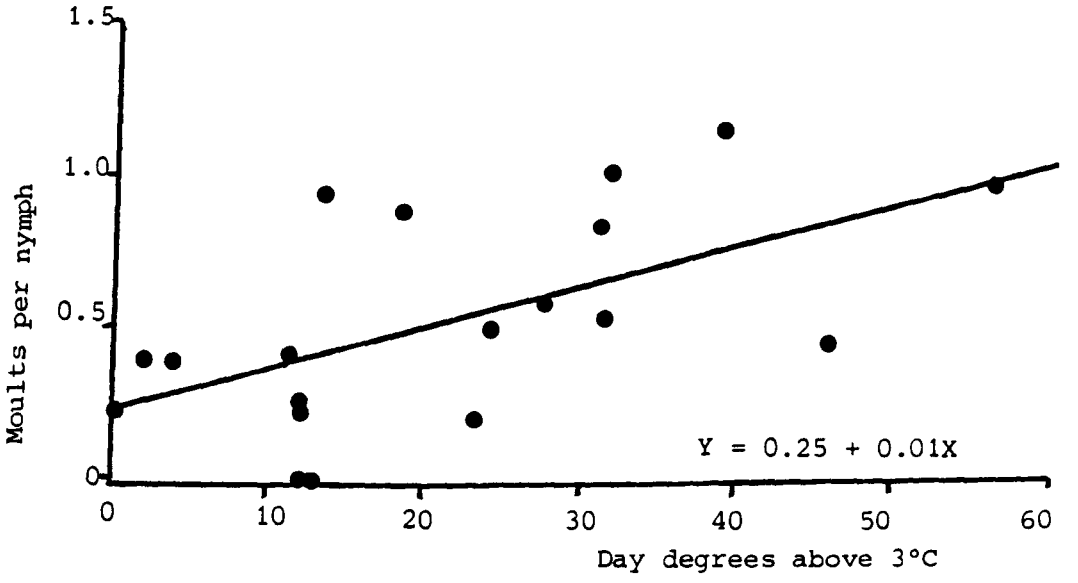

Figure 2. The number of S. avenae moults per nymph during each sample period plotted against the number of day degrees above 3°C for the same period.

The mortality of S. avenae was also inversely proportional to temperature with a marked increase in the mortality at temperatures below about -8°C (Figure 3). The mortality was measured as the rate of population change; the daily rates of change, $(\log N_2 - \log N_1)/t$ (where N_1, and N_2 are the number of aphids present in the first and second observations, respectively, of each consecutive pair and t is the number of days between samples) were regressed against the lowest grass minimum temperature recorded between consecutive samples. No correlations could be found between mortality, development or reproduction with either precipitation or wind speed.

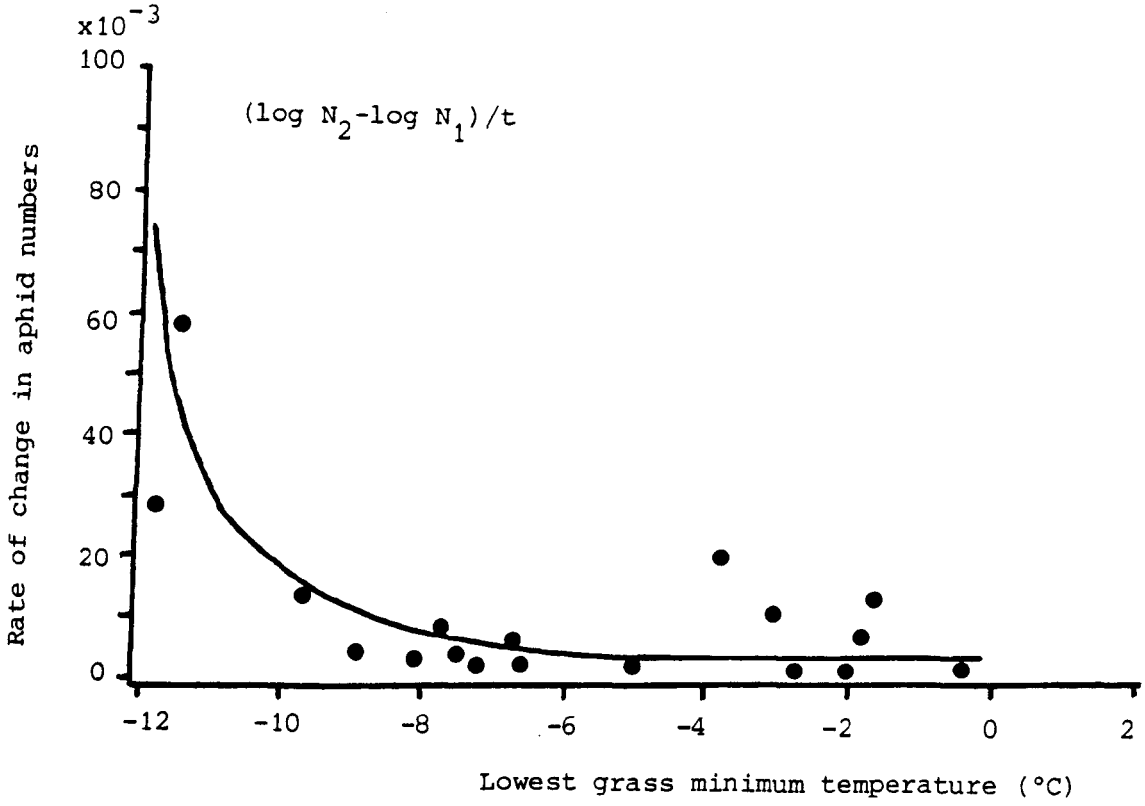

Figure 3. Rate of change of aphid numbers of S. avenae with lowest grass minimum temperature.

Discussion

This study has shown that anholocyclic overwintering is successful for S. avenae in the North of England and that reproduction and development continue in all but the most severe conditions. The overwintering success of S. avenae appears to be dependent only upon temperature and not precipitation or wind speed. However, this may be due to the protection afforded by clip cages which would exclude the worst effects of wind and rain.

Previous laboratory experiments showed the grain aphid to have LT_{50} (the temperature required to kill 50% of a sample) values of -8.1°C for adults and -11.2°C for nymphs (Knight and Bale, 1986). These values correspond well to the temperatures recorded in the field when the population declined most markedly, and are therefore a good index of the cold hardiness of S. avenae.

The adult aphids responded rapidly to short periods of milder weather by reproducing more quickly; this would lead to an increase in the number of

nymphs in the field. Since the nymphs appear to be more cold hardy than adults, in terms of LT_{50} values, such rapid reproduction will ensure a higher rate of survival of aphids. Therefore, it is possible that isolated periods of mild weather are important for the success of anholocyclic overwintering of S. avenae.

The quick response to warmer conditions also ensures that in spring, nymphs will be born and able to develop much in advance of populations derived from overwintering eggs. This could lead to an early build up in aphid numbers, early migration and damage to the cereal crop if predators, parasites and diseases fail to regulate the aphid population.

In severe winters when temperatures are consistently much below the LT_{50} values then the more cold hardy eggs will ensure the survival of the species, but the spring population build up and migration will be later due to the extra time required for the eggs to hatch, nymphs to develop and reproduction to occur.

The data from this study confirm prior investigation and speculation on the overwintering biology of S. avenae. A comprehensive and thorough knowledge of the aphid's biology is required before the forecasting of outbreaks and the need for control can be accurately made.

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Cold hardiness and overwintering of the grain aphid *Sitobion avenae*

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ABSTRACT. 1. Cold hardiness as measured by supercooling ability in the active stages of the grain aphid *Sitobion avenae* (F.) decreased progressively with maturation.

2. Aphids showed no acclimation response when maintained at low temperatures.

3. Starvation did not improve supercooling ability.

4. In a single exposure, surface moisture caused inoculation above the inherent supercooling point in a small proportion of a population.

5. Field populations show a seasonal change in supercooling ability, which is at a maximum in summer and a minimum in late winter.

6. It is concluded that the act of feeding on healthy plant tissue may confer extensive supercooling ability.

Key words. Grain aphid, *Sitobion avenae*, cold hardiness, overwintering, freeze susceptible, supercooling point.

Introduction

Over forty species of aphid have been recorded on cereals and grasses in Europe (Müller, 1964) but only three are generally regarded as important pests of these crops in Britain. *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.) can cause significant yield reduction by feeding at high density (Rabbinge & Mantel, 1981; Vereijken, 1979) while *Rhopalosiphum padi* (L.) is primarily important as a vector of barley yellow dwarf virus (BYDV) (Vickerman, 1977). All three species have anholocyclic (asexual) strains which overwinter as active stages on available hosts.

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With the increase in land under cereals in the United Kingdom, currently about 4 million hectares (Anon., 1982) and in the proportion of winter sown crops, particularly barley, these aphids have become major pests both as direct feeders and virus vectors. Current research has shown that alates colonize winter barley soon after emergence; the apterae which subsequently develop can produce a large number of nymphs before low temperatures halt reproduction. The fate of these aphids is important because they can transmit virus in the winter crop before low temperature prevents movement and if they overwinter successfully they give rise to an early migration of potentially viruliferous aphids to spring barley. Studies elsewhere have shown that aphids can be found on emerging spring crops before individuals are caught in suction traps (Taylor, 1974) and addi-

tionally, because of the time requirement for virus transmission tests with oat seedlings by enzyme-linked immunosorbent assay, it is likely that substantial colonization and transmission of virus may occur in both winter and spring crops before adequate control measures are taken. The overwintering success of these aphids is therefore fundamentally important in determining their abundance and damage.

S.avenae was chosen for study since it was known to overwinter anholocyclically in the locality and had been recently implicated as a vector of severe strain BYDV (Anon., 1983).

Insects exhibit two distinct strategies to survive at low temperatures; freeze-tolerant species can survive the formation of ice in the body tissues and fluids and often contain nucleating agents to initiate freezing (to advantage) at temperatures around −5°C which minimizes tissue disruption. Freeze-susceptible species die if the tissues and fluids freeze and for these insects the only protection against a freezing death is the ability to supercool; the process of supercooling maintains the tissue and fluids in a liquid state below the equilibrium freezing point. The supercooling point of such species is therefore the instantaneous low temperature death point and a convenient basis on which to assess cold hardiness and compare similar species (Sømme, 1982); for this reason in this paper the terms supercooling ability and cold hardiness are used synonymously.

This study was undertaken to assess the cold hardiness of *S.avenae* both in the field and laboratory and to investigate the effects of sustained low temperatures (acclimation), starvation and surface moisture on the inherent cold hardiness.

Materials and Methods

Determination of supercooling points

In each of the experiments involving the measurement of supercooling ability the following procedure was employed. The aphid to be supercooled was attached to a thermocouple with a spot of petroleum jelly, and the two together placed in a chamber in an aluminium block mounted on a Peltier device. The thermocouple was interfaced to a microcomputer, via a thermocouple converter, for the sensing and recording of supercooling points. The Peltier device had an automatic temperature controller for the maintenance of a constant cooling rate of 1°C per minute. The system and methods used are described in detail by Bale *et al.* (1984).

The system described by Bale *et al.* (1984) has been slightly modified to reduce the effects of mains electrical interference which could cause a small rise in temperature (0.2°C) sufficient for the system to record a supercooling point. Plotting of the differential temperature between a reference thermocouple and that carrying an insect ensures that only true supercooling points are recorded.

Laboratory experiments

The laboratory experiments were designed to test the effects of developmental stage, acclimation, starvation and surface moisture on the supercooling ability of *S.avenae* compared to the 'inherent' supercooling potential when maintained at 20°C.

Stock cultures of aphids were clonal and maintained on young winter wheat plants (var. Avalon) at 20±2°C with a photoperiod of 18L:6D. All nymphs used in the experiments were born overnight so were of approximately the same age (within 15 h).

The inherent supercooling ability of each developmental stage was assessed by supercooling twenty individuals of each instar shortly after they had moulted. The aphids were reared on strips of wheat leaves in illuminated incubators (18L:6D) at 20±1°C.

Acclimation responses were investigated by placing first instar nymphs or newly moulted

TABLE 1. Supercooling ability (mean ±SE and range °C, N=20) of developmental stages of *Sitobion avenae* maintained at 20°C. (Means followed by the same letter do not differ significantly at 5% level.)

First instar	Second instar	Third instar	Fourth instar	Apterous adult	Alate adult
−27.0 ±0.2	−25.9 ±0.2	−25.2 ±0.2	−24.4 ±0.3	−24.2 ±0.3	−23.2 ±0.3
−25.6/−28.6	−23.8/−26.8	−23.0/−26.4	−18.8/−25.8	−20.2/−25.9	−20.5/−26.6
			a	a	

TABLE 2. Supercooling ability (mean ±SE and range °C, $N=20$) of first instar *Sitobion avenae* after acclimation at (a) 0°C or (b) 5°C and combined starvation and acclimation at (c) 0°C and (d) 5°C. (Means followed by the same letter do not differ significantly at the 5% level.)

Acclimation					
	Days of acclimation				
Temperature	2	7	14	21	28
(a) 0°C	−25.8 ±0.2 −23.5/−26.9 a	−26.0 ±0.1 −25.1/−26.8 a	−26.1 ±0.1 −25.2/−27.8 a	All dead	All dead
(b) 5°C	−26.2 ±0.2 −24.4/−26.9 ab	−26.4 ±0.1 −24.6/−27.1 a	−26.2 ±0.2 −24.1/−27.8 ab	−25.6 ±0.3 −22.0/−27.2 b	−26.1 ±0.2 −24.6/−27.1 ab
Acclimation and starvation in combination					
	Days of acclimation and starvation				
Temperature	2	7	14	21	28
(c) 0°C	−26.0 ±0.1 −24.2/−27.2	−25.9 ±0.2 −24.8/−27.6	All dead	All dead	All dead
(d) 5°C	−25.4 ±0.2 −23.3/−26.4	−25.4 ±0.2 −24.2/−26.5	All dead	All dead	All dead

TABLE 3. Supercooling ability (mean ±SE and range °C, $N=20$) of adult apterous *Sitobion avenae* after acclimation at (a) 0°C or (b) 5°C and combined starvation and acclimation at (c) 0°C and (d) 5°C. (Means followed by same letter do not differ significantly at 5% level.)

Acclimation					
	Days of acclimation				
Temperature	2	7	14	21	28
(a) 0°C	−23.1 ±0.5 −17.9/−25.0 ab	−24.1 ±0.5 −16.4/−25.7 a	−24.5 ±0.3 −21.0/−26.1 a	−22.2 ±0.3 −20.1/−24.5 b	All dead
(b) 5°C	−24.0 ±0.3 −20.6/−25.3 b	−25.0 ±0.2 −21.7/−26.3 a	−24.4 ±0.3 −21.1/−26.3 ab	−23.9 ±0.2 −20.8/−25.7 b	−24.4 ±0.2 −21.3/−25.6 ab
Acclimation and starvation in combination					
	Days of acclimation and starvation				
Temperature	2	7	14	21	28
(c) 0°C	−24.2 ±0.2 −21.4/−25.8 ab	−23.5 ±0.3 −19.8/−25.1 ab	−24.2 ±0.1 −22.6/−25.0 a	−21.7 0.7 −14.9/−25.2 b	−22.3 ±0.6 −16.7/−25.0 b
(d) 5°C	−23.8 ±0.2 −21.2/−25.2 a	−23.2 ±0.6 −14.9/−25.3 ab	−23.3 ±0.4 −19.1/−25.0 ab	−22.1 ±0.3 −18.8/−24.4 b	All dead

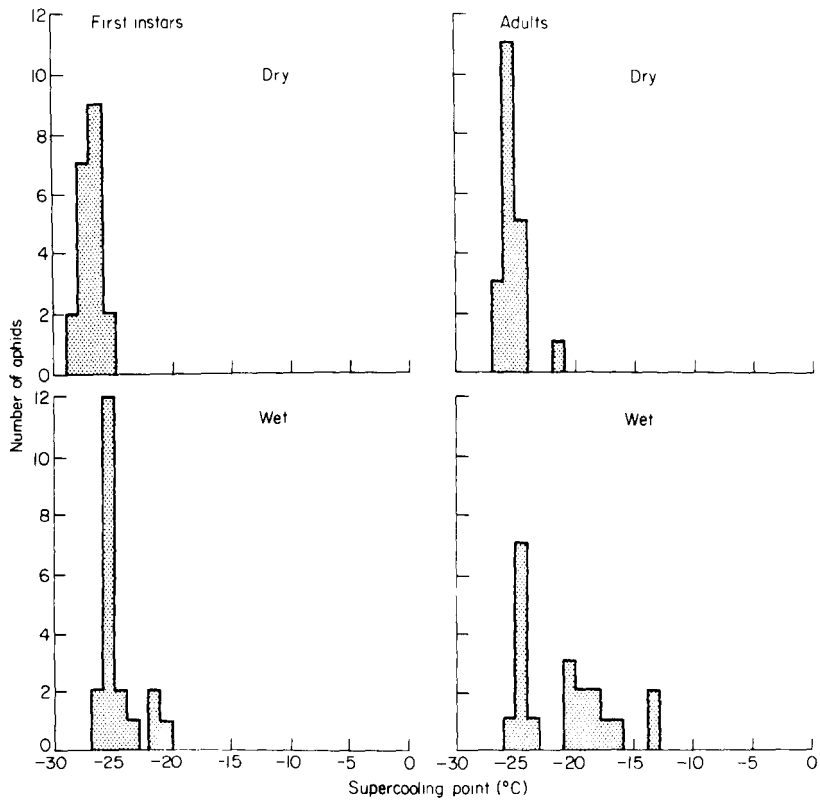

FIG. 1. Supercooling point distribution histograms of first instar nymphs and adults of *Sitobion avenae* when dry and after wetting with water.

adult apterae on leaf strips in illuminated incubators at 5±1°C and 0±1°C for 2, 7, 14, 21 or 28 days, after which the aphid supercooling points were determined. The effects of starvation and acclimation in combination were studied as above except that the aphids were maintained on damp tissue paper rather than leaf strips. To determine the effects of surface moisture on supercooling ability newly moulted adult apterae and first instar nymphs were first moistened with a wet paintbrush, after being placed on the thermocouple, and then supercooled as normal.

Field experiment

The field experiment monitored (i) the change in density of a population of *S. avenae* in a field of winter barley (var. Igri) and (ii) the seasonal variation in supercooling ability of first and second instar nymphs and adults drawn from the population. The experiment was conducted at the University of Leeds Field Station near Tadcaster, North Yorkshire, from November 1983 until July 1984.

The population density was estimated at fortnightly intervals by examining 200 tillers, in groups of twenty selected at random, within an area of approximately 12×160 m, and recording the number of aphids present.

The aphids used in the supercooling point determination were collected by hand at monthly intervals, with a target sample size of forty adults and forty nymphs. When the population density was low, fewer aphids could be collected in the time available so smaller samples were used. At one point the population density was so low that a vacuum insect sampler (Burkhard) was used to obtain sufficient aphids. The use of this machine coincided with a marked decrease in supercooling ability so a controlled experiment was run to assess the effect of such mechanical sampling. Laboratory reared aphids were vacuum sampled from plants and their mean supercooling point compared with those of hand collected aphids from the same plants.

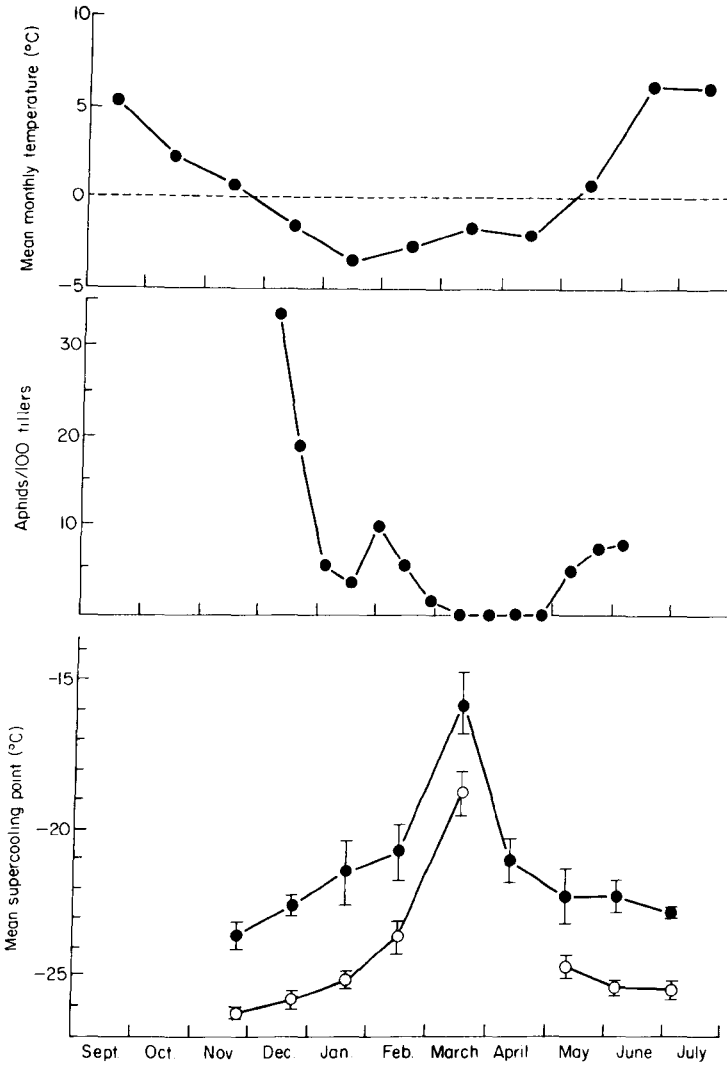

FIG. 2. (a) Mean monthly grass minimum temperature recorded at the University field station, September 1983 to July 1984. (b) Number of *Sitobion avenae* found per 100 tillers on each sampling occasion. (c) Mean supercooling point (±SE) of adult apterae (●) and nymphs (○) of *Sitobion avenae* collected from the field.

Metereological data were collected at the University farm weather station approximately 500 m from the experimental site. Statistical analysis of the results consisted of linear regression and one-way analysis of variance followed by the Student-Newman-Keuls (SNK) test where appropriate.

Results

Laboratory experiments

The laboratory experiments showed that all developmental stages have a mean supercooling point below −20°C and show little intra-stage variation. A progressive loss of cold hardiness is exhibited with increasing age, the first instars being significantly more cold hardy than the fourth instars or adult morphs ($F=29.80$, 5, 114 d.f., $P<0.001$) (Table 1).

In general, acclimation did not improve cold hardiness and the few significant differences found were so small as to be of no biological importance in the field (Tables 2a, 2b, 3a, 3b). Aphids with access to food died at 0°C when exposed for more than 14 days but mortality occurred after 7 days when starved at the same

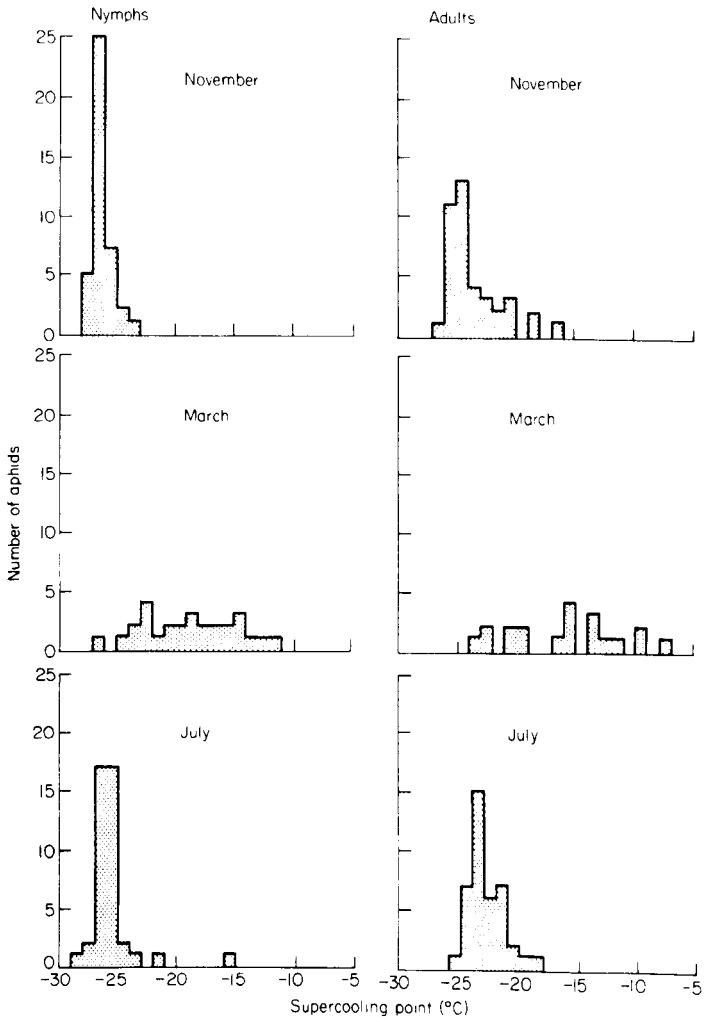

FIG. 3. Supercooling point distribution histograms of *Sitobion avenae*, nymphs and adult apterae, showing seasonal changes.

temperature (Table 2c). At 5°C starved nymphs also began to die after only 7 days (Table 2d).

Nymphs in general showed no change in cold hardiness in these experiments, whereas adults show a loss in cold hardiness with increased exposure to the low temperatures of acclimation and starvation in combination; the mean supercooling point after 21 days at 0°C is significantly less than that after 2 days but it improves again by 28 days ($F=6.14, 4, 95$ d.f., $P<0.001$) (Table 3c). Starvation at 5°C resulted in a significant loss of cold hardiness in the adults after 21 days compared to that at 2 days ($F=3.44, 3, 76$ d.f., $P<0.05$) (Table 3d).

Surface moisture caused a significant loss of cold hardiness in a proportion of both adult apterae and first instar nymphs ($t=5.96$, 30 d.f., $P<0.001$ and $t=3.70$, 23 d.f., $P<0.01$ respectively) although in both cases the mean supercooling point did not rise above −20°C (Fig. 1).

Field experiments

The mean monthly grass minimum temperature and the change in population density with time are shown in Figs. 2(a) and 2(b) respectively. Adults and nymphs collected from the field showed a marked seasonal variation in

mean supercooling point (Fig. 2c). In November and December and from April to July the adults had a mean supercooling point below −20°C, whilst the mean supercooling point of the nymphs remained below −20°C for all months except March. No nymphs could be found in April.

Frequency histograms of supercooling points in monthly samples for winter and early spring were skewed, but there were no significant deviations from normal (Fig. 3).

The mean monthly grass minimum temperature was positively and linearly related to both the adult mean monthly supercooling point and the population density occurring 2 months later (r=0.93, 3 d.f., P<0.05 and r=0.92, 3 d.f., P<0.05 respectively) for the first 5 months of the experiment. No significant relationship was found between the mean monthly grass minimum temperature and the nymphal mean monthly supercooling point or between either of the mean monthly supercooling points and population density. There was no significant relationship between any of the aphid parameters measured and mean monthly rainfall or mean monthly windspeed.

The control experiment showed that there was no significant difference between mean monthly supercooling points of laboratory reared aphids collected by hand and those collected by the vacuum sampler (t=1.85, 32 d.f.).

Discussion

Supercooling points indicate the variation in the instantaneous low temperature death point of a population and are widely used as a convenient laboratory method to assess and compare the cold hardiness of freeze-susceptible species (Sømme, 1982). The results for *S. avenae* suggest that its supercooling ability is adequate to survive freezing temperatures in an instantaneous exposure in all stages of development, and in all but the most severe British winters.

Freezing-susceptible insects share a number of common features of cold hardiness based on supercooling point data: (i) a seasonal pattern of cold hardiness which is at a maximum in winter; (ii) an acclimation response induced by laboratory exposure to low temperatures; (iii) inoculative freezing above the supercooling point induced by body surface moisture; and (iv) reduced supercooling ability when feeding associated with nucleators in the gut (Sømme, 1982). This study suggests that *S. avenae* is atypical in all of these features. Thus the aphids showed maximum cold hardiness in summer, little or no acclimation response, a low percentage mortality above the inherent supercooling point even when wet, and were most cold hardy when feeding on healthy plants. While atypical for other freeze-susceptible species, these features are apparently common to anholocyclic aphids including *Myzus persicae* (O'Doherty & Bale, 1985).

Natural summer field populations feeding on healthy plants or laboratory samples reared on leaf strips at 20°C showed maximum cold hardiness. This suggests that the diet of aphids may confer high levels of supercooling ability without the risk of nucleator contamination. Plant phloem sap contains polyols which are known to be cryoprotectants in other insects (Danks, 1978) and it appears that feeding may be essential to maintain this source of antifreeze agents which may act directly without the need for intermediary metabolism; as such the freezing protection of aphids is simply an artefact of their specialized feeding habit, but to be effective it does impose the need for continued feeding during winter. At temperatures below −4°C *S. avenae* are totally immobile (Smith, 1981) and aphids may then become progressively starved until temperatures rise again. A similar effect may occur when aphids are dislodged from host plants by wind and rain and are then prevented from recolonizing the host (Harrington & Xia-Nian, 1984).

If aphid cold hardiness is based largely on a quantitatively and qualitatively adequate diet, rather than an internal seasonal synthesis of cryoprotectants, then it is unlikely that acclimation will occur and no responses of any biological significance were observed in extensive experiments at 0°C and 5°C. The results do, however, provide additional evidence for the importance of feeding in aphid cold hardiness since under conditions of starvation the distribution of supercooling points was different to that of feeding aphids, but was similar to that of winter field samples which are likely to be starved, or feeding at reduced levels.

In some freezing susceptible insects the action of body surface moisture reduces inherent supercooling ability dramatically and consis-

tently as with the beech weevil *Rhynchaenus faqi* (Bale, 1980). Overwintering aphids on cereal plants would encounter rain, dew and frost and although only a small proportion of laboratory *S.avenae* were affected by surface moisture in a single exposure it is likely that in the repeated frosts of winter considerable cumulative mortality may occur.

Laboratory research on insect cold hardiness based on supercooling point data assesses the ability of a species to avoid a freezing death and inevitably isolates factors which in nature act in combination. The field experiments show a strong correlation between mean monthly grass minimum temperature and both the mean adult supercooling ability and changes in population density occurring 2 months later. Under conditions of decreasing temperature, aphids will move and feed less actively and host plants will decrease in quality; at times the aphids will be completely immobile and the plants frozen. In such circumstances a lag relationship may be expected between temperature and its influence on metabolic related processes such as supercooling ability. It is unlikely, however, that changes in population density can be attributed entirely to reduced supercooling ability and freezing deaths. The lowest temperature in the study period was −12.1°C in February 1984, whereas the majority of the population died in December when supercooling ability was far in excess of environmental temperatures. While it is possible that some aphids may have accumulated a 'cold dose' leading to a freezing death (Salt, 1950) it is more likely that a proportion of aphids died for reasons other than freezing and at temperatures much above their supercooling point. Indeed recent experiments, to be reported elsewhere, have shown that some aphids die during the course of a supercooling experiment at −10°C even though their supercooling point is below −20°C. This apparently non-freezing mortality above the supercooling point has been described for *S.avenae* and other cereal aphids (Williams, 1984). When winter cold causes mortality by both freezing and non-freezing death, supercooling studies alone will not provide an accurate index of low temperature mortality.

There is no doubt that aphid populations decline markedly in mid-winter and that some mortality may occur through freezing and inadequate supercooling. Some of the observed mortality may be due to 'cold-injury' (Ring, 1980) but the mortality factors are complex and may involve membrane disruption, starvation and desiccation and studies in this laboratory are currently investigating the interrelationships between these factors. In contrast to the active stages of aphids, eggs are very cold hardy with supercooling points as low as −43°C and also show acclimation at low temperatures (James & Luff, 1982). Thus, although the adults and nymphs may die due to a lack of supercooling ability or cold injury, the eggs of the holocyclic strains will ensure overwintering survival of the species through severe winters.

The distinct seasonal changes in supercooling ability of *S.avenae* may be of more value as an indicator of population fitness rather than a basis for predicting winter mortality. At present it is concluded that aphids represent a highly atypical group of freezing susceptible insects whose cold hardiness is based on their specialized diet. The relationship between this cold hardiness and winter mortality is an area of current research with considerable implications for the prediction of aphid abundance and virus transmission.

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