EFFECTS OF SHORT EXPOSURE PERIODS TO LOW TEMPERATURES ON THE BIOLOGY OF *TETRANYCHUS URTICAE*

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TETRANYCHUS URTICAE
SURVIVAL AT LOW
TEMPERATURES
DEVELOPMENTAL CYCLE

SUMMARY: All stages of *Tetranychus urticae* Koch (Acari: Tetranychidae) were subjected to short periods (0–16 hours) of exposure to low temperatures (0, -5 and -10°C), followed by rearing at 20°C. Adult survivorship was greater than that of the juvenile stages, but there was no difference between survival rates of the two sexes; duration of exposure had more effect than the actual temperature experienced. Many surviving juvenile mites did not successfully complete their development to adult: 8 hours at 0°C was sufficient to prevent almost all development. The longevity of surviving treated adults was reduced, but there was little reduction in the adult longevity of surviving treated juvenile stages. Both duration and level of low temperature exposure affected the fecundity of treated adults. Treatment at the deutonymph stage also affected the fecundity of the resulting adults. Male fertility was unaffected by low temperature exposure, except possibly at the longest exposure durations. Development of the surviving treated immature stages was retarded; the temperature experienced had more effect than the duration of exposure.

TETRANYCHUS URTICAE
SURVIE A BASSES
TEMPERATURES
CYCLE DU DEVELOPPEMENT

RÉSUMÉ : Tous les stades du développement de *Tetranychus urticae* Koch (Acari: Tetranychidae) ont été exposés pendant de courtes périodes (0–16 heures) à de basses températures (0, -5 et -10°C), suivies par un élevage à 20°C. La survie chez les adultes est plus grande que chez les jeunes, mais il n'y a aucune difference entre le taux de survie des deux sexes; la durée de l'exposition a davantage d'effet que la température. Beaucoup de jeunes acariens survivants n'atteignent pas le stade adulte: un traitement de 8 heures à 0°C est suffisant pour empêcher presque tout développement. La longévité des adultes traités survivants est réduite mais il y a très peu de réduction de la longévité chez les adultes provenant de jeunes traités survivants. La durée et l'exposition à basses températures affectent la fecondité des adultes traités. Le traitement au stade deutonymphe agit également sur la fécondité des adultes. L'exposition à basses températures ne modifie pas la fertilité des mâles, lorsque la durée de cette exposition est brève. Le développement des jeunes traités survivants est retardé; la température a sur eux plus d'effet que la durée de l'exposition.

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Introduction

There is little precise information on mortalities of non-insectan arthropods in relation to intensity of cold and duration of exposure; differences in susceptibility to cold are known to be associated with differences in the annual extreme low temperature of the area concerned (MacPhee, 1961). Contributions by van de Vrie *et al.* (1972), Jeppson *et al.* (1975) and Tanigoshi *et al.* (1976) have been directed toward a better understanding of the effects of temperature on spider mites (Tetranychidae), including the two spotted mite *Tetranychus urticae* Koch, which has become the choice experimental mite for many ecological and biological studies because of its polyphagous habits, rapid rate of development and resulting economic importance (Alford, 1984). The life cycle of *T. urticae* consists of egg, larva, protonymph, deutonymph and adult (Cagle, 1949).

All controlled, experimental studies of the thermal response of *T. urticae* have been based on constant temperatures. The most detailed studies of the biology of *T. urticae* under precisely controlled, constant conditions of temperatures are those of Nuber (1961), Laing (1969), Shih *et al.* (1976), Herbert (1981), Trichilo & Leigh (1985) and Lin (1989). In reality, however, mites are exposed to fluctuating temperatures, and these may include sub-lethal extreme conditions whose effects are not known. Stenseth (1965) investigated the low-temperature mortality of *T. urticae* in Norway, but with particular regard to long periods of exposure, and differences between diapausing and non-diapausing females, and cold-hardiness of diapausing *T. urticae* was also studied by Cone & Wildman (1989).

In none of the studies reported was there an impact of short exposure periods of low temperature on the non-diapausing stages of *T. urticae*: these have not previously been carried out. Therefore the present paper reports the findings of laboratory studies conducted in 1993 on the effect of short exposure periods to low temperatures on some aspects of the biology of *T. urticae*.

Materials and methods

A stock culture of *Tetranychus urticae* (Natural Pest Control Ltd., Sussex, England) was maintained on dwarf beans (*Phaseolus vulgaris*), in the laboratory at about 20°C ± 2°C, and long photoperiod. This strain of mites had been obtained by the suppliers from commercial cucumber glasshouses some years previously, and maintained ever since on bean plants. The mites were capable of being reared continuously under long day conditions without entering diapause, although short day conditions did produce diapausing females. The plants were changed when heavy infestation depleted their foliage, as indicated by the presence of migrating mites.

1. *Adult mites*

The leaf disc method was adopted from that used by Rodriguez *et al.* (1971) and Dabrowski & Rodriguez (1971). Leaves from the upper half of the bean plant were used to make leaf discs, punched out of the leaves by a 20 mm diameter cork borer, and placed in 90 mm diameter petri dishes on wet cotton wool. Each disc was inoculated with one teneral *T. urticae* male or female. The dishes were covered with lids, and distributed among three incubators with temperatures 0, -5 and -10°C for exposure periods of 0, 1, 2, 4, 8 and 16 hours respectively. Ten replicates of each sex were used for each treatment combination and their progeny. The mites were then examined and their survival assessed. The surviving female mites were then kept at 20°C and examined daily, counting the eggs on each disc, in order to assess their fecundity and longevity; larger numbers of replicates were not used due to the time taken to count and rear all the survivors.

The surviving male individuals were then reared at 20°C after adding one untreated adult female to each disc. Each adult female was then allowed to lay a number of eggs (total number of eggs about 100 per ten replicates). The mites on each disc were examined daily until all the surviving males and all eggs had reached the nymphal stage in order to determine the sex ratio of the new progeny. As only fertilised eggs produce female progeny, the resultant sex ratio served

as an indication of any low temperature exposure effects on the fertility of the treated male mites.

2. *Immature stages*

The immature feeding stages (larvae, protonymphs and deutonymphs) were treated in the same way on leaf discs as in the previous experiment. Ten replicates were taken for each immature stage at each temperature level and each exposure period After an initial assessment of survival, they then were kept at 20°C, and mortality was estimated at two day intervals until all immatures were either dead or had reached the adult stage. The resultant newly moulted females were then examined daily and the eggs counted until all the adult females were dead. This procedure enabled estimation of the percentage completion of each developmental stage, as well as the fecundity of those individuals that reached the adult stage.

For the egg stage, ten healthy, newly deposited eggs were removed from bean plants and placed in petri dishes (90 mm diameter) on wet filter paper. Ten such dishes were used as replicates in each treatment as in the previous experiments, and were then examined at two day intervals until all the eggs had either hatched and reached the adult stage or were dead. The fecundity of the newly moulted adult females was measured as in the previous experiment.

Results

1. *Survivorship*

Survivorship was dependent on both duration and level of low temperature exposure, as well as on the stage of the mites that was treated (Table 1). 30% of adult mites of both sexes survived even the most extreme conditions, whereas most larvae died after eight or more hours at -5°C or below. Treatment of the eggs caused no immediate apparent mortality at all, but many did not subsequently hatch, and were assumed to have been killed by the treatments. Overall percentage survival varied significantly according to stage (Fig 1a), with juveniles being less cold-hardy than the adults, but there was no significant difference between the survivorship of the two sexes. Temperature (Fig 1b) had less effect than the duration of exposure (Fig. 1c).

Temp	Duration in h	Stage treated					
		Female	Male	Deutonymph	Protonymph	Larva	Egg
Control		10	10	10 (8)	10 (7)	10 (7)	10 (8)
0°C	1	10	10	10 (8)	10 (6)	10 (7)	9 (8)
	2	10	10	10 (7)	10 (6)	10 (5)	9 (7)
	4	10	10	9 (4)	8 (4)	7 (3)	7 (4)
	8	9	10	7 (4)	7 (1)	5 (0)	6 (1)
	16	9	9	4 (0)	3 (0)	5 (0)	4 (1)
-5°C	1	10	10	10 (6)	10 (7)	10 (6)	9 (7)
	2	10	10	10 (6)	10 (6)	10 (4)	7 (5)
	4	9	10	8 (4)	8 (3)	8 (0)	5 (1)
	8	9	9	6 (1)	5 (0)	3 (0)	3 (0)
	16	4	4	0 (0)	0 (0)	0 (0)	2 (0)
-10°C	1	10	10	10 (6)	10 (5)	10 (6)	8 (7)
	2	10	10	10 (5)	9 (4)	10 (2)	6 (2)
	4	9	9	7 (3)	6 (2)	5 (0)	4 (0)
	8	7	8	4 (0)	2 (0)	2 (0)	1 (0)
	16	3	3	0 (0)	0 (0)	0 (0)	1 (0)

Table 1: Numbers of surviving mites (out of 10 treated) after exposure to low temperatures (°C) for durations of up to 16 hours. Numbers in parentheses after each immature stage are the numbers of mites that successfully reached the adult stage.

Although many juvenile immature mites survived the immediate effects of the cold treatments, Table 1 also shows that they suffered excessive further mortality during development, and many did not successfully reach the adult stage. Exposure durations of eight hours or more, even only as low as 0°C, prevented almost all immature stages from reaching the adult stage.

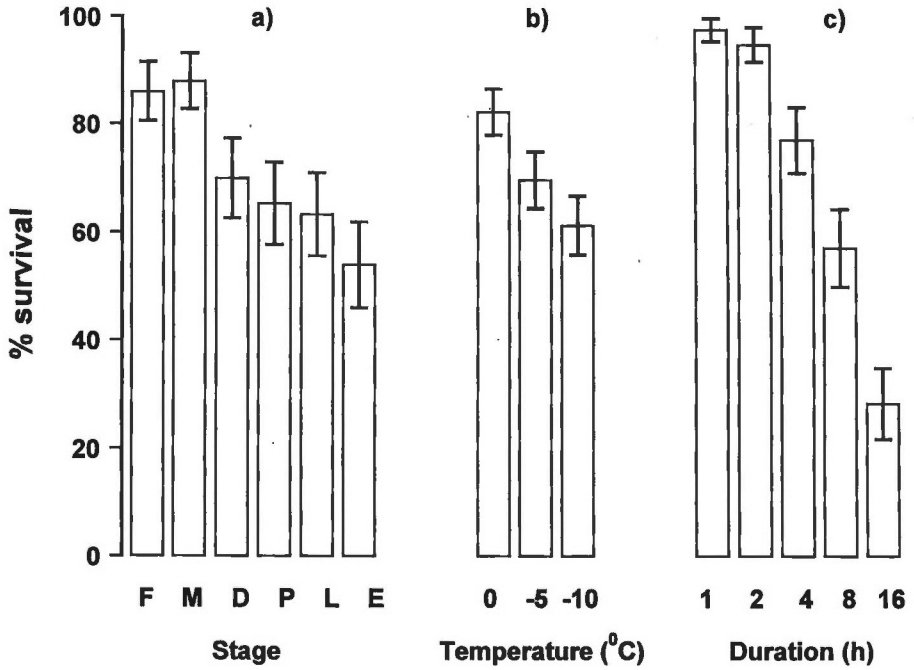

FIG. 1: Overall means of % survival (with s.e. bars) of *Tetranychus urticae* after exposure to short periods of low temperatures. (a) by stages — F female adult; M male adult; D deutonymph; P protonymph; L larva; E egg; (b) by temperatures; (c) by durations of exposure.

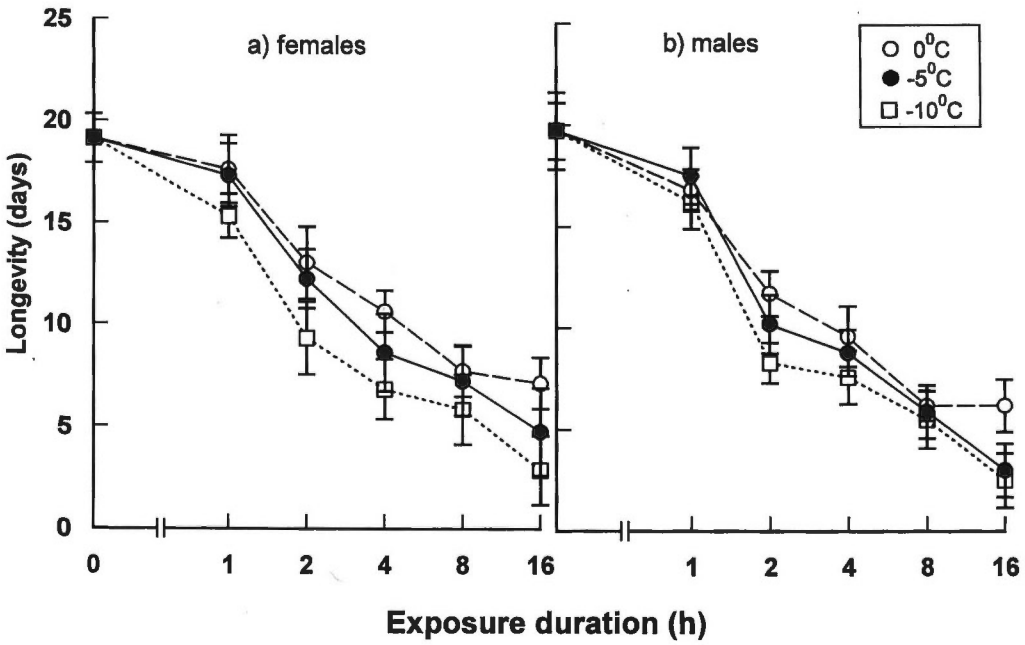

FIG. 2: Longevity of female (a) and male (b) adult *T. urticae* after exposure to low temperatures. (a) deutonymph; (b) protonymph; (c) larva; (d) egg.

2. *Longevity*

The overall mean longevity (in days) of adult female *T. urticae* decreased with lowering temperatures from 0 to -10°C and also by increasing exposure durations from 0 to 16 hours (Fig 2a). There were highly significant differences between temperatures ($F_{2, 162} = 4.9$, $P < 0.01$) and between exposure durations ($F_{5, 162} = 31.6$, $P < 0.001$), but no significant interaction between temperature and duration.

The longevity of adult male *T. urticae* (Fig. 3b) was similarly affected, with further significant differences between temperatures ($F_{2, 172} = 3.57$, $P < 0.05$) and between exposure durations ($F_{5, 172} = 52.65$, $P < 0.001$). The responses of each sex were very similar, and did not differ significantly.

The above analyses include all treated adults, some of which were in fact killed by the treatment, and therefore had zero longevity. A further analysis, including only those adults that survived the initial treatment, showed significant effects of duration of exposure on both females ($F_{5, 141} = 25.94$, $P < 0.001$) and males ($F_{5, 144} = 51.46$, $P < 0.001$), but the effect of the level of temperature was insigificant for both sexes.

When the immature stages had been subjected to low temperature treatment, the adult longevity of those that survived to maturity showed some tendency to be reduced after the more extreme treatments (Fig. 3), but this effect was much less marked than in the treated adults. Only one-way analyses of variance were carried out because many combinations of temperature and duration of exposure resulted in no mites surviving to the adult stage, as already mentioned. The only significant effect was that of duration of exposure on the adult longevity of those mites that were treated as deutonymphs ($F_{3, 63} = 6.20$, $P < 0.001$): their longevity was noticeably reduced after exposures of 4-8 hours (Fig 3a).

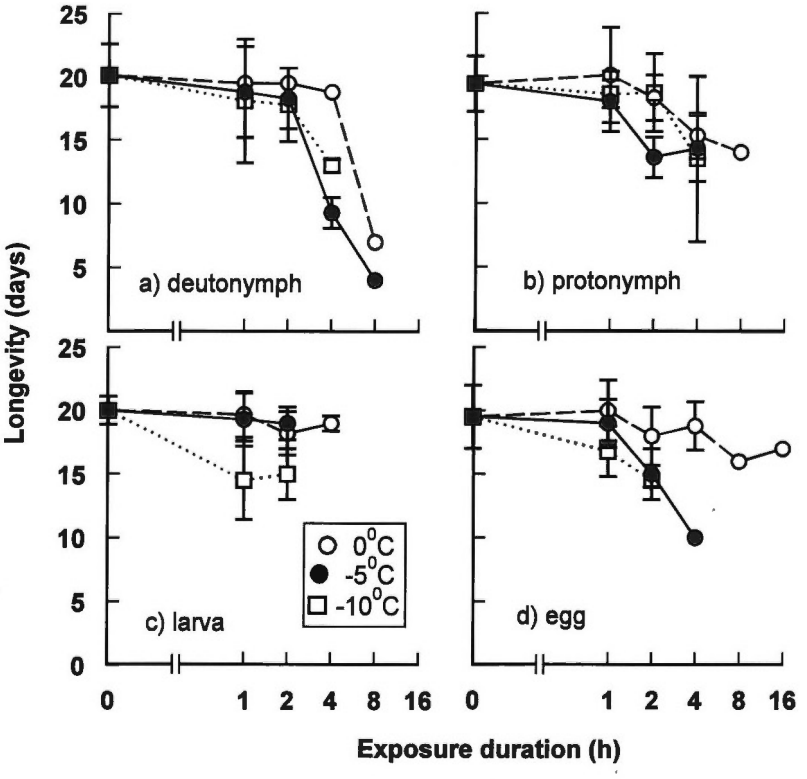

FIG. 3: Longevity of immature stages of *T. urticae* after exposure to low temperatures.

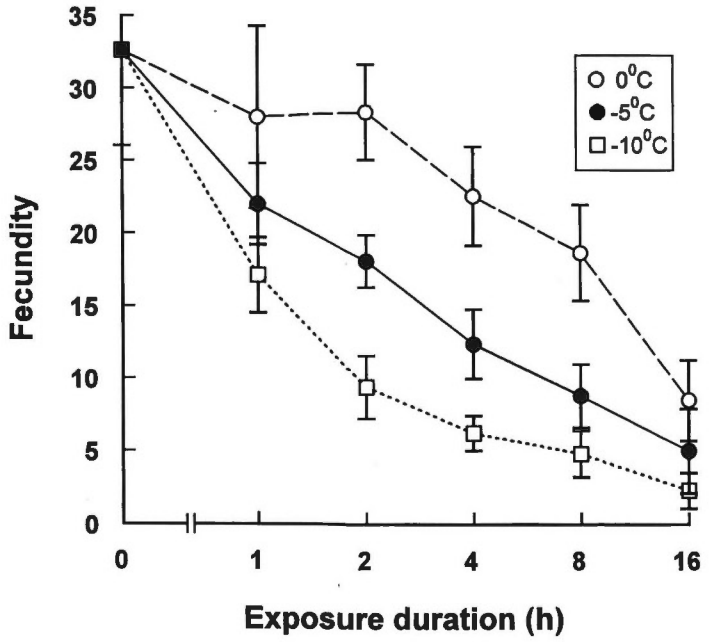

FIG. 4: Fecundity of adult *T. urticae* after exposure to low temperatures.

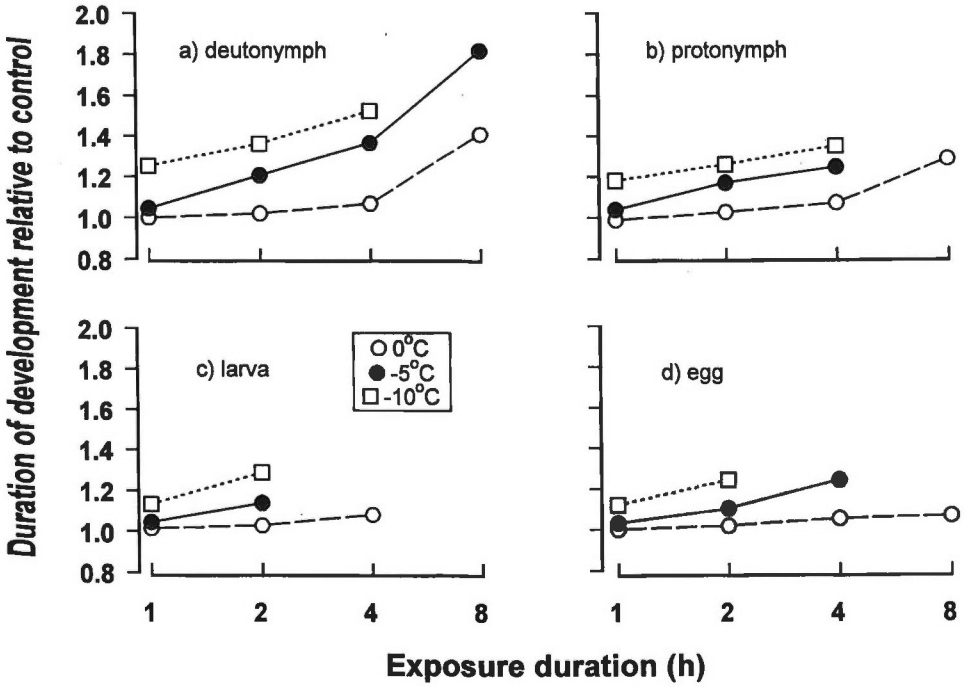

FIG. 5: Duration of development of immature stages of *T. urticae* after exposure to low temperatures, expressed relative to the developmental duration of control (untreated) mites at each stage. (a) deutonymph; (b) protonymph; (c) larva; (d) egg.

3. *Fecundity*

The overall mean number of progeny from treated adult females clearly decreased with increasing exposure periods from 0 to 16 hours at each temperature level and also with decreasing temperature (Fig. 4). There were highly significant differences between temperatures ($F_{2,\ 142} = 9.86$, $P < 0.001$) and exposure periods ($F_{5,\ 142} = 13.11$. $P < 0.001$). There was also a significant interaction ($F_{10,\ 142} = 0.76$, $P < 0.05$) between temperature and exposure, with fecundity differing more between short exposures at -10°C and between the longer exposures when the mites were treated at 0°C (Fig. 4).

When surviving treated immature stages were reared to adult, the fecundity of those treated in the deutonymph stage was also affected (Table 2). In particular, there was a tendency for the deutonymphs that survived the more extreme treatments to become adult but then to lay no eggs. Treatment in the earlier stages had little or no effect (Table 2), although the small numbers of individuals that reached the adult stage make it difficult to draw any firm conclusion. The only statistically significant effect was that of duration of exposure on the fecundity of surviving deutonymphs ($F_{4,\ 63} = 12.68$, $P < 0.001$).

Temp.	Duration in h	Stage treated			
		Deutonymph	Protonymph	Larva	Egg
Control		32.1 (5.6, 8)	31.4 (3.7, 7)	30.0 (6.1, 3)	27.0 (3.2, 3)
0°C	1	28.5 (4.5, 8)	31.5 (5.0, 6)	32.0 (, 4)	27.1 (5.2, 6)
	2	22.8 (7.5, 7)	29.5 (2.9, 6)	29.0 (3.0, 2)	31.2 (3.9, 4)
	4	9.5 (4.0, 7)	30.5 (6.2, 4)	27.0 (—, 1)	26.0 (1.0, 2)
	8	0 (0, 4)	28.0 (—, 1)	—	—
	16	—	—	—	28.0 (—, 1)
-5°C	1	24.6 (4.4, 6)	26.1 (4.3, 7)	29.8 (4.0, 4)	25.5 (5.2, 4)
	2	20.8 (4.5, 6)	28.6 (5.1, 6)	30.5 (4.0, 2)	26.5 (8.5, 2)
	4	0 (0, 4)	32.0 (4.9, 3)	—	—
	8	0 (—, 1)	—	—	—
-10°C	1	24.1 (5.2, 6)	28.8 (4.1, 5)	24.6 (9.3, 5)	32.6 (3.1, 5)
	2	16.0 (4.3, 5)	27.7 (7.9, 4)	—	26.0 (—, 1)
	4	0 (0, 3)	30.5 (0.5, 2)	—	—

TABLE 2: Mean numbers of eggs laid by surviving adult mites after exposure to low temperatures in the immature stages (with s.e.'s and numbers of females in parentheses). Note that no females survived to lay eggs after 8 or 16h exposure at -5 or -10°C

4. *Fertility*

Fertility of adult male *T. urticae* was not obviously affected by short exposure periods to low temperatures (Table 3). The overall progeny from treated male parents comprised 411 males and 821 females (ratio of males to females = 0.50), or almost exactly one third males. There were no significant differences between the relative numbers of males and females resulting from male parental exposure to different low temperature levels ($\chi^2_2 = 0.824$, $P > 0.1$) or durations ($\chi^2_5 = 7.68$, $P > 0.1$). The only indication of any effect was at the longest duration of exposure, when the ratio of males to females rose to 0.73 ($\chi^2_1 = 3.59$, $0.1 > P > 0.05$). This suggests that there might be a slight reduction in male fertility if low temperatures persist for more than 16 hours.

5. *Development Rate*

There was some evidence that the rate of development was retarded in those individuals that survived the initial cold, but the time taken to develop only increased by a maximum factor of about 1.25 (eggs), 1.29 (larvae), 1.35 (protonymphs) and 1.52 (deutonymphs) (Fig. 5). This suggests, however, that the later the stage that was subjected to cold, the greater was the resulting slowing of the remaining period of development.

Temperature	Duration in h	Male	Female
Control		33	60
0°C	1	25	55
	2	28	51
	4	24	48
	8	18	72
	16	24	30
-5°C	1	29	65
	2	31	40
	4	20	49
	8	32	62
	16	13	19
-10°C	1	20	52
	2	28	55
	4	22	52
	8	35	40
	16	8	12

TABLE 3: Numbers of male and female mites resulting from low temperature treatment of male parents.

DISCUSSION

Observation on the responses of *T. urticae* to temperature have been reported to a limited extent, and all controlled, experimental studies of the thermal response of *T. urticae* have been based on constant temperatures. This work, however, has emphasised the effect of short exposure periods to low temperatures on biological characteristics (longevity, fecundity, fertility, % survival, % egg hatching, and % of immatures successfully reaching adult stage) of each *T. urticae* stage (egg, larva, protonymph, deutonymph, female, and male). Previous studies have also not considered all these feature at all developmental stages.

The general conclusion of this study is that short periods of low temperature may have a very marked effect of the population performance of *T. urticae*. When not in diapause, the mite normally exists in a multi-stage population, so that sudden, unseasonal cold periods are liable to affect all stages of the life cycle, with corresponding effects on the population rate of increase which can be calculated through life tables (eg. CAREY & BRADLEY, 1982).

The comparable previous work on *T. urticae*, by STENSETH (1965), considered longer exposures to mostly lower temperatures (-5 to -19°C). At -10°C, STENSETH found the LT50 of adult female mites to be greater than 15 days: this contrasts markedly with the present work, in which only 30% of adults survived even 16h exposure at this temperature. This discrepancy could be caused by either strain of mite used, or the actual experimental conditions. STENSETH (*l.c.*) showed that high humidities were important to the survival of the mites at low temperatures, but in the present work all treated individuals were exposed to low temperatures in containers lined with moist cotton wool, so that low humidities were unlikely to have contributed to the mites' poor survival. It seems more likely that the strain of *T. urticae* studied in Norway by STENSETH was inherently more cold-hardy than the English glasshouse strain used in this paper. In the cold-hardiness classification as applied to insects by BALE (1993), the Norwegian mites were moderately chill-tolerant, whereas those in the present work were chill susceptible.

In the basic studies of insects, SALT (1961) remarked that, as the temperature falls, the needs of insects change from those of activity, growth, reproduction, etc. to those of survival. Temperature and time are here inseparable, for, in general, cold injury becomes more severe as either the temperature is lower or the time of exposure is increased. MACPHEE (1961) in his study on fruit tree red spider mite, *Panonychus ulmi* (Koch), pointed out that the duration of exposure has to be considered when estimating mortality in the field. It follows that weather records should include the duration of low temperatures to be most useful for making estimates of pest mortality. MACPHEE (*l.c.*) also showed that the difference in mortality resulting from an exposure lasting 15 minutes compared to one lasting 4 hours might be as much as 40%; and the duration effect was equivalent to an increase in mortality of about 10% each time the duration of exposure was doubled after initial 15 minutes exposure. *P. ulmi* differs from *T. urticae* in that it overwinters solely in the (diapausing) egg stage, so that the effect of low temperatures on other developmental stages is less likely than in *T. urticae*, which has both diapausing and non diapausing strains.

One might expect diapause and cold-hardiness to be associated in *T. urticae*, as in aphids (JAMES & LUFF, 1982) who studied the effect of low temperatures on hatching of *Rhopalosiphum insertum* (Walker) eggs, and found significant differences between per-

centage of hatching at 0°C and -5°C. These authors concluded, however, that diapause and cold-hardiness were determined by separate environmental factors, and STENSETH (1965) found no difference between the cold-hardiness of diapausing and non-diapausing females of *T. urticae*. In similar studies to the present work, MOHAMED (1984) found significant effects of low exposures to low temperatures (0, -5 and -10°C) on the hatching of *R. insertum* eggs, as well as on the performance of later stages of the aphids' life cycle.

The importance of *T. urticae* in temperate regions depends in part on its ability to survive outside during the winter, as well as in protected environments such as glasshouses, as the species infects a range of outdoor horticultural crops (ALFORD, 1984). Although reducing daylength normally ensures that the winter is passed as diapausing females, unseasonal cold conditions, or the failure of heating in controlled environments can lead to short exposure of all developmental stages to low temperatures. The work presented here suggests that this can have a marked and to some degree delayed effect on all the life history stages of the mite, and may justify the further investigation of into the use of short-term cold treatment as a preventative measure against *T. urticae* infestation.

REFERENCES

ALFORD (D. V.) 1984. — Fruit Pests. Their recognition, biology and control. — Wolfe Publishing, London: 320pp.

BALE (J. S.) 1993. — Classes of insect cold hardiness. — Functional Ecology, **7**: 751-753.

CAGLE (L. R.), 1949. — Life history of the two spotted spider mite. — Tech. Bull. Virginia Agric. Exp. Sta., **113**: 1-31.

CAREY (J. R.) & BRADLEY (J. W.), 1982. — Developmental rates, vital schedules, sex ratios, and life tables for *Tetranychus urticae*, *T. turkestani* and *T. pacificus* (Acarina: Tetranychidae) on cotton. — Acarologia, **23**: 333-345.

CONE (W.W.) & WILDMAN (T.E.), 1989. — Cold hardiness of diapausing two-spotted spider mite on hops in the Yakima Valley. — VII Int. Congr. Acarol., **2**: 5-9.

DABROWSKI (Z. T.) & RODRIGUEZ (J. G.), 1971. — Studies on resistance of strawberries to mites. 3. Preference and non preference responses of *T. urticae* and *T. turkestani* to essential oils of foliage. — J. econ. Entomol., **64**: 387-391.

HERBERT (H. J.), 1981. — Biology, life tables and innate capacity for increase of the two spotted spider mite, *Tetranychus urticae* (Acarina: Tetranychidae). — Can. Ent., **113**: 371-378.

JAMES (B. D.) & LUFF (M. L.), 1982. — Cold-hardiness and development of eggs of *Rhopalosiphum insertum* (Walker). — Ecol. Entomol., **7**: 277-282.

JEPPSON (L. R), KEIFER (H. H.) & BAKER (E. W.), 1975 — Mites injurious to economic plants. — Univ. California Press: 614 pp.

LAING (J. E.), 1969. — Life history and life table of *Tetranychus urticae* (Koch). — Acarologia, **11**: 32-42.

LIN (T. S.), 1989. — Effect of host plant on the development and reproduction of two spotted spider mite, *T. urticae* (Koch). — Bull. Taich. Distr. Agric. Improv. Station., **22**: 49-55.

MACPHEE (A. W.), 1961. — Mortality of winter eggs of the European red mite, *Panonychus ulmi* (Koch), at low temperatures, and its ecological significance. — Can. J. Zool. **39**: 229-243.

MOHAMED (A. M.), 1984. — Effects of physical environmental factors on some aphid populations. — Ph.D. Thesis, Univ. Newcastle upon Tyne, U.K.: 317 pp.

NUBER (K.), 1961. — Overwintering of the red spider mite, *T. urticae* (Koch) in hop gardens. — Höfchenbrie, **14**: 6-15.

RODRIGUEZ (J. G.), DABROWSKI (Z. T.), STOLTZ (L. P.), CHAPLIN (C. E.) & SMITH (W. O.), 1971. — Studies on resistance of strawberries to mites. 2. Preference and non preference of *T. urticae* and *T. turkestani* to water-soluble extracts of foliage. — J. econ. Entomol., **64**: 383-387.

SALT (R. W.), 1961. — Principles of insect cold-hardiness. — Annu. Rev. Entomol., **6**: 55-74.

SHIH (C. I. T), POE (S. L.) & CROMROY (H. L.), 1976. — Biology, life table and intrinsic rate of increase of *T. urticae* (Acarina: Tetranychidae). — Ann. entomol. Soc. Amer., **69**: 362-364.

STENSETH (C.), 1965. — Cold hardiness in the two-spotted spider mite (*Tetranychus urticae* Koch). — Entomol. exp. appl., **8**: 33-38.

TANIGOSHI (L. K.), BROWNE (R. W.), HOYT (S. C) & LAGIER (R .F.). 1976. — Empirical analysis of variable temperature regimes on life stage development and population growth of *T. mcdanieli* (Acarina: Tetranychidae). — Ann. entomol. Soc. Am. **69**: 712-716.

TRICHILO (P. J.) & LEIGH (T. F.), 1985. — The use of life tables to assess varietal resistance of cotton to spider mites. — Entomol. Exp. Appl. **39**: 27-33.

VAN DE VRIE (M.), MCMURTRY (J. A.) & HUFFAKER (C. B.), 1972. — Ecology of tetranychid mites and their natural enemies. A review. III. Biology, Ecology and pest status, and host-plant relations of tetranychid mites. — Hilgardia, **41**: 343-342.