COLD HARDINESS AND COLD STORAGE OF *PHYTOSEIULUS PERSIMILIS* AND *AMBLYSEIUS CUCUMERIS* (ACARINA: PHYTOSEIIDAE)

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

The spider mite predator *Phytoseiulus persimilis* Athias-Henriot and the thrips predator *Amblyseius cucumeris* (Oudemans) are both commercially mass-reared for use as biological control agents for greenhouse pests. The ability to stockpile these mites in cold storage would greatly facilitate economical mass-production and distribution. In addition, these two species provide an opportunity for comparative studies of cold hardiness because *P. persimilis* originated in subtropical Mediterranean type climates and is thought to be incapable of entering diapause whereas *A. cucumeris* is widespread in temperate zones where adult females enter a reproductive diapause for overwintering.

Temperature/mortality curves confirmed that both species are freezing intolerant in the traditional sense that supercooling points (SCPs), at which freezing of body fluids occurs, represent absolute lower lethal temperatures. Both species were capable of moderate supercooling, into the range of –20°C to –30°C, and both showed a trend of increasing SCP temperatures during development from egg to adult that suggested an inverse relationship between supercooling capacity and body size within species. The only exception to this trend was adult female *A. cucumeris*, which may be significant because this is the only life stage that is capable of diapause or survival of temperate winters. On the other hand, diapause induction and low temperature acclimation had little or no effect on supercooling capacity, and survival of nonacclimated mites at subzero temperatures above their SCPs was limited to very short periods of exposure, suggesting that the SCP represents a physical property of the mites rather than an adaptation for survival of exposure to subzero temperatures.

Cold-storage survival of both species was optimum at 7.5°C, was greatly enhanced when a source of moisture was provided, and was enhanced even further when food was provided even though the mites were held at temperatures below their

theoretical temperature threshold for development. Under these conditions, survival of *P. persimilis* was 80% after six weeks whereas that of *A. cucumeris* was only 35% after the same period of time. Furthermore, longevity and fecundity of *P. persimilis* after eight weeks of cold storage were comparable to mites taken directly from rearing cultures whereas oviposition by *A. cucumeris* after six weeks of cold storage was low and irregular compared to previously reported values.

Temperature data from areas where *P. persimilis* survives outdoors indicate that this species is capable of surviving prolonged exposure to temperatures below 10°C and occasional exposure to subzero temperatures, and apparently does so without diapausing. On the other hand, *A. cucumeris* may be capable of surviving prolonged exposures to subzero temperatures, but only as diapausing adult females. The results of the current studies are consistent with these ideas and further suggest that nondiapausing *A. cucumeris* are less cold hardy than *P. persimilis*.

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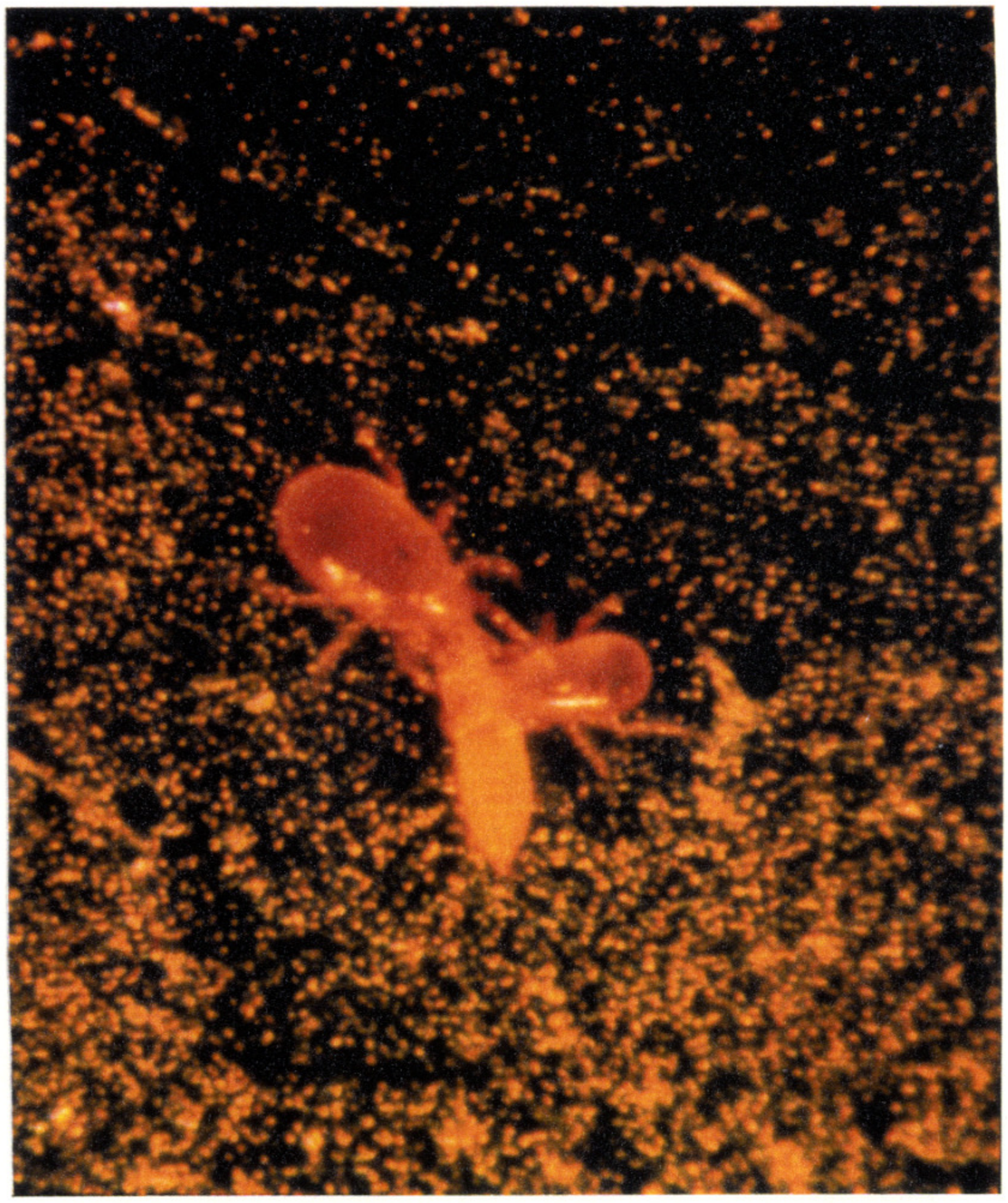

DEDICATION

This thesis is dedicated to my parents, Harry and Paula Morewood, who have always encouraged me to pursue my interests and have provided personal, financial, and logistic support for the many years of my academic endeavors.

INTRODUCTION

Cold hardiness of terrestrial arthropods is a relatively new field of biology and one that is being studied with increasing intensity. Stimulated by the pioneering work that R.W. Salt began in the 1930s (reviewed by Ring and Riegert 1991), researchers had published almost 700 papers on studies of cold hardiness in terrestrial arthropods, including 39 reviews, prior to 1986 (Baust *et al*. 1982, Lee *et al*. 1986) and these efforts are continuing to expand (*cf*. Lee and Denlinger 1991). In addition to providing insights into the physiology and ecology of terrestrial arthropods, studies of cold hardiness yield information of value for such applied areas as pest management and cryopreservation of living systems.

Cold hardiness of terrestrial arthropods is generally divided into two different strategies: freezing tolerance and freezing intolerance or susceptibility (Salt 1961, Asahina 1969, Block 1982, Zachariassen 1985, Storey and Storey 1988, Lee 1991). Although questions have been raised about the distinctiveness of these two categories (*eg*. Baust and Rojas 1985, Bale 1987), they still serve as useful generalizations and are worthy of discussion.

Freezing tolerance is defined as the ability to survive the formation of ice in body fluids (Salt 1961). Typically, although not exclusively (*cf*. Ring 1982), ice formation in freezing tolerant species is initiated in extracellular fluids at relatively high subzero temperatures (*i.e*. above –10°C), usually with the aid of special ice-nucleating proteins (Duman 1982, Zachariassen 1982, Duman and Horwath 1983, Duman *et al*. 1991). In this way the freezing event is controlled, allowing time for osmotic adjustments at the cellular level and helping to prevent intracellular freezing, which is generally thought to be lethal (Storey and Storey 1988, Duman *et al*. 1991). Both the rate of freezing and the amount of ice formed at a given temperature are decreased by the presence of low molecular weight polyols and sugars, which increase viscosity, interact directly with water molecules, and colligatively depress the melting point (Baust 1973, Lee 1991). Once a freezing tolerant arthropod is frozen, thermal hysteresis proteins may act to

inhibit recrystallization that would otherwise lead to damaging growth of ice crystals in the frozen tissues, especially during thawing (Knight and Duman 1986).

Freezing intolerance or susceptibility, more appropriately termed "freezing avoidance" as a strategy, refers to adaptations of the arthropods for which any ice formation in body fluids is lethal (Salt 1961). In this case freezing is avoided primarily by supercooling (lowering the freezing point of body fluids) with the removal or masking of potential ice-nucleating agents (Zachariassen 1982) and with the accumulation of biochemicals that help to prevent freezing (Duman *et al.* 1982). Low molecular weight polyols and sugars serve to depress the freezing point of freezing intolerant arthropods and this depression may be as much as twice the associated depression in the melting point (Duman *et al.* 1982, Zachariassen 1985). Thermal hysteresis proteins also depress the freezing point several degrees below the melting point (Duman *et al.* 1982 and 1991) and may also stabilize the supercooled state by adsorbing to embryonic ice crystals and preventing their growth (Zachariassen and Husby 1982). Freezing intolerant arthropods typically supercool into the range of –10°C to –40°C (Sømme 1982); however, a few arctic species have been found to remain unfrozen at temperatures below –60°C (Ring and Tesar 1981, Miller 1982).

A separate discussion of lower lethal temperatures for cold hardy arthropods is warranted because this parameter is not as simply defined as might be implied by the nominal strategies of freezing tolerance and freezing intolerance. In freezing intolerant species, the lower lethal temperature might be assumed to correspond with the temperature at which ice formation is initiated, the supercooling point (SCP). Although this is true to the extent that the SCP represents the absolute lower temperature limit to survival, it has been shown that some insects are killed by exposure to temperatures well above their SCP (Knight *et al.* 1986, Bale *et al.* 1988), particularly if such exposure is prolonged (Turnock *et al.* 1983 and 1985, Lee and Denlinger 1985). Similarly, freezing tolerance should not be taken to imply that an arthropod, once frozen, is able to survive any exposure to subzero temperatures. Rather, survival is limited to the freezing of a certain proportion of body water and this proportion

increases both with decreasing temperature and with length of exposure to a given subfreezing temperature (Lee 1991). Thus, determination of lower lethal temperatures should be made independently of both SCPs and freezing tolerance/intolerance in order to provide a meaningful estimate of cold tolerance.

The Experimental Animals

Phytoseiulus persimilis Athias-Henriot and *Amblyseius cucumeris* (Oudemans) are both mites (Class Arachnida, Order Acarina) belonging to the family Phytoseiidae. Phytoseiid mites are generally known as predators of phytophagous mites and are regarded as the most important natural enemies of spider mites (Acarina: Tetranychidae), some of which are major agricultural pests of worldwide importance (*cf*. Helle and Sabelis 1985). At least 30 phytoseiid species have been reported to show potential for biological control of mite pests (McMurtry 1982) and five species have been found to prey on thrips (Thysanoptera: Thripidae) that have considerable significance as agricultural pests (MacGill 1939, Ramakers 1978, Tanigoshi *et al*. 1983).

Because of their economic importance, phytoseiid mites have been studied extensively during the past few decades with respect to their basic biology and potential application as biocontrol agents (reviewed in Hoy 1982, Helle and Sabelis 1985). All phytoseiids have five life stages: the egg, a six-legged larva, eight-legged protonymph and deutonymph stages, and the adult (Sabelis 1985). Development is typically quite rapid, with reported mean egg-to-egg developmental periods above 20°C being less than two weeks for almost all species studied (reviewed by Tanigoshi 1982), and successive generations are produced continually as long as conditions remain favourable. In temperate zones, short daylengths and relatively cool temperatures induce a reproductive hibernal diapause in adult females after mating, which represent the only life stage that overwinters (Overmeer 1985a). Overwintering phytoseiid mites have been collected mainly from fruit trees where they are found in bark crevices and under insect scales (Homoptera: Coccidae) (Putman 1959, Chant 1959, Knisley and

Swift 1971).

Diapause induction has been investigated in a number of temperate species (reviewed by Overmeer 1985a; also, van Houten 1990, Morewood and Gilkeson 1991); however, only four studies have addressed cold hardiness in phytoseiids (MacPhee 1963, Knisley and Swift 1971, Wysoki 1974, van der Geest *et al.* 1991). Similarly, mass-rearing systems have been developed for phytoseiid mites (*eg*. Ramakers and van Lieburg 1982, King and Morrison 1984), leading to commercial production both in Europe (Hussey and Scopes 1985) and in North America (Steiner and Elliott 1987); however, only four studies have been published that address cold storage of these mites (Scopes 1968, Hamamura *et al.* 1978, Schliesske 1980 (cited in Overmeer 1985c), Gillespie and Ramey 1988), a potentially valuable component of mass-production systems.

Phytoseiulus persimilis Athias-Henriot

Phytoseiulus persimilis was originally described from specimens collected in association with *Tetranychus urticae* Koch (Acarina: Tetranychidae) on greenhouse roses in Algeria (Athias-Henriot 1957). Soon after, *Phytoseiulus riegeli* Dosse was described as a new species from Chile (Dosse 1958) but was then provisionally synonymized under *P. persimilis* by Chant (1959). Both names were used for several years, *P. riegeli* mainly in Europe and *P. persimilis* mainly in North America, until biosystematic studies confirmed the synonymy (Kennet and Caltagirone 1968). The natural geographic range of *P. persimilis* appears to be confined to below 45° latitude, both north and south of the equator, with collection records from several countries bordering on the Mediterranean Sea as well as scattered locations in the southern hemisphere (western South America, South Africa, Reunion Island, and southeastern Australia; see Figure 1). In addition, releases of this predator have reportedly led to established populations in coastal Ventura County, California (McMurtry *et al.* 1978).

Mean egg-to-adult developmental times determined for *P. persimilis* range from

Figure 1. Geographical distribution of *P. persimilis* and *A. cucumeris*, compiled from de Moraes *et al.* (1986) with additional records from Womersley (1954), Duso and Liguori (1984), Ridland *et al.* (1986), Gutierrez and Etienne (1986), Pande *et al.* (1989), and McMurtry and Bounfour (1989).

AITOFF'S PROJECTION
Interrupted by J. Johnson
Adapted and compressed by
R.M. Basile

A. cucumeris:

Nations, states, or provinces with collection records shaded.

P. persimilis:

⊙ collection locality.

○ locality unavailable.

● introduced.

Both species reported: ⊠

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MILES

just over seven days at 20°C to less than four days at 30°C (Hamamura *et al.* 1976a, Sabelis 1981). Estimates of mean female longevity typically range from 30 to 70 days, during which time most individuals lay between 50 and 80 eggs with a sex ratio of 85% females on average (reviewed by Sabelis 1981). Adult females require only a single mating to realize their maximum fecundity (Amano and Chant 1978, Schulten *et al.* 1978) and are thought to be incapable of entering diapause (Overmeer 1985a).

Prey preferences of *P. persimilis* are very specific, with suitable prey species limited almost exclusively to *T. urticae* and a few other members of the genus *Tetranychus* Dufour (Hamamura *et al.* 1976a, Amano and Chant 1977, Denmark and Schicha 1983, Overmeer 1985b, Rasmy *et al.* 1991). As a result, mass-rearing of this predator requires at least three greenhouses: one for growing host plants, a second for rearing the prey, and the third for the predators. Soon after its discovery, *P. persimilis* was shown to be very efficient in controlling populations of *T. urticae*, a major pest species (Chant 1961). This fact, combined with the occurrence of pesticide resistance in *T. urticae*, stimulated the return to biocontrol in greenhouse pest management that began in the late 1960s (van Lenteren and Woets 1988). Since then, use of *P. persimilis* has expanded to more than 5000 ha of greenhouses worldwide and biocontrol has become a well established pest management strategy (van Lenteren and Woets 1988, Gilkeson 1991).

Amblyseius cucumeris (Oudemans)

Amblyseius cucumeris was originally described without illustration from specimens collected in association with *Tetranychus* mites on melon [*Cucumis melo* L. (Cucurbitaceae)], and was placed in the genus *Typhlodromus* Scheuten (Oudemans 1930). It was later redescribed as a new species, *Typhlodromus thripsi* MacGill, when it was found preying on *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in glasshouses in England (MacGill 1939). Nesbitt (1951) published Oudemans' drawings along with an English translation of the original description, leading to

recognition that *T. thripsi* was a junior synonym of *T. cucumeris* (Evans 1952). Generic revisions of the family later placed the species in the genus *Amblyseius* Berlese (Chant 1965). More recently, *A. cucumeris* has been placed in the genus *Neoseiulus* Hughes (de Moraes *et al.* 1986, McMurtry and Bounfour 1989), but the details of this more recent generic revision of the family have yet to be published (Denmark, personal communication, 1991).[1] The geographic range of *A. cucumeris* is widespread, with collection records from throughout North America, Europe, and the Mediterranean region, as far east as India, and also from Australia and New Zealand (Figure 1). The commercially mass-reared mites used in this study originated in The Netherlands.

Mean egg-to-adult developmental times determined for *A. cucumeris* range from 11.1 days at 20°C to 6.3 days at 30°C (Gillespie and Ramey 1988). The only published estimate of mean adult female longevity is 40 days, during which time each individual laid between 25 and 39 eggs (El-Badry and Zaher 1961). Other estimates of mean fecundity range from 29 to 60 eggs/female with a sex ratio of *ca.* 65% females (reviewed by Castagnoli and Simoni 1990). Some species of *Amblyseius* require multiple matings to maintain fecundity whereas others can realize maximum fecundity after only a single mating (Schulten 1985); this aspect of reproduction has not been studied in *A. cucumeris*. Adult females are induced to enter diapause if exposed to daylengths of less than 12.5 h during their juvenile development but this response may be prevented if temperatures remain above 20°C (Morewood and Gilkeson 1991). The only published record of overwintering *A. cucumeris* is for a small number of adult females collected from the bark of peach tree trunks in Ontario (Putman 1959).

As a predator, *A. cucumeris* is a generalist. Food sources reported to be suitable include a variety of phytophagous mites (El-Badry and Zaher 1961, Burrell and McCormick 1964), mites of stored food (Ramakers and van Lieburg 1982, Morewood and Gilkeson 1991), thrips (Gillespie and Ramey 1988, Castagnoli *et al.* 1990), and even pollen (Overmeer *et al.* 1989, Castagnoli and Simoni 1990). Thus, *A. cucumeris*

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can be mass-reared relatively easily and without the greenhouses that would otherwise be required to grow plants and rear prey. With the return to biocontrol in greenhouses stimulated by the discovery of *P. persimilis*, there arose a need to control other greenhouse pests without the use of pesticides. A survey for natural enemies of *T. tabaci* turned up several prospects (Ramakers 1978), of which *A. cucumeris* proved to be the most promising as a biocontrol agent for both *T. tabaci* and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (de Klerk and Ramakers 1986, Ramakers 1988, Gillespie 1989).

Objectives

The ability to stockpile biocontrol agents in cold storage would help to mediate the inevitable differences between supply and demand, and would provide some insurance against unforeseen problems in mass-production, such as disease epidemics. Although much more space and labour is required for mass-production of *P. persimilis* than *A. cucumeris*, cold storage of either species for even one month would be beneficial. In addition, the phylogenetic relationship of these species, combined with their contrasting ecological backgrounds, provides an opportunity for comparative studies of cold hardiness.

The objectives of the current study were to determine and compare basic cold hardiness parameters for *P. persimilis* and *A. cucumeris*, including SCPs, freezing tolerance *vs*. intolerance, susceptibility to cold shock and chilling injury, and lower lethal temperatures, and to determine optimum temperatures and conditions for cold storage of these predaceous mites.

MATERIALS AND METHODS

Mites were obtained from Applied Bio-Nomics Ltd., Sidney, British Columbia. *Phytoseiulus persimilis* were mass-reared on *T. urticae*-infested bean plants as described by King and Morrison (1984). *Amblyseius cucumeris* were mass-reared in coarse wheat bran as described by Ramakers and van Lieburg (1982) with mold mites [*Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae)] as prey. In the laboratory, both predators were maintained on *T. urticae*-infested bean leaves in small rearing cages, as described by Overmeer (1985c), and *A. cucumeris* were also simply kept in their bran rearing culture in small cardboard shipping containers. Unless otherwise specified, mites selected for experiments were healthy, gravid adult females. All handling of mites and their eggs was done under a stereomicroscope using fine sable paintbrushes.

Diapausing *A. cucumeris* were obtained by rearing these mites in a controlled environment chamber under diapause inducing conditions. These conditions consisted of a short day photoperiod of Light:Dark (LD) 10:14 h combined with a thermoperiodic cycle of Thermophase:Cryophase (TC) 20:15°C that was timed to coincide with the photoperiod [thermophase with photophase (L), cryophase with scotophase (D)]. Mites were reared on large (*ca.* 10 cm diameter) floating arenas of thin plastic foam cut from meat packing trays and anchored on cotton pads in 15 cm petri dishes of water. Either *T. putrescentiae* (mostly nymphs) or eggs of *T. urticae* were provided as prey.

To monitor survival and fecundity, mites were placed individually on floating arenas consisting of thin plastic disks (*ca.* 1.5 cm diameter) anchored with short pieces of thread in 15 cm petri dishes of water. Eggs of *T. urticae* were provided as prey and 3 x 3 mm pieces of felt were provided for shelter and oviposition sites.

Statistical procedures used were among those described by Zar (1984).

Cold Hardiness

Supercooling points were determined in a Neslab (Neslab Instruments Inc., Portsmouth, New Hampshire) U-tainer B stirred cryobath containing 95% ethanol. Cooling was provided by a Neslab CC-100 immersion cooler and controlled by a Neslab Exatrol and ETP-3 temperature programmer, producing an average cooling rate of 0.75°C/minute. This rate was as close to the recommended standard cooling rate of 1°C/minute (Salt 1966b) as could be obtained. Temperatures were monitored using 30-gauge copper-constantan thermocouples set inside 3 ml glass shell vials that were then placed inside glass test tubes suspended in the cryobath. One thermocouple was connected to a Bailey Instruments BAT-5 cryothermometer (Bailey Instruments Inc., Saddle Brook, New Jersey), to monitor the temperature of the cryobath, and two others were connected to a Heath Schlumberger (Heath Company, Mississauga, Ontario) strip chart recorder (EU-205-11) through a potentiometric amplifier (EU-200-01) and DC offset module (EU-200-02), to detect the heat of fusion released by specimens when they froze. This apparatus was very similar to that described and illustrated by Block and Young (1979). Specimens were attached to thermocouples with a small amount of petroleum jelly and SCP temperatures were recorded from the cryothermometer when indicated by deflections on the chart recorder.

Supercooling points were determined for all life stages of both species taken directly from mass cultures and for adults acclimated to 7.5°C for one week, with the exception of adult male *P. persimilis*. In addition, *A. cucumeris* were reared on *T. putrescentiae* under diapause inducing conditions and SCPs of the adults were determined before, during, and after sequential acclimation to 7.5°C, 2.5°C, and –1°C for one week at each temperature. Sample sizes ranged from 20 to 36 for each life stage and/or treatment. Student's *t* test as well as analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) multiple comparison were used to test for significant differences in SCPs among different life stages and among adults subjected to different diapause and/or acclimation regimes.

Lower lethal temperatures were determined for adult females of both species taken directly from mass cultures, and for diapausing *A. cucumeris* reared on *T. urticae* eggs, by constructing temperature/mortality curves. The temperature that was acutely lethal to 50% of the sample population (LT_{50}) was then calculated by interpolation. Groups of mites were placed in gelatin capsules that were placed inside the shell vials in the SCP apparatus. The mites were then cooled to a range of temperatures, at 1°C intervals, that corresponded to the range of individual SCPs determined previously. Mites were removed from the cryobath as soon as the desired temperature was reached and survival was assessed after one hour at room temperature. Sample sizes ranged from 60 to 63 for each species at each temperature.

Short term survival and fecundity of both species reared on *T. urticae* were assessed after cooling to –15°C in the SCP apparatus. After cooling, the mites were placed on individual arenas and checked after 48 h at room temperature for survival and numbers of eggs laid. Control groups for both species were taken directly from mass cultures for comparison of short term survival and fecundity. Sample sizes ranged from 22 to 26 in each case. Student's *t* tests were used to check for significant differences in mean numbers of eggs laid by treated *vs*. control groups.

The ability of these mites to survive exposure to subzero temperatures above their SCPs was tested for both species by placing groups of mites in gelatin capsules and then exposing them to –12.5°C for periods ranging from 0 to 90 min. After being returned to room temperature, the mites were allowed 12 h to recover before survival was assessed. This experiment was replicated three times, giving cumulative sample sizes of 105 for *P. persimilis* and 95 for *A. cucumeris* for each period of exposure. To determine whether mortality resulting from exposure to –12.5°C could have been due to freezing, mites were attached to thermocouples in the SCP apparatus and cooled to –15°C. After being held at that temperature for a minimum of two hours, with continuous monitoring on the chart recorder, the mites were further cooled until freezing was detected. Sample sizes for this delayed freezing experiment were 12 for *P. persimilis* and 13 for *A. cucumeris*.

Cold Storage

In initial trials, mites were taken from their respective mass cultures and held individually in clear gelatin capsules (No. 00, T.U.B. Enterprises, North Augusta, Ontario). Temperatures tested were room temperature (22 ± 1°C), 7.5°C, and 2.5°C; survival was checked daily. These trials were initiated with 80 *P. persimilis* and 66 *A. cucumeris* at each temperature. In subsequent trials, both species were reared on *T. urticae* and then held individually in 50 x 9 mm Falcon® petri dishes with tight fitting lids (Becton Dickinson and Company, Lincoln Park, New Jersey). The above temperatures were tested again with mites in Falcon dishes provisioned with moist filter paper or with *T. urticae* eggs that had been washed from plants and collected on filter paper; survival was checked weekly. These trials were initiated with 30 *P. persimilis* and 20 *A. cucumeris* in each treatment at each temperature.

The 15 *P. persimilis* that survived eight weeks in cold storage were removed from the Falcon dishes and placed on individual arenas at room temperature (22 ± 1°C). Eggs of *T. urticae* were provided as prey while survival and fecundity were assessed daily until all of the predators had died. A control group of 21 *P. persimilis*, taken directly from a mass culture, were also placed on individual arenas and were monitored concurrently with the cold-storage survivors. *Amblyseius cucumeris* that survived six weeks in cold storage were placed on individual arenas as above and were monitored for five weeks.

To test the effect of bran and vermiculite (used as distribution media) on cold-storage survival of *P. persimilis*, mites were placed individually in Falcon dishes loosely filled with either moist wheat bran or moist vermiculite and provisioned with *T. urticae* eggs on filter paper. In the first trial, the mites were agitated (to simulate shipping) by inverting the Falcon dishes rapidly several times and then held at 6°C. Agitation was repeated after five days and survival was assessed after a total of ten days in cold storage. In the second trial, the mites were held at either 5°C or 8°C in either bran or vermiculite and survival was assessed after four weeks. Chi-square

contingency table analysis was used to test for significant differences in survival among treatments.

RESULTS

Cold Hardiness

Mean SCPs of adult females taken directly from mass-rearing cultures were –22.5°C for *P. persimilis* and –20.7°C for *A. cucumeris* (Table 1), a small but statistically significant difference ($t_{(2)48} = 7.7157$, $P < 0.001$). Supercooling point temperatures increased slightly during development from egg to adult in *P. persimilis* (Figure 2, top), with statistically significant differences (ANOVA, $F_{4,137} = 23.440$, $P < 0.0005$) between larvae and protonymphs and between deutonymphs and adult females (SNK, $P < 0.05$). Adult female *P. persimilis* acclimated to 7.5°C for one week showed no change in their mean SCP ($t_{(1)43} = 0.2788$, $P > 0.25$). Supercooling point temperatures increased markedly during development from egg to deutonymph in *A. cucumeris* but dropped again in adults (Figure 2, bottom), with statistically significant differences (ANOVA, $F_{5,176} = 92.674$, $P < 0.0005$) between all life stages except adult males and protonymphs (SNK, $P < 0.05$).

The mean SCP of adult female *A. cucumeris* (Figure 3, top) acclimated to 7.5°C for one week was slightly, but significantly, lower than that of adult females taken directly from mass-rearing cultures ($t_{(1)43} = 5.2800$, $P < 0.0005$) and did not differ significantly from that of adult females reared under diapause inducing conditions ($t_{(2)48} = 1.4575$, $0.10 < P < 0.20$). Diapausing adult females acclimated sequentially to 7.5°C, 2.5°C, and –1°C for one week at each temperature showed no change in their mean SCPs (ANOVA, $F_{3,105} = 1.001$, $P > 0.25$). Adult males (Figure 3, bottom) showed no change in their mean SCP when acclimated to 7.5°C for one week ($t_{(1)40} = 0.5441$, $P > 0.25$) or when reared under diapause inducing conditions ($t_{(2)42} = 1.9243$, $0.05 < P < 0.10$). Similarly, adult males reared under diapause

inducing conditions showed no change in their mean SCP after acclimation to 7.5°C for one week ($t_{(1)42}$ = 1.6700, 0.05 < P < 0.10). The mean SCP of diapausing adult female *A. cucumeris* reared with *T. urticae* eggs as prey was significantly ($t_{(2)56}$ = 12.1709, P < 0.001) lower than that of mites reared under the same conditions but with *T. putrescentiae* as prey (Table 1).

Temperature/mortality curves for adult females showed a marked increase in mortality through a range of temperatures corresponding very closely to the range of individual SCP temperatures (Figures 4 to 6). Lower lethal temperatures (LT_{50}s) interpolated from these curves were –21.6°C for *P. persimilis*, –20.3°C for *A. cucumeris*, and –23.8°C for diapausing *A. cucumeris*, which corresponded well with mean (± SD) SCPs of –22.5 ± 0.90°C for *P. persimilis*, –20.7 ± 0.77°C for *A. cucumeris*, and –24.9 ± 0.90°C for diapausing *A. cucumeris*.

After a brief exposure to –15°C, *P. persimilis* laid an average (± SD) of 5.2 ± 1.9 eggs over a 48 h period whereas mites taken directly from mass cultures laid an average (± SD) of 5.4 ± 1.8 eggs during the same period, a difference that was not statistically significant ($t_{(1)45}$ = 0.4481, P > 0.25). In contrast, *A. cucumeris* exposed briefly to –15°C laid an average (± SD) of 1.3 ± 1.0 eggs over a 48 h period whereas mites taken directly from mass cultures laid an average (± SD) of 2.7 ± 0.6 eggs during the same period, a highly significant difference ($t_{(1)46}$ = 5.6479, P < 0.0005).

Mortality of both species increased rapidly with length of time exposed to –12.5°C, with no mites surviving a 90-min exposure (Figure 7); however, mites held at –15°C in the SCP apparatus for a minimum of two hours did not freeze until they were further cooled into the range of temperatures of their previously determined SCPs.

Cold Storage

Survival of mites held in empty gelatin capsules was inversely related to temperature; however, at no temperature did any mites survive longer than two weeks (Figure 8). Similarly, none of the *P. persimilis* held in empty Falcon dishes survived

even two weeks whereas 7% and 43% of those provided with moist filter paper or *T. urticae* eggs, respectively, survived eight weeks of storage at 7.5°C. Survival at this temperature was much better than at either 2.5°C or room temperature (Figure 9). At 7.5°C with food, survival was 97% after four weeks and 80% after six weeks of cold storage. In contrast, a few *A. cucumeris* survived up to six weeks in empty Falcon dishes at 7.5°C, and although survival was better when the mites were provided with moist filter paper or *T. urticae* eggs (Figure 10), the improvement was not as great as for *P. persimilis*. Survival of *A. cucumeris* also was better at 7.5°C than at 2.5°C or room temperature but even at 7.5°C with food, survival dropped to 75% after four weeks and 35% after six weeks of cold storage.

The 13 *P. persimilis* that survived eight weeks at 7.5°C with food showed subsequent survival and fecundity comparable to 21 others taken directly from rearing cultures (Figure 11), as did the two individuals that survived eight weeks at 7.5°C with moisture only (data not shown). Due to the small number of *A. cucumeris* that survived six weeks of cold storage, analysis of subsequent survival and fecundity was limited. All of the eight individuals that survived more than one week after cold storage resumed laying eggs within one week of being returned to room temperature; however, oviposition was irregular and fecundity varied widely from 0.4 to 1.4 eggs/female/day over a two-week period after all individuals had resumed laying eggs.

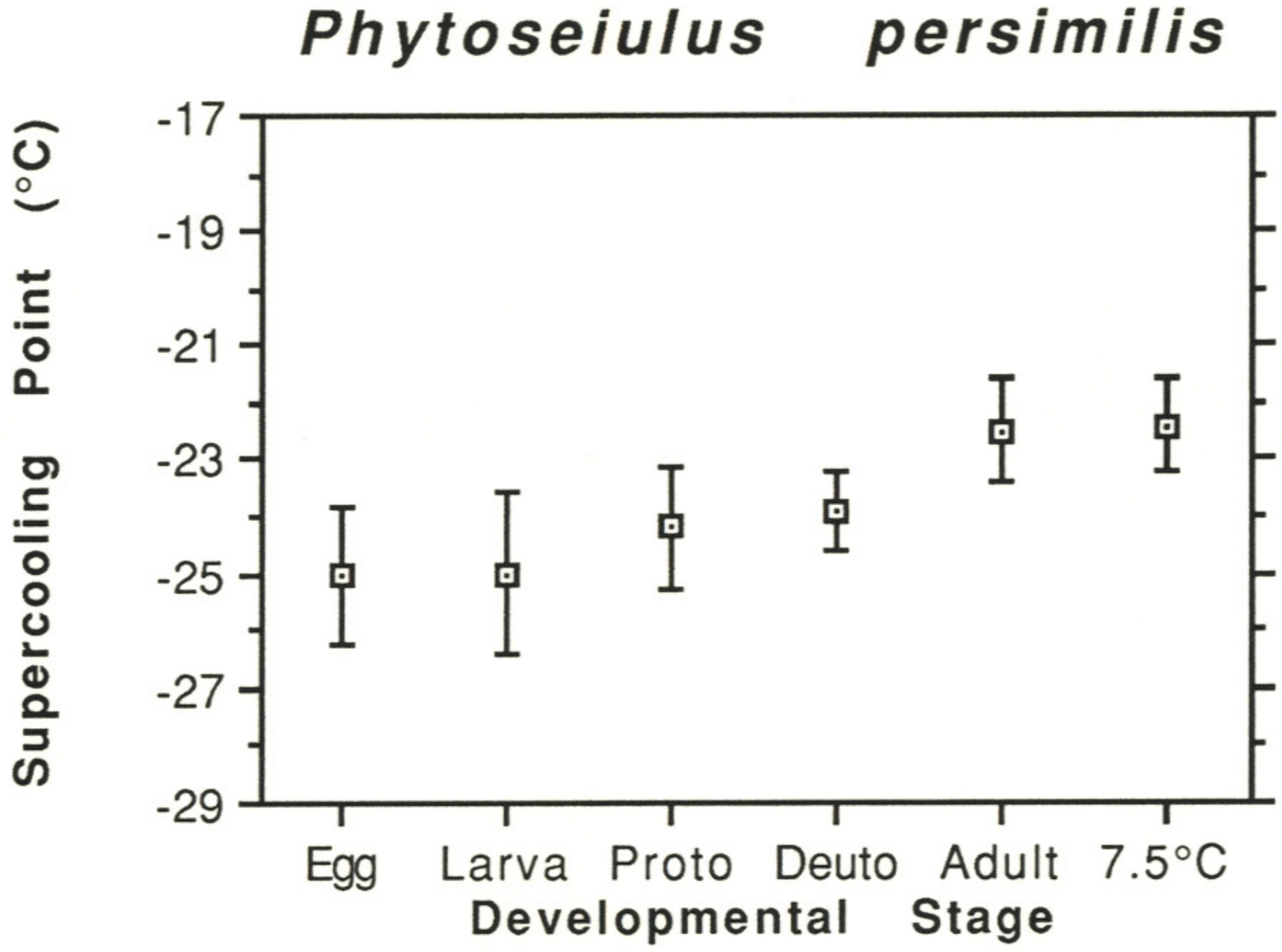
Survival of *P. persimilis* held for ten days at 6°C was good and showed no significant differences whether the mites had been stored in bran or vermiculite and whether or not they had been agitated (Table 2). Mites held for four weeks at 5°C or 8°C showed no significant differences in survival according to temperature or storage medium; however, survival was poor in all cases (Table 3).

Table 1. Supercooling points for life stages of *P. persimilis* and *A. cucumeris*, including adults that had been reared under diapause inducing conditions (LD 10:14 h, TC 20:15°C) and/or acclimated sequentially for one week at each of the indicated temperatures. Quotation marks reflect the fact that males do not enter diapause.

	Mean ± SD (–°C)	Range (–°C)	Sample Size
P. persimilis			
eggs	25.0 ± 1.22	23.3 - 27.2	30
larvae	25.0 ± 1.41	22.3 - 28.4	30
protonymphs	24.2 ± 1.09	21.8 - 26.2	28
deutonymphs	23.9 ± 0.71	22.2 - 25.0	29
adult females	22.5 ± 0.90	20.0 - 23.8	25
7.5°C	22.4 ± 0.84	20.8 - 24.3	20
A. cucumeris			
eggs	27.1 ± 1.23	24.9 - 29.4	34
larvae	25.7 ± 1.73	21.6 - 28.6	30
protonymphs	22.0 ± 2.43	17.6 - 27.9	36
deutonymphs	19.4 ± 1.96	13.7 - 23.7	35
adult females			
nondiapause	20.7 ± 0.77	19.4 - 22.1	25
7.5°C	22.1 ± 1.05	20.1 - 23.8	20
diapause	21.6 ± 1.40	17.4 - 23.6	30
7.5°C	21.8 ± 0.85	20.7 - 23.3	30
2.5°C	21.3 ± 1.27	19.6 - 24.5	25
–1°C	21.6 ± 0.64	20.5 - 22.7	24
diapause[1]	24.9 ± 0.90	22.9 - 26.4	28
adult males			
"nondiapause"	21.9 ± 1.63	18.8 - 26.0	22
7.5°C	21.6 ± 2.14	14.4 - 24.9	20
"diapause"	22.9 ± 1.70	18.2 - 25.7	22
7.5°C	23.7 ± 1.60	21.0 - 26.0	22

[1]Reared on *T. urticae* eggs rather than *T. putrescentiae*.

Figure 2. Mean (± SD) SCPs for developmental stages of *P. persimilis* and *A. cucumeris* (n = 20 to 36 in each case). "Proto" and "Deuto" indicate nymphal stages, "Adult" represents females only, and "7.5°C" refers to adult females acclimated to this temperature for one week before supercooling.


Phytoseiulus persimilis
Supercooling Point (°C)
-17
-19
-21
-23
-25
-27
-29
Egg
Larva
Proto
Deuto
Adult
7.5°C
Developmental Stage

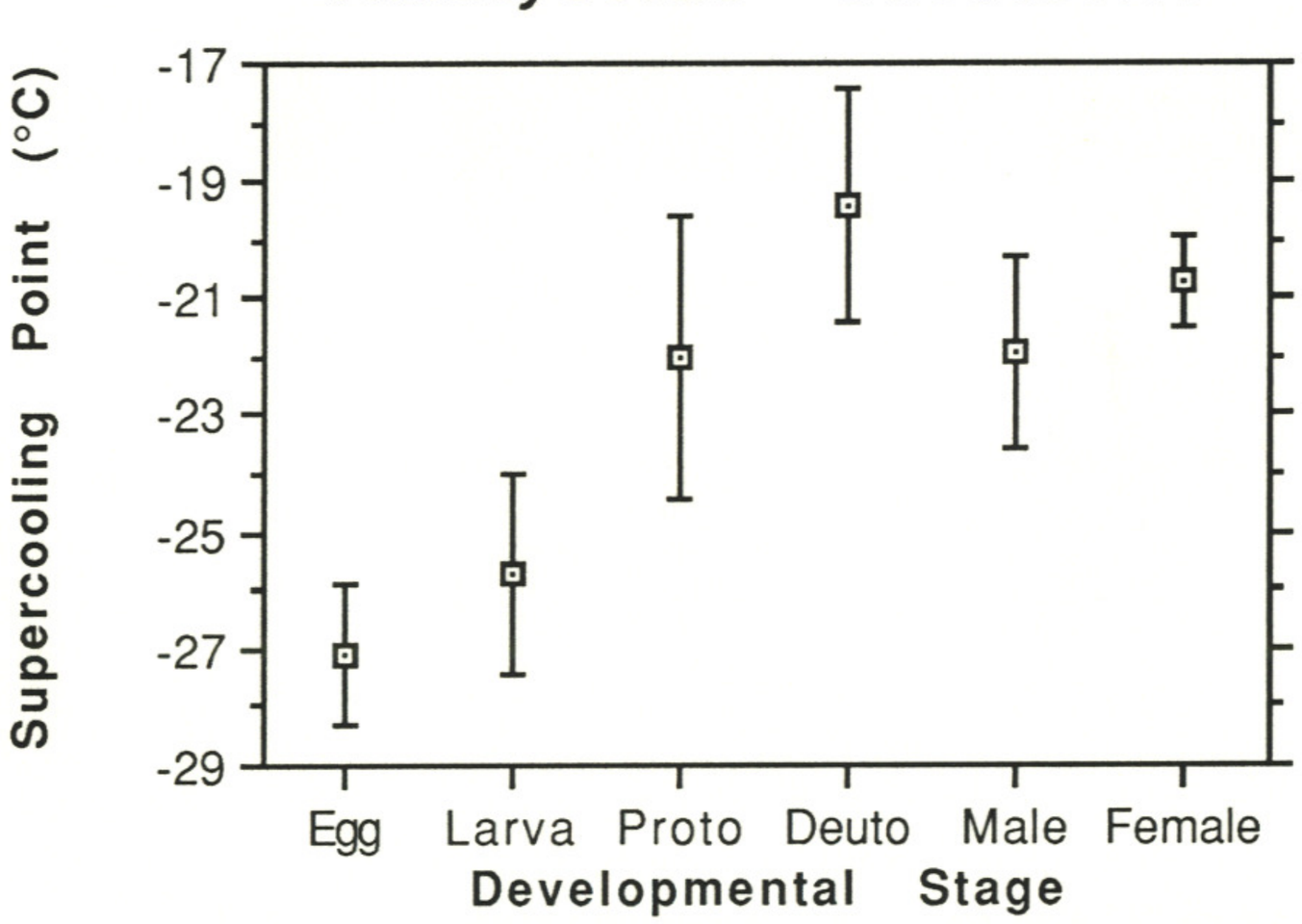
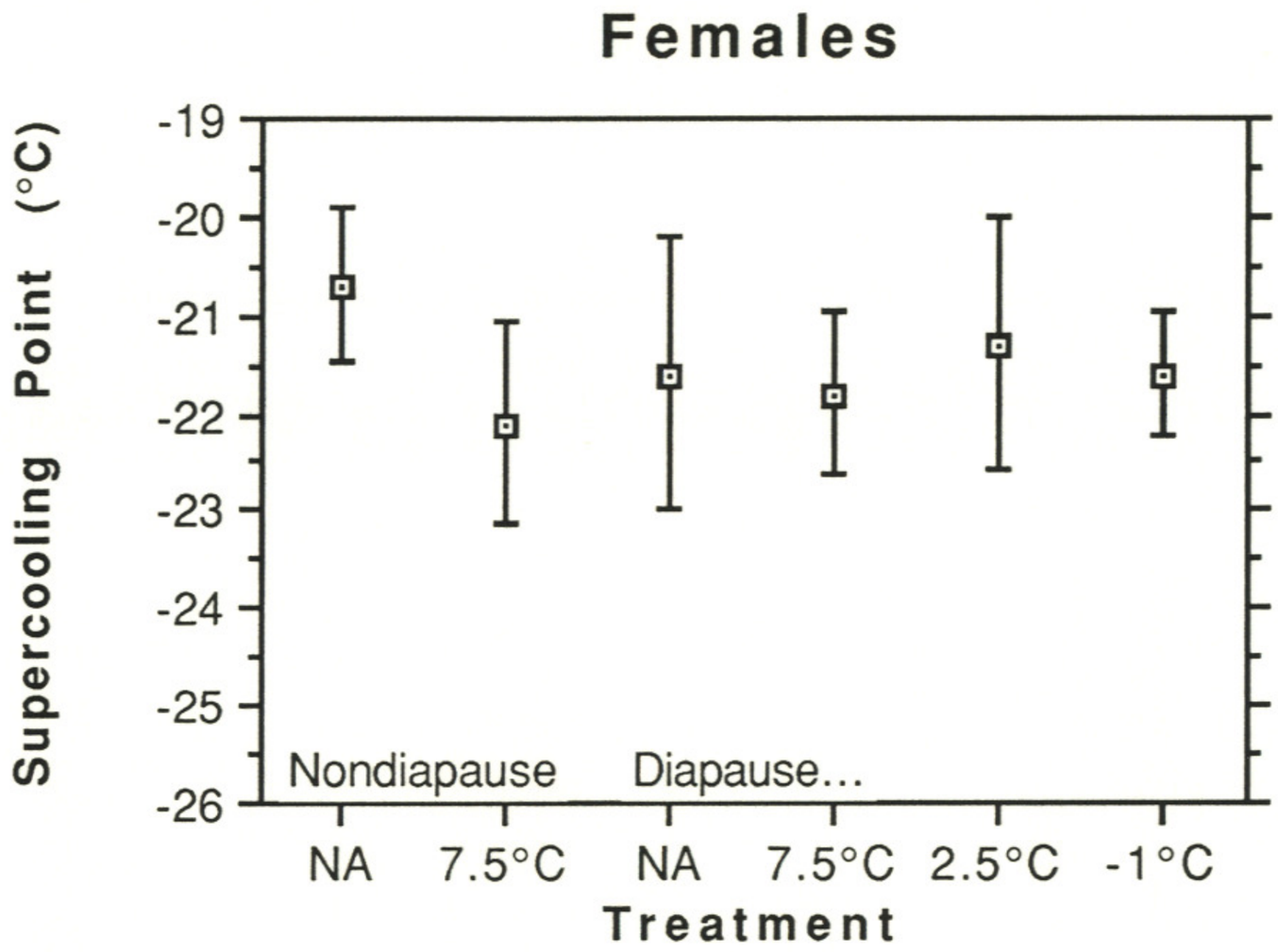

Amblyseius cucumeris
Supercooling Point (°C)
-17
-19
-21
-23
-25
-27
-29
Egg
Larva
Proto
Deuto
Male
Female
Developmental Stage

Figure 3. Mean (± SD) SCPs for adult *A. cucumeris* reared under diapause inducing conditions (LD 10:14 h, TC 20:15°C) and/or acclimated for one week at each of the indicated temperatures (n = 20 to 30 in each case). NA = not acclimated. Quotation marks reflect the fact that males do not enter diapause.


Females
Supercooling Point (°C)
-19
-20
-21
-22
-23
-24
-25
-26
Nondiapause
Diapause...
NA
7.5°C
NA
7.5°C
2.5°C
-1°C
Treatment

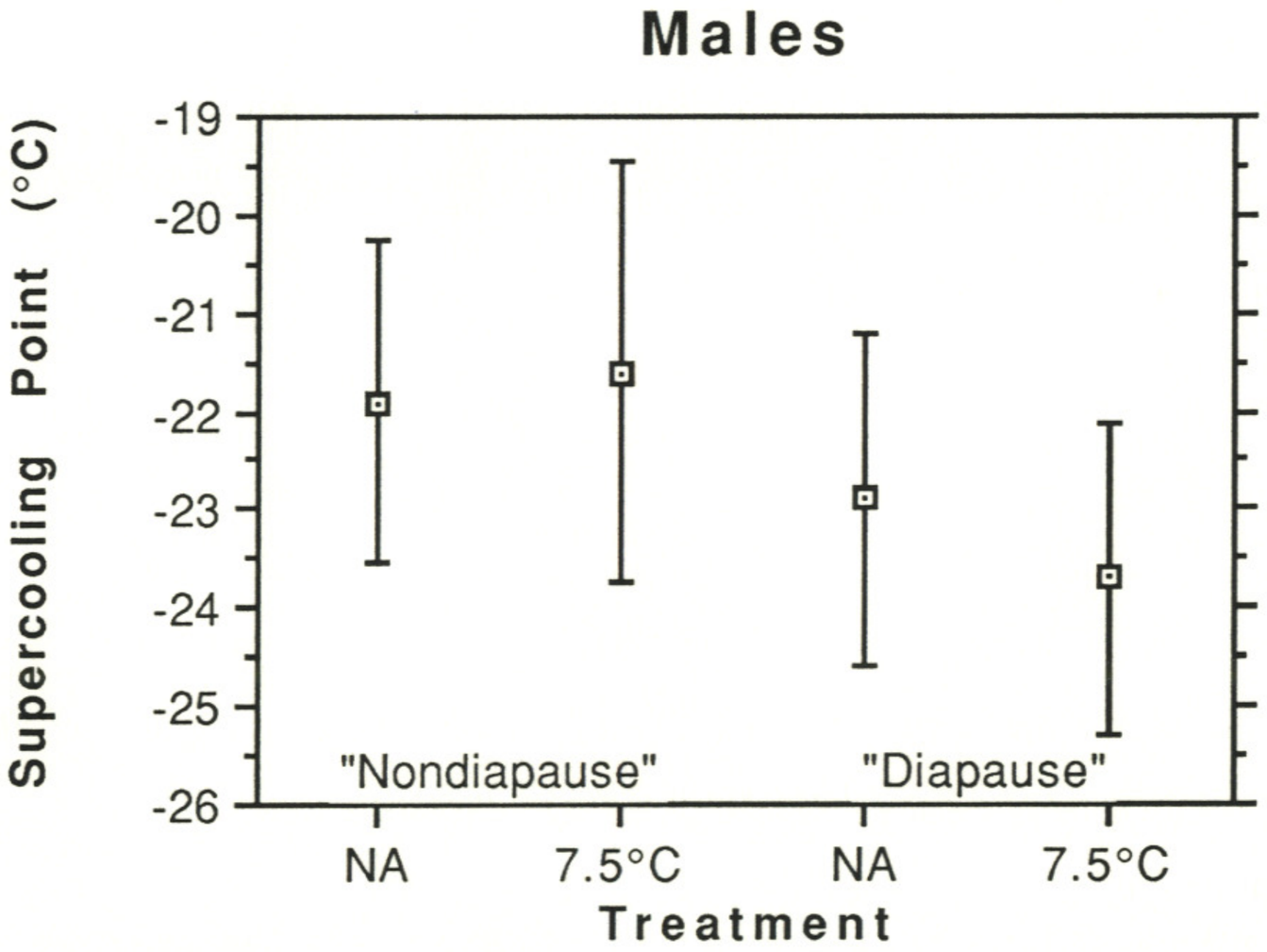
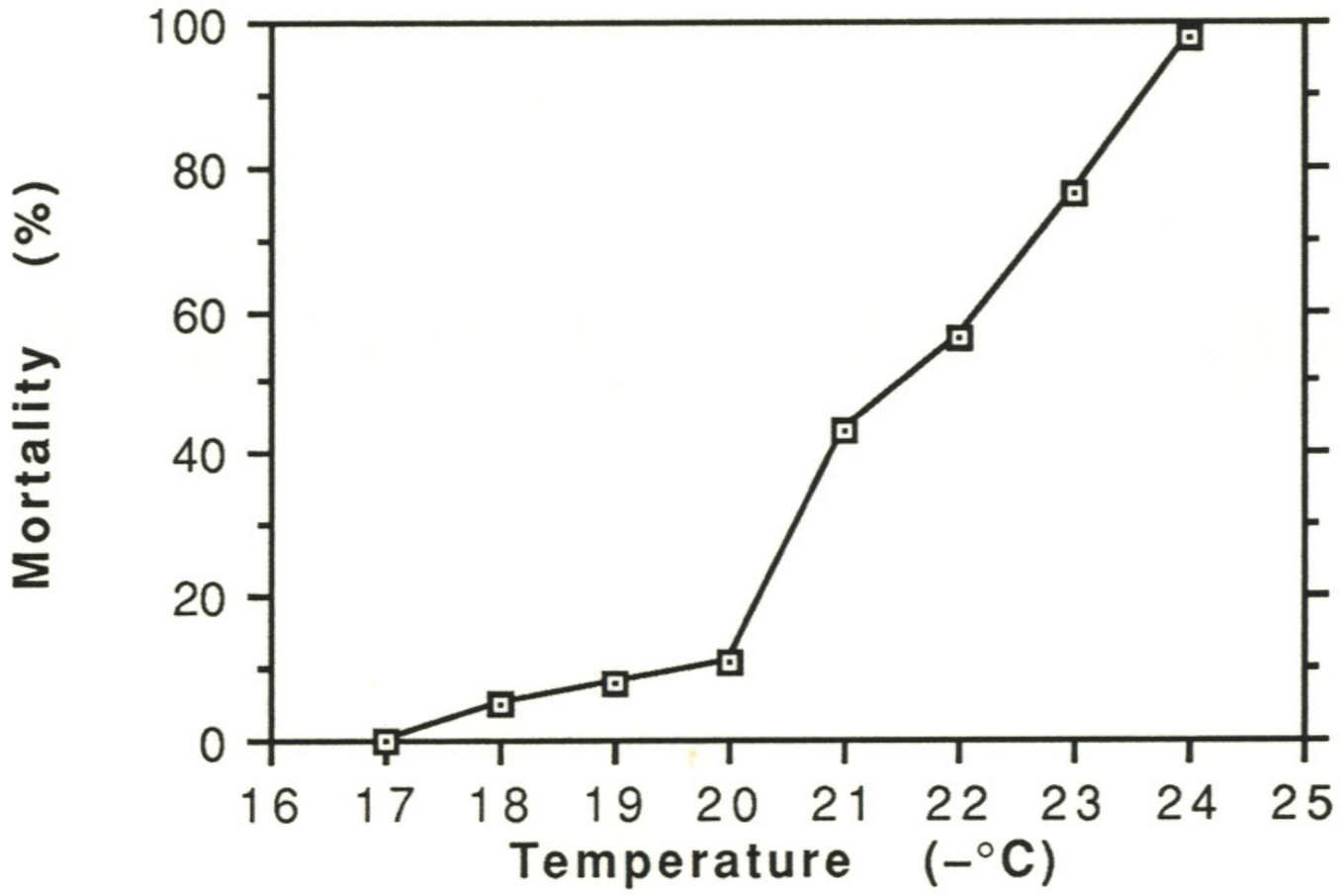

Males
Supercooling Point (°C)
-19
-20
-21
-22
-23
-24
-25
-26
"Nondiapause"
"Diapause"
NA
7.5°C
NA
7.5°C
Treatment

Figure 4. Temperature/mortality curve for adult female *P. persimilis* (n = 61 to 63 at each temperature). Mortality was assessed one hour after cooling to each indicated temperature using SCP protocol. A cumulative frequency distribution of individual SCPs (n = 25) is plotted below to show the close relationship between acute mortality and freezing.


Mortality (%)
100
80
60
40
20
0
16 17 18 19 20 21 22 23 24 25
Temperature (–°C)

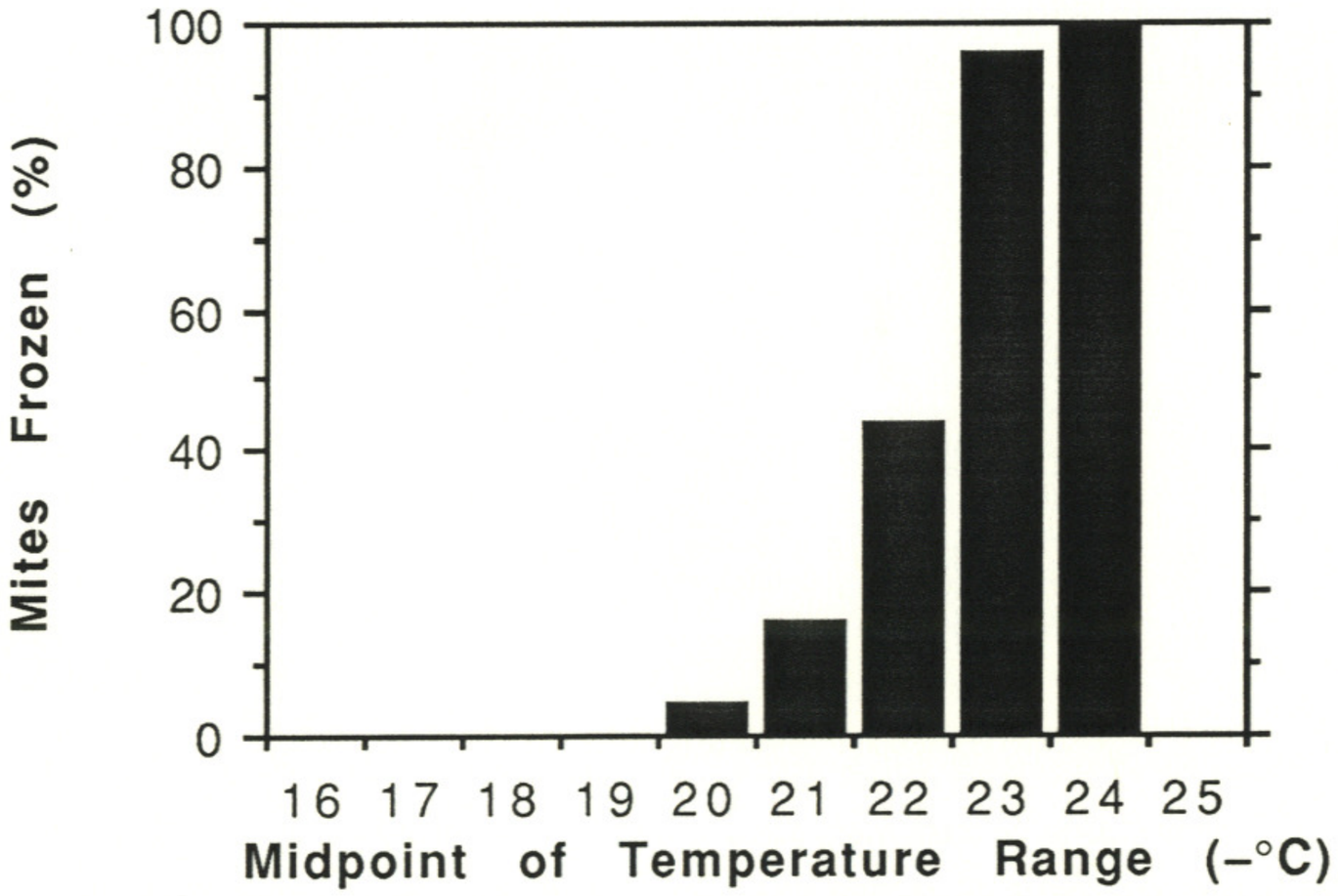
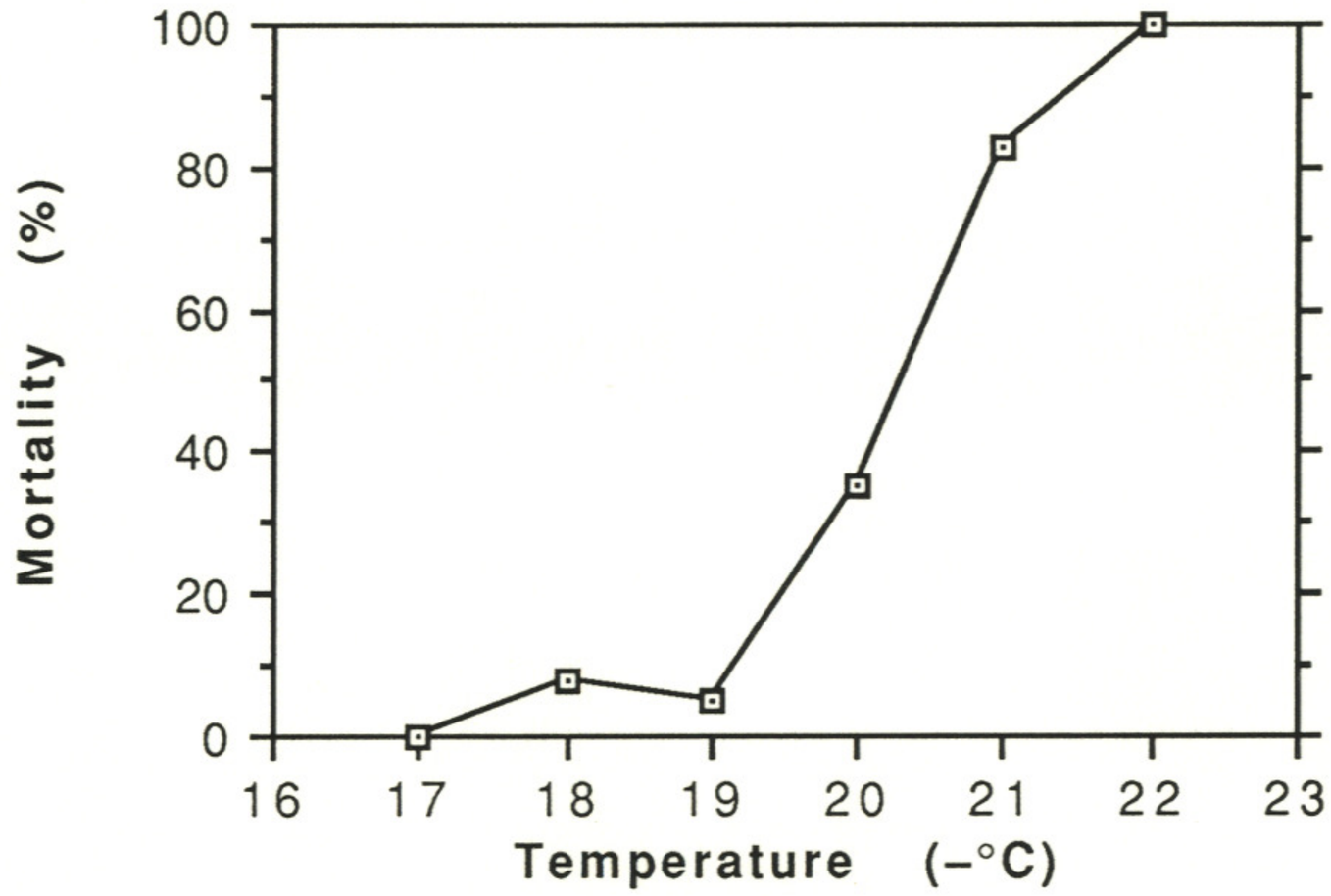

Mites Frozen (%)
100
80
60
40
20
0
16 17 18 19 20 21 22 23 24 25
Midpoint of Temperature Range (–°C)

Figure 5. Temperature/mortality curve for adult female *A. cucumeris* (n = 63 at each temperature). Mortality was assessed one hour after cooling to each indicated temperature using SCP protocol. A cumulative frequency distribution of individual SCPs (n = 25) is plotted below to show the close relationship between acute mortality and freezing.


Mortality (%)
100
80
60
40
20
0
16
17
18
19
20
21
22
23
Temperature (–°C)

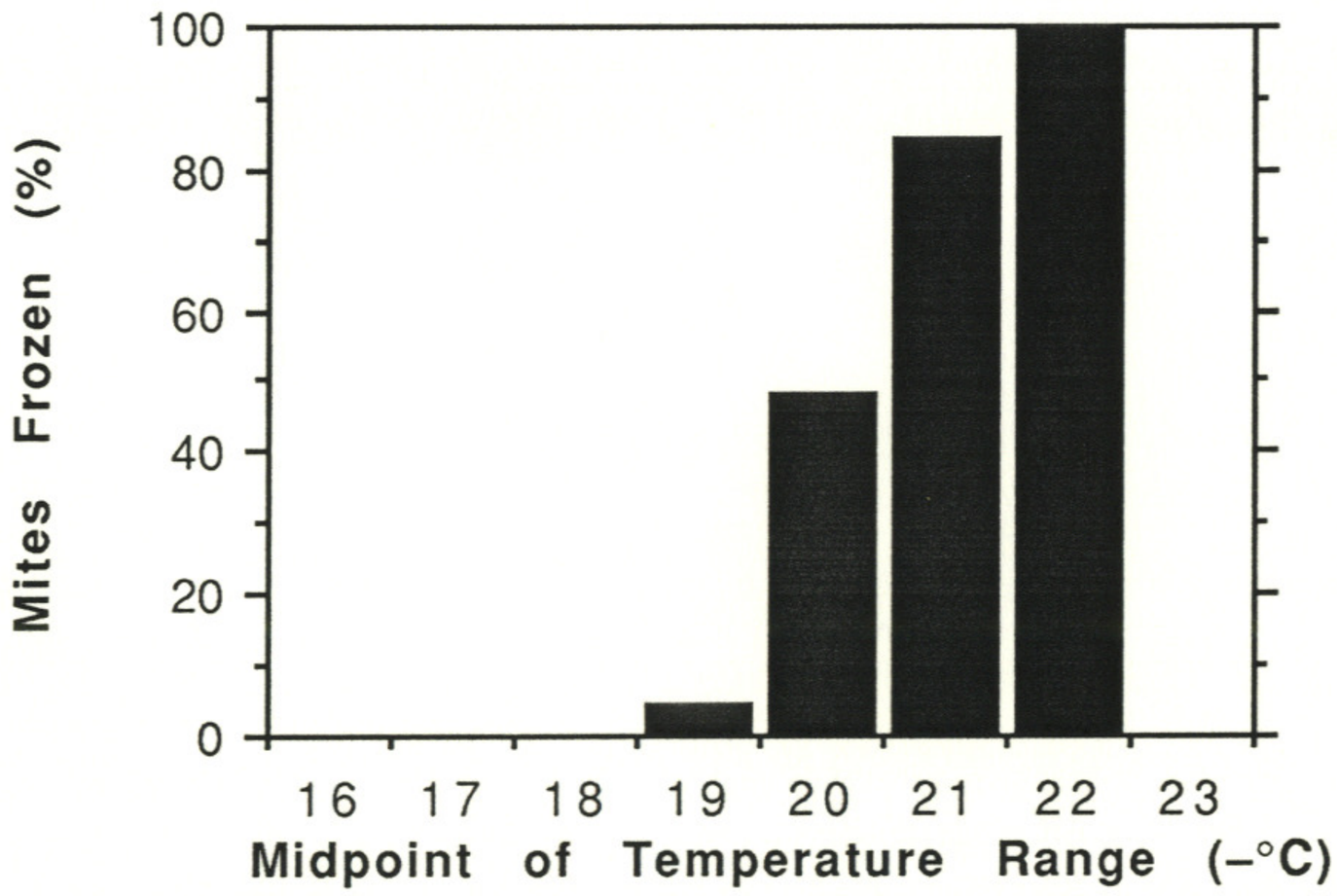
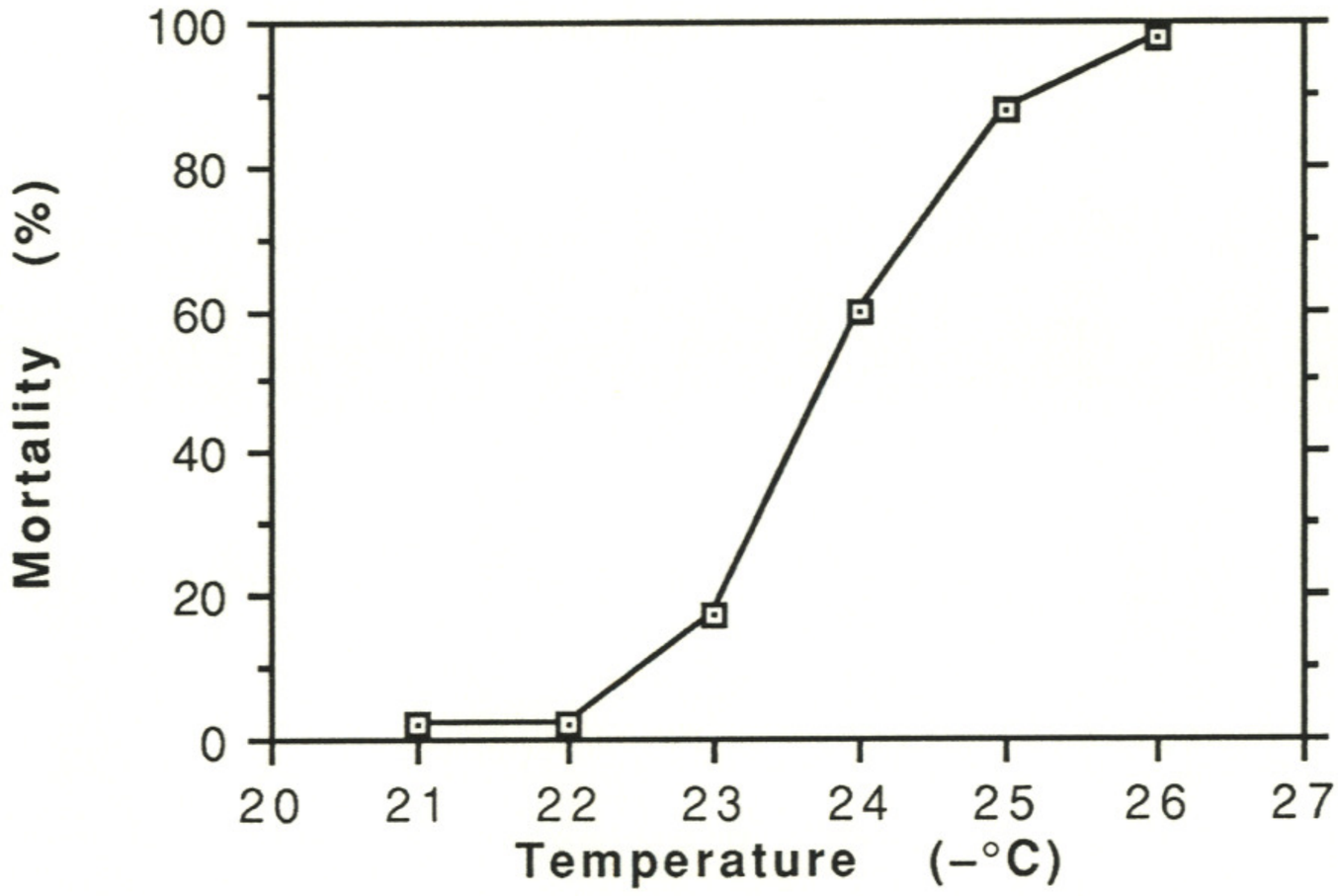

Mites Frozen (%)
100
80
60
40
20
0
16
17
18
19
20
21
22
23
Midpoint of Temperature Range (–°C)

Figure 6. Temperature/mortality curve for diapausing adult female *A. cucumeris* (n = 60 at each temperature). Mortality was assessed one hour after cooling to each indicated temperature using SCP protocol. A cumulative frequency distribution of individual SCPs (n = 28) is plotted below to show the close relationship between acute mortality and freezing.


Mortality (%)
100
80
60
40
20
0
20
21
22
23
24
25
26
27
Temperature (–°C)

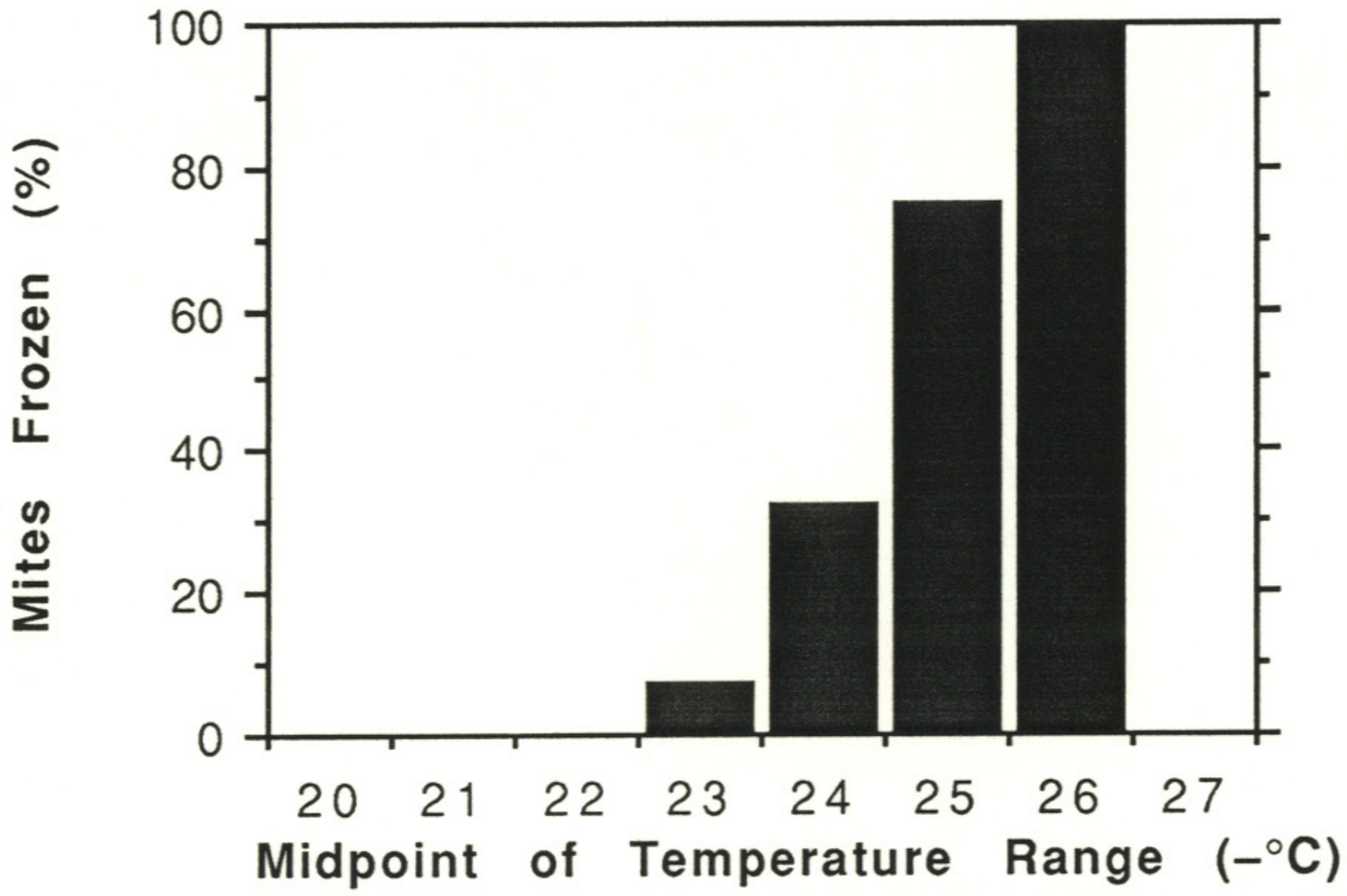
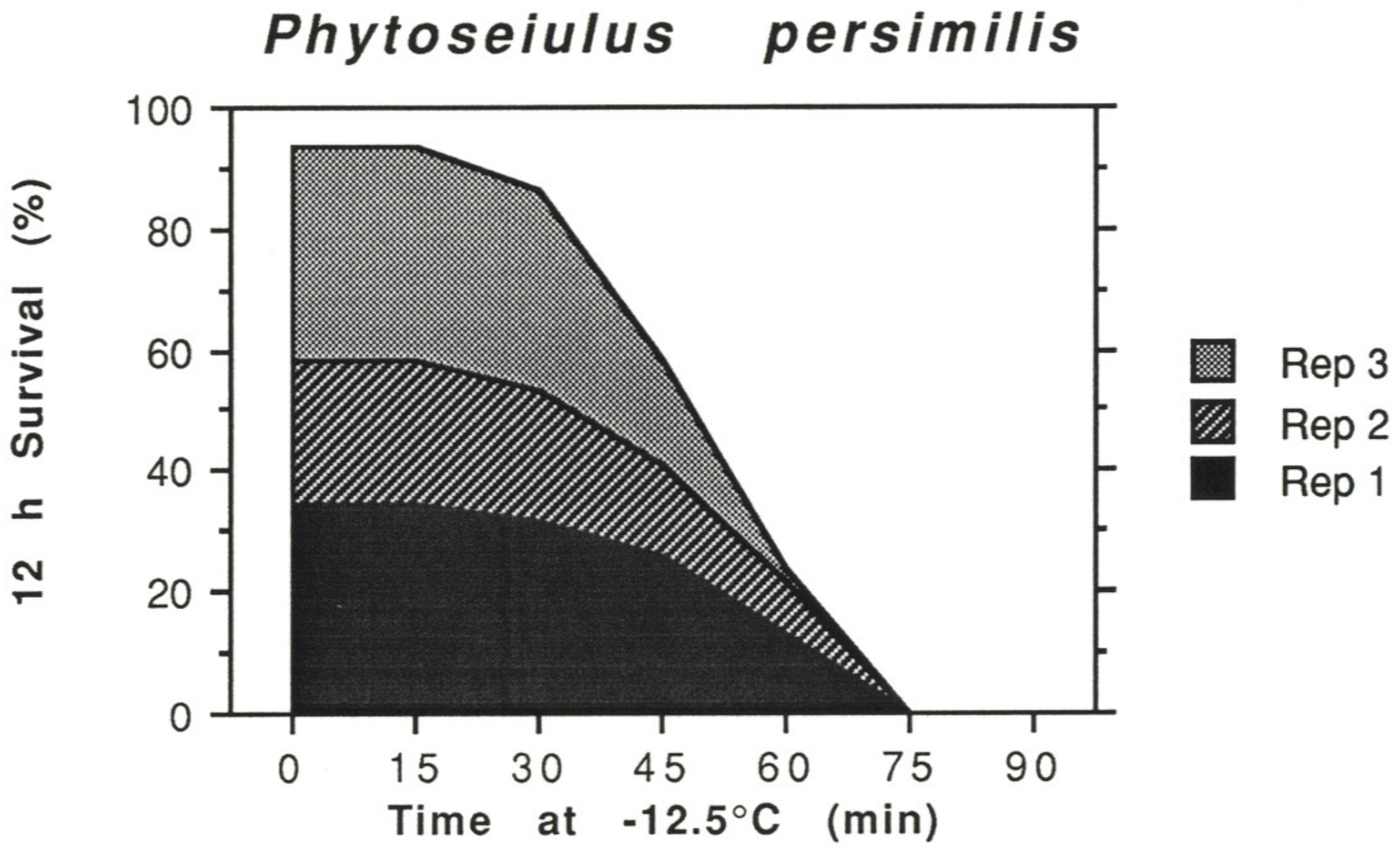

Mites Frozen (%)
100
80
60
40
20
0
20
21
22
23
24
25
26
27
Midpoint of Temperature Range (–°C)

Figure 7. Survival of adult female *P. persimilis* and *A. cucumeris* 12 h after exposure to –12.5°C for up to 90 min. The experiment was replicated three times and the results plotted cumulatively (n = 24 to 38 in each replicate for each length of exposure).


Phytoseiulus persimilis
12 h Survival (%)
100
80
60
40
20
0
0 15 30 45 60 75 90
Time at -12.5°C (min)
Rep 3
Rep 2
Rep 1

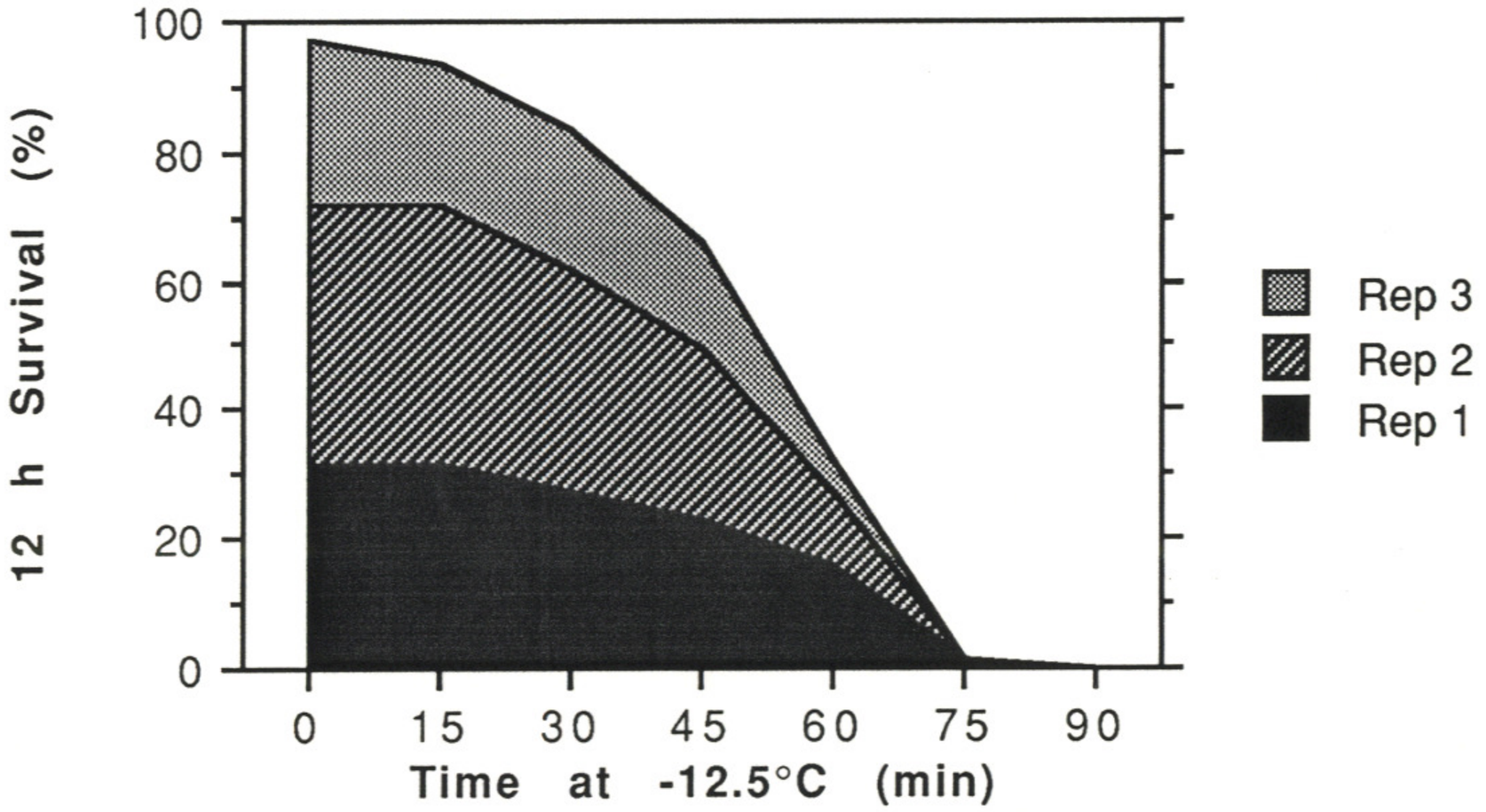
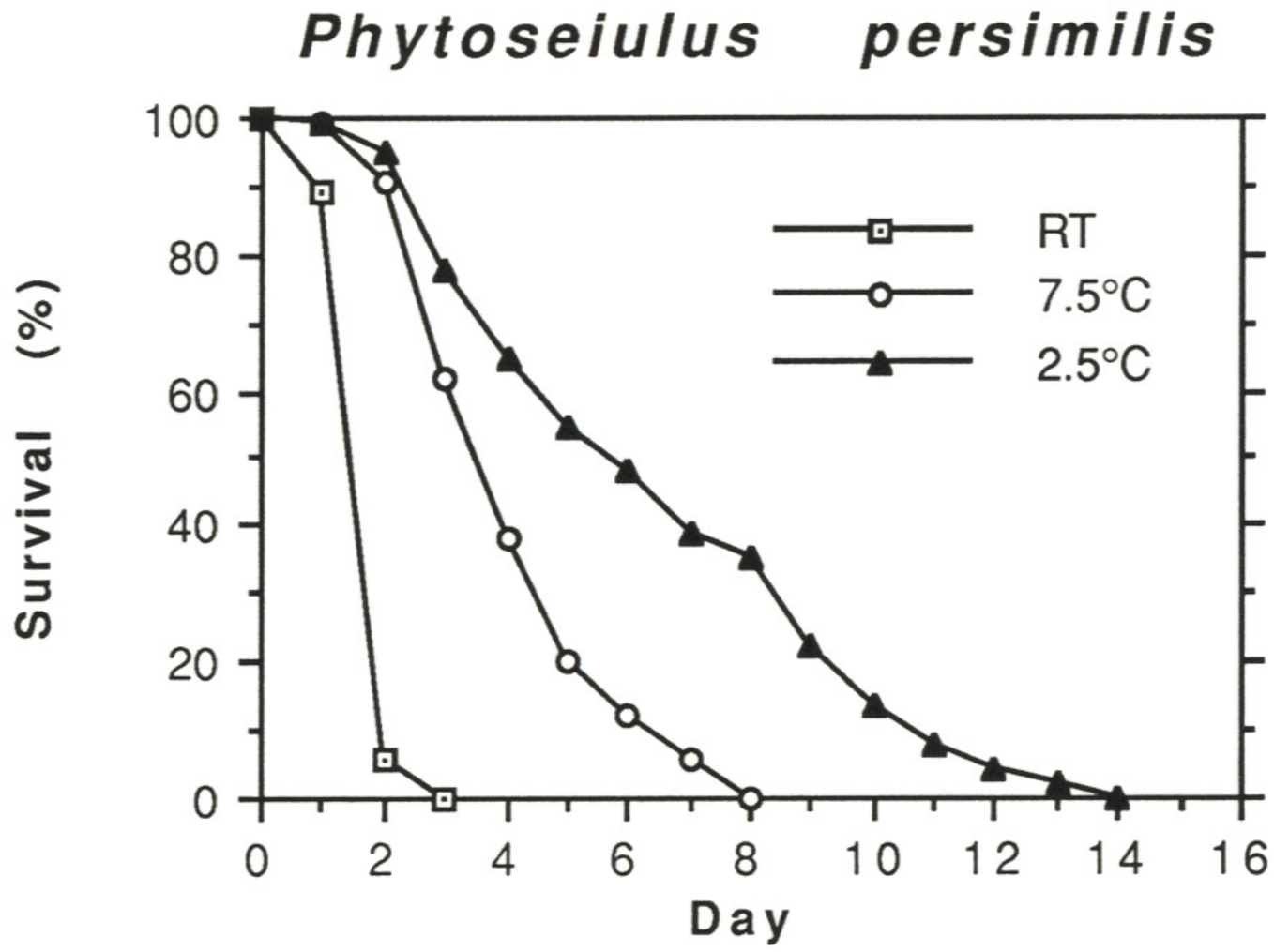

Amblyseius cucumeris
12 h Survival (%)
100
80
60
40
20
0
0 15 30 45 60 75 90
Time at -12.5°C (min)
Rep 3
Rep 2
Rep 1

Figure 8. Survival of adult female *P. persimilis* and *A. cucumeris* held individually in empty gelatin capsules at room temperature (RT = 22 ± 1°C), 7.5°C, or 2.5°C (initial n = 80 *P. persimilis* and 66 *A. cucumeris* at each temperature).


Phytoseiulus persimilis
Survival (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
Day
RT
7.5°C
2.5°C

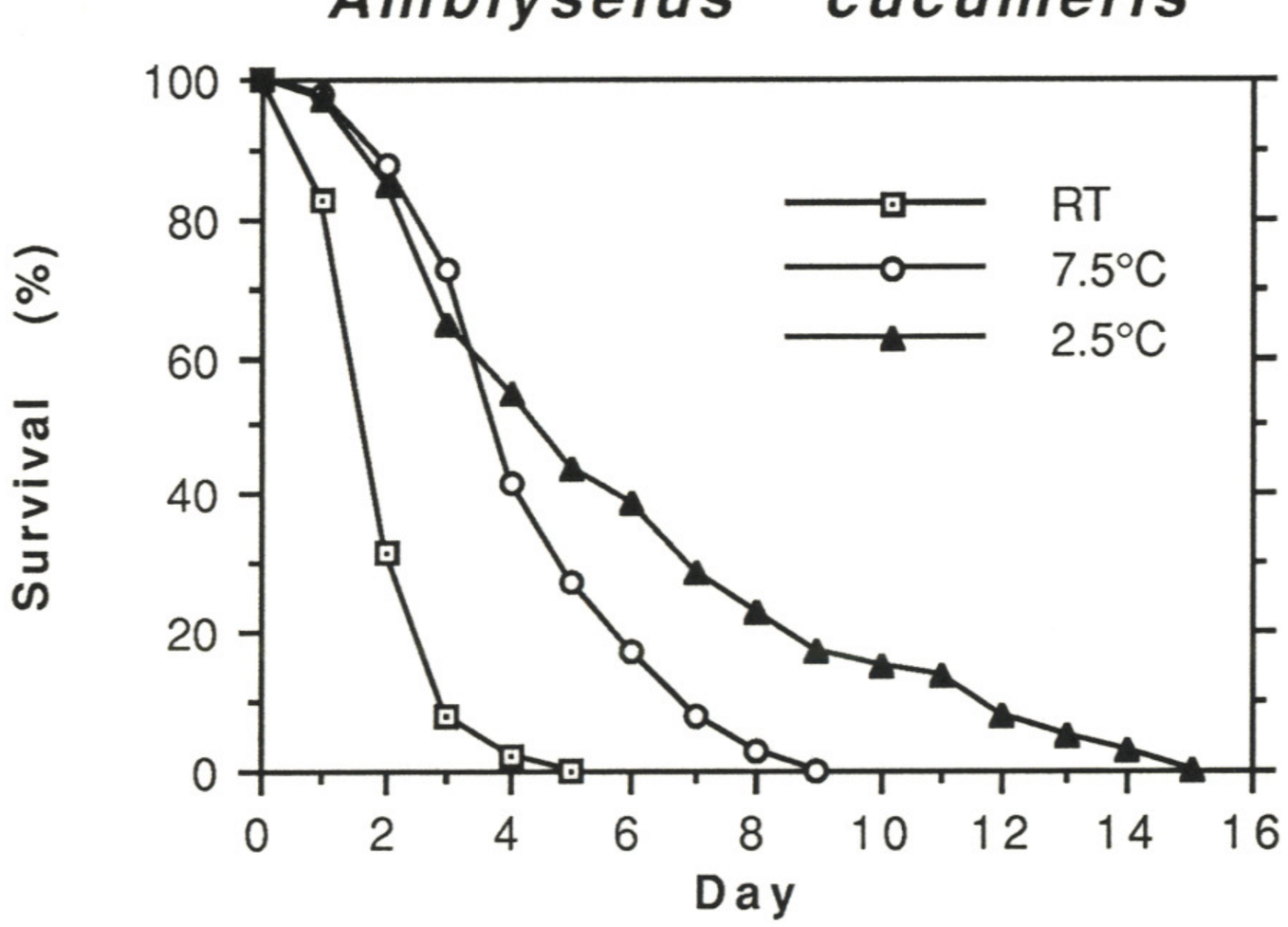

Amblyseius cucumeris
Survival (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
Day
RT
7.5°C
2.5°C

Figure 9. Survival of adult female *P. persimilis* held individually in Falcon dishes at room temperature (22 ± 1°C), 7.5°C, or 2.5°C (initial n = 30 in each treatment at each temperature). Filter paper wetted with distilled water provided moisture and *T. urticae* eggs washed from plants and collected on filter paper provided food.

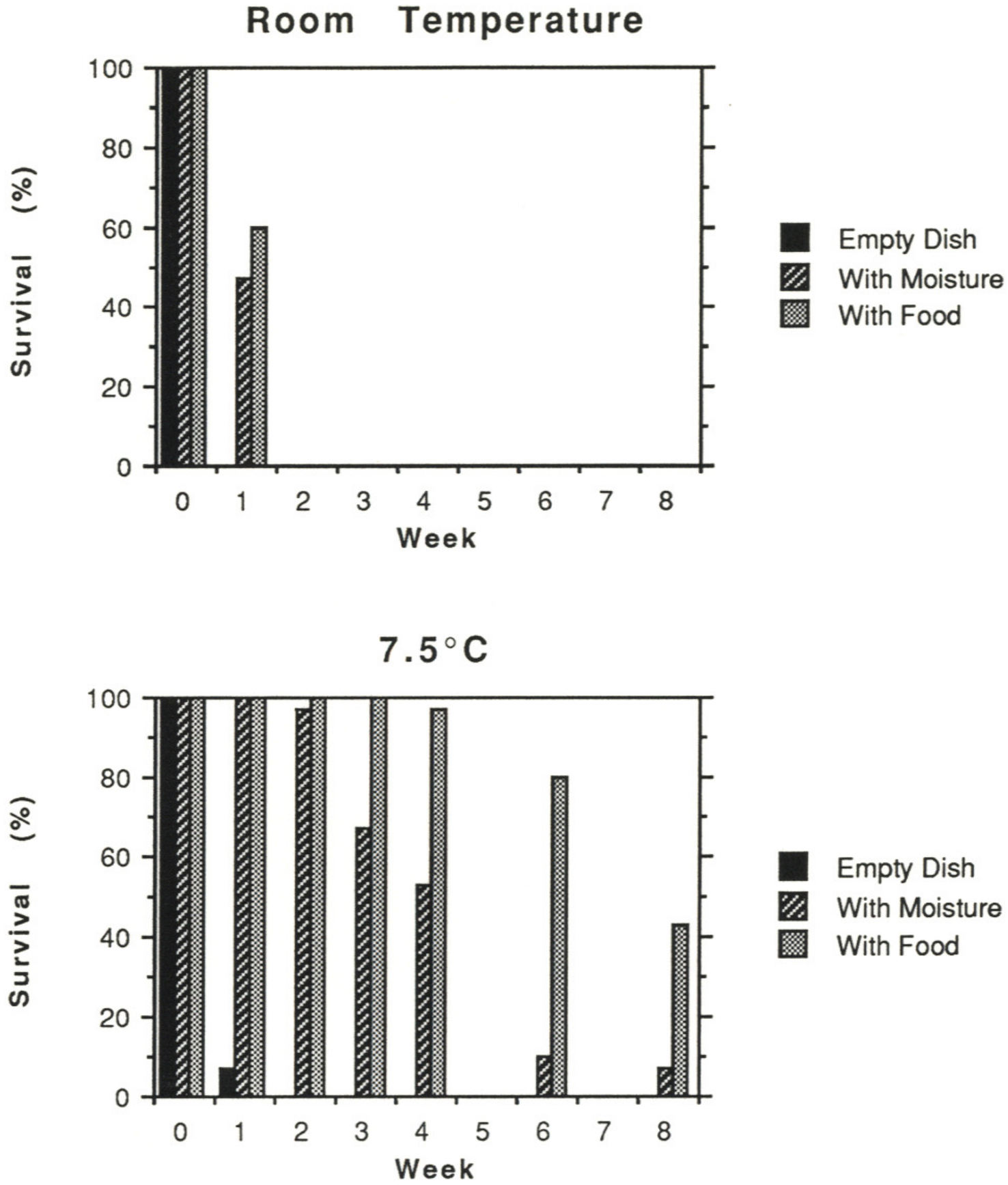

Room Temperature
Survival (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8
Week
Empty Dish
With Moisture
With Food
7.5°C
Survival (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8
Week
Empty Dish
With Moisture
With Food
2.5°C
Survival (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8
Week
Empty Dish
With Moisture
With Food

Figure 10. Survival of adult female *A. cucumeris* held individually in Falcon dishes at room temperature (22 ± 1°C), 7.5°C, or 2.5°C (initial n = 20 in each treatment at each temperature). Filter paper wetted with distilled water provided moisture and *T. urticae* eggs washed from plants and collected on filter paper provided food.

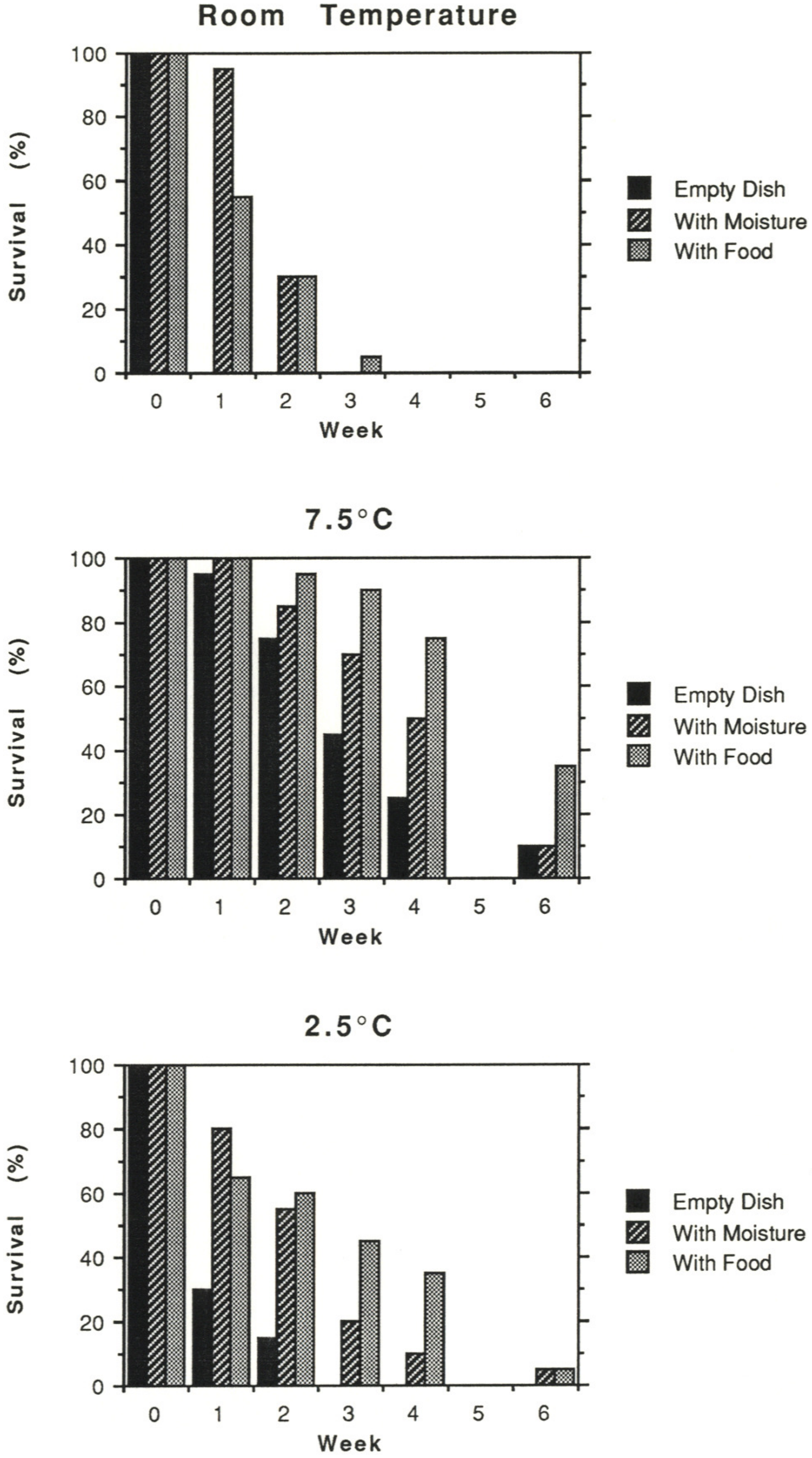
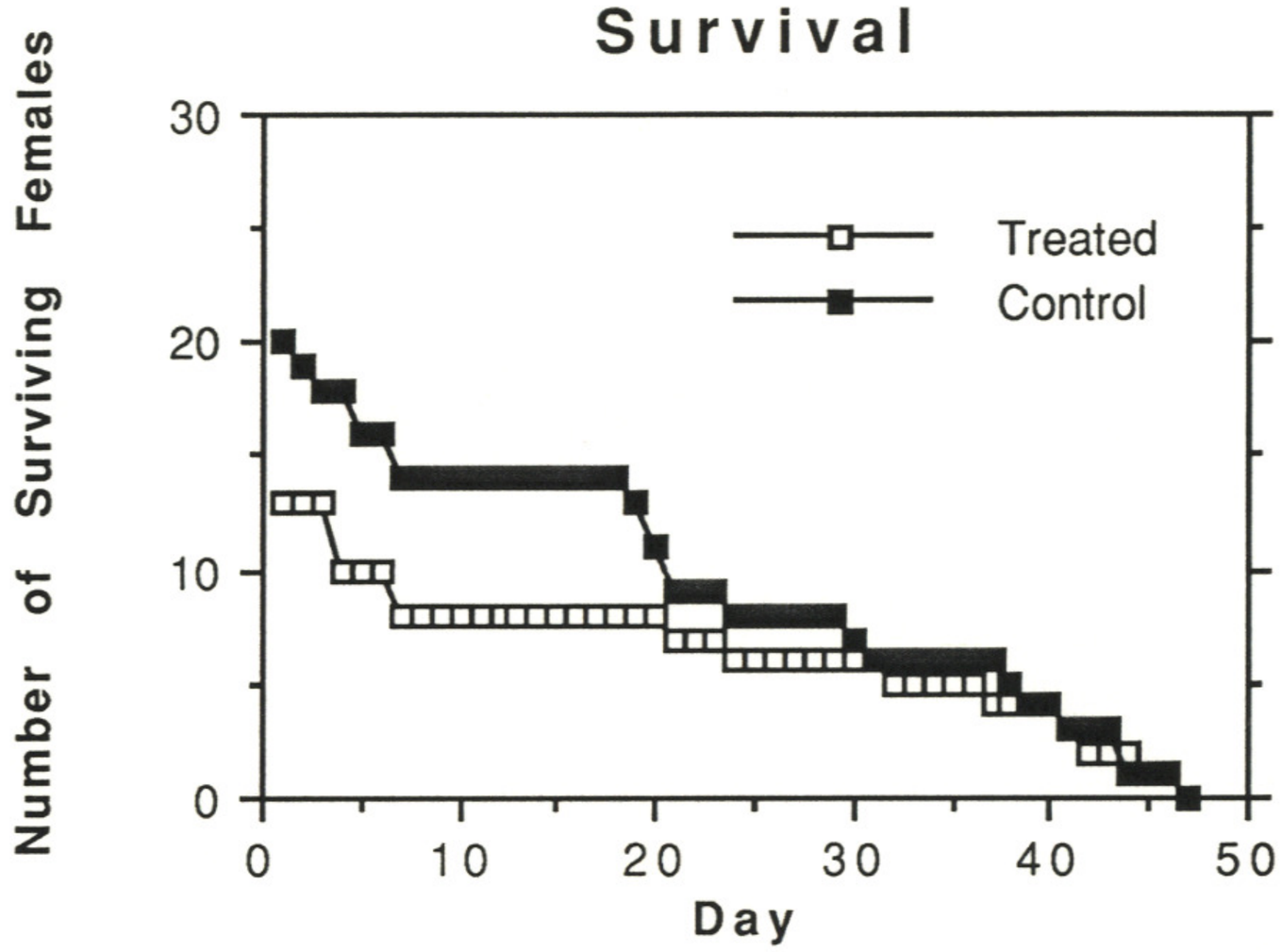

Room Temperature
7.5°C
2.5°C
Survival (%)
100
80
60
40
20
0
0 1 2 3 4 5 6
Week
Empty Dish
With Moisture
With Food

Figure 11. Survival and fecundity of adult female *P. persimilis* held individually on arenas at room temperature (22 ± 1°C) and provided with *T. urticae* eggs. "Treated" mites had been held individually in Falcon dishes with food at 7.5°C for eight weeks (Figure 9); "Control" mites were taken directly from mass-rearing cultures.


Survival
Number of Surviving Females
30
20
10
0
0
10
20
30
40
50
Day
Treated
Control

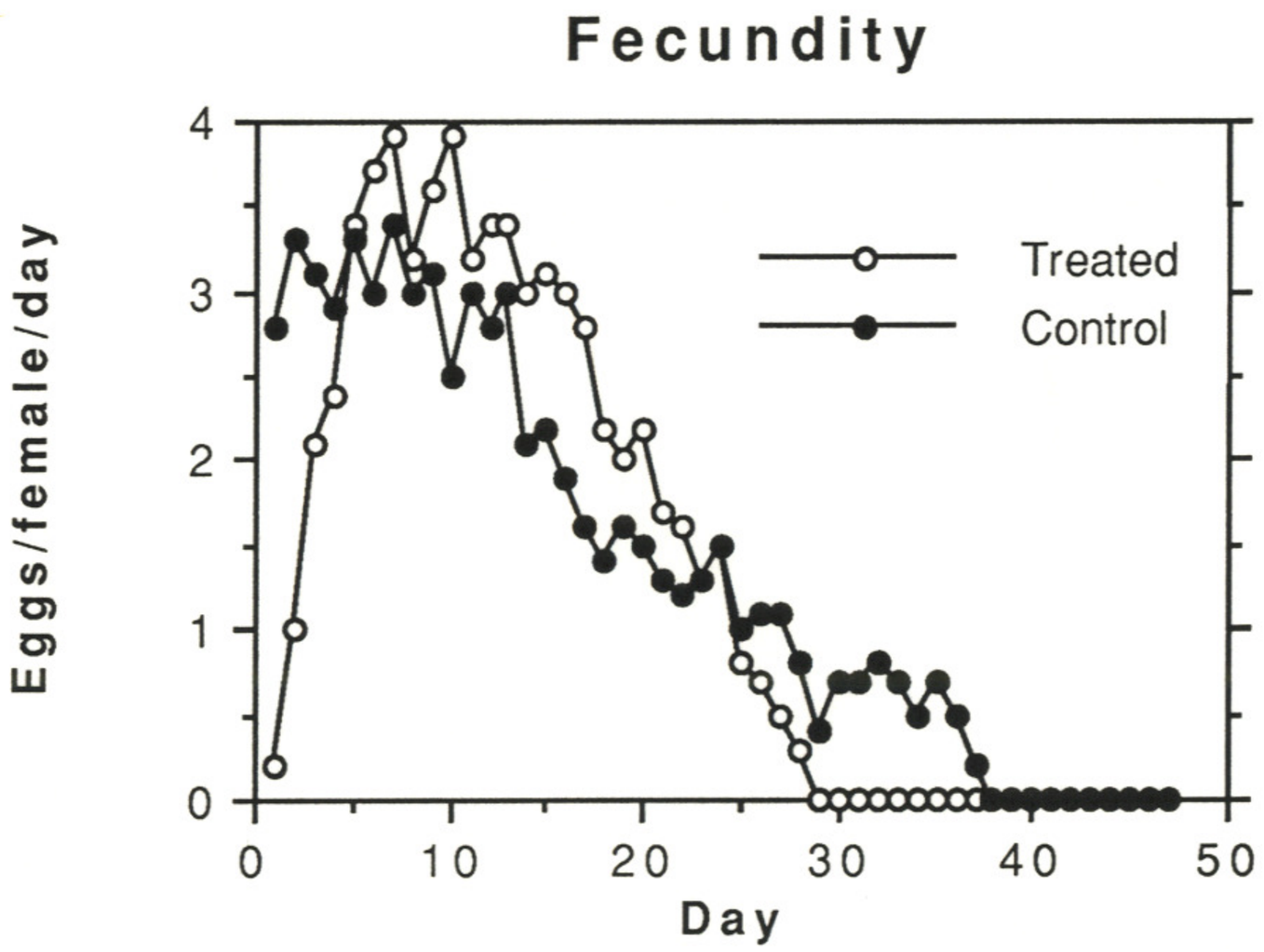

Fecundity
Eggs/female/day
4
3
2
1
0
0
10
20
30
40
50
Day
Treated
Control

Table 2. Survival of adult female *P. persimilis* held individually in Falcon dishes loosely filled with moist bran or vermiculite and provisioned with *T. urticae* eggs on filter paper. Agitation was accomplished by rapidly inverting the Falcon dishes several times immediately prior to cold storage and again after five days. Survival was assessed after a total of ten days at 6°C.

Treatment	Survival (n = 40)[1]
Bran	
Agitated	82%
Control	85%
Vermiculite	
Agitated	80%
Control	75%

[1]No significant differences among treatments ($\chi^2 = 1.4004$, df = 3, $0.50 < P < 0.75$).

Table 3. Survival of adult female *P. persimilis* held individually in Falcon dishes loosely filled with moist bran or vermiculite and provisioned with *T. urticae* eggs on filter paper. Survival was assessed after four weeks of cold storage.

Treatment	Survival (n = 36)[1]
Bran	
5°C	0
8°C	11%
Vermiculite	
5°C	17%
8°C	19%

[1]No significant differences among treatments ($\chi^2 = 7.6702$, df = 3, $0.05 < P < 0.10$).

DISCUSSION

Cold Hardiness

Nonacclimated adult females of both *P. persimilis* and *A. cucumeris* showed a remarkable ability to supercool, which might be expected largely because of their small size. Among freezing intolerant terrestrial arthropods, smaller species or life stages tend to supercool to a greater extent than larger ones (Sømme 1982, Lee 1991), with mean SCPs reported for mites generally in the range of –20°C to –30°C (Sømme 1982). The only SCPs published for phytoseiid mites are mean values of –28.9°C, –30.0°C, and –31.4°C for overwintering adult females of *Typhlodromus pyri* Scheuten, *Amblyseius finlandicus* (Oudemans), and *Typhlodromus rhenanus* Oudemans, respectively, collected from apple trees in Nova Scotia (MacPhee 1963). The fact that adult females of *P. persimilis* supercooled to a significantly greater extent than those of *A. cucumeris* was unexpected because *P. persimilis* is by far the larger of the two species and also originated from a milder climatic zone than the *A. cucumeris* population used in this study. However, the difference in mean SCP between the two species was less than 2°C and therefore may be of little or no evolutionary significance in spite of its statistical significance.

Both species also showed a trend of increasing SCPs during development from egg to adult, with the notable exception of adult female *A. cucumeris* discussed below. Again, the trend may be a reflection of the fact that a smaller body will supercool to a greater extent than a larger body, other factors being equal, because of the probabilistic nature of nucleation in the supercooled state (Salt 1966a). A positive relationship between SCP temperature and droplet size has been shown repeatedly for pure water (Angell 1982), and in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), SCP temperatures were found to be significantly correlated with weight (Johnston and Lee 1990). In various aphids (Homoptera: Aphididae), first instar nymphs are known to have lower SCPs than adults (O'Doherty and Ring 1987) and Stenseth (1965) reported

that *T. urticae* larvae supercooled to a greater extent than adult females and that eggs supercooled to a greater extent than larvae. Similarly, mean SCPs determined for different instars of *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) showed a trend of increasing temperature in older instars and a significant correlation with mean weights of the different instars (Butts, in press).

The increase in mean SCP from egg to deutonymph of 7.7°C for *A. cucumeris* but only 1.1°C for *P. persimilis* is not likely to be a result of differential increases in mass between the two species. Mass has not been determined for *A. cucumeris*; however, Sabelis (1981) measured stage-specific fresh weights of *P. persimilis*, *Amblyseius andersoni* (Chant) [=*Amblyseius potentillae* (Garman) (Chant and Yoshida-Shaul 1990, Messing and Croft 1991)], *Amblyseius bibens* Blommers, and *Typhlodromus occidentalis* Nesbitt and found that mass increased by a factor of four to five times from egg to adult female in all four species, despite large differences in size among species. Interestingly, Sabelis (1981) also found that in both *P. persimilis* and *T. occidentalis* (only eggs and adult females were weighed for the *Amblyseius* spp.), adult males had virtually the same mass as protonymphs, suggesting that the nonsignificant difference in SCPs between these two stages in *A. cucumeris* would fit the pattern of SCPs being directly related to body mass.

In contrast, adult females are considerably larger than deutonymphs but in *A. cucumeris* the mean SCP of adult females was significantly lower than that of deutonymphs. What makes this exception noteworthy is that the adult female of *A. cucumeris* is the only life stage in this study that normally overwinters. Furthermore, adult female *A. cucumeris* apparently responded to one week of acclimation at 7.5°C with a small, but significant, decrease in mean SCP. This response did not occur in adult female *P. persimilis* or in adult male *A. cucumeris*, neither of which diapause or generally survive temperate winters. The mean SCP of adult female *A. cucumeris* after the above acclimation did not differ significantly from that of diapausing mites or even that of diapausing mites that had been acclimated for up to three weeks at temperatures down to –1°C. This indicates that the ability of these

mites to increase their supercooling capacity in response to diapause inducing conditions or low temperature acclimation is very limited. This, in turn, indicates that these mites do not accumulate biochemical antifreezes in preparation for overwintering. In spite of its statistical significance, the difference in mean SCP of nonacclimated *versus* acclimated or diapausing mites was less than 2°C and therefore may be of little consequence for overwinter survival. Rather, the small increase in supercooling capacity is probably a result of other physiological adjustments associated with preparation for overwintering, such as an accumulation of energy reserves in the form of lipids and a decrease in size and water content associated with decreased feeding and suspended reproductive activity (*cf*. Morewood 1989).

The relationship between SCP temperature and size is by no means precise. Variation both within and among species arises from other physical and chemical factors, such as the presence or absence of nucleating agents and the levels and combinations of biochemical antifreeze compounds. For example, in the Antarctic mite *Alaskozetes antarcticus* (Michael) (Acarina: Podacaridae), nymphs were found to supercool to a greater extent than adults, both of which are overwintering stages. This was attributed to both the smaller size of the nymphs and their higher concentrations of glycerol (Young and Block 1980). Feeding status is often cited as having a profound effect on supercooling capacity due to the presence or absence of nucleating agents associated with food in the gut (Salt 1953 and 1968, Block 1981, Zachariassen 1982, Cannon and Block 1988). For example, in *A. antarcticus* the distribution of SCPs has been divided into a "high group" and a "low group", corresponding to the presence or absence of gut contents (Young and Block 1980, Block and Sømme 1982, Cannon 1983). If this was the case for *P. persimilis* or *A. cucumeris*, there should be a distinct decrease in supercooling capacity between the larval and protonymphal stages because these mites only begin feeding once they have become protonymphs. The fact that this did not occur suggests that their essentially liquid diet, which consists of body fluids drained from their prey, does not affect their ability to supercool the way that solid food does in other terrestrial arthropods.

There are a number of parallells in the supercooling capacity of phytoseiid mites and that of an unrelated, but in some ways similar, group of terrestrial arthropods. Aphids are relatively small, feed on a liquid diet of plant sap, and are freezing intolerant. As noted above, differences in mean SCPs among instars have been related to differences in size. Nonacclimated, actively feeding aphids will supercool to below –20°C (Knight and Bale 1986, O'Doherty and Ring 1987, Bale *et al*. 1988) and do not show any increase in supercooling capacity after low temperature acclimation (Knight and Bale 1986). Similarly, feeding status *per se* does not affect supercooling capacity in aphids the way that it does in terrestrial arthropods that have a diet of solid food; however, the source of food (herbaceous *vs*. woody plants) has been shown to cause differences on the order of 10°C in mean SCPs of aphids (O'Doherty 1986). Although the reason for this effect has not been established, another example of it may be the difference in mean SCPs of diapausing *A. cucumeris* that were reared on different prey species.

Both *P. persimilis* and *A. cucumeris* were determined to be freezing intolerant in the traditional sense; that is, they did not survive freezing of their body fluids, as illustrated by the close relationship between SCPs and acute mortality, and therefore the SCP represents the absolute limit of low temperature survival. It is noteworthy that diapausing *A. cucumeris* were also freezing intolerant because some insects that are considered freezing tolerant only survive freezing in their overwintering life stage [*eg*. third larval instar of *Eurosta solidaginis* Fitch (Diptera: Tephritidae) Baust and Nishino 1991, pupae of *Hyalophora euryalus kasloensis* (Cockerell) (Lepidoptera: Saturniidae) Morewood 1991 and unpublished observations]. In contrast with insects, which may be freezing tolerant or intolerant and which may even change from one "overwintering strategy" to the other (Horwath and Duman 1984, Duman 1984, Kukal and Duman 1989), no mites have yet been found to survive freezing and it has been suggested that the necessary mechanisms have not evolved in this group (Cannon and Block 1988).

Several authors have recommended, usually in reference to freezing tolerance,

that assessment of survival ideally should include the ability to continue development and/or reproduce rather than being limited to the resumption of coordinated activity upon rewarming (*eg*. Ring 1980, Baust and Rojas 1985, Lee 1991). For this reason, survival and fecundity of adult female *P. persimilis* and *A. cucumeris* were monitored for 48 h after cooling to –15°C to check for detrimental effects of supercooling in the absence of freezing, which was already found to be lethal. Surprisingly, *P. persimilis* showed no such adverse effects whereas the *A. cucumeris* that had been supercooled laid only half the number of eggs laid by the control group. This may represent cold shock injury not suffered by *P. persimilis* or it may indicate that *A. cucumeris* take longer to recover from chill coma, or both. The *A. cucumeris* used for this experiment were reared on *T. urticae* eggs rather than on *T. putrescentiae* because, in preliminary trials, the predators were apparently killed and eaten by the mold mites before recovering from chill coma. Although they are nominally and perhaps predominantly fungivorous, *T. putrescentiae* are also known to be carnivorous when "defenceless" prey, such as insect eggs, is available (*eg*. Brust and House 1988).

Although both *P. persimilis* and *A. cucumeris* were able to survive brief cooling to subzero temperatures above their SCPs, their ability to survive exposure to such temperatures for extended periods of time was extremely limited. Again because of the probabilistic nature of nucleation in the metastable supercooled state, a supercooled body can be expected to eventually freeze at any temperature below its melting point, with a positive relationship between freezing time and temperature (Salt 1950 and 1966c). The limited survival of supercooled *P. persimilis* and *A. cucumeris* was apparently not due to freezing, however, and therefore must be ascribed to what has been termed "pre-freeze mortality" (Knight *et al*. 1986). The SCP of a freezing intolerant arthropod may provide a benchmark as the absolute limit to low temperature survival; however, it cannot be assumed that such an arthropod will survive as long as it is not exposed to temperatures below its SCP. Such an assumption may be valid in some cases that show a profound increase in supercooling capacity associated with overwintering [*eg*. *Epiblema scudderiana* (Clemens) (Lepidoptera: Tortricidae) Rickards

et al. 1987, *A. antarcticus* Cannon 1987]. There is a growing number of examples, however, where death occurs long before the SCP is reached or occurs even if the SCP is never approached (Bale 1991). Phytoseiid mites are among these freezing intolerant terrestrial arthropods that suffer pre-freeze mortality. For example, adult female *T. pyri* collected during winter from apple trees in Nova Scotia supercooled to almost –29°C on average but none survived eight hours at –25°C, and survival of *Phytoseius macropilis* (Banks) and *Amblyseius fallacis* (Garman) was equally limited at similar temperatures (MacPhee 1963). Survival of other phytoseiid species, for which SCPs have not been determined, at temperatures ranging from –1°C to –11°C has been reported in terms of only hours or days, even for diapausing mites which were consistently more cold hardy than nondiapausing mites (Knisley and Swift 1971, Wysoki 1974, Hamamura *et al.* 1976b, van der Geest *et al.* 1991). Thus it may not be surprising that estimates of natural winter mortality for various species of phytoseiid mites have ranged from almost 80% to well over 90% (Chant 1959 and 1963, Herbert 1962, Knisley and Swift 1971).

It appears that the ability of phytoseiid mites to supercool has little to do with cold hardiness. That is, their supercooling capacity may simply be a reflection of their inherent physical and biochemical characteristics rather than an adaptation for survival of exposure to subzero temperatures. The SCP as a cold hardiness parameter for these mites is useful in defining the absolute low temperature limit of cold tolerance as the point at which freezing of body fluids occurs. This may be of some physiological interest; however, it is of little ecological relevance for overwinter survival because death often occurs at temperatures well above this absolute limit. Overwinter survival may be contingent on other physiological adjustments, particularly those associated with diapause such as altered metabolism and accumulation of energy reserves, rather than simply the ability to survive or, in this case, avoid freezing.

1975). Hamamura *et al.* (1976b) reported survival of *P. persimilis* overwintered on potted strawberry plants in the field at Hiroshima, Japan, where mean weekly temperatures ranged between 0°C and 10°C from January to March and extreme lows approached –5°C. It appears, then, that *P. persimilis* is capable of surviving substantial periods at moderately cool temperatures and brief exposures to subzero temperatures without subsequent adverse effects on longevity or fecundity, and without the benefit of a diapause.

In contrast, published collection records for *A. cucumeris* suggest a broad distribution throughout favourable areas of North America and Europe, including areas classified as Subarctic (average temperature of the coldest month below 0°C, average temperature of the warmest month above 10°C *cf.* Boucher 1975). In northern populations, female *A. cucumeris* respond to short daylengths and cool temperatures by entering a reproductive diapause as adults in preparation to overwinter (Overmeer *et al.* 1989, Morewood and Gilkeson 1991); however, this may not be true in other parts of their range. For example, McMurtry *et al.* (1976) reported that in another widespread species, *A. andersoni*, individuals from the Netherlands entered diapause after being reared under short day photoperiods, but individuals from Italy did not. Similarly, several phytoseiid species have been reported to remain reproductively active throughout the winter in Israel, with all life stages present on sampled plants (Wysoki and Swirski 1971a and 1971b). Although diapause is necessary for overwinter survival in colder temperate areas, the Mediterranean climate is apparently favourable enough to allow phytoseiid mites to overwinter without diapausing.

Diapausing females of *Amblyseius umbraticus* (Chant) (Knisley and Swift 1971), *Phytoseius finitimus* Ribaga (Wysoki 1974), and *A. andersoni* (van der Geest *et al.* 1991) all survived much longer at subzero temperatures than nondiapausing stages. This indicates that diapause is a requisite for optimum cold hardiness of the phytoseiid species in which it occurs. Given that diapause prevents development of the ovaries in female phytoseiids (Wysoki 1974), it is interesting that chilling injury of nondiapausing *A. cucumeris* was expressed as reduced and irregular oviposition. This information

implies that, by preventing development of the ovaries, diapause provides protection from adverse effects of low temperature exposure on subsequent fecundity.

Studies on the relationship between diapause and cold hardiness in terrestrial arthropods have demonstrated that either may occur independently of the other whereas in some cases cold hardiness is a component of the diapause program (reviewed by Denlinger 1991). Although the results of such studies involving phytoseiid mites imply a strong link between diapause and cold hardiness, *P. persimilis* was found here to show a considerable degree of cold hardiness in the absence of any diapause. In fact, *P. persimilis* was more cold hardy than nondiapausing *A. cucumeris* even though the latter species would have to withstand longer periods of time at lower temperatures in order to survive in the northern parts of its range. Expression of such enhanced cold hardiness may be dependent on diapause, considering that only diapausing mites overwinter. The results presented here are consistent with this conclusion but do not provide a firm foundation for it; rather, more comprehensive studies are required to clarify the relationship between diapause and cold hardiness. Phytoseiid mites could prove to be an ideal group for such studies because their diapause is facultative, allowing for comparison of cold hardiness in nondiapausing and diapausing individuals from the same population, and because some species or populations do not diapause, allowing for comparative studies of diapause and cold hardiness at both interspecific and interpopulation levels.

CONCLUSIONS

Both *P. persimilis* and *A. cucumeris* were capable of moderate supercooling and were freezing intolerant, which was also true of diapausing adult female *A. cucumeris*. A gradual decrease in supercooling capacity during development from egg to adult occurred in both species, probably as a function of increasing body mass. The single exception to this trend was noteworthy because it occurred in the only life stage that diapauses and overwinters in temperate climates (*ie*. adult female *A. cucumeris*). Feeding status, diapause, and low temperature acclimation all had little or no effect on supercooling capacity, suggesting that the SCP is largely a physical characteristic of the mites and that supercooling capacity *per se* does not represent an adaptation for survival of exposure to low temperatures.

Although the SCP clearly represented the absolute lower temperature limit for survival, the ability of nonacclimated mites to survive supercooling was limited to very short periods of time. Diapause and acclimation did not enhance cold hardiness of the mites by lowering their SCPs, but probably do facilitate overwintering through metabolic adjustments that allow survival for longer periods of time at a given low temperature.

Of the conditions tested for cold storage, 7.5°C with food and moisture available was the most favourable for both species. Adult female *P. persimilis* may be held for four to six weeks under these conditions with survival of 80% or more and without adverse effects on subsequent longevity or fecundity. They should not, however, be packaged in distribution media during cold storage because the media provide a substrate for the growth of mold, which appears to be detrimental to the mites' survival. Adult female *A. cucumeris*, on the other hand, may only be held for two to three weeks before survival drops below 80% and also appear to be susceptible to chilling injury which is expressed as lowered and variable rates of oviposition.

An explanation for the unexpected difference in survival during cold storage might be found in the contrasting ecological backgrounds of the two species and their

corresponding adaptations. Although *P. persimilis* is thought to be incapable of entering diapause, a certain level of cold hardiness would be necessary for overwintering even in the Mediterranean climates where the species is native. In contrast, *A. cucumeris* ranges into Subarctic climates where the species overwinters as diapausing adult females. It may be that in *A. cucumeris*, cold hardiness has become associated with diapause, leaving nondiapausing individuals with less ability to tolerate cold than, for example, *P. persimilis*. Of course, this explanation is largely speculative and further research is required to clarify the relationship between diapause and cold hardiness in phytoseiid mites.

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