ASPECTS OF THE OVERWINTERING SURVIVAL STRATEGY OF *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) ON APPLE TREES IN NOVA SCOTIA

by

Debra Lynn Moreau
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Scanned electron micrograph of adult female *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae). Magnification: 150x. (Photograph by S. Carbyn and D. Moreau)

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ABSTRACT

Mean density counts, on trees previously inoculated with the New Zealand strain of *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in 1993, showed that this predator had overwintered successfully and established a viable population still present in 1998. The proportion of adult *T. pyri* surviving winter, based on the ratios of the peak October density on leaves to the peak May density of adults on leaves were c.12.5% and 42.9% in two orchard plots, at Sheffield Mills, Nova Scotia. Low temperature trials were conducted throughout the winter of 1997-1998 to determine the response of overwintering female *T. pyri* collected from the field to exposure at low temperatures and the influence of substrate, rate of cooling, and acclimation regime on mortality. Analysis of variance showed a significant effect of temperature on mortality in the December 1997 trial. On average, mortality after 24 h was higher for mites exposed to -15°C (100%) versus -5°C (27%), -7.5°C (37%), and -10°C (13%). In February 1998, temperature and substrate had a significant effect on mortality and there was a significant interaction between temperature and substrate (wood versus plastic). Mortality of mites on a twig was no higher at -15°C (31%) than at -10°C (33%), whereas in plastic vials, mortality was significantly higher at -15°C (85%) than at -10°C (40%). In April 1998, daily changes in live mites on the arena (survival), mortality and dispersal, were recorded for unfed overwintered female *T. pyri* maintained on small arenas (5 x 8 cm) delimited using petroleum jelly to prevent escapes. Mites on arenas were exposed to two temperatures (5°C and 20°C), three relative humidity (RH) levels (20%, 70% and 95%) and two water treatments (with or without available water). Survival, mortality and dispersal were

followed for six-24 hour sampling intervals. Linear and non-linear models were fitted to the data. *T. pyri* showed low mortality (< 7%) where RH was ≥ 70% for both temperatures. After 6 d, the greatest mortality (33%) was observed for mites exposed to 20% RH at 20°C, where free water was not available. Mortality was lower (c.7%-14%) at all humidity levels at 20°C where free water was available. In contrast, at 5°C, there was no observed mortality at 70% and 95% RH (without free water) or at any of the RH levels when water was available. On average, the number of mites dispersing was higher at 20°C than 5°C and also increased when free water was not available. After 6 d, the greatest dispersal (67%) was recorded for mites exposed to 20°C, 70% RH and without available water. At 5°C, dispersal was < 30% except for the driest regime, 20% RH with no water, where 51% of the mites had dispersed 6 d after the trial began. Although a significant increase in dispersal (51%) was observed for mites exposed to 20% RH with no available water, at 20°C, no such trends were evident in dispersal. Overall, survival was higher for mites maintained at 5°C than 20°C with the greatest survival (84%) recorded for 70% RH with no water. In contrast, the harshest regime was at 20°C, 20% RH and without free water, where there was 0% survival after 5 d of observations. Responses to low temperature, humidity and water availability are discussed in relation to the effectiveness of *T. pyri* as a biological control agent for spider mite pests.

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INTRODUCTION

Effective pest management practices have become a necessity with our growing awareness of the over-reliance on insecticides, increasing cases of pesticide resistance, increasing costs of pesticides, environmental pollution and adverse impacts on human health; all resulting from the injudicious use of insecticides (Luckmann and Metcalfe, 1982; Shipp *et al.*, 1991; Hardman, 1992). Research since the 1990's, in the Annapolis Valley, Nova Scotia, Canada, has led to the refinement of current Integrated Pest Management (IPM) programs that involve consideration of biological, behavioural and cultural controls (Hardman & Bent, 1992), and provide practical alternatives to the current dependence on chemical control methods.

Typhlodromus pyri Scheuten (Acari, Phytoseiidae), is a widely distributed predacious mite found in various fruit crop agroecosystems of North America, Europe, and New Zealand (Collyer, 1964; Ramsy & MacPhee, 1970; Markwick, 1986; Hayes, 1988; Hardman & Rogers, 1991; Dunley & Croft, 1992; Blommers, 1994). In the 1980's, researchers in New Zealand developed a strain of *T. pyri* that had 28 times greater resistance to pyrethroids than the native susceptible population of *T. pyri* (Markwick, 1993). Since 1993, this New Zealand strain of *T. pyri* was used extensively to inoculate commercial apple orchards throughout the Annapolis Valley, Nova Scotia (Hardman & Bent, 1992; Hardman *et al.*, 1995). This predatory mite is considered an effective biological control agent for one of the most serious mite pests in apple orchards, the European red mite *Panonychus ulmi* (Koch) (Acari, Tetranychidae) (Hardman & Gaul,

1990; Hardman & Rogers, 1991). *P. ulmi* is a phytophagous mite that is resistant to most organophosphate insecticides and to synthetic pyrethroids (Solomon and Fitzgerald, 1984; van de Vrie, 1985; Hardman & Rogers, 1991). Effective biological control of *P. ulmi* is demonstrated by maintaining pest densities below the economic threshold, which is the level at which pest densities exceed economic injury levels and warrant control action (Luckmann & Metcalf, 1982). The effectiveness of the New Zealand *T. pyri* strain for biological control of *P. ulmi* is influenced by several environmental factors.

In many insects and mites, overwintering mortality is considered to be of great importance in controlling population levels and can often be an important factor in keeping pest populations below economic thresholds or outbreak levels (Bale, 1991). However, conditions experienced in winter can also influence mortality of natural enemies living within the same system as the pest species. In cooler climates, such as that experienced in Nova Scotia, overwintering survival is considered an important component of the effectiveness of biological control agents. In particular, the overwintering generation of adult females plays a key role in the development of field populations of *T. pyri*, as they are gradually activated in the spring and initiate the predator-prey relationship which then develops in the growing season (Zacharda, 1989). In late summer and in the fall, inseminated adult female *T. pyri* enter a facultative reproductive diapause: they arrest egg production, and by October or November are slightly paler, flatter and less active than females not in diapause (Fitzgerald and Solomon, 1991; Overmeer, 1985a). The severity and length of the winter can greatly affect the initial size of the female population in the spring. The size of the spring

population will then affect dynamics for the whole season (Walde *et al.*, 1992).

Temperature and humidity may affect the mite directly through their influence on survival and water loss. However, suitable overwintering sites can ameliorate these effects on mites. Severe physiological stress is placed on terrestrial arthropods in the winter (Bale, 1987), and overwintering sites that are above the soil surface are generally colder and experience more variable temperature regimes than those below the surface (Leather *et al.*, 1993). Overwintering sites are chosen specifically on the basis of certain characteristics such as position, shelter and moisture content, and in well-adapted organisms, are located before the onset of severe conditions (Danks, 1978; Leather *et al.*, 1993). These sites can often protect the organism from the climatic extremes of the winter months. Female *T. pyri* that overwinter above ground on the trunks and limbs of apple trees, are thus subjected to 'winter factors' such as low temperature, dampness, drought, wind, precipitation and freeze-thaw events, and are dependent on finding a suitable winter micro-habitat. Since the adult female *T. pyri* are free-living they are able to move to sites with greater protection, in response to changes in temperature and moisture. However, mites face greater risks in moving to more favourable conditions during the winter months. In Nova Scotia they may be exposed to temperatures as low as -30°C although winter minima are typically not much below -20°C (meteorological office, Agriculture & Agri-Food Canada, Kentville, Nova Scotia). Predicted mortality for *T. pyri* exposed to -10°C for 48 hours was approximately 78% in February and 95% in November for trials conducted in the 1995-1996 winter (Moreau, 1996). Herbert (1962), reported that overwintering mortality for native *T. pyri* in Nova Scotia, may be as high as 80% to 90%

due to exposure to low temperatures.

Seasonal patterns of movement have been noted, where, at the onset of winter, mites move down the tree trunk (Zemek, 1993). *T. pyri* will often aggregate in the fibers of bands of fabric that are placed near the base of the tree to collect samples during the winter months (personal observations). *T. pyri* have also been found during the winter in the splintered ends of drying twigs, in deep crevices surrounding old scars (Chant, 1959), and under bark scales and canker wounds on the tree trunk or larger branches (Herbert, 1952; Fitzgerald & Solomon, 1991; MacPhee, 1964; Ramsy & MacPhee, 1970; Nyrop *et al.*, 1994).

In the growing season, humidity and temperature within the boundary layer of the apple leaf are probably the most important abiotic factors regulating mite population dynamics (Ferro *et al.*, 1979). Humidity in the stirred air layer around the leaf is usually higher than the ambient atmospheric humidity (van Dinh *et al.*, 1988), and the relative humidity on the surface of leaves where *T. pyri* naturally occurs can be close to 100% (Hayes & McArdle, 1987). This is important because *T. pyri* are often observed on the undersurface of leaves sampled and disperse over wood to move from leaf to leaf. During winter, the lowest temperatures over the surface of the tree, on or in the bark, where a number of mite species overwinter, are often quite similar to the lowest ambient air temperatures (MacPhee, 1964), although temperatures of overwintering sites (such as twigs and under bark) are influenced by orientation (Danks, 1978). The lowest temperature on the tree trunk near the ground can be several degrees warmer than ambient air temperatures, when low temperature extremes are of short duration (MacPhee, 1964).

Tauber *et al.* (1998), reported that solid substrates, such as wood, frequently exhibit distinct patterns of seasonal variation in moisture: because short-term fluctuations are dampened, conditions on the substrate reflect long term average conditions.

Temperature and moisture, are widely regarded as the main abiotic environmental parameters governing arthropod development, population dynamics (Cloudsley-Thompson, 1962; Ferro *et al.*, 1979; Holtzer *et al.*, 1988; Tauber *et al.*, 1998), and survival (Sabelis, 1985), and undoubtedly affect the overwintering success of *T. pyri*. Another important factor is the quality and availability of food resources during the growing season, including pre- and post-diapause periods. Tauber *et al.* (1986) reported that the activities of arthropods are synchronized with the seasonal variation in availability of food and moisture. During the winter months, *T. pyri* must depend on internal reserves, accumulated from feeding during the fall, since the overwintering females stop feeding and empty their guts to prepare for diapause. The elimination of possible ice-nucleators in the gut enables freeze-avoidant organisms, such as *T. pyri*, to supercool and avoid injury or death from intracellular freezing upon exposure to low temperatures (Salt, 1961; MacPhee, 1964; Block, 1982; Moreau, 1996). Furthermore, since female *T. pyri* overwinter in the adult stage and are not able to oviposit without prior feeding, the availability of food sources early in the season is likewise crucial (Eichhorn & Hoos, 1989; Engel. 1989). During the growing season, if prey density is low, generalist predators such as *T. pyri* (McMurtry, 1992), can feed on alternate foods such as pollen and fungi, allowing the predator to maintain itself in a locality when spider mite numbers are low (Blommers & van Arendonk, 1979; Overmeer, 1985b; Nyrop *et al.*,

1994; Walde *et al.*, 1992). Nyrop *et al.* (1994), reported that *T. pyri* did not disperse or starve when tetranychid prey densities were low. Furthermore, Engel (1989), found that in the absence of mite prey *T. pyri* reproduced, and increases in population size were obtained when fed a variety of wind-borne pollen species. Another important alternative food source for predacious mites early in the season is the apple rust mite, *Aculus schlechtendali* Nalepa (Acari: Eriophyidae), because it is active much earlier in the season than *P. ulmi* (Overmeer, 1985b; Walde *et al.*, 1992). *T. pyri* have also been reported to feed on *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (Chant, 1959; Herbert, 1956), on tarsonemid mites and on fungi such as powdery mildew (*Uncinula necator*) (Eichhorn & Hoos, 1989). The occurrence of another generalist predator mite, such as *Zetzellia mali* Ewing (Acari: Stigmaeidae), that feeds on the eggs of *T. pyri and* competes for prey such as *P. ulmi* and *A. schlechtendali* (Lawson & Walde, 1993), also tends to reduce populations of *T. pyri* during the growing season and reduces numbers of *T. pyri* that enter diapause (Croft & MacRae, 1992).

Availability of water obtained from prey or other food can also minimize the effects of low humidity on the survival of mobile stages during diapause or pre-moult stages (van Dinh *et al.*, 1988). Organisms the size of a phytoseiid mite (*T. pyri* has a body length of about 400 μm, Appendix A) have a ratio of surface area to volume which imposes severe stress when they are exposed to a vapour deficit (Cloudsley-Thompson, 1962; van der Geest, 1985). When food sources are scarce, low humidity may be associated with low water availability, so that adaptations to cope with the effects of these factors may be expected in the mobile stages of predatory mites (van Dinh *et al.*, 1988).

Several studies have assessed the influence of temperature and humidity on the predatory mite, *T. pyri*, (Croft *et al.*, 1993; Hardman & Rogers, 1991), however, little work has been done to examine the influence of temperature and humidity on the overwintering female *T. pyri* at spring emergence. The purpose of this research was to examine the ability of overwintering *T. pyri* to survive exposure to low temperatures under varying conditions of substrate, relative humidity and availability of water. Factors governing the number of *T. pyri* that survive to early spring (before *P. ulmi* eggs hatch in mid-May) would include (1) mortality in winter from cold, (2) mortality in autumn or spring because of low humidity or high temperatures when a more preferred habitat (leaves in a developed canopy) is not available, or (3) starvation in the spring.

The objectives of this study were to: (1) monitor population densities of *T. pyri* within an experimental orchard through the summer months, in 1997, and in late spring, 1998, and evaluate overwintering survival and timing of spring emergence; (2) examine components of winter mortality by studying the effects of substrate, acclimation, rate of cooling and duration of exposure to low temperatures on mortality of mites collected from the field; (3) assess seasonal variation in the structural and ultrastructural morphology of mites as influenced by environmental stresses, specifically exposure to low temperature and humidity; and (4) to assess the effects of temperature, relative humidity and availability of water on survival of *T. pyri* which have successfully overwintered until early spring.

MATERIALS AND METHODS

Mite populations in the orchards

From June to October 1997, mite densities were monitored on a weekly basis, on 11 year old semidwarf McIntosh apple trees maintained by Agriculture & Agri-Food Canada (AAFC) in an IPM orchard at Sheffield Mills, Nova Scotia. The 2 ha IPM orchard included alternating double rows of Royal Court 'Cortland' apple trees (planted in 1995) and Summerland 'McIntosh' trees (planted in 1987) in north-south rows on MM 111 semi-dwarf rootstocks at a spacing of 4.3 x 6.1 m and a density of 383 trees per ha. The orchard was divided into five southern plots (A-E from west to east) and five northern plots (F-J from west to east). Each 0.2 ha plot included two parallel rows of Cortland trees to the west and two parallel rows of McIntosh trees to the east. There were 18-19 trees per row and samples were taken from trees in the middle of the row and only included McIntosh. Since 1992, these trees have been treated annually, with either a full recommended rate (Ripcord 400 EC, 50g [A.I.]/ha) or dilute rates (5g A.I./ha) of the pyrethroid, cypermethrin. In 1996 there was no application of cypermethrin. Additional insecticide treatments were applied (1997 and 1998), to control orchard insects (Hardman *et al.*, 1997), and fungicides were applied to control apple scab, *Venturia inaequalis* (Cooke) Wint. These fungicides (listed in Appendix B) are considered innocuous to *T. pyri*. In July 1993, trees were inoculated with New Zealand *T. pyri* derived from potted trees maintained in an outdoor insectary (Hardman *et al.*, 1997). Weekly sampling was done from June 1997 through to October 1997 and again in May 1998. Sampling

involved collecting 20 leaves from each of five trees from a southern and a northern plot (labelled D and I respectively). Leaves were placed in plastic bags and refrigerated at c.2°C for up to two days and examined individually under a binocular microscope at 12x. Densities of egg, nymph and adult life stages of predator (*T. pyri* and *Z. mali*) and prey mites, *P. ulmi*, were counted and recorded. Densities of eggs and motile stages were counted for *T. urticae*. For *A. schlechtendali*, tydeids, and tarsonemids only motile stages were counted and recorded.

Mean densities per leaf and standard errors for individual life stages of phytophagous and predator mites were calculated for each plot on each sampling day. The proportion of mites to have successfully overwintered was calculated by dividing *T. pyri* densities per leaf for 20 May 1998 by those determined for 08 October 1997. The 08 October 1997 date was chosen since the observed decrease in mite numbers on the last subsequent sampling dates may have been the result of mites moving to overwintering sites. Herbert (1962), reported that female *T. pyri* migrate from foliage to overwintering sites at the end of September. Likewise, densities per leaf for 20 May 1998 were used, since the trees may have had a more fully developed canopy, as compared with earlier sampling dates in May, and it has been reported that overwintered *T. pyri* become active on the trees about the middle of May (Herbert, 1962; Fitzgerald and Solomon, 1991). Graphical representations for this and the following trials were done using Slide Write Plus 4.1 graphics (Advanced Graphics Software, Inc., 1997).

Trends in temperatures at overwintering sites

In October 1997, a single velvet band (12 by 55 cm) was stapled loosely to the base of the trunk, below the lowest whorl of branches (c.45 cm above ground level), on every tree located in Plots D and I. On January 30, 1998, weatherproof data loggers, Minilogs® (VEMCO Ltd., Halifax, Nova Scotia), were placed several centimeters above the velvet band with the thermistor approximately 2-3 mm inside a bark crevice. Loggers were secured on the north and south side of each of two apple trees located in plot D and temperature was monitored for the month of February 1998.

Trials involving exposure to low temperature

Source of mites for low temperature trials

Low temperature trials conducted in late 1997 and early 1998, involved mites collected from velvet fabric bands taken from Plot D. Bands were stored in plastic bags and refrigerated at c.2°C for up to two days and examined individually under the microscope for live *T. pyri* adult females (Hardman *et al.*, 1995). Details of these trials involving exposure to low temperature are outlined later in this section.

Effect of temperature, December 1997

The influence of duration of exposure to low temperature was examined on New Zealand *T. pyri*. Mites were exposed to various temperatures found to be above the lowest mean supercooling point (SCP), recorded for February 1996, of -28.9°C (Moreau, 1996). The trial was conducted between December 29 and 31, 1997, in which 4 replicates (one

plastic vial with 5 mites was considered a replicate) were tested at each combination of time intervals (1, 3, 6, 16, 24 h) and temperatures (-5°C, -7.5 °C, -10°C and -15 °C). All replicates were cooled at a rate of 1.0 °C/minute. To minimize variability between treatments and across regimes a complete randomized block design was used to allocate treatments for all temperatures. The substrate consisted of twigs taken from secondary branches that were cropped from apple trees, located within the same plots that mites were collected from, and maintained at 1 °C for up to two hours, until cut up into smaller pieces (diameter of c.5mm and length of 15mm). Twigs cut from secondary branches had either smooth or rough bark surfaces. Their surface roughness was not recorded despite the fact that the rough surfaced wood could have provided refuges (bud scale or crevice) not offered by smooth wood. Moreover, newly cut ends were not sealed, allowing separation of the bark from the cambium that may act as an additional refuge for the mites. In later trials, surface features were standardized between different substrates and cut ends were sealed. All branch pieces were then examined for motile animals, which, if found, were then removed. European red mite eggs were not removed because they do not act as a food resource to overwintering *T. pyri* (Herbert, 1956). Five mites were transferred from velvet bands to each twig using a dry, single hair paintbrush. Each twig was then placed vertically in a Corning™ 2.0 ml cryovial with screw-on cap. A strip of Parafilm® was wrapped around the edge of the cap and vial. to ensure that mites could not escape. Temperature regimes were established using commercial freezers, a FTS-Systems ultra-low temperature bath (Stone Ridge, NY) and Haake G cooling bath with Haake D8 circulating pump (Berlin, Germany). The baths were interfaced with a 486

DX2 IBM Compatible computer using virtual instrumentation (VI) Lab View4 (National Instruments, Austin, TX) software and hardware (DAQPad/1200; National Instruments, Austin, TX). Fluctuations in temperature regimes were monitored throughout the experiments using a Fluke 52 K/J Thermometer with a type T Thermocouple (Everett, Washington).

Effect of substrate, January 1998

In January 1998, velvet bands were collected from the field and examined for *T. pyri.* Mites were left on bands which were then placed in plastic bags and maintained at c.2°C, for up to two days. Trials were initiated over a five-day period using a complete randomized block design for treatments. Eight replicates for each of four substrates (i.e., 40 mites per day) were subjected to 24 hours exposure to -10°C, on January 28 and 30, 1998 (Table 1). For each substrate, four replicates (20 adult female *T. pyri* per regime) were treated to each of two acclimation regimes and cooling rates (Table 1). Mites that had been acclimatized were exposed to 1.0°C for four hours prior to being cooled to -10°C, whereas, non-acclimatized mites were immediately cooled to -10°C. One vial with 5 mites on one of four substrates (1, no substrate; 2, leaves; 3, smooth twigs; and 4, rough twigs) was considered a replicate.

For substrate 1, five mites were placed into each of four empty Corning ™ 2.0 ml cryovials. For substrate 2, leaves from Jonagold potted trees maintained in an indoor greenhouse at AAFC, were collected and placed in a plastic bag and stored at c.2°C for no longer than four hours. Leaves were inspected at 12x under a binocular microscope, and cleaned of fauna and debris that would otherwise act as a possible food source and

increase the risk of nucleation. The latter could result in increased mortality from chill injury. Five adult female *T. pyri* were transferred from velvet bands to each cleaned leaf. Individual leaves were placed in plastic vials (9.0 cm in length by 2.5 cm in diameter)

Table 1. Experimental design and pattern of replication for a laboratory trial where field-collected, diapausing adult female *T. pyri* were cooled to -10°C in January 1998. Each replicate included 5 mites on a specific substrate.

	Acclimatized		**Not acclimatized**	
Substrate	**0.1°C/minute**	**1.0°C/minute**	**0.1°C/minute**	**1.0°C/minute**
Vial	4	4	4	4
Leaf	4	4	4	4
Smooth twig	4	4	4	4
Rough twig	4	4	4	4

with plastic screw tops. Leaves were rolled up and placed gently in each vial ensuring that the underside of the leaf faced inward so as to not touch the sides of the container, as it has been observed that *T. pyri* predominantly stay on the lower surface of the leaf (personal observations).

For substrates 3 and 4, secondary branches were cropped from apple trees located within the same plots from which mites were collected. Smooth twigs consisted of wood that had no cracks, crevices, or bud scales, and rough twigs were taken only from the terminal ends of spur wood and I ensured that the surface had cracks or crevices and at least one bud scale (Figure 1). Branches were cropped in the orchard and maintained at

1 °C for up to two hours, until cut up into smaller pieces (diameter of c.5mm and length of 15mm). Cut ends of each twig were sealed using a heated glue stick to prevent the mites from accessing areas that would not usually be available (e.g. gaps under the bark). Smooth twigs (substrate 3) had both cut ends sealed, whereas, rough twigs (substrate 4) only had one end sealed since the other end was not cut for access to the bud scale (Figures 1a-b). All branch pieces were then examined for motile animals, which, if found, were then removed. European red mite eggs were not disturbed since they do not act as a food resource to overwintering *T. pyri* (Herbert, 1956). Five mites were transferred from velvet bands to each twig using a dry, single hair paintbrush. Each twig was then placed vertically in a Corning ™ 2.0 ml cryovial with a screw-on cap. A strip of Parafilm® was wrapped around the edge of the cap and vial, to ensure that mites could not escape. Two control vials for each substrate, with five *T. pyri* each, were held at room temperature (c.20°C and 65%RH) throughout the course of the experiment.

Based on start time of trial for each vial, replicates were removed from each regime upon completion of specific treatment. After removal, mites were allowed to recover in a favourable temperature regime (20°C, 65%RH) for one hour. Each leaf and twig was then removed from the vial and placed on a clean Watson Filter Paper No.5 to be examined under a binocular microscope at 12x. Vials and lids were also examined. Leaves and twigs were examined for up to 20 minutes and the number of live, dead or missing mites were recorded. Mites were classed as dead if they failed to move their appendages after being prodded with a fine probe. Individual leaves were removed from vials and examined following the same procedure as was used for the December trial, with

numbers of live, dead and missing mites counted and recorded.

Effect of low temperatures, February 1998

In February 1998, a final trial was established that examined the rate of mortality of mites on two substrates (empty vials and rough twigs), exposed for 24 hours to two temperature regimes (-10°C and -15°C). Rough twigs used as substrate were pieces of branches (ca. 5 mm in diameter and 15 mm in length) taken only from the terminal ends of spur wood collected from the same trees as those that had been sampled for mites and had the same characteristics as the rough wood used in the January 1998 trials. Methods and procedures previously outlined for January 1998 trials were followed for trials conducted in February 1998.

Statistical analysis of the data

In the December, January, and February trials, estimates of mortality for individual treatments in each temperature regime were calculated by dividing the total number of dead mites by the total number of live and dead mites combined. Counts of missing mites were not used. With data for mean mite mortality, analysis of variance (ANOVA) was done with the SAS GLM procedure (SAS Institute 1990, pp. 898-908) after arcsine transformation of the square root of mortality data. For the interaction between time and temperature and their influence on mortality, logit analysis was done using the SAS procedure PROC PROBIT (SAS Institute 1990, pp. 1327-1350). Using the coefficients for intercept and slope generated by SAS, best fit curves were generated using Slide

Write Plus graphics (Advanced Graphics Software, Inc., 1997).

Desiccation and starvation trial, April 1998

Mites were collected from velvet bands on trees in plot 'D' located at the experimental orchard at Sheffield Mills. The trial was started from April 17 through 18, 1998, where five replicates (one black, plastic 5x8 cm arena with five mites was considered a replicate) were treated with one of two experimental conditions (free water available and no free water), and then were placed in one of three relative humidity (RH) treatments (18-30% RH, 70-80% RH and 95-99% RH) and exposed to one of two temperature regimes (5°C and 20°C), as shown in Table 2.

Table 2. Experimental design and pattern of replication for a laboratory trial where field-collected, diapausing adult female *T. pyri* were exposed to one of two temperatures, with or without free water and at one of three relative humidity levels in April 1998. Each replicate included 5 mites on an arena.

	Treatment			
	5°C		**20°C**	
Humidity	**No water**	**Free water**	**No water**	**Free water**
20%	5	5	5	5
70%	5	5	5	5
95%	5	5	5	5

To simplify reporting, relative humidity ranges will be referred to as follows: 20% RH

(18-30% RH), 70% RH (70-80%RH) and 95% RH (95-99% RH). Temperature regimes were established using growth chambers that were maintained at 5°C and 20°C, with 50% RH and 24 h light. These temperatures were chosen because mites were expected to be active and feeding at 20°C but below the feeding threshold at 5°C (Walde *et al.*, 1992). Relative humidity regimes were established in 2.4L plastic containers, using of $CaSO_4$ (20% RH), saturated salt solution of NaCl (70% RH) (Winston and Bates, 1960), and water-saturated cotton for the 95% RH regime, and were equilibrated for 48 hours prior to use. Fluctuations in temperature and humidity regimes were monitored throughout the experiment using Smart LCD Indoor Thermo-Hygrometers (InterTAN Canada Ltd., Barrie, Canada). Five adult female *T pyri* were placed on a black plexiglass arena (5x8 cm) with the edges delimited by petroleum jelly and a water-saturated small wad of cotton in one corner. Full saturation was when a small pool of water formed around the cotton. Five arenas were then placed in a large plastic box (2.4L capacity) and covered with plastic wrap and secured with two elastic bands for a 24-hour period. This system allowed for repeated observations that would not affect humidity levels. After 24 hours with a free water source available, cotton balls and excess water were removed from one half of the arenas, those classified ad lacking free water. Arenas were then transferred to one of six plastic containers, for each temperature regime, where a specific humidity regime had been previously established. Transfer of arenas with mites was conducted under a fume hood and processed as quickly as possible. Relative humidity was measured to monitor changes during the experiment due to air transfer. Containers were again covered with plastic wrap and secured with two elastic bands. Lids were then

placed on top to maximize seals. Six containers each housing five arenas (20% RH with free water, 20% RH with no water, 70% RH with free water, 70% RH with no water, 95% RH with free water, 95% RH with no water) were placed in each of two growth chambers set at 5°C and 20°C. Temperature, relative humidity and availability of drinking water were randomized for each test replicate. Containers were maintained at their respective temperature regime for the duration of the study, as mites were examined inside the chambers to minimize disturbance. Mites were examined in situ on arenas every 24 hours for six days, using a binocular microscope at 12x. Every 24 hours, a micropipette was inserted through a small opening in the plastic wrap covering the top of the box so additional water could be added to the cotton balls for those arenas that had free water.

Dispersal and Mortality

To examine the influence of experimental factors on mortality of individuals during the desiccation experiments, live mites were distinguished from those found motionless (dead) and those mites found in the petroleum jelly (either alive or dead), used to delimit the arenas. Those in the petroleum jelly were categorized as dispersers and were analyzed as a separate category. Live mites that had not dispersed were classed as survivors.

Statistical analysis

Means and standard errors were calculated for the proportions of live, dead and dispersing mites found on arenas. The choice of the appropriate function to describe the effect of time in a regime on mortality, dispersal or survival was based on visual

assessment, using the equations shown in Table 3. The function chosen was one that would generate a curve that most closely approximated the temporal pattern shown by the data. Coefficients for the functions were generated with the SAS NLIN and STEPWISE procedures (SAS Institute 1990, pp. 1358-1369). The intercept was set at zero for all linear regression functions. Comparison of linear regression parameters indicated whether significant differences existed among regimes in rates of mortality, dispersal and total declines in survival ($|Z| < 1.96$; [SAS Institute 1990]). Using the coefficients generated for each function by SAS, curves of best fit were created using Slide Write Plus 4.1 graphics (Advanced Graphics Software, Inc., 1997).

RESULTS

Mite populations in the IPM orchard

Mean density counts, on trees inoculated with the New Zealand strain of *T. pyri* in 1993, showed that the predators had overwintered successfully and established a viable population that was still present in 1998. By mid- July 1997, motile stages of *T. pyri* (nymphs and adults) reached a peak density of approximately 1 mite per leaf in plots D and I (Figures 2 and 3), despite low prey densities (Figure 3). Peak densities of motile stages of *P. ulmi* were less than 2/leaf in plot D and less than 1/leaf in plot I (Figures 2 and 3), and peak densities for tydeids and tarsonemids were less than 4/leaf and 1/leaf, respectively, for plots D and I (Figures 2 and 3). Even in October 1997 there was still a significant number of adult *T. pyri* remaining on the leaves in both plots. These may be underestimates of the *T. pyri* numbers since mites may already have moved from leaves to overwintering sites on the wood.

Mean density counts on leaves taken in May 1998, showed that low numbers of overwintering *T. pyri* had emerged, having survived the winter (Figures 2 and 3). However, the numbers of *T. pyri* in spring may also be underestimated, because some mites may still have been moving out from their overwintering sites to leaves. The proportion of adult *T. pyri* surviving winter, based on the ratios of the peak May density on leaves to the peak October density of adults on leaves, were approximately 12.5% and 42.9% for plots D and I, respectively (Figures 2 and 3). Densities of motile stages of *T. pyri* were highest in early July 1997, and then declined in late September and early October as they dispersed to their winter habitat. By late May 1998, just before bloom,

motile stages of *T. pyri* were found on leaves.

Trends in temperatures at overwintering sites

During the month of February 1998, ambient temperature, hours of daily sunshine, and temperatures inside bark crevices were recorded for the north and south side of each of two apple trees. Trends in ambient temperature and hours of bright sunshine fluctuated widely and within short time intervals (Figure 4). Mean ambient temperature ranged from -11.6 to 4.5°C; daily maximum temperature ranged from -8.9 to 8.3°C; and daily minimum temperature ranged from -14.6 to 3.9°C (Figure 4A). Hours of bright sunshine ranged from no sunshine to 8.6 hours within one 24-hour period (Figure 4B).

Temperatures experienced at the level of an overwintering site, located above the ground on the tree trunk, showed a wide range in daily temperatures that often followed ambient trends (Figure 4, 5). Similar sites that only differ in positioning on the tree trunk showed variation in temperatures, where south-facing sites reached temperatures exceeding 15°C on three occasions (Figure 5B). The lowest daily minimum temperatures and greatest fluctuations in daily temperature changes were observed on the south-facing bark surface, where temperatures went from 15.6°C to -7°C, in one six hour period (Figure 5B). Whereas, the greatest fluctuation experienced at a north-facing site, over one six hour period, ranged from -5°C to -16°C (Figures 5A, 5D). Minimum temperatures recorded for the north and south-facing bark surfaces fell below -15°C on three occasions (Figure 5), however, temperatures were at -15°C for periods $\leq$ 12 hours (Figure 5).

Mortality on exposure to low temperature

Effect of temperature and duration of exposure, December 1997

Mites collected from velvet bands on trees in plot D in December 1997, were placed on twigs and exposed to sub-zero temperatures for varying lengths of time (one to 24 hours) to assess rates of mortality. The mean mite mortality (± SE) for each exposure time at each of four temperature regimes (-5°C, -7.5°C, -10°C and -15°C) is shown in Table 4. Analysis of variance showed no significant effect of temperature or duration of exposure on mortality, but the interaction between temperature and duration of exposure did show a significant effect on mortality. On average, mortality was higher for mites exposed to -15°C than other temperatures (Table 4).

Observed mortality resulting from increased exposure time averaged over the four temperature regimes for the December 1997 trial is shown in Figure 6A. Mortality increased with increasing exposure to cold (Figure 6). I fitted a logit function which is best suited to data where mortality (which follows the binomial distribution) increases with time, dosage or concentration (Robertson and Preisler, 1992). Estimated coefficients were generated with logistic regression analysis of the observed mortality data as influenced by the interaction of exposure times at all four temperature regimes, as shown in Table 5. Best fit curves were generated using the estimated coefficients (Table 5) for the observed mortality at the four temperature regimes (Figure 6);

$$\text{logit}(\rho) = \frac{e^{(\beta_0 + \beta_1 * \ln(t)*T)}}{1 + e^{(\beta_0 + \beta_1 * \ln(t)*T)}} \quad (1)$$

where ρ_i = proportion dead at time $_i$

$$\text{logit}(\rho) = \log \frac{\rho}{1 - \rho_i}$$

β_0 = intercept,

β_1 = slope of the regression of mortality on interaction between temperature and natural logarithm of duration of exposure,

$\ln(t)$ = logarithm of duration (hours) of exposure

T = temperature (°C)

Chi-square (χ^2) tests for the average of all four temperature regimes indicate that both the intercept and slope were different from zero (Table5). Mortality of *T. pyri* increased with increasing exposure to cold (Figure 6A). The relationship between observed and predicted mite mortality with increasing exposure times to low temperatures (Figure 6B) was significant ($0.01 > P > 0.005$).

Effect of substrate on mortality at -10 °C, January 1998

Mortality on different substrates as influenced by acclimation and rate of cooling, was examined in the January 1998 trials. The mean mite mortality (± SE) observed on four substrates (leaf, vial, rough twig and smooth twig) are shown in Table 6. Analysis of variance for the whole data set indicated no significant effect of substrate on mortality (*F*

= 2.76; $df = 3, 51$; $P = 0.052$). Acclimation regime and rate of cooling also did not significantly affect mortality ($F = 0.37$; $df = 1, 51$; $P = 0.548$ and $F = 1.66$; $df = 1, 51$; $P = 0.203$, respectively). Correspondingly, no significant interactions were found between acclimation regime, type of substrate and rate of cooling ($F = 1.40$; $df = 3, 51$; $P = 0.254$; $F = 0.28$; $df = 1, 51$; $P = 0.601$; and $F = 1.63$; $df = 3, 51$; $P = 0.195$). Comparison between December 1997 (Table 4) and January 1998 trials at -10°C, after 24 hours exposure, showed higher observed mortality of mites in January. Mean mortality was 0.13 (± 0.13) for December 1997 and 0.32 (± 0.14) for mites on smooth twigs, not acclimated and cooled at 1°C/minute, for January 1998 trials (Table 6).

Effect of low temperatures and substrate, February 1998

Twenty-four hour tests were conducted in February 1998 to compare the effect of exposure to -10°C and -15°C on mortality of overwintering *T. pyri* placed on two substrates (vial and rough twig). Mean mite mortality (± SE) for both temperatures is shown in Table 7. Analyses indicated that temperature and substrate had a significant effect on mortality ($F_t = 4.93$; $df = 1, 12$; $P = 0.046$; $F_s = 5.65$; $df = 1, 12$; $P = 0.035$). There was also a significant interaction between temperature and substrate ($F = 5.66$; $df = 1, 12$; $P = 0.035$) which is evident from the mean mortalities shown in Table 7. Mortality on the twig was no higher at -15°C than at -10°C, whereas in the plastic vial, mortality more than doubled when temperature was -15°C compared with -10°C (Table 7).

Desiccation and starvation trials, April 1998

Trends for mortality

Mite mortality was affected by relative humidity, temperature and water availability (Figures 7, 8). The relationships between mortality and duration of exposure to varying levels of relative humidity were determined using linear regression of the form shown in Table 3. Coefficients for curves of best fit are shown in Table 8. At 5°C with no water available, mortality was observed only for those mites exposed to 20% RH, with approximately 27% found dead by day six (Figure 7A). No mortality was observed until the fourth day of the trial and then increased from day four to day six (Figure 7A). In contrast, no mites were found dead on arenas when water was available at 5°C, at all three relative humidity levels (Figure 7B).

When free water was available less mortality was also observed at 20°C, except for mites exposed to 95% RH which showed no mortality when water was not available (Figure 8). At 20% RH, 20°C without water, mortality increased to 33% by day two with no change for the duration of the trial (Figure 8A). In contrast, no mortality was observed for mites exposed to 70% RH until the fifth day of the trial (Figure 8A). Comparison across all regimes showed that mites exposed to 20% RH, at 20°C with no free water, had the highest rate of mortality by day six (approximately 33%) which took place within the first two days of the trial (Figure 8A). At 20°C and without or with available water, significant contrasts ($|Z| > 1.96$) were found between calculated slopes, for mortality, observed between 20% RH and 70% RH (Table 8). The greatest mortality was at 20% RH when water was not available and the least mortality was observed at 95% RH when

mites had no access to free water. However, the low number of replicates (four sets of five mites per regime) and relatively high variation in mortality between humidity regimes, precluded statistically significant treatment effects.

Trends for dispersal into petroleum jelly

The relationship between relative humidity and dispersal for all treatments, except for 20°C without available water, were determined using linear regression of the form shown in Table 3. Calculated coefficients for curves of best fit are shown in Tables 9 and 11. By day 6, at 5°C with no available water, 51% of the mites exposed to 20% RH were found in the petroleum jelly, whereas only 18% of the mites held at 70% RH and 27% of those held at 95% RH were found in the petroleum jelly (Figure 9A). Significant differences in slopes ($|Z| > 1.96$), indicated most rapid dispersal at 20% RH and least rapid at 70% RH (Table 9). At 5°C with available water (Figure 9B), slopes for 20% RH and 95% RH were not significantly different (Table 9), whereas the slope at 70% RH was significantly lower ($|Z| > 1.96$) than for the other two humidities (Table 9).

At 20°C with no free water the cumulative proportion of *T. pyri* dispersing showed an initial acceleration phase of rapid increase followed by deceleration to a plateau (Figure 10A). For this reason I used the Gompertz function with the slope $c > 0$ to describe the data (see Table 3 for the form of the equation). Estimated coefficients for the Gompertz functions are shown in Table 10. Comparison of coefficient d, regarded as the inflexion point, indicated a more rapid increase in dispersal at 20% RH compared with 70 and 95% RH, which were similar. However, the lower asymptote (coefficient b) for 20% RH

indicates that the expected final value for proportion dispersing would be at a lower value than for 70% RH and 95% RH (Table 10). At 20°C with no water, dispersal of mites was inversely proportional to the relative humidity. Relative humidity and rate of dispersal into petroleum jelly showed positive correlations for 20% RH ($F = 154.88$; $df = 3, 32$; $P < 0.001$), 70% RH ($F = 49.37$; $df = 3, 32$; $P < 0.001$) and 95% RH ($F = 42.56$; $df = 3, 32$; $P < 0.001$). When water was present at 20°C, dispersal was higher at 70% RH (Figure 10B), although mortality was higher at 20% RH (Figure 8B). When water was made available, the slope at 20% RH was significantly less than ($|Z| > 1.96$) for the other two humidities, whereas slopes for 70 and 95% RH were similar (Table 11). The most rapid dispersal was at 70% RH and the least dispersal observed at 20% RH (Figure 10B).

Trends for total attrition ('declines in survival')

At 5°C with no available water, the relationship between attrition and 20% RH was described using a quadratic function (Table 3) because the rate of loss accelerated over time. In Table 12 the only significant regression coefficient, -0.022, was associated with time squared. For 20% RH, the quadratic regression response was significant ($P = 0.0001$; $R^2 = 0.73$). Relationships between attrition and relative humidity at 70% and 95% RH were described using linear regression with coefficients for curves of best fit as shown in Table 13. All of the decrease in survival for mites exposed to 70% RH and 95% RH resulted from all mites having dispersed into the petroleum jelly (Figures 7A, 9A, 11A). Whereas, at 20% RH, the decrease in survival resulted from both mortality and mites having dispersed into the petroleun jelly (Figure 13). Slopes for total attrition, at

5°C without water, were similar ($|Z| > 1.96$) between 70 and 95% RH (Table 13). When water was available at 5°C, there were no significant differences ($|Z| > 1.96$) between all three relative humidity levels (Table 13). The observed decrease in survival (Figure 11B), for all three relative humidity regimes, resulted from mites dispersing into the petroleum jelly (Figure 9B), since no mites were found desiccated on the arenas (Figure 7B).

At 20°C with no available water, the relationship between attrition and 20% relative humidity was described using a negative exponential function (Table 3) because the instantaneous rate of decrease does not change with time. For 20% RH, the negative exponential response was significant ($P = 0.0001$; $R^2 = 0.64$), as shown in Table 14. Relationships between attrition and relative humidity (70% and 95% RH) were described using linear regression with coefficients for curves of best fit as shown in Table 13. Mite survival at 70% RH and 95% RH decreased linearly with $P = 0.0037$; $R^2 = 0.22$ and $P = 0.0001$; $R^2 = 0.36$, respectively. Slopes for total attrition, at 20°C without water, were similar ($|Z| > 1.96$) between 70 and 95% RH (Table 13). Nonetheless, at 95% RH there was a slower decrease in rate of change in survival compared with attrition at 70% RH (Figure 12A). The decrease in survival at 95% RH resulted from mites having dispersed into the petroleum jelly (Figure 12A). However, attrition at 20% RH and 70% RH resulted from both mortality and mites having dispersed into the petroleum jelly (Figure 14).

In contrast, when water was available at 20°C, the relationship between attrition and 20% RH was described using a quadratic function (Table 3) because the rate of loss accelerated over time. For 20% RH, the fit was good ($P = 0.0001$; $R^2 = 0.70$). In Table 15

the regression coefficient, -0.016, was associated with time squared. Relationships between attrition and relative humidity (70% and 95% RH) were described using linear regression with coefficients for curves of best fit as shown in Table 13. Mite survival at 70% RH and 95% RH decreased linearly with $P = 0.0003$; $R^2 = 0.32$ and $P = 0.0001$; $R^2 = 0.43$, respectively. Slopes for total attrition, at 20°C with water, were similar ($|Z| > 1.96$) between 70 and 95% RH (Table 13). However, there was a more rapid attrition in survival for mites at 95% RH versus 70% RH but the difference was not significant (Table 13). The decrease in survival at all three relative humidity regimes from both mortality and mites having dispersed into the petroleum jelly (Figure 15).

TABLES

Table 3. Linear and nonlinear equations used to generate curves of best fit to survival, mortality and dispersal rates in the desiccation trial.

Curve of Best Fit	**Equation**
Linear	$y = a + bx$
Quadratic	$y = a + bx + cx^2$
Gompertz (right, $c > 0$)	$y = a + b \exp[-\exp(-c(x - d))]$
Negative Exponential	$y = a * \exp(-b * x)$

Table 4. Mortality ($\bar{x} \pm$ S. E.) of adult female *Typhlodromus pyri* as a function of exposure to low temperature. Overwintering female *T. pyri* were collected from the field in December 1997 and placed on twigs.

Temp (°C)	Time (h)	N	Mean	SE
-5	1	4	0.40	0.40
-5	3	4	0.20	0.20
-5	6	4	0.00	0.00
-5	16	4	0.17	0.17
-5	24	4	0.27	0.67
-7.5	1	4	0.00	0.00
-7.5	3	4	0.00	0.00
-7.5	6	4	0.13	0.13
-7.5	16	4	0.25	0.25
-7.5	24	4	0.37	0.033
-10	1	4	0.43	0.18
-10	3	4	0.13	0.13
-10	6	4	0.63	0.13
-10	16	4	0.54	0.21
-10	24	4	0.13	0.13
-15	1	4	0.58	0.08
-15	3	4	0.50	0.30
-15	6	4	0.88	0.13
-15	16	4	1.00	0.00
-15	24	4	1.00	0.00

Table 5. Coefficients for the logistic function fitted to mortality data averaged over all four temperature regimes (-5°C, -7.5°C, -10°C, -15°C). Coefficients were computed iteratively by the SAS NLIN procedure (SAS Institute 1990, pp. 1135-1193).

Variable	Coefficient	*df*	Estimate	Std Err	χ^2	Pr>χ^2
Intercept	β_0	1	-1.64	0.28	33.65	0.0001
Slope	β_1	1	-0.07	0.01	24.83	0.0001

Table 6. Mortality ($\bar{x}$ ± S. E.) of acclimated and non-acclimated adult female *Typhlodromus pyri* that were exposed to -10°C for 24 hours, at different rates of cooling and on four different substrates. Overwintering female *T. pyri* were collected from the field in January 1998. Substrates: plastic vial, leaf, "smooth" twig, "rough" twig.

Substrate	Acclimation	Cooling rate	N	Mean	SE
vial	no	fast	4	0.23	0.13
leaf	no	fast	4	0.16	0.055
smooth twig	no	fast	4	0.32	0.14
rough twig	no	fast	4	0.44	0.21
vial	no	slow	4	0.00	0.00
leaf	no	slow	4	0.61	0.16
smooth twig	no	slow	4	0.20	0.071
rough twig	no	slow	4	0.16	0.10
vial	yes	fast	4	0.15	0.09
leaf	yes	fast	4	0.23	0.08
smooth twig	yes	fast	4	0.28	0.11
rough twig	yes	fast	4	0.40	0.20
vial	yes	slow	4	0.050	0.050
leaf	yes	slow	4	0.050	0.050
smooth twig	yes	slow	4	0.35	0.09
rough twig	yes	slow	4	0.25	0.15

***Cooling rates: Fast = 1°C/min; Slow = 0.1°C/min.**

Table 7. Mortality ($\bar{x} \pm$ S. E.) of acclimated adult female *Typhlodromus pyri* in plastic vials and on rough surface twigs, cooled at 0.1 °C/min, and exposed to -10 °C and -15 °C. Mites were collected from the field in February, 1998. Substrates: plastic vial, "rough" twig.

Temp (°C)	Substrate	N	Mean	SE
-10	vial	4	0.40	0.16
-10	rough twig	4	0.33	0.047
-15	vial	4	0.85	0.15
-15	rough twig	4	0.31	0.036

Table 8. Linear regression coefficients for mortality of adult female *T. pyri* exposed to 20°C, at three relative humidities (20%, 70% and 95%), with presence or absence of available water. Calculated slopes with the same letter are not significantly different ($|Z| < 1.96$, $P > 0.05$). * Asterisks denote regression coefficients without standard errors that were generated by Slide Write Plus graphics (Advanced Graphics Software, Inc., 1997) because only two of the observation values differed.

Regime	r^2	Slope ± SE	$Pr > F$
20°C, No Water			
20% RH	0.83	0.17 ±0.020a	0.0001
70% RH	0.25	0.014 ±0.0063b	0.0478
20°C, Free Water			
20% RH	0.49	0.024 ±0.0042a	0.0001
70% RH	0.18	0.0095 ±0.0034b	0.0093
95% RH	0.37 *	0.010 *	0.0054 *

Table 9. Estimates of linear regression parameters for attrition due to dispersing into petroleum jelly of adult female *T. pyri* exposed to 5°C, at three relative humidities (20%, 70% and 95%), with presence or absence of available water. Calculated slopes with the same letter are not significantly different ($|Z| < 1.96$, $P > 0.05$).

Regime	r^2	Slope ± SE	$Pr > F$
5°C, No Water			
20% RH	0.75	0.084 ± 0.0083a	0.0001
70% RH	0.52	0.033 ± 0.0054c	0.0001
95% RH	0.83	0.051 ± 0.0039b	0.0001
5°C, Free Water			
20% RH	0.66	0.040 ± 0.0053a	0.0001
70% RH	0.40	0.031 ± 0.0065b	0.0001
95% RH	0.58	0.042 ± 0.0061a	0.0001

Table 10. Estimates of nonlinear regression parameters, using Gompertz curve equation (Table 7), for dispersal into petroleum jelly by adult female *T. pyri* exposed to varying levels of relative humidity while maintained at 20°C and without available water. The y intercept, a, was always set to zero which was the observed dispersal rate at time zero.

Regime	Coefficient	Estimate	Std Err
20°C, No Water			
20% RH	a	0	
	b	0.65	0.040
	c	1.76	0.72
	d	1.08	0.15
70% RH	a	0	
	b	0.96	0.37
	c	0.52	0.32
	d	3.04	0.96
95% RH	a	0	
	b	0.79	0.32
	c	0.48	0.32
	d	2.80	1.10

Table 11. Estimates of linear regression parameters for attrition due to dispersing into petroleum jelly of adult female *T. pyri* exposed to 20°C, at three relative humidities (20%, 70% and 95%), with available water. Calculated slopes with the same letter are not significantly different ($|Z| < 1.96$, $P > 0.05$).

Regime	r^2	Slope ± SE	$Pr > F$
20°C, Free Water			
20% RH	0.74	0.061 ±0.0062a	0.0001
70% RH	0.83	0.11 ± 0.0090b	0.0001
95% RH	0.81	0.086 ±0.0072b	0.0001

Table 12. Coefficients for quadratic functions (Table 7), describing survival of adult female *T. pyri* exposed to 5°C, at 20% relative humidity, with presence or absence of available water.

Regime	r^2	Intercept ± SE	Slope (c) ± SE	*Pr > F*
5°C, No Water				
20% RH	0.73	0.99 ± 0.041	-0.022 ± 0.0023	0.0001

Table 13. Coefficients for straight lines fitted to rates of survival of adult female *T. pyri* exposed to two temperatures (5°C and 20°C), at three relative humidities (20%, 70% and 95%), with presence or absence of available water. For each regime of temperature and water supply, calculated slopes with the same letter are not significantly different ($|Z| < 1.96$, $P > 0.05$).

Regime	r^2	Slope ± SE	$Pr > F$
5°C, No Water			
70% RH	0.63	-0.20 ±0.026a	0.0001
95% RH	0.58	-0.18 ±0.026a	0.0001
5°C, Free Water			
20% RH	0.64	-0.18 ±0.025a	0.0001
70% RH	0.61	-0.20 ±0.028a	0.0001
95% RH	0.59	-0.19 ±0.027a	0.0001
20°C, No Water			
70% RH	0.22	-0.093 ±0.030a	0.0037
95% RH	0.36	-0.12 ±0.028a	0.0001
20°C, Free Water			
70% RH	0.32	-0.11 ±0.027a	0.0003
95% RH	0.43	-0.14 ±0.028a	0.0001

Table 14. Estimates of nonlinear regression parameters, using a negative exponential curve equation (Table 7), for survival of adult female *T. pyri* exposed to 20% relative humidity at 20°C and without available water.

Regime	r^2	Scaling parameter	Slope ± SE	$Pr > F$
20°C, No Water				
20% RH	0.64	-0.53 ± 0.38	-0.81 ± 0.11	0.0001

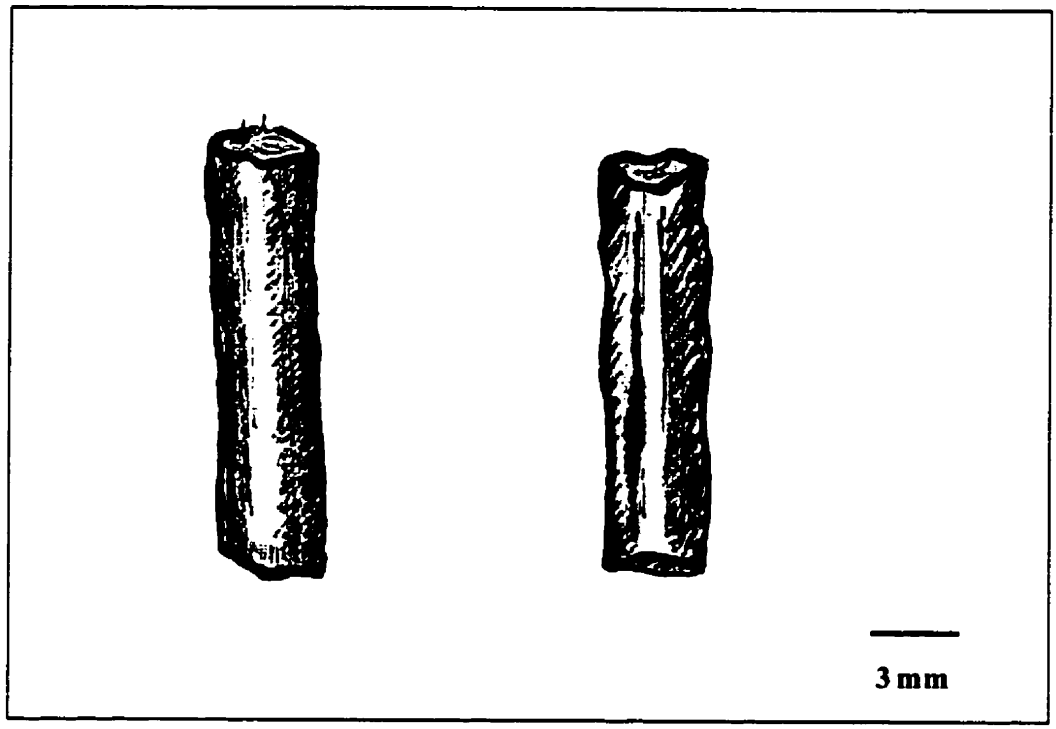
Table 15. Coefficients for quadratic functions (Table 3), describing survival of adult female *T. pyri* exposed to 20°C, at 20% relative humidity, with available water.

Regime	r^2	Intercept ± SE	Slope (c) ± SE	*Pr > F*
20°C, Free Water				
20% RH	0.70	0.97 ± 0.033	-0.016 ± 0.0018	0.0001

FIGURES

Figure 1a. Biological drawings depicting surface texture of pieces of branches, cropped from 11 year old apple trees, referred to as 'smooth twigs' substrate for low temperature trials conducted in January 1998.

Figure 1b. Biological drawings depicting surface texture of pieces of branches, cropped from 11 year old apple trees, referred to as 'rough twigs' substrate for low temperature trials conducted in January 1998.


3 mm

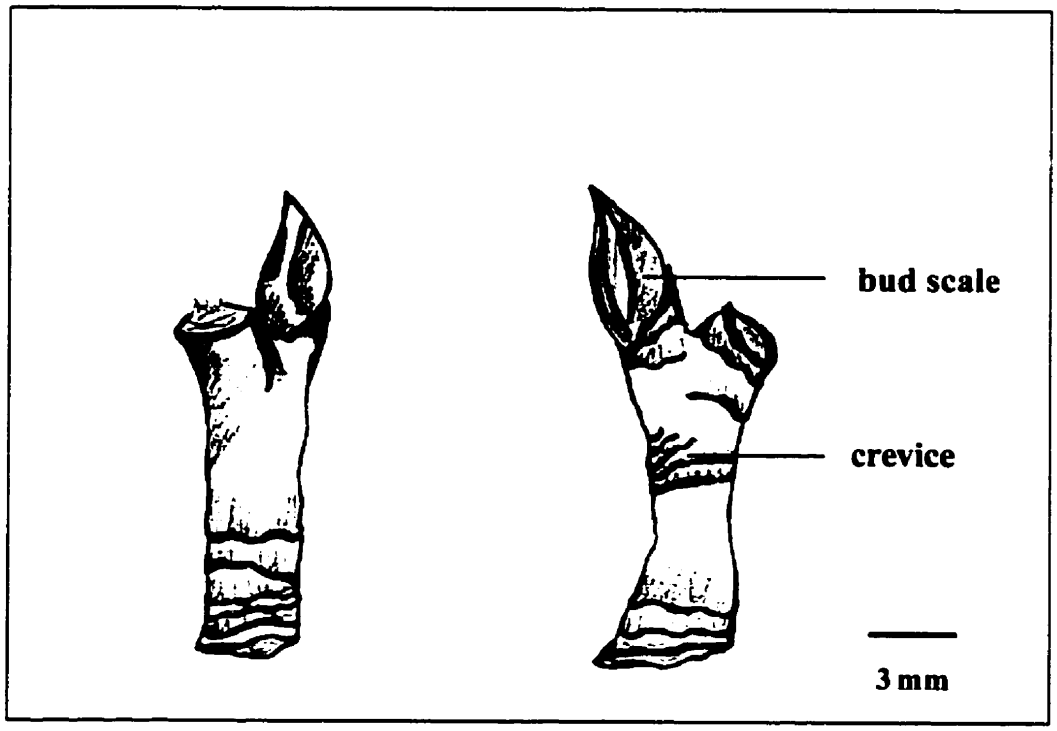
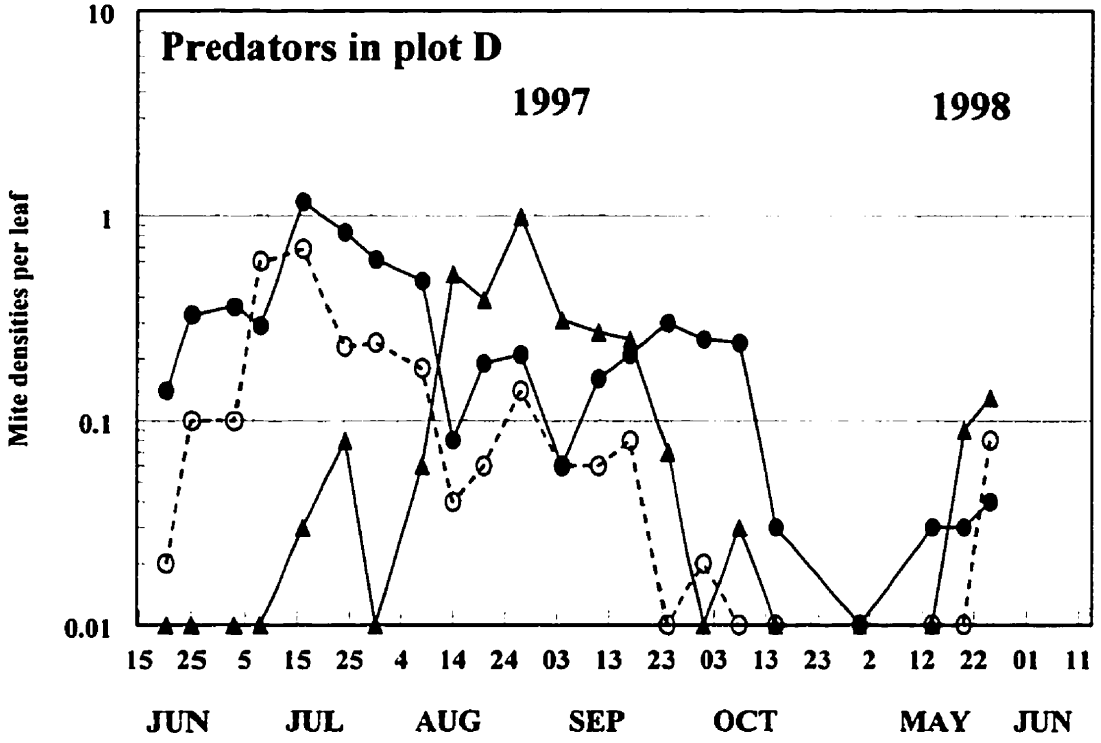

bud scale
crevice
3 mm

Figure 2. Mean densities for predatory (A) and pest (B) mites counted on 100 leaves, including *P. ulmi* and *T. pyri*, sampled in 1997 and early 1998 from experimental plot 'D'. Predators: *T. pyri* nymphs (○), *T. pyri* adults (●) and *Z. mali* adults (▲). Prey: *P. ulmi* motiles (●), tydeids (◊) and tarsonemids (▼).


Predators in plot D
1997
1998
Mite densities per leaf
10
1
0.1
0.01
15 25 5 15 25 4 14 24 03 13 23 03 13 23 2 12 22 01 11
JUN JUL AUG SEP OCT MAY JUN

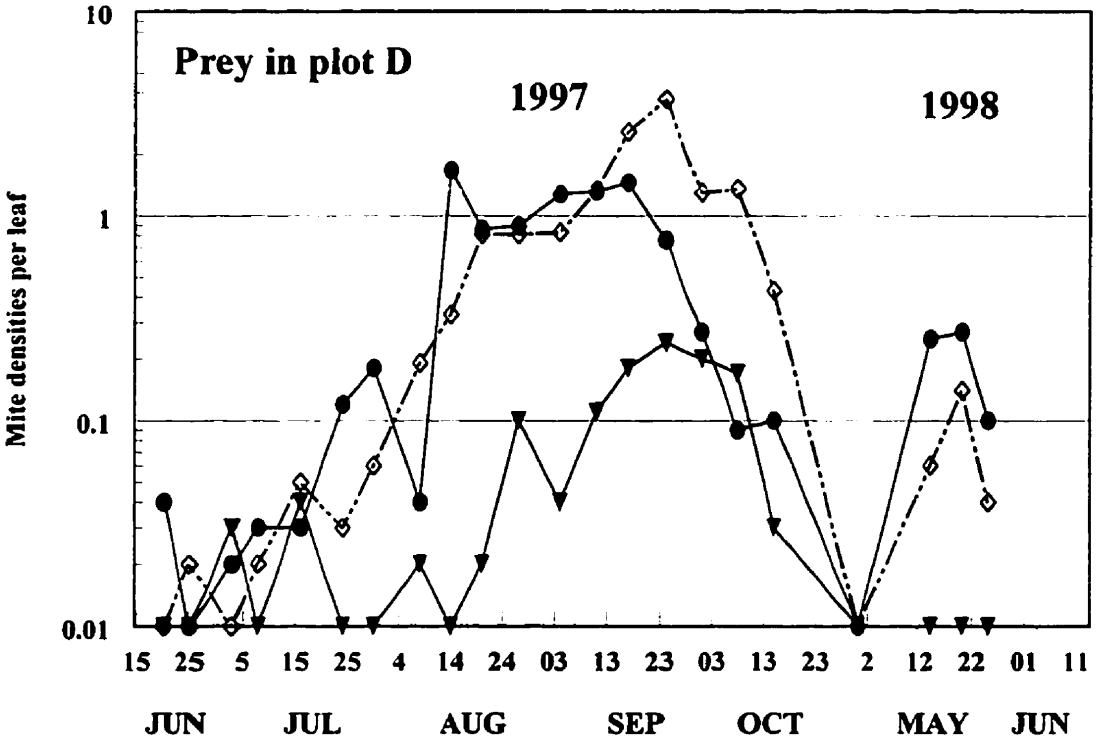
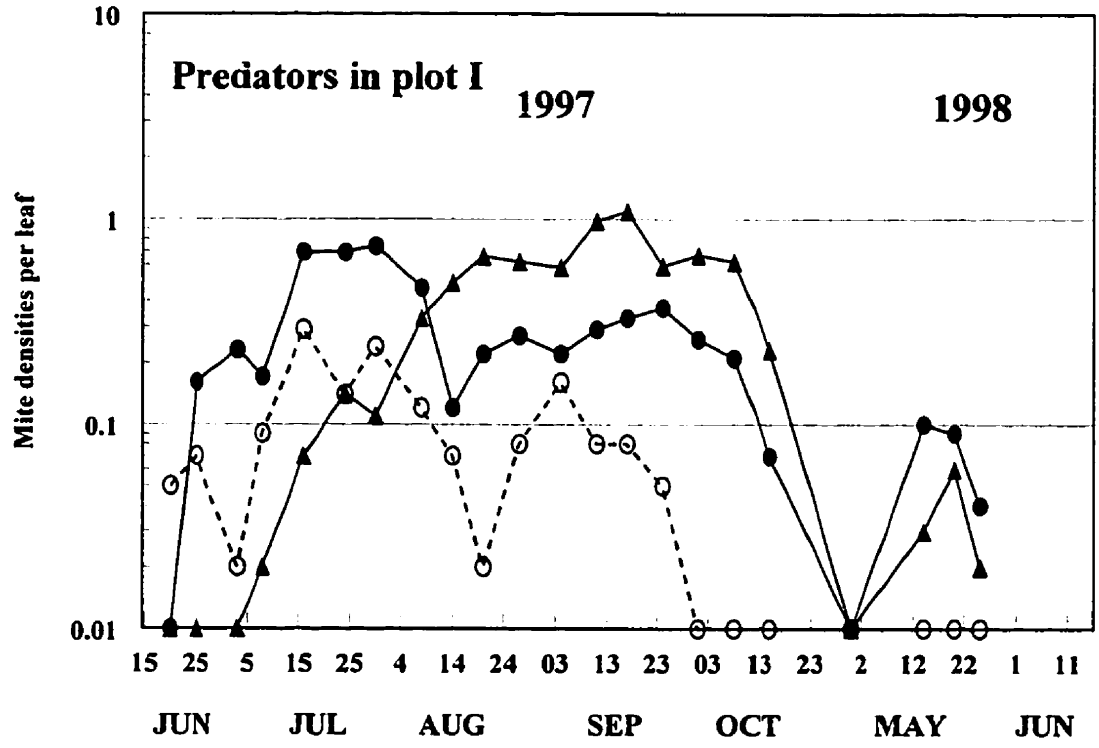

Prey in plot D
1997
1998
Mite densities per leaf
10
1
0.1
0.01
15 25 5 15 25 4 14 24 03 13 23 03 13 23 2 12 22 01 11
JUN JUL AUG SEP OCT MAY JUN

Figure 3. Mean densities for predatory (A) and pest (B) mites counted on 100 leaves, including *P. ulmi* and *T. pyri*, sampled in 1997 and early 1998 from experimental plot 'I'. Predators: *T. pyri* nymphs (○), *T. pyri* adults (●) and *Z. mali* adults (▲). Prey: *P. ulmi* motiles (●), tydeids (◇) and tarsonemids (▼).


Predators in plot I
1997
1998
Mite densities per leaf
10
1
0.1
0.01
15 25 5 15 25 4 14 24 03 13 23 03 13 23 2 12 22 1 11
JUN JUL AUG SEP OCT MAY JUN

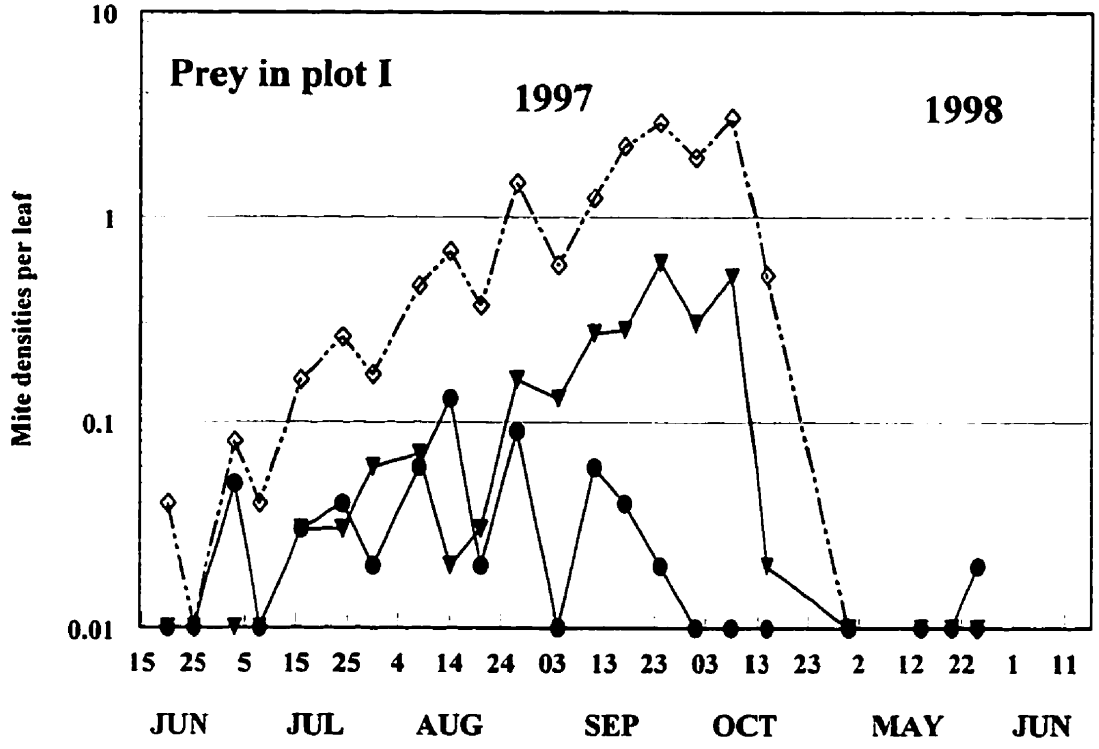
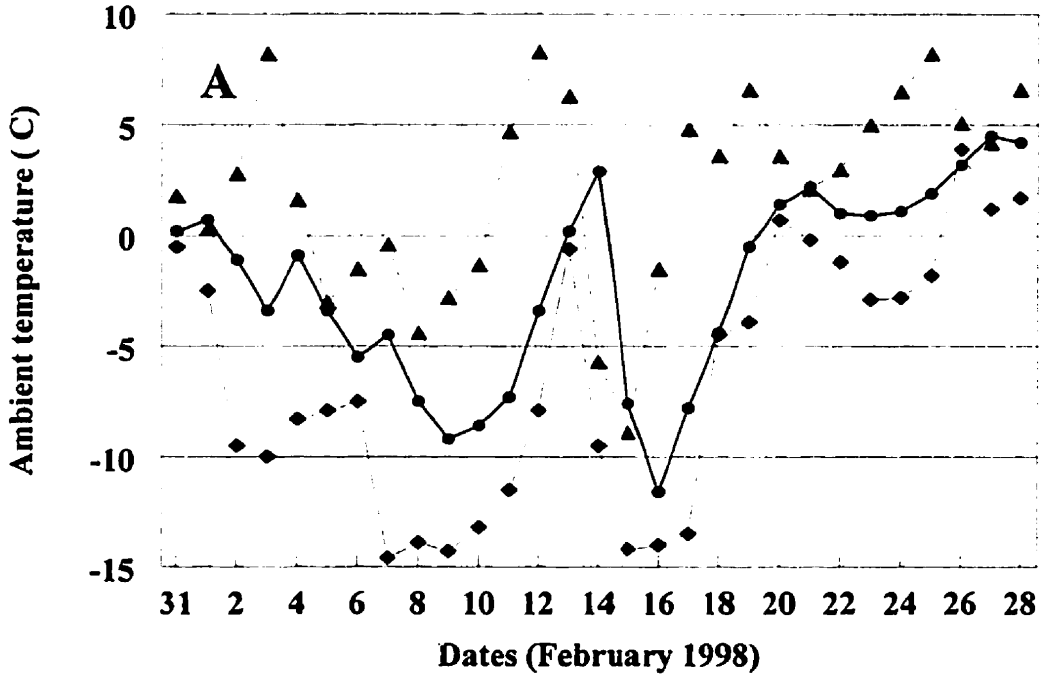

Prey in plot I
1997
1998
Mite densities per leaf
10
1
0.1
0.01
15 25 5 15 25 4 14 24 03 13 23 03 13 23 2 12 22 1 11
JUN JUL AUG SEP OCT MAY JUN

Figure 4. Variation in daily mean ambient temperature (A) and daily mean hours of sunshine (B) for the month of February 1998. Mean ambient temperature (●), maximum temperature (▲), and minimum temperature (♦).


A
10
5
0
-5
-10
-15
Ambient temperature (C)
31 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Dates (February 1998)

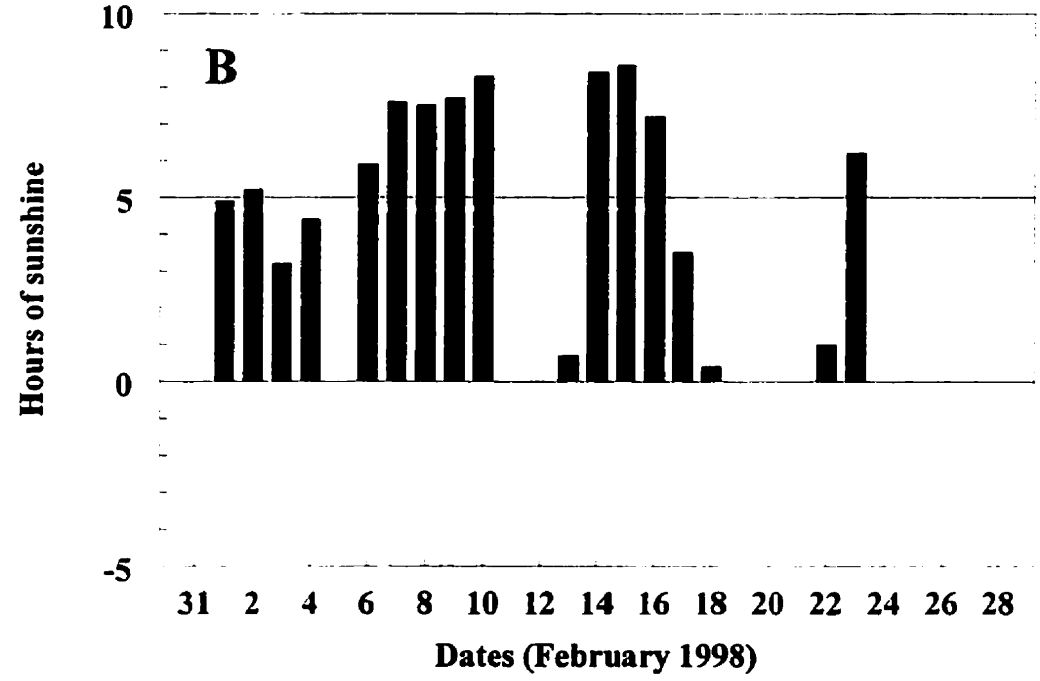
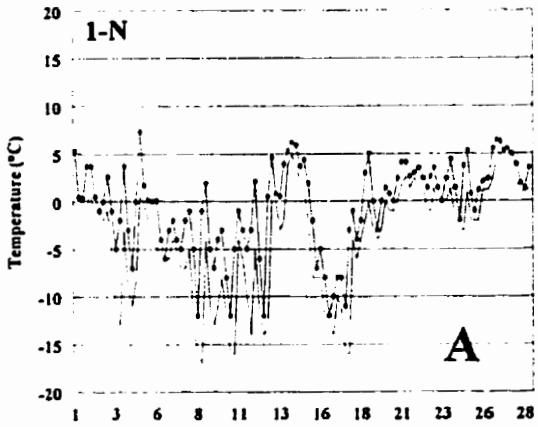
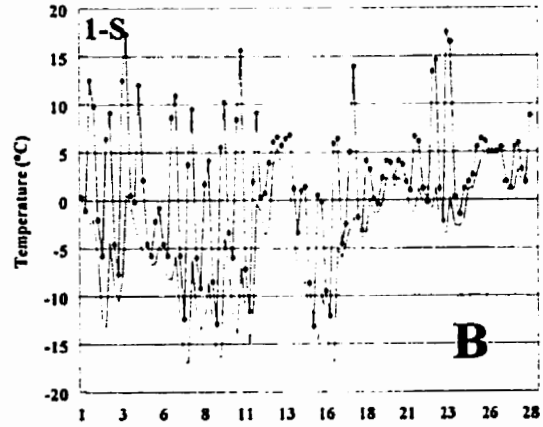

B
10
5
0
-5
Hours of sunshine
31 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Dates (February 1998)

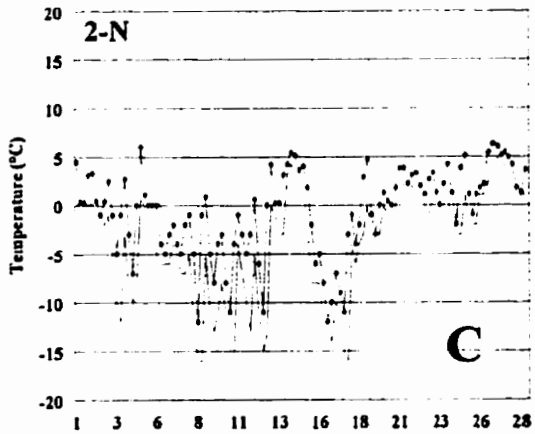
Figure 5. Temperature profiles of south-facing and north-facing sites on trunks of apple trees, observations represent four averages over six hour periods, for the month of February 1998. Minima are represented by solid lines and maxima are represented as lines with solid circles.



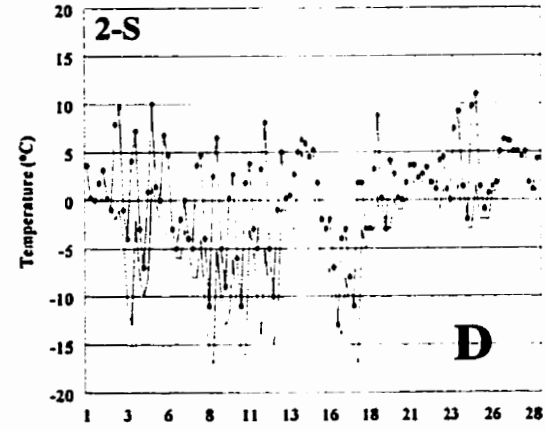
Date (February 1998)



Date (February 1998)

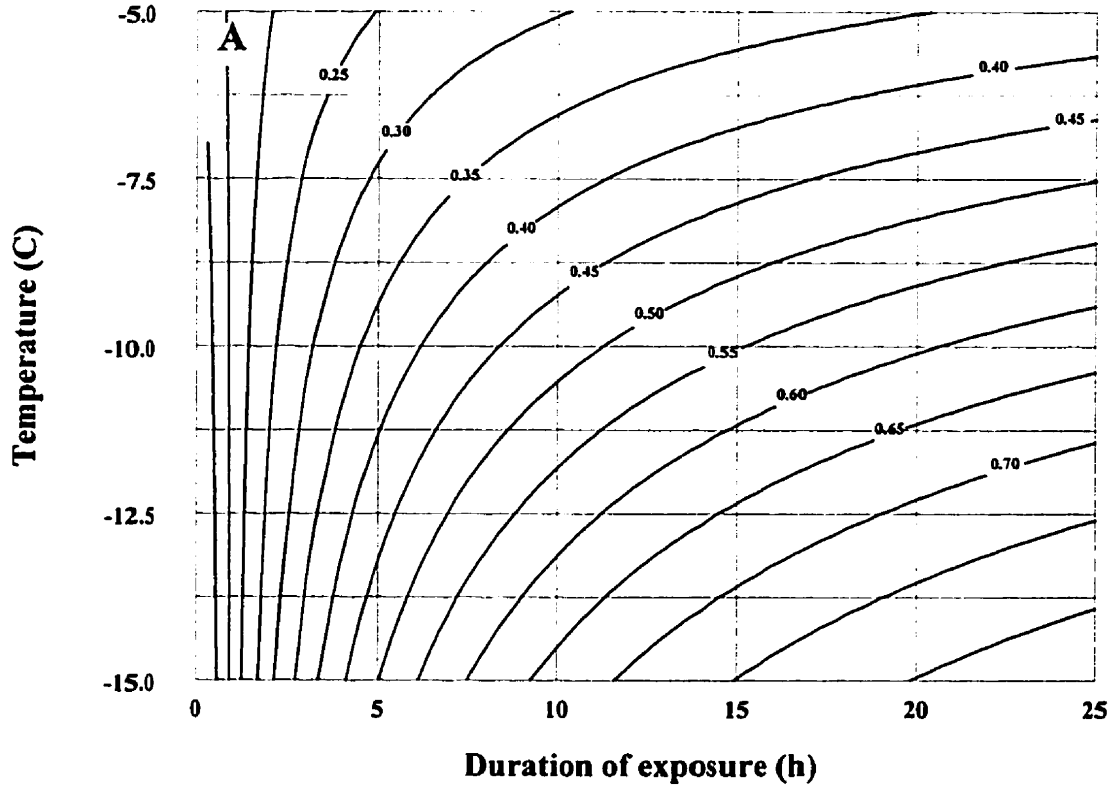


Date (February 1998)



Date (February 1998)

Figure 6. (A) Contour map of differences in mortality for New Zealand *T. pyri* in relation to exposure to low temperatures (-5°C, -7.5°C, -10°C, -15°C) as influenced by duration of exposure. Mites were collected from the field in December 1997. Proportions of mite mortality were based on fitting logistic regression coefficients shown in Table 4 to equation 1 (see equation 1 in text). (B) Relationship between observed and expected mortality for adult, female *T. pyri* after varying duration of exposure to low temperatures (-5°C, -7.5°C, -10°C, -15°C). Solid line is where observed and expected values would be equal.


A
-5.0
-7.5
-10.0
-12.5
-15.0
Temperature (C)
0
5
10
15
20
25
Duration of exposure (h)
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70

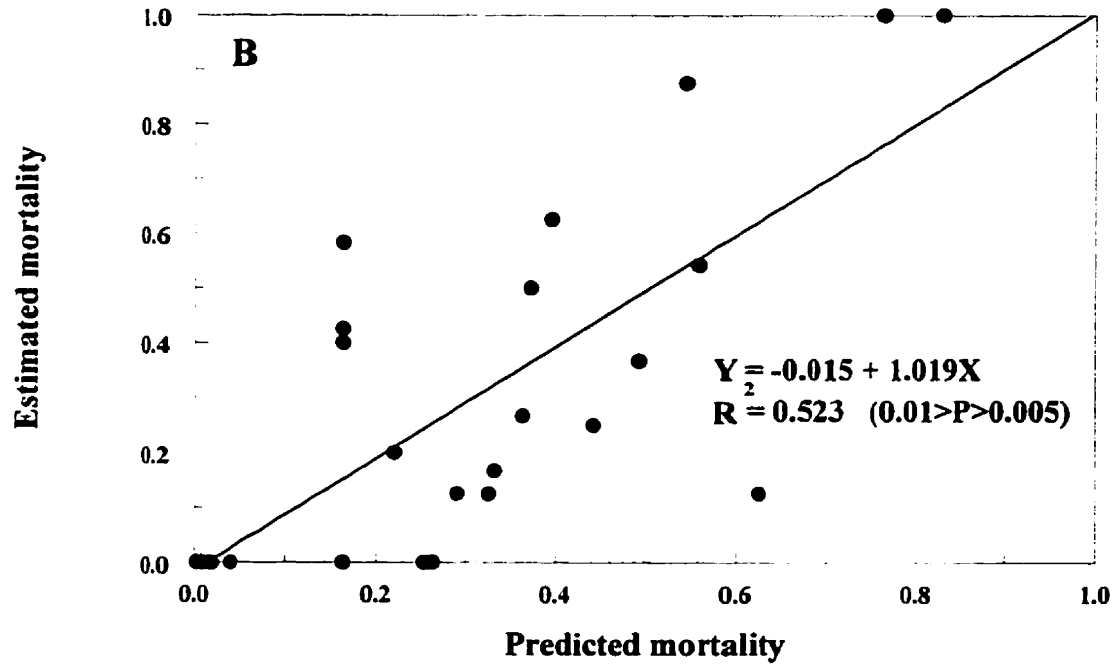
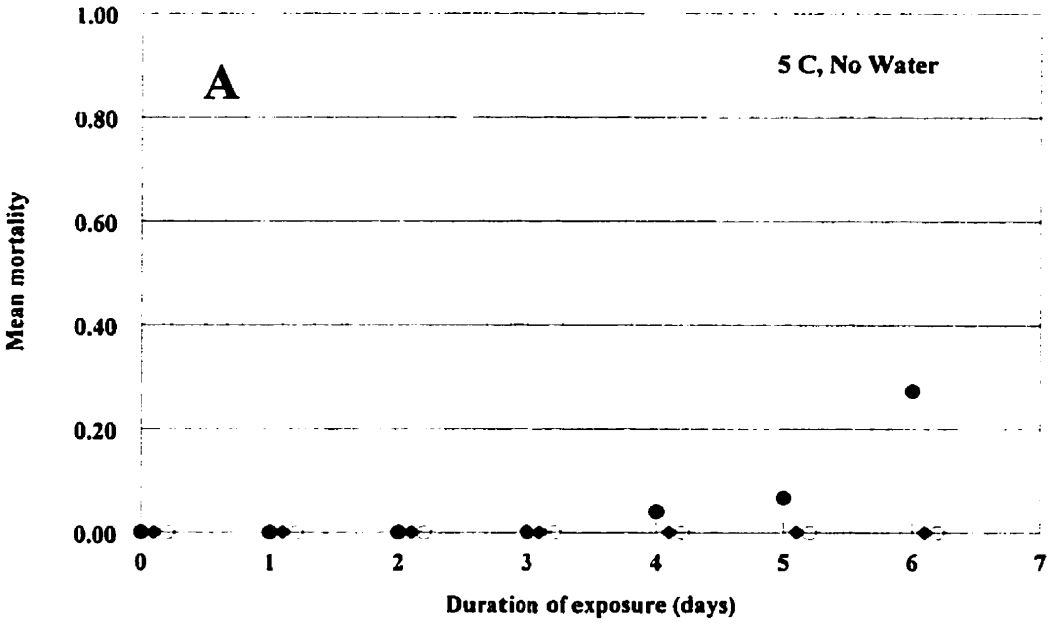

B
1.0
0.8
0.6
0.4
0.2
0.0
Estimated mortality
0.0
0.2
0.4
0.6
0.8
1.0
Predicted mortality
Y = -0.015 + 1.019X
R² = 0.523 (0.01>P>0.005)

Figure 7. Relationship between mortality of the New Zealand strain of *T. pyri* and duration of exposure at 5°C across different humidity regimes, without (A) or with (B) free water. The mites were collected in the field in April 1998. Humidities were 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles).


A
5 C, No Water
Mean mortality
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

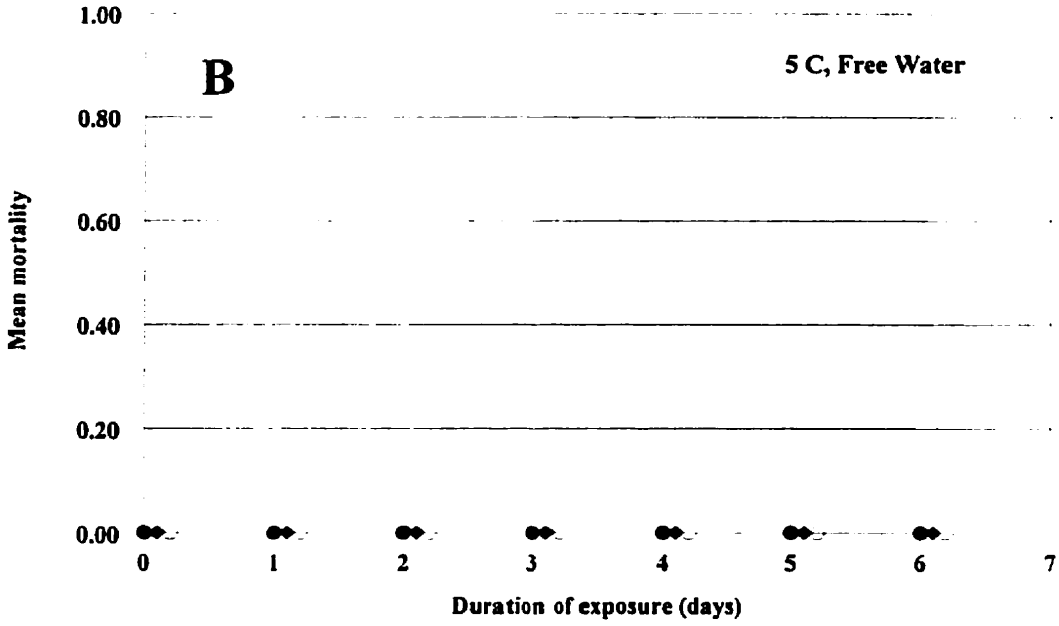
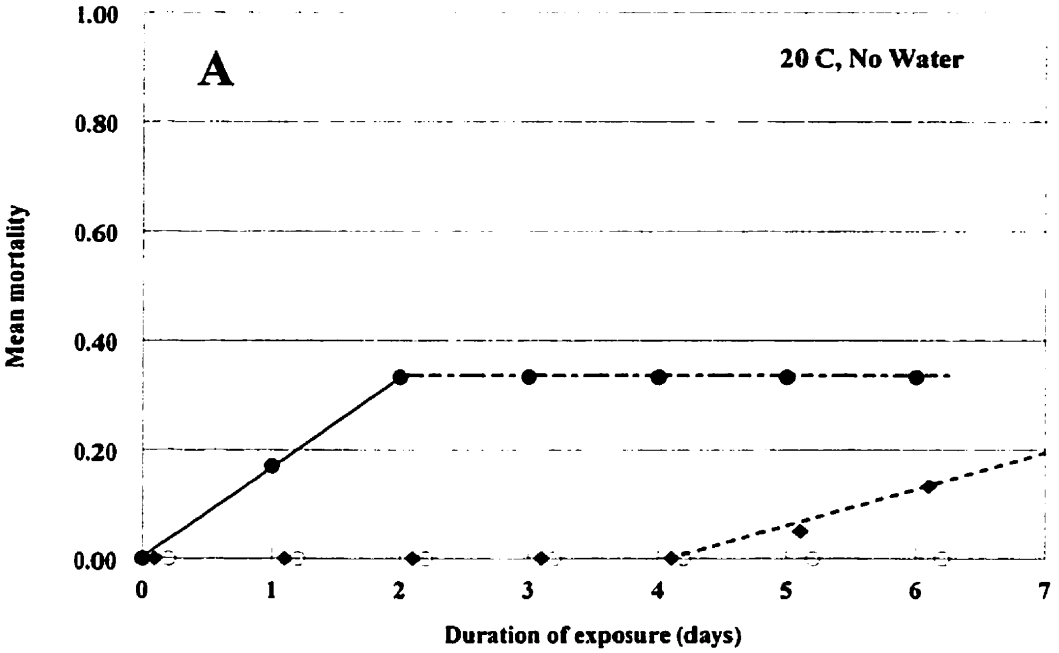

B
5 C, Free Water
Mean mortality
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 8. Relationship between mortality of the New Zealand strain of *T. pyri*, and duration of exposure at 20°C across different humidity regimes without (A) or with (B) free water. The mites were collected in the field in April 1998. Mean observed mortality for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles) is fitted with curves generated using linear regression coefficients. Curve represents 20% RH (solid line), 70% RH (broken line) and 95% RH (broken line with dash).


A
20 C, No Water
Mean mortality
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

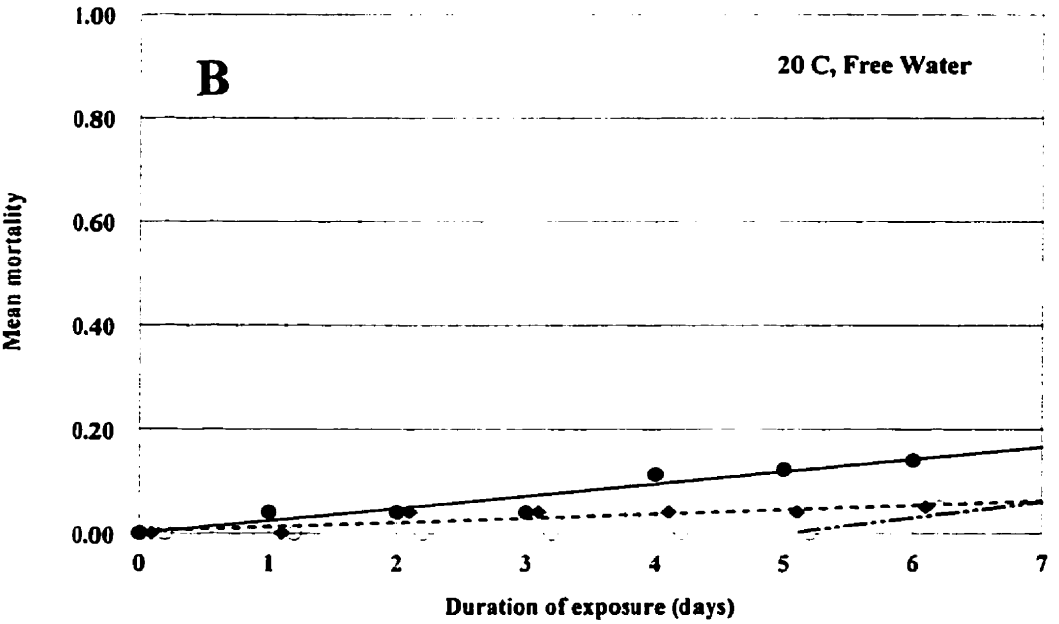
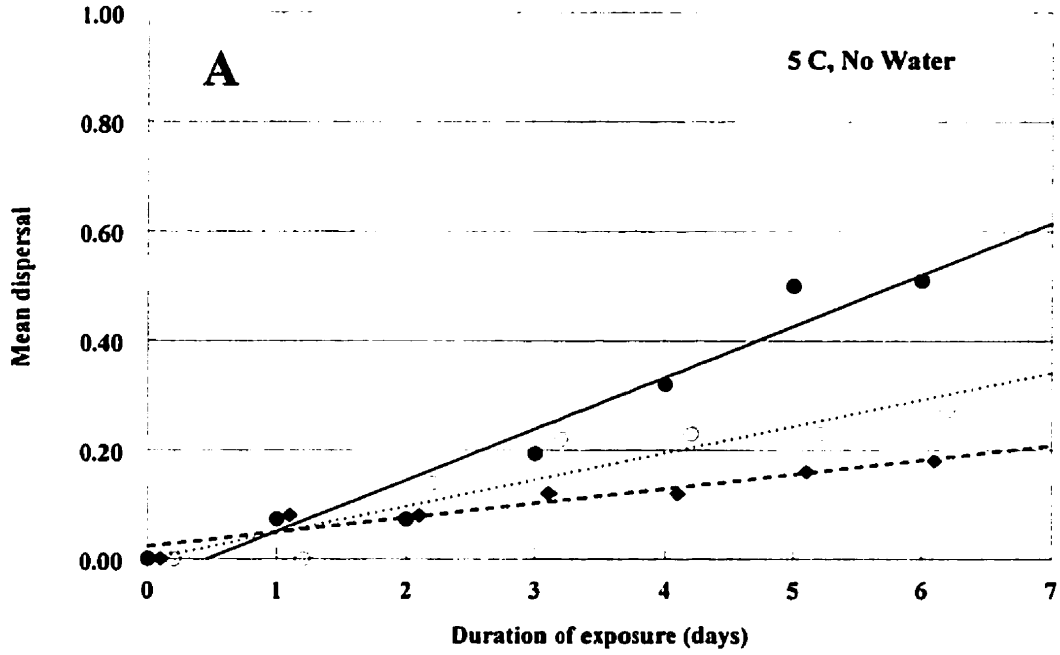

B
20 C, Free Water
Mean mortality
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 9. Relationship between the proportion of New Zealand *T. pyri* observed in the petroleum jelly, and duration of exposure at 5 °C across different humidity regimes and without (A) or with (B) free water. The mites were collected in the field in April 1998. Observed dispersal for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles). Curves generated using linear regression are for 20% RH (solid line), 70% RH (broken line) and 95% RH (broken line with dash).


A
5 C, No Water
Mean dispersal
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

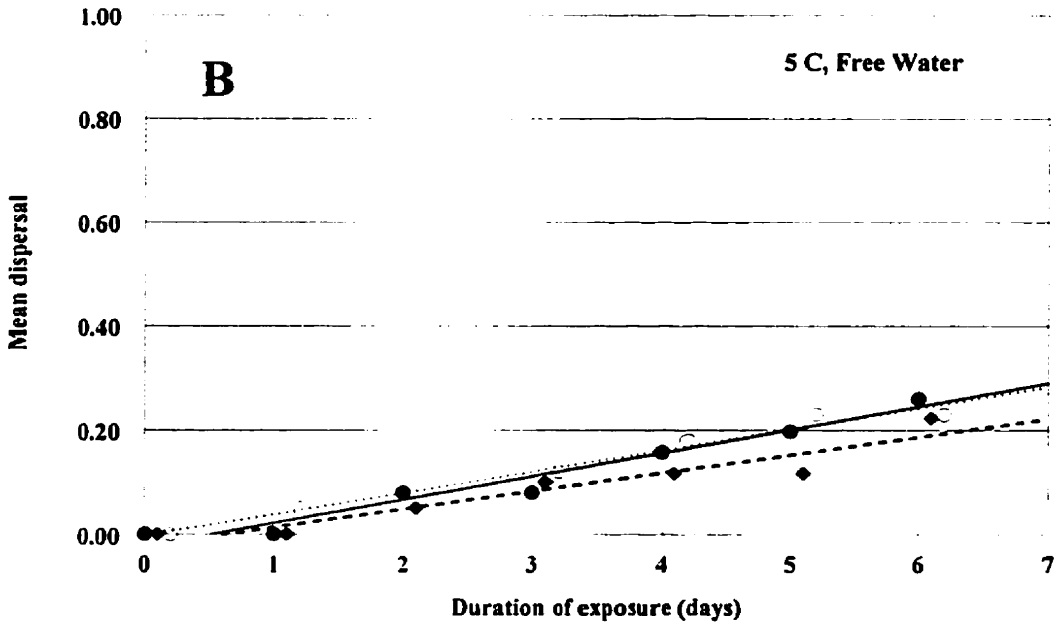
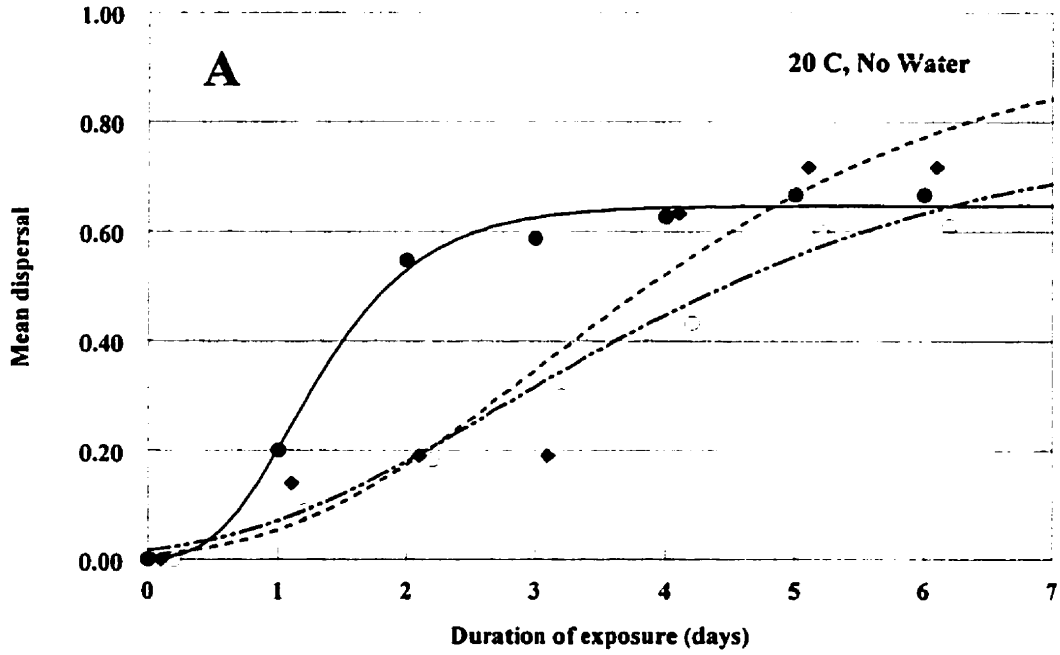

B
5 C, Free Water
Mean dispersal
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 10. Relationship between the proportion of New Zealand *T. pyri* observed in the petroleum jelly and duration of exposure at 20°C across different humidity regimes and without (A) or with (B) free water. The mites were collected in the field in April 1998. Observed dispersal is represented for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles). When water was not available, curves were generated using a Gompertz function (Table 3) for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles). Curves generated using linear regression are for 20% RH (solid line), 70% RH (broken line) and 95% RH (broken line with dash).


A
20 C, No Water
Mean dispersal
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

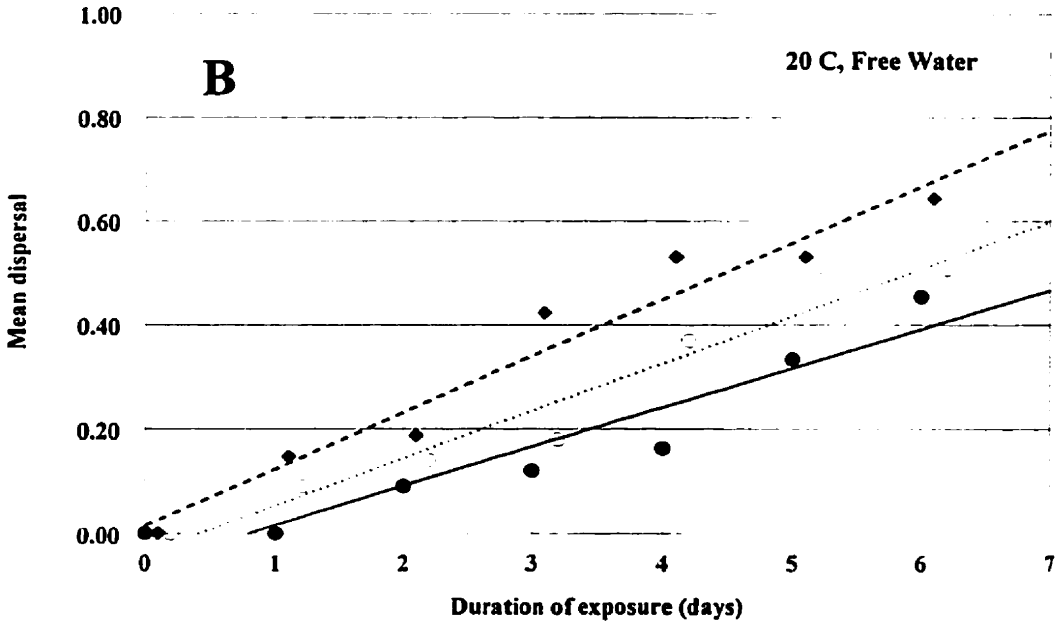
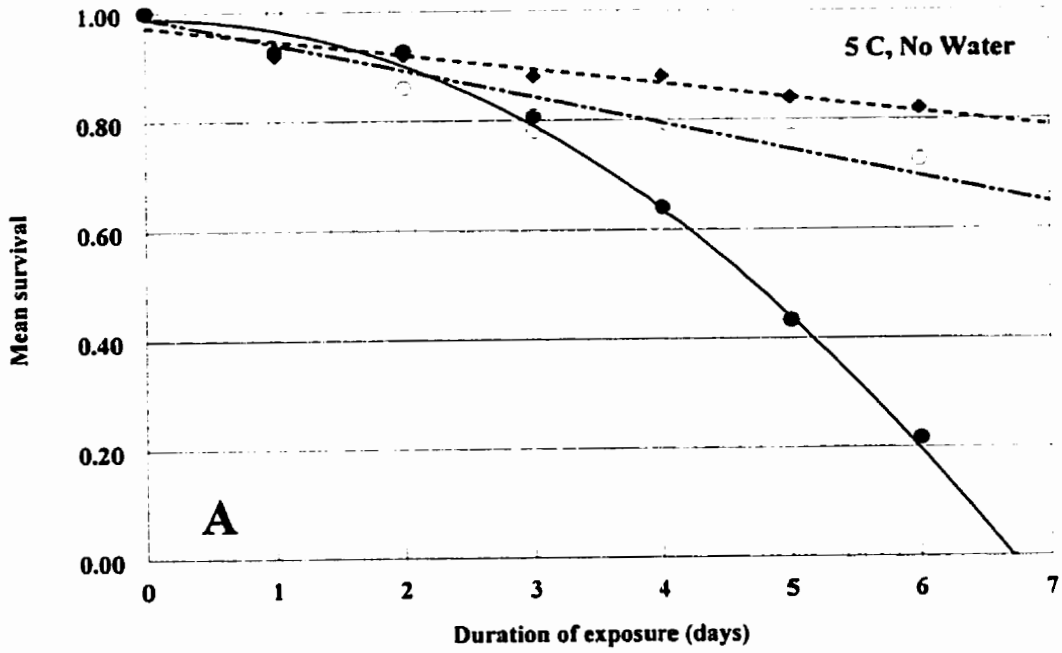

B
20 C, Free Water
Mean dispersal
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 11. Relationship between survival of New Zealand *T. pyri*, and duration of exposure at 5°C across different humidity regimes and without (A) or with (B) free water. The mites were collected in the field in April 1998. Mean observed survival is shown for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles). At 20% RH without water, the curve of best fit was generated using a quadratic function (Table 3). Curves generated using linear regression are for 20% RH with available water, 70% RH and 95% RH. Curves represent 20% RH (solid line), 70% RH (broken line) and 95% RH (broken line with dash).


1.00
0.80
0.60
0.40
0.20
0.00
Mean survival
5 C, No Water
A
0
1
2
3
4
5
6
7
Duration of exposure (days)

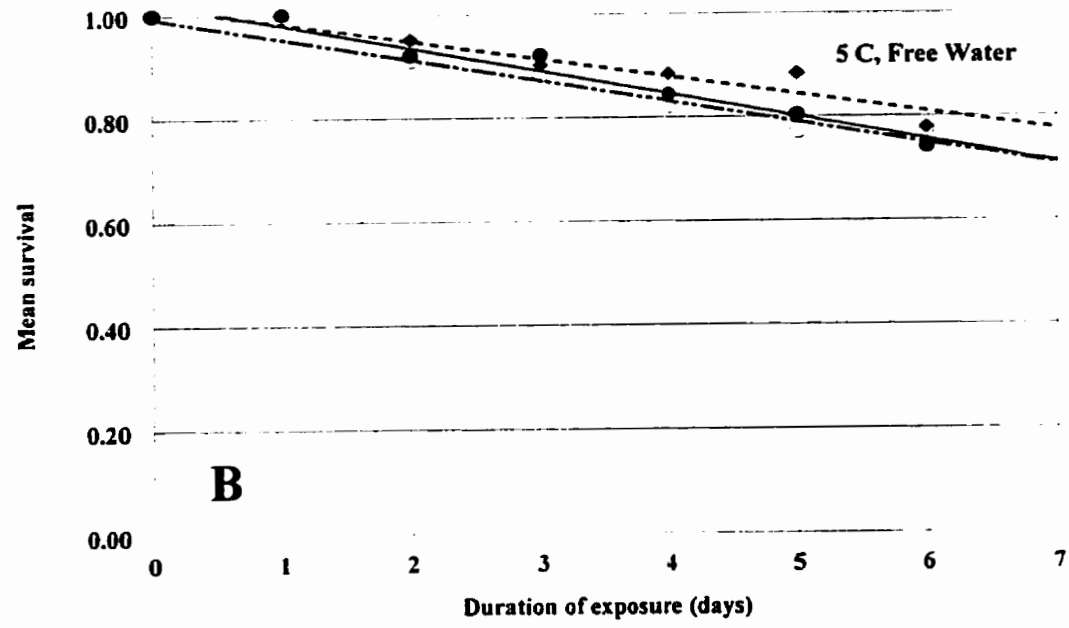
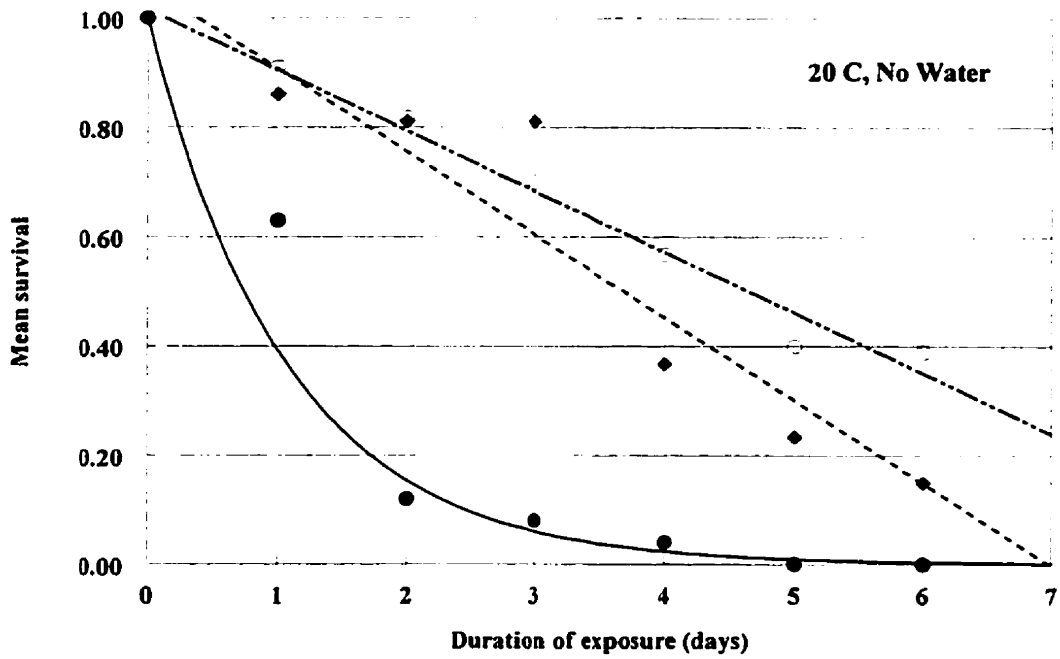

1.00
0.80
0.60
0.40
0.20
0.00
Mean survival
5 C, Free Water
B
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 12. Relationship between survival of New Zealand *T. pyri*, and duration of exposure at 20°C across different humidity regimes and without (A) or with (B) free water. The mites were collected in the field in April 1998. Mean observed survival is shown for 20% RH (solid circles), 70% RH (diamonds) and 95% RH (hollow circles). When water was not available at 20% RH (solid circles), the curve was generated using negative exponential function (Table 3). Curves generated using linear regression are for 70% RH and 95% RH with or without available water. With coefficients generated using the quadratic function (Table 3), the curve was generated for mean observed survival at 20% RH (solid circles) and with available water. Curves represent 20% RH (solid line), 70% RH (broken line) and 95% RH (broken line with dash).


20 C, No Water
Mean survival
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

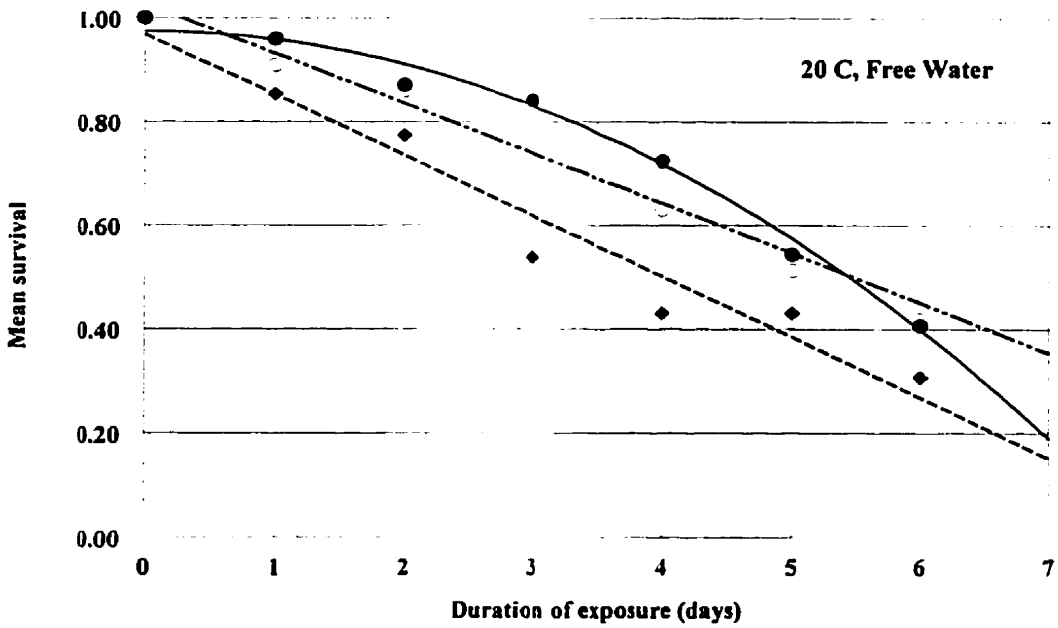

20 C, Free Water
Mean survival
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
Duration of exposure (days)

Figure 13. Relationship between observed decrease in survival and numbers of mites found desiccated on arenas versus in the petroleum jelly, for mites exposed to 20% RH (solid circles) at 5°C and with no water available. Observations were taken every 24 hours and the last sampling day is indicated by the number six.

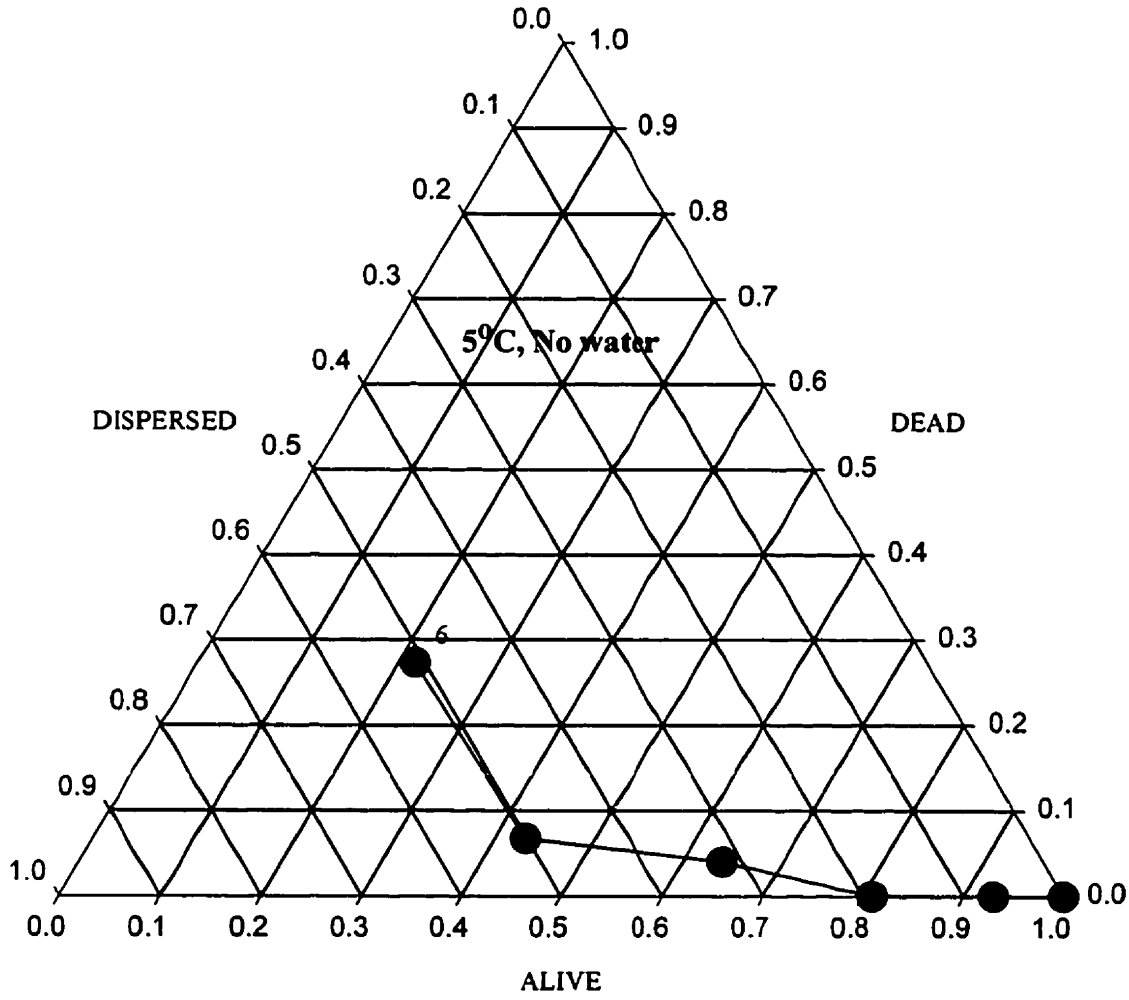

5°C, No water
DISPERSED
DEAD
ALIVE
6
0.0
1.0
0.1
0.9
0.2
0.8
0.3
0.7
0.4
0.6
0.5
0.5
0.6
0.4
0.7
0.3
0.8
0.2
0.9
0.1
1.0
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Figure 14. Relationship between observed decrease in survival and numbers of mites found desiccated on arenas versus in the petroleum jelly, for mites exposed to 20% RH (solid circles) and 70% RH (hollow circles) at 20°C and with no water available. Observations were taken every 24 hours and the last sampling day is indicated by the number six.

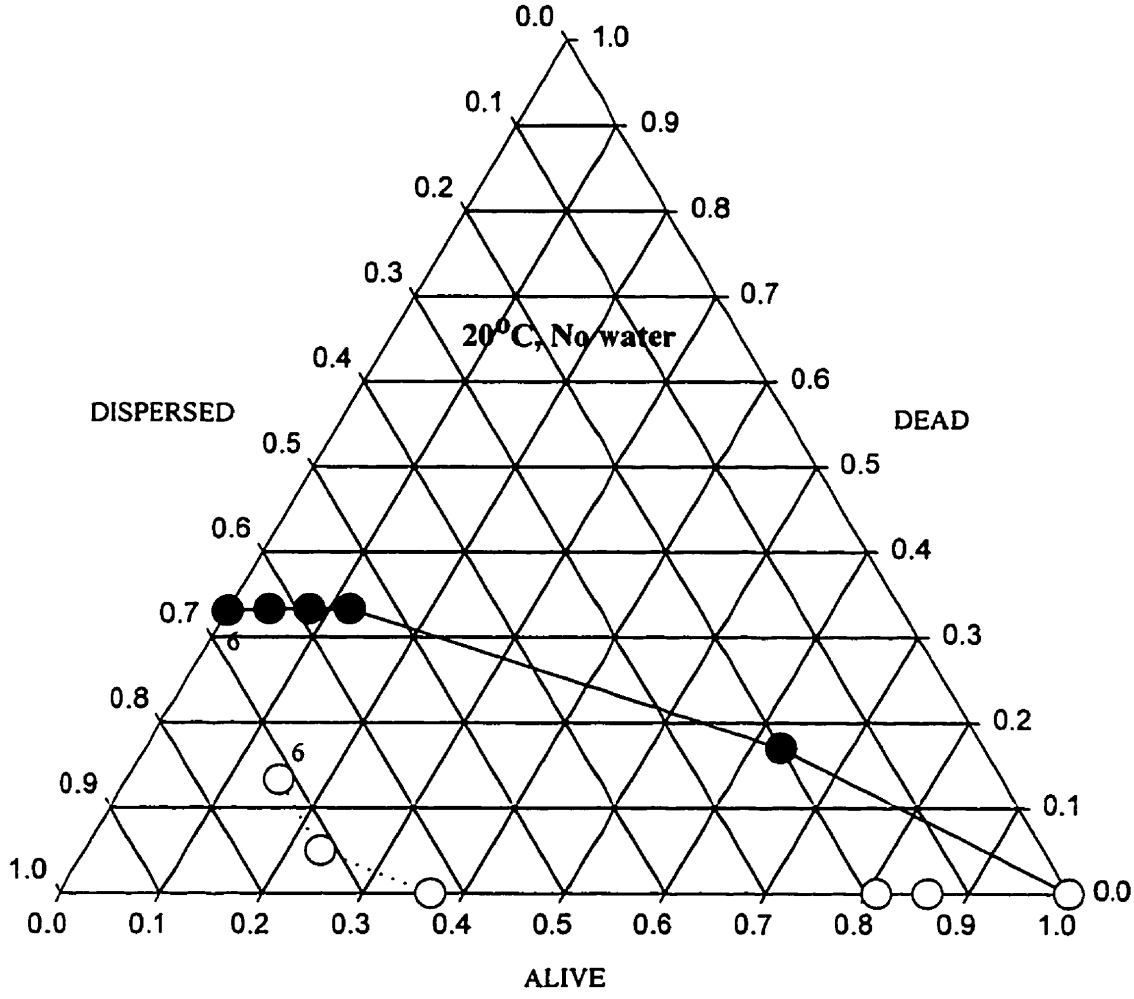
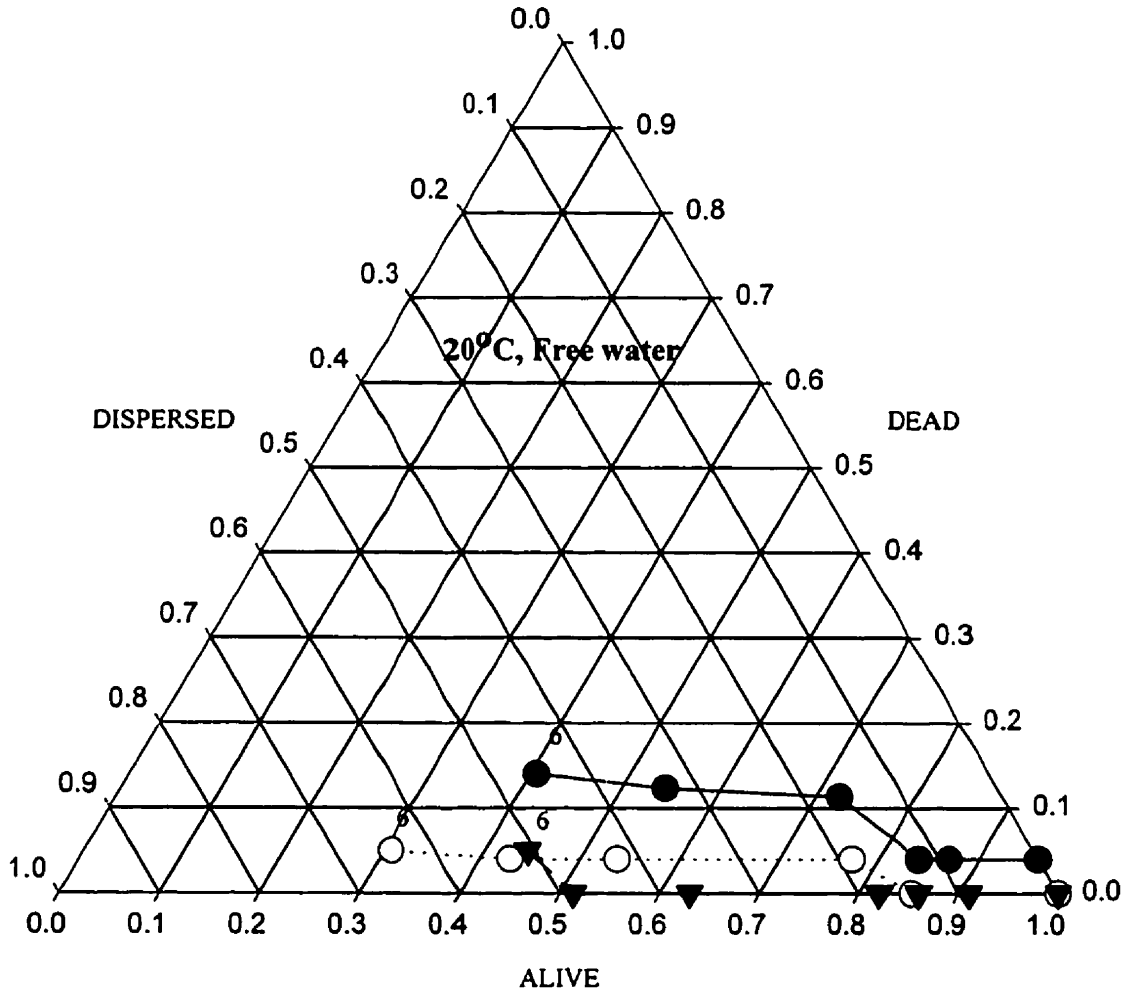

0.0
1.0
0.1
0.9
0.2
0.8
0.3
0.7
0.4
0.6
0.5
0.5
0.6
0.4
0.7
0.3
0.8
0.2
0.9
0.1
1.0
0.0
20°C, No water
DISPERSED
DEAD
6
6
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
ALIVE

Figure 15. Relationship between observed decrease in survival and numbers of mites found desiccated on arenas versus in the petroleum jelly, for mites exposed to 20% RH (solid circles), 70% RH (hollow circles) and 95% RH (diamonds) at 20°C and with water available. Observations were taken every 24 hours and the last sampling day is indicated by the number six.


20°C, Free water
DISPERSED
DEAD
ALIVE
0.0
1.0
0.1
0.9
0.2
0.8
0.3
0.7
0.4
0.6
0.5
0.5
0.6
0.4
0.7
0.3
0.8
0.2
0.9
0.1
1.0
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
6
6
6

DISCUSSION

In 1997, the predator mite *T. pyri* maintained densities in the range of approximately 0.1 - 1 motile *T. pyri* per leaf throughout the growing season (June to October), in spite of low numbers of *P. ulmi*, the preferred prey (Figures 2, 3). Furthermore, sampling in early May 1998 showed that *T. pyri* had successfully overwintered (Figures 2, 3). Casual examination of material bands also revealed surviving adult female *T. pyri* throughout the winter months. In the growing season, the predator's diet can be supplemented by wind-borne pollen (Eichhorn and Hoos, 1989; Engel, 1989). This may have provided an additional alternate food resource, from late June to late July, when peak densities were observed for adult and nymphal *T. pyri* in plot D (Figures 2,3) in spite of low densities (< 0.1 per leaf) of motile stages of prey (*P. ulmi*, tydeids and tarsonemids) as shown in Figure 2. Peak densities of 1 motile *T. pyri* per leaf in plot I were from early July to early August when *P. ulmi* and tarsonemids were < 0.1 per leaf and tydeids were considerably < 1 per leaf (Figure 3). Consumption of phytoseiid eggs by *Z. mali* represents a potentially important source of mortality for *T. pyri* (Clements and Harmsen, 1990).

Densities of female *T. pyri* observed in October 1997, may also reflect an underestimate in mite numbers due to leaf fall and individuals moving to overwintering sites on branches and the trunk. By May 1998, low numbers of motile *T. pyri* were observed, however, these densities may also be an underestimate because part of the population that has not yet moved from overwintering sites on wood to the summer habitat on leaves. However, there may have been less foliage in May than in October resulting in a lower estimate of actual mortality because the mites would be concentrated

on fewer leaves in the spring and hence appear to be more abundant. The decrease in survivorship observed in the field may have been caused by a combination of overwintering mortality, spring mortality during dispersal and starvation in the spring. Initially, low numbers of *T. pyri* disperse on the tree in the spring and exploit available food sources while a portion of the population remains in winter shelters and will emerge later.

Zacharda (1989) suggested that overwintering *T. pyri* populations are gradually reactivated over a period of several weeks in the spring. In spite of lower prey densities in the spring in plot I, *T. pyri* numbers appeared similar to those observed for plot D where live prey reached higher densities by mid-May. The presence of tydeid and tarsonemid mites (Zacharda, 1989; Fitzgerald & Solomon, 1991), and pollen (Engel, 1989; Walde *et al.*, 1992) may have provided an alternate food source for *T. pyri* when faced with low *P. ulmi* numbers early in the season. Windborne pollen, which is especially abundant in spring and early summer, is a major food source for *T. pyri* and is one primary reason for the success of this predator in suppressing spider mites (Eichhorn & Hoos, 1989; Engel, 1989). *T. pyri* were found to be effective biological control agents on Canada's west coast because of their ability to survive cool conditions and low prey densities experienced in late and early season (Helle & Sabelis, 1985). Furthermore, Croft *et al.* (1993), reported that *T. pyri* eggs and larvae have the capacity to persist in drier environments such as 50% RH when maintained at 20°C.

The combined influence of several climatic variables interacting simultaneously affects the energy, water balance and survival of terrestrial arthropods living in their

microenvironment (Shipp *et al.*, 1988). Experiments on the effect of substrate, temperature, humidity and availability of water on survival of overwintering female *T. pyri*, also showed that no single variable acted in isolation on overwintering survival (Tables 4, 6-7) (Figures 7-15). When temperatures were decreased in the December 1997 trial, from 10°C to -15°C, mites faced a greater risk of mortality at shorter exposure times (Table 4). Few organisms can tolerate prolonged exposure to low temperatures, since low temperature-induced mortality is also dependent on time (Salt, 1966a). However, in December, the results did not clearly show an interaction between decreasing temperature and longer exposure times. Although temperature showed a significant effect on mortality at -5°C, -7.5°C, -10°C and -15°C regimes, there was no overall significant effect of duration of exposure on mortality nor was there any interaction between temperature and duration. On average, mortality was higher for mites exposed to -15°C compared with other temperatures (Table 4). At -5°C and -10°C there was no significant relationship between mortality and duration of exposure.

Trends in the data for the effect of temperature and duration of exposure on mortality may have been masked by random variation, possibly the result of low replication. Results reported conflicting mortality rates for mites exposed to -10°C, with 62% dead after six hours and only 12% after 24 hours. Results did show a significant time trend for mortality with for all four temperature regimes as shown in Figure 4A. However, at -15°C the mites may not have had sufficient time to find shelter before being affected by the severe cold, whereas at -10°C, the mites may have been able seek more favourable conditions in the splintered ends of the twig. The availability of shelter underneath the

bark layer could have reduced mortality compared with *T. pyri* that had been left on exposed surfaces. This factor, however, was not recorded; hence, it is an uncontrolled source of variation. A second bias was in recovery time since mites were found crawling out of the splintered ends of the twigs after the initial half-hour assessment. This could have resulted in an overestimate of actual mortality due to my underestimating the number of mites that actually survived.

Because of anomalous results in December, the ends of the pieces of branches were sealed and recovery time was increased to one hour for January and February 1998 trials. The 1 °C/minute rate of cooling may have been sufficiently slow to enable individuals to adjust to -10°C and survive exposure in the December, January and February trials (Tables 4, 6-7). A standard rate of cooling of 1°C/minute is recommended to reduce the probability of ice nucleus formation (Salt, 1966b). The difference between slow and fast cooling rates used in this trial may not have been large enough to exert an observable influence over the rather short time interval of 24 hours. Results from trials conducted in January 1998 to assess the influence of substrate on low temperature induced mortality, showed no significant difference for the four types of substrate on mortality at the fast or slow rate of cooling and regardless of acclimation regimes (Table 6). The absence of significant differences in mortality across regimes, after 24 hours of exposure at -10°C, suggests that these factors may not independently influence the survival of overwintering *T. pyri*, under controlled conditions (Table 6). Furthermore, the results of trials conducted in December 1997 and January 1998 may suggest that the -10°C regime was not sufficiently extreme to assess low temperature-induced mortality of mites that had been

provided with a substrate that offered refuge. Trials conducted in February, 1998, indicated that substrate at -15°C was indeed significant. However, at -10°C, mortalities did not differ for mites on the rough twig substrate and those on plastic vials (Table 7).

Overwintering adult female *T. pyri* often experience sub-zero temperatures during a winter in Nova Scotia and the annual variation in temperatures can exert a considerable influence on the population dynamics of the mites. In temperate climates where there can be extended periods of moderate winter temperatures and only occasional cold snaps, the overwintering survival of the organism may be higher (Moore & Lee, 1991). For example, in 1993 the coldest minimum temperature dropped to -30°C (Hardman *et al.*, 1997), and during the winter of 1994 and 1995, the daily minimum temperature was below 0°C for 116 days from November to March. On two occasions only, in January and February 1995, did temperatures remain below -10°C for a 48-hour period. In contrast, the ambient air temperature reported during the winter of 1997 and 1998 was below 0°C for 125 days and fell below -10°C on 28 occasions, below -15°C on 8 occasions and below -20°C once. However, only on one occasion in December 1997 did temperature remain below -10°C for 24 hours. Temperatures during a single winter month in Nova Scotia can fluctuate widely and within short time intervals (Figure 4). Furthermore, temperatures experienced at the level of an overwintering site, located above the ground, can also show a wide range in daily temperatures that often follow ambient trends (Figure 5). However, Holtzer *et al.* (1988), reported that the effective environment (chiefly temperature and humidity) of spider mites and phytoseiid mites can be quite different from representative, ambient conditions. Similar sites that only differ in

positioning on a tree trunk also show variation in temperatures, as shown in Figure 5. North and south-facing surfaces on the bark of a tree can be quite different due to the effects of the sun, which varies diurnally and seasonally (Cloudsley-Thompson, 1962).

Thus, location and positioning can provide a higher temperature in a local microclimate than that of the surrounding air. Tauber *et al.* (1998), reported that when wood is unsaturated, capillarity allows the slow redistribution of water throughout, and the resulting changes to the local microclimate are dampened. The lowest daily mean temperatures and greatest fluctuations in daily temperature changes were observed on the south-facing bark surface (Figure 5). A reduction in the range over which temperature varies can minimize the adverse influence for *T. pyri* overwintering in Nova Scotia apple orchards since the vulnerability to extreme winter cold is affected by duration of exposure (MacPhee, 1964). Minimum temperatures recorded for the north and south-facing bark surfaces, in February 1998, showed that arthropods overwintering on the trunk would have been exposed to -15°C on three occasions (Figure 5). At these times, temperatures remained at or below -15°C for periods ≤ 12 hours (Figure 4). Low temperature trials in December 1997 and February 1998 assessed mortality at several temperatures, including -15°C, however, in the January 1998 trial the mites were only exposed to -10°C. Data for ambient air temperature and bark surface temperature indicate that mites can be exposed to daily maximum temperatures below -10°C (Figure 5). In February 1998, after 24 hours, exposure at -15°C, there was approximately 31% mortality for mites on twigs and approximately 81% for those individuals in plastic vials (Table 7). Although the temperatures in small exposed twigs can fluctuate rapidly with changes in ambient

temperature (Danks, 1978), twigs used as substrate in the lab bioassays for our study evidently gave some protection since they lacked the full severity of wind, etc. but offered less shelter than other field overwintering sites (cankers, crevices, pruning wounds).

The desiccation trials revealed that the interactions between temperature, availability of free water and varying levels of relative humidity can influence survival in overwintered mites. The effects of low and high temperature, influence of access to free water and relative humidity levels on mortality, dispersal and survival, are shown in Figures 7-15. Temperature appeared to play a significant role in the survival and dispersal of mites when subjected to limiting levels of relative humidity and there is no source of free water. Comparison of survival at low versus high temperatures showed that higher numbers of mites survived and remained in place at all three levels of relative humidity when exposed to 5°C as compared to 20°C (Figures 11-12).

One cannot easily conclude that the decrease in survival for mites maintained at 20°C was directly influenced by dispersing and desiccation because starvation may have also been an important factor. Everson (1980) stated that an increase in food (prey) consumption compensates for the greater energy demands of the predator at higher temperatures. In the absence of food resources the primary cause of attrition observed at 20°C is difficult to discern. Although there was evidence of fat body tissue in sections taken from overwintering *T. pyri* from the field and individuals maintained in a greenhouse, one cannot conclude that starvation is not an important influence on mortality due to inconsistencies with sampling and staining techniques (Appendix A).

Humidity influences the water content of an organism, and, provided this can be kept within certain limits, exposure to extremely dry or extremely humid conditions may not be harmful (Bursell, 1974). The microclimate in which an arthropod lives is a critical parameter since the loss of water is primarily related to air moisture content and the severity of the gradient between air and internal fluids (Shipp *et al.*, 1991). Exposure to low relative humidity with no available water caused the most dramatic decrease in survival at both 5°C and 20°C (Figures 11-12). However, most of this attrition was from mites dispersing into the petroleum jelly (Figures 9-10, 13-14). Croft *et al.* (1993), reported that since the larval stages of *T. pyri* do not require feeding, motile stages could withstand exposure to 50% relative humidity at 20°C, when live prey are low in number. In my study mites exposed to higher temperatures were found both desiccated and having dispersed, with the majority found in the petroleum jelly. Van Dinh *et al.* (1988), reported that low atmospheric humidity associated with high temperature is a significant selective factor in phytoseiid populations.

In February 1998 at Kentville, mean daily maximum temperatures recorded on the bark surface of apple trees, on the north-side, were as high as 7°C (Figure 5). In contrast, south-side temperatures exceeded 15°C on several occasions (Figure 5). At 5°C the changes in survival numbers resulted primarily from mites dispersing into the petroleum jelly (Figure 11). This supports Croft *et al.*'s (1993) contention that nymphs and adults are probably less susceptible than eggs to low humidity since they can ingest free water and can also move to areas of higher humidity. To limit losses of water, terrestrial arthropods must seek rather cool or very moist conditions (Shipp *et al.*, 1991). As

discussed earlier, movement under severe conditions experienced during the winter months may prove detrimental to the overwintering mites. During this time the ability to withstand severe water stress becomes critical to the diapausing females to ensure overwintering survival. Shipp *et al.*, (1991) also reported that increased activity, at lower humidity, is probably the result of an avoidance response as the mites attempt to seek a less desiccating environment. In contrast, Mori and Chant (1966), reported that at high humidity predator mites ceased activity over time, while at low humidity they were active all the time. When water was available at either temperature, higher numbers of live mites were found on the arenas by day 6 of the trial, with the most effect of free water observed at 20% RH (Figures 11-12). Access to drinking water can increase survival and override the effect of low humidity in predator mites (Mori and Chant, 1966; van Dinh *et al.*, 1988; Blommers, 1994). The mites used in April 1998 trial had already overwintered and their survival, at various relative humidity regimes and temperatures, reflects conditions encountered early in the spring rather than winter conditions. A reduction in body water in autumn, suggesting partial dehydration, is considered a favourable adaptation that contributes to a seasonal increase in cold-hardiness by increasing supercooling points and reducing the risk of nucleation in the digestive system (Leather *et al.*, 1993). Conversely in the spring, sustained periods of partial dehydration may be detrimental to the emerging overwintering mite. However, the results of the desiccation trial need to be interpreted with caution since the relative humidity regimes at 5°C versus 20°C would have exerted different influences on the mites.

Conclusions

This study demonstrates that populations of *T. pyri* overwinter and provide effective biological control of *P. ulmi* on apple trees under temperate climatic conditions and when faced with fluctuations in availability of preferred prey. In cases where *T. pyri* are not present *P. ulmi* can reach densities > 100 per leaf (Hardman and Gaul, 1990), which are well above the predetermined economic threshold of < 2.5 *P. ulmi* per leaf before July, < 5 per leaf during July, and < 7.5 per leaf in August (Breth *et al.*, 1998).

Overwintering mortality has frequently been measured in introduced species that are not necessarily in long-term balance with regional conditions (Danks, 1978). It is hoped that an assessment of the overwintering survival of this predator will provide some indication of its respective ecological requirements, as well as form a basis for further studies on their cold resistance. Results presented in this study contribute to our understanding of the effect of temperatures, humidity, availability of water and alternate food resources on survival of *T. pyri*. When combined with weather predictions, at the ambient and microclimate levels, this additional information will strengthen pest management decisions and contribute to the conservation of effective natural enemies. There is a great need for comprehensive micro-climate studies that employ an integrative assessment of the relationship between ambient climatic factors, microclimatic measurements and mite population dynamics. Additional studies of mortality at low temperature will provide a clearer understanding of the mechanisms and strategies of survival, and the relation to the ecology and performance of the populations in the field (Bale, 1987).

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APPENDICES

APPENDIX A

Light and electron microscopy

Materials and Methods

Mites were collected from leaves and velvet bands from potted apple trees located in a greenhouse at the AAFC, Kentville and an IPM orchard at Sheffield Mills, Nova Scotia, from December 1997 through to April 1998. Preservation of mites for light microscopy was done by methods outlined in Crooker *et al.* (1985). Adult, female *T. pyri* were fixed in a water-based fixative Sanfelice (1% chromic acid, 40% formaldehyde, glacial acetic acid; 16:8:1) and an alcohol-based fixative Carnoy (absolute alcohol, chloroform, glacial acetic acid; 6:3:1). They were then dehydrated in an alcohol series, and embedded in paraffin for sectioning (Crooker *et al.*, 1985). Material was sectioned at 8 - 10 μm (usually 10 μm) with a rotary microtome, and stained with sudan black B (Bayliss High & Lake, 1996). Sections were then examined and photographed using a compound microscope at 100x.

Preservation of mites for scanning electron microscopy was done by methods outlined in Crooker *et al.* (1985). Specimens were examined using a Jeol JSM-T330A Scanning electron microscope (Tokyo, Japan) and photomicrographs taken on 35 mm black and white film. Additional mites were transferred from the leaf to a small leaf disk or piece of filter paper and immediately plunged in liquid nitrogen, prior to examination with the scanning electron microscope. Specimens were examined, *in vivo*, using an Oxford CT1500 cryotransfer system (Oxford Instruments Inc., England) attached to the scanning electron microscope. This system avoided some of the problems associated with

handling small organisms and fixation artifacts.

Results and Discussion

Using light microscopy, overwintering versus nondiapause female *T. pyri* were assessed for differences in fat body content. Overwintering mites were collected from the field (December 1997, January and February 1998) and further exposed to sub-zero temperatures (-5°to -15°C) for 24 hours in a controlled chamber, prior to fixation. Sections of overwintering mites showed the presence of fat bodies (Figure 1), suggesting that mites were probably not dying from having utilized all of their stored reserves in response to exposure to low temperatures. However, caution must be taken when making these inferences. In my study, the presence of small amounts of fat body tissue was not considered a positive indicator that the mite had used up its reserves and died of starvation, due to the possibility of fixation artifacts and difficulties associated with preparation of large numbers of mites. The staining technique may not have been the most suitable since sudan black B will stain most neutral fats. Since the amount fat body tissue was assessed by the size of stained regions, stained neutral fats could result in an overestimate of actual fat body quantity.

Photomicrographs from adult female *T. pyri* examined using the scanning electron microscope did not reveal any distinct differences in the external morphology of active mites versus overwintering mites. Since mites were frozen while moving around on the SEM stage, it proved difficult to capture individuals in a position that allowed for measurement of abdomens between adult female *T. pyri* in order to compare seasonal variations in size. Overwintering mites during the winter months appear to be somewhat

more flattened (Figure 2a) when compared with adult female mites collected from leaves in a greenhouse (Figure 2b). Due to the inconsistencies associated with specimen orientation, it is not possible to suggest that these differences are a result of the depletion of reserves during the overwintering period.

Figure 1. Whole-mite longitudinal section of overwintering *T. pyri* (400 x) with anterior end shown. M, midgut with stained fat body tissue. Mite fixed in alcohol-based fixative, February 1998. Samples fixed in water-based fixative are not shown due to sectioning problems.

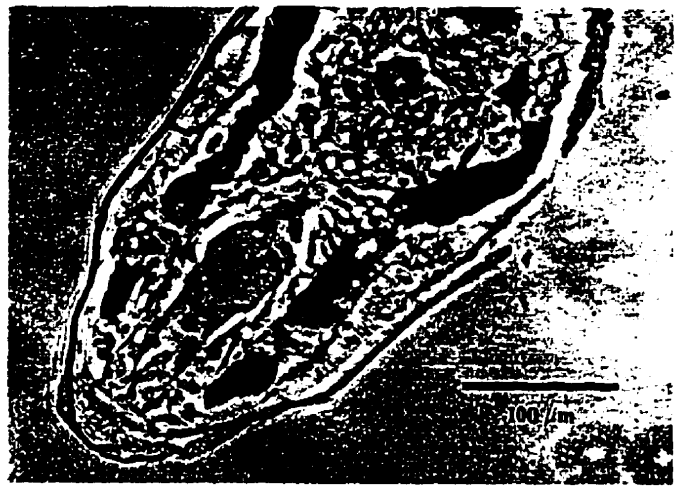

100 μm

Figure 2a. Scanning electron micrograph of an overwintered adult, female *T. pyri* collected from the field in February 1998. Magnification: 200 x. (Photograph by S. Carbyn and D. Moreau)

Figure 2b. Scanning electron micrograph of an adult, female *T. pyri* collected from a greenhouse in February 1998. Magnification: 350 x. (Photograph by S. Carbyn and D. Moreau)


100 μm

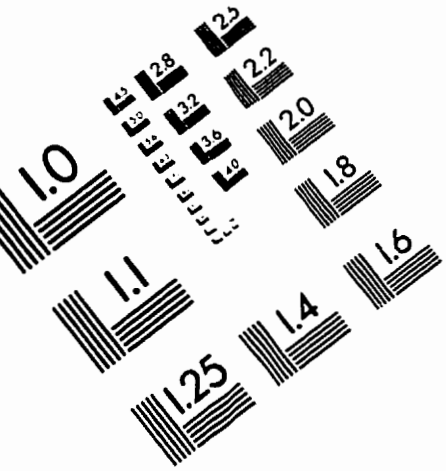
APPENDIX B

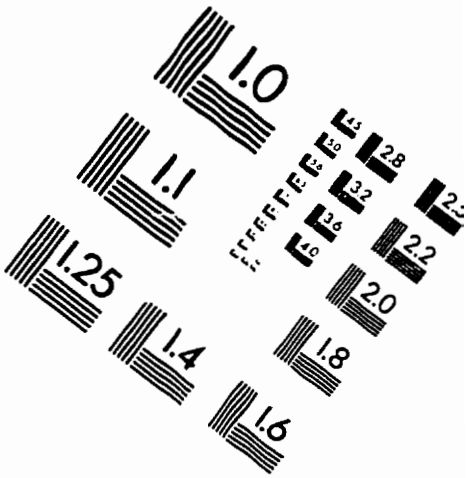
Chemical and pesticide applications for plots D and I in 1997 and 1998.

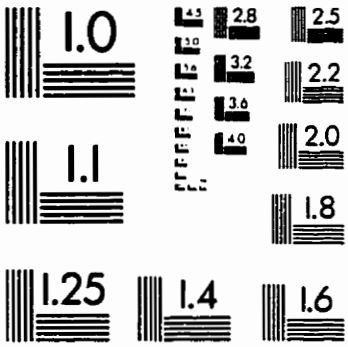
Date	Plot	Material	Concentration
18-06-97	D	Tebufenozide 240 F	170 ml/200 L
18-06-97	D	*Bacillus thuringiersis* var *burstaki* WP 560	187 g/200 L
		Cypermethrin 400 EC	4.2 ml/200 L
07-07-97	D	Malathion 25 WP	1.17 kg/350 L
07-07-97	D	Pirimicarb 50 DF	496 g/350 L
07-07-97	I	Imidacloprid 240 FLO	111 ml/350 L
22-07-97	D	Phosmet 50 WP	2.2 kg/ha
19-08-97	D	Pirimicarb 50 DF	850 g/ha
19-08-97	D	Phosmet 50 WP	4.0 kg/ha
19-08-97	I	Imidacloprid 240 FLO	380 ml/ha
13-05-98	I	Tebufenozide 240 F	1 L/ha
13-05-98	D	Cypermethrin 400 EC	125 ml/ha
28-05-98	I	Pirimicarb 50 DF	1.42 g/L
28-05-98	D	Imidacloprid 240 FLO	88 ml/L
11-06-98	I	Carbaryl XLR	2.5 L/ha
23-06-98	I	Pirimicarb 50 DF	850 g/ha
23-06-98	D	Imidacloprid 240 FLO	231 ml/ha
23-06-98	D	Cypermethrin 400 EC	125 ml/ha
03-07-98	I	Phosalone FLO	600 ml/800 L
03-07-98	D	Phosmet 50 WP	1.12 kg/700 L
21-07-98	I	Phosalone FLO	1.8 L/ha
21-07-98	D	Phosmet 50 WP	2.2 kg/ha

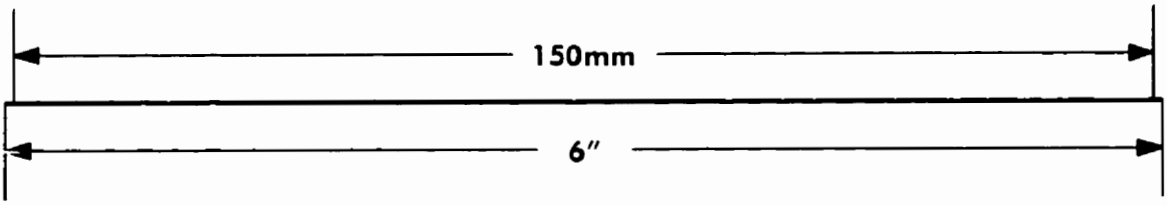
In 1997, fungicides including Maestro (captan) and Nova (myclobutanil) were applied 15 and 8 times respectively. In 1998, Maestro, Nova and Equal (dodine) were applied 12 times, twice and once respectively. There was one treatment of urea in 1997 and again in 1998 to promote leaf nitrogen levels. To ensure fruit firmness, calcium chloride was applied 3 times in 1997 and again in 1998.

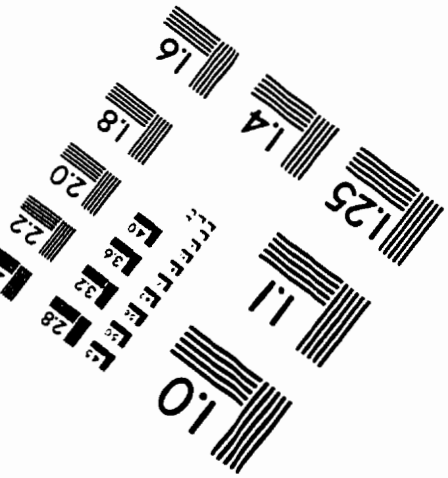
IMAGE EVALUATION
TEST TARGET (QA-3)

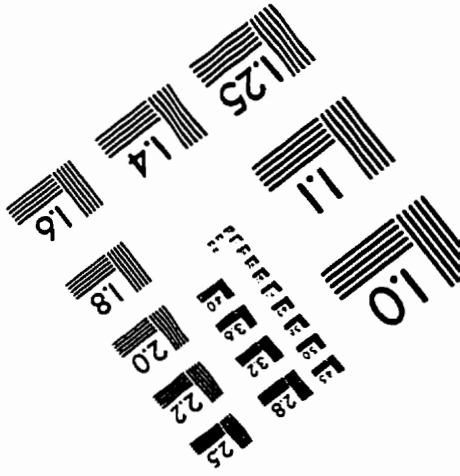












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989