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Environmental Factors Affecting Longevity, Adaptability and Diapause Termination of *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae): A Predator of *Lepidosaphes ulmi* L. (Hemiptera: Diaspididae) on Apple

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Abstract.- Adults *Exochomus quadripustulatus* L. were utilized to determine the effect of various environmental factors (mainly temperature) on the longevity, adaptability (hysteresis) and diapause termination either under controlled laboratory conditions or field insectary conditions. Newly eclosed male and female adults *E. quadripustulatus* from previously field collected pupae were studied for the lifespan of adults under different constant temperatures (15° and 20°C) and naturally fluctuating (ambient) temperatures using starved (no food), liquid diets (water, and 10% honey solution) and natural prey (mussel scale *Lepidosaphes ulmi* L.) on apple. Access to distilled water compared to starvation did not increase the longevity of adult but significantly prolonged the life span when honey solution was provided. Longevity in constant conditions and ambient depended on both temperature and food. Coccinellid *E. quadripustulatus* survived longer time (≥10 months) under field conditions on natural prey *L. ulmi* than under constant temperature (≤4 months). Prey *L. ulmi* was the best food for survival than 10% honey solution. The degree of adaptability (hysteresis) of adult *E. quadripustulatus* at alternating temperature regimes (ascending and descending) showed significant differences in feeding at decreasing temperature than increasing ones. The results indicate that, in natural conditions, the adult beetles may be well adapted if they are introduced to different climatic regions where it has not yet been tested. Still more detailed information is to be investigated to prove this hypothesis. Results on diapause termination of female adult *E. quadripustulatus* showed that temperature did not effect diapause termination under the present experimental conditions. This may be because this species is of univoltine strain in the north-east England.

Key words: Coccinellidae, *Exochomus quadripustulatus*, mussel scale, *Lepidosaphes ulmi*, hysteresis, diapause, apple.

INTRODUCTION

The environmental temperature has most important influence on the life of both plant and animal (Precht *at al*., 1973). Porter (1995) cited that the temperature-induced stress can cause changes in plant physiology resulting in changes in the levels or chemical or morphological defenses and/or nutritional quality of the host. As a result of these changes, insect feeding, on the plants would have altered growth, development, reproduction, survival and/or behaviour. Precht *at al.* (1973) described that life span of poikilothermic animals, depends on the environmental temperature from the embryo to egg laying and to the death of adult, at the given temperature. For example, temperature is considered as the most important abiotic factor affecting biology of *Trichogramma* species, influencing the developmental rate, fecundity, longevity, sexual ratio size and morphology (cited in Cônsoli and Parra, 1995). Constant experimental temperature compared to fluctuating temperature may not allow any assertions to be made about the environmental requirement of a species (Precht *et al*., 1973) as was found in *Coccinella septempunctata* L. that consumed double food under naturally fluctuating temperatures compared at different constant temperatures (Hodek, 1957 cited in Hodek, 1973). Similarly, Cônsoli and Parra (1995) found shorter developmental time of *Trichogramma galloi* Zuchi at alternating temperature compared to constant temperature. Therefore, adaptation to changing environment for any selective advantage may be considered as useful character of a species in question.

Coccinellids, as natural enemies of aphids and scales, are well studied with various aspects particularly development, predation and reproduction

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under different temperatures, natural prey, artificial diets, etc. (Hodek and Honěk, 2009; Eliopoulos *et al*., 2010; Jalali *et al*., 2009; Koch, 2003; Katsarou *et al*., 2005; Michaud and Qureshi, 2005). Among predacious coccinellids, *Exochomus quadripustulatus* L. (Col., Coccinellidae) is a polyphagous predator of several homopteran pests including aphids (Iperti *et al.*, 1977; Radwan and Lövei, 1983), scales (Uygun, 1978), whiteflies (Eshmatov and Saidova, 1993), coccids (Sengonca and Arnold, 2003; Hodek and Honěk, 2009). Although some aspects of biology as aphidophagous and coccidophagous species have been studied, no information as predator of diaspidid (armoured scale) is available about some other biological characteristics (longevity, degree of adaptability and diapause termination) under different environmental conditions with particular reference to mussel scale *Lepidosaphes ulmi* L. on apple.

For better understanding and usefulness of *E. quadripustulatus* as biocontrol agent of scale insects, the present study was conducted to determine the effect of different constant and fluctuating temperatures on the longevity, adaptability of adult to changing environment and the influence of the photoperiod and temperature requirement for the termination of diapause in adult beetles.

MATERIALS AND METHODS

Longevity of adults

Longevity of adult *E. quadripustulatus* was determined using four diets (i.e; starvation, distilled water, honey solution (10% honey + water) and natural prey, mussel scale (*L. ulmi*) under three environmental conditions. Two constant (15 and 20°C; 16L: 8D) and one fluctuating (ambient) temperature were maintained to determine the life span of adult beetles. The experimental design was based on 3 temperatures x 2 sexes x 4 diets x 5 replicates. 120 adult *E. quadripustulatus* (60 male, 60 female) immediately after emergence (within 24h) were collected from previously field collected pupae. As all the unfed and similar ages of adult beetles were not simultaneously available in the field at the commencement of the experiment, recently emerged individuals were placed systematically into different treatments in order to keep the experimental errors at minimum level which may result from the effect of age, sex, and time of emergence. These individuals were divided into three main groups (Groups 1, 2, & 3), each comprising of 40 beetles (half males and half females). Each of these three was further sub-grouped into four with 10 adults (five males and five females). The representative of each individual group was confined to a separate transparent plastic cage (5 cm diameter, 3 cm deep) with a lid on the top having gauze-covered window (2x2 cm) and provided with four different diets. The liquid diets such as water, and honey solutions were given with an eye dropper by wetting the cotton ball previously fixed to the lid of the containers as source of food. These balls before getting dark were replaced with new ones as necessary. Two to three scale infested spurs measuring 3-5 cm long were provided as natural prey for food to each individual. Liquid diets were replaced after every 24h till the death of the beetles, whereas prey was replaced at 2-3 days interval depending upon the rate of consumption. Initially, observations were made at regular intervals of 24h to see if there was any mortality but were delayed to a week interval when few individuals were left under insectary conditions. The experiment was commenced on 20 July 1994 and terminated on 17 May 1995.

Adaptation (Hysteresis) of E. quadripustulatus *to modified environment*

Ten adults (three males and seven females) *E. quadripustulatus* used in this experiment were collected from the apple trees on 21st March 1994. These trees previously had been planted at Close House, Heddon-on-the-Wall, Northumberland, NGR NZ 131660, England. All these beetles were placed individually in each plastic container as described above. At the beginning of experiment 30 gravid female (GF) scales, *L. ulmi*, removed and ventrally exposed from the infested spur were provided as food to each individual. As the food consumption increased the prey numbers were also increased from 30 to 40 at the time when temperature was set at 16°C. The containers were placed in a plastic tray covered with a transparent polythene bag in order to maintain humidity and then was kept in an incubator. The incubator was sequentially set at two altering temperature regimes in ascending (0, 2, 4, 6, 8, 10, 12, 14 and 16°C) and descending (14, 12, 10, 8, 6, 4, 2 and 0°C) orders. The experiment was commenced under the altering environmental conditions similar to field (12L:12D) from 0 to 16°C with increasing 2 degrees after every 48h and then decreasing 2 degrees at the same time interval from 16 to 0°C. On the completion, the experiment was terminated after 34 days on 24th April 1994.

Observations were made on the feeding rate of adult *E. quadripustulatus* after every 48h. The data pertaining to egg consumption was analysed as described below:

Estimation of egg density

Adult female scale, *L. umli*, lay eggs on the underside of the scale covering and the eggs are completely concealed on the substrate (twig, branch, leaf-petiole, trunk and, or fruit) of the apple. Prior to the assessing egg consumption, estimation of the number of eggs per scale, a total of 150 healthy GF scales previously collected at random from apple during the population study of *L. ulmi* were measured individually using binocular stereo-microscope with a graduated micrometer eye-piece. With relation to length and breadth of the scale, all the eggs laid by each female insect were also counted and recorded individually. The relationship between size and number of eggs was determined using regression equation ($Y=25.46X-7.93$, $r=0.70$, $df=149$, $F=146$, $P< 0.001$) and used in subsequent studies.

Assessment of egg consumption

The egg-bearing female scales were carefully removed from the stocked or freshly available spurs and placed ventrally exposed to feeding in the containers. On each observation day, the empty gravid female coverings were collected in a small glass tube for estimating the average numbers of egg eaten in relation to the body measurements. The body size (length and breadth) of the female coverings was measured and recorded under the stereo microscope (as described above) in the field laboratory. At a later stage, the numbers of GF consumed were calibrated and transformed into number of eggs per scale using the above regression equation for determining the amount and number of eggs eaten. The data were recorded as the numbers of GF consumed per predator rather than the number of eggs. It was impossible to record the amount of eggs consumed from the whole GF without the known quantity. Where the ladybirds had nibbled some or none of eggs, the observation was either recorded zero or delayed until the female-shell was almost all eaten by the predator. After predation, the female body covering was collected from the container only when all the eggs were either eaten or ≤5 eggs were left inside the container. In the later stage, the remaining eggs were emptied into the pot and new food was presented to equalize the numbers of GF as provided at onset of the experiment. Dislodged, particularly damaged eggs (*e.g.*, dead, dried, shriveled, etc.), if found, were replaced by healthy eggs.

Diapause termination experiment

Female adults of *E. quadripustulatus* of mixed ages were collected from the field on 21 September 1994. They were divided into two main groups, each consisting of 36 female adults. All the adult beetles were weighed in order to keep homogenous individuals prior to commencing the experiment. Each main group of adult was further subdivided into three sub-groups, each with 12 female adults. They were introduced individually into the container (as described above) and each container was allocated a code number for future reference. Female adults of Group-1 were kept in three different incubators with constant temperature set at -5, 0 and 5°C in order to investigate the influence of different temperatures on the induction of diapause and complete chilling period. The ovipositional behaviour of this species takes several months to undergo in dormant period thus they were kept for 90 days under the above temperature conditions whilst the individuals of Group-2 were also kept in the same incubator for 120 days of chilling period. At the end of these periods, all the adults *E. quadripustulatus* of Group-I and Group-II were transferred to 20°C (16L: 8D) after 90 and 120 days, respectively. Each beetle was offered 1-3 pieces of scale infested spur as food till the termination of experiment. Thus from 12 to 17 weeks of chilling followed by up to 7 weeks of long-day warm conditions, observations were continued at three-day interval at initial stage then after every week in order to see if they laid any eggs.

RESULTS

Adult's longevity

An ANOVA test for longevity of adult *E. quadripustulatus* studied under constant (15°C and 20°C) and ambient temperature showed some significant differences. The influence of temperature and diet on longevity of these adults was highly significant *i.e;* $F_{2,119}$=18.06, P<0.001 and $F_{3,119}$=61.97, P<0.001, respectively. Although an interaction between temperature and diet was significant ($F_{6,119}$=13.91, P<0.001), no interaction ($F_{2,119}$=0.16, P>0.05) was found between temperature and sex. The longevity of both sexes did not differ significantly ($F_{2,119}$=1.33, P>0.05), nor was there any interaction between sex and diet ($F_{3,119}$=0.44, P>0.05).

Effect of starvation

When no food was provided, all the beetles (on average) died due to starvation within 14-28 days both at constant and fluctuating temperatures (Table I, Fig. 1A,B). Both male and female adult beetles survived longer period (22-28 days) at 15°C than 20°C and ambient temperatures (14-23 days). Apparently some female adults survived longer (28 days) than male (22) but did not differ significantly.

Effect of water

Adult beetles fed on distilled water lived a few days more than when starved. All the beetles died on average between 16-31 days at constant temperatures. Although no interaction between sex and temperature was found, females died later than the males at constant temperatures compared to ambient conditions.

Effect of honey solution

Adult *E. quadripustulatus* fed on honey solution lived longer than without food or with water. Adult beetles survived on average between 21-70 days. Temperature showed a significant (P<0.001) effect on the longevity of adult beetles. For instance, at high temperature 20°C, all the male and females died within an average of 21-29 days compared to 50-70 days at low 15°C and fluctuating temperatures. Honey solution appeared as a second best alternate dietary source of survival after *L. ulmi* to the beetles (Table I).

*Effect of natural prey (*L. ulmi*)*

The survival period on average was greatest between 188-238 days when fed on natural scale insects rather than other liquid diets or starvation.

Effect of temperature

Overall, mortality occurred earlier in *E. quadripustulatus* under constant laboratory conditions, than fluctuating conditions. The effect of temperature on the life span of adults was obvious when all the male and female beetles died within 12-134, and 22-115 days at 15°C, respectively, between 12-122 and 15-145 days at 20°C, whereas mortality in males occurred in 11-279 days and 11-317 days in females at ambient conditions. The longest period of survival in adult beetles was recorded ≥ 10 months under ambient conditions compared to ≥ 4 months at constant temperatures. Overall, temperature and diet had highly significant effects on the longevity of adult *E. quadripustulatus.* Most beetles lived longer on natural prey when starved or on liquid diets under ambient conditions than constant temperature regimes (Fig.1, Table I).

Hysteresis of E. quadripustulatus *to altering temperature*

An experiment was conducted on the degree of adaptability of adult *E. quadripustulatus* predation on gravid female *L. ulmi* to an altering environment (Fig. 2). From the ANOVA test, it appeared that there was a significant ($F_{1,164}$=5.45, P<0.05) effect of both temperature regimes (ascending and descending order) on the food consumption of predator *E. quadripustulatus.* It clearly indicates that the influence of modified environment enhances the predation rate and led the *E. quadripustulatus* gradually to adapt to change with delayed time intervals. Sex and temperatures also showed a highly significant ($F_{1,164}$=12.77, P<0.001 and $F_{8,164}$=32.28, P<0.001, respectively) effect on predation. For example, predators of both

Table I.- Mean longevity (days±SE) of adult *Exochomus quadripustulatus* at different temperature regimes it close house during 1994 and 1995.

Diet and replicates		Temperatures					
		15°C		20°C		Ambient	
		Male	Female	Male	Female	Male	Female
Starved	1	12	33	12	17	16	17
	2	28	22	13	15	11	26
	3	28	29	13	18	17	14
	4	21	26	20	22	17	11
	5	25	30	14	15	20	14
Mean ± SE		22.28±2.99[ab]	28.00±1.87 [ab]	14.40±1.44 [ab]	23.60±5.71 [ab]	16.20±1.46 [ab]	16.40±2.58 [ab]
Water	1	32	26	20	24	20	18
	2	25	42	21	38	37	16
	3	28	35	13	21	32	32
	4	24	31	13	17	17	31
	5	20	25	17	25	30	30
Mean ± SE		25.80±2.01 [ab]	31.80±3.12 [ab]	16.80±1.69 [ab]	25.00±3.54 [ab]	27.20±3.76 [ab]	25.40±3.46 [ab]
Honey+ Water	1	53	80	33	21	94	77
	2	48	48	30	23	75	74
	3	50	84	28	21	28	71
	4	50	76	25	20	58	15
	5	53	63	33	22	64	48
Mean ± SE		50.80±0.97 [ab]	70.20±6.58 [ab]	29.80±1.53 [ab]	21.40±0.51 [ab]	63.80±10.80 [ab]	57.00±11.70
Prey (*L. ulmi*)	1	74	85	122	145	257	287
	2	134	106	28	116	67	317
	3	39	115	79	35	98	280
	4	33	42	121	34	279	218
	5	89	33	45	86	242	92
Mean ± SE		73.80±18.3 [ab]	76.20±16.60 [ab]	79.00±19.20 [ab]	83.20±22.00 [ab]	188.60±44.00 [ab]	238.80±40.10 [ab]

a, Significant variation exists with respect to temperatures; b, In-significant variation between sexes

sexes ate a negligible amount of food under low temperatures (0 to 8°C) compared to high temperature (10-16°C). No significant ($P>0.05$) interaction between regimes and sexes was observed, which indicates that both male and female adult consumed proportionally increased numbers of prey with regard to their sexes under altering environmental conditions. A significant interaction ($F_{8,164}=2.06$, $P<0.05$) between regimes and temperature was found which shows the variations in feeding to occur somewhere between 10 to 16°C. Both sexes and temperature also showed a highly significant ($F_{8,164}=3.44$, $P<0.001$) interaction. For instance, the female adults consumed within 48 h more food (>600 eggs) than did males (>300 eggs) under 16^{o}C (Fig.2).

Diapause termination

The termination of female adult diapause in *E. quadripustulatus* was attempted in to rapidly order obtain some fecund adults. The surviving of the females is shown in Figure 3. After 60-80 days of feeding under warm conditions (20°C), none of the females examined at any temperature had broken diapause. Both groups of female adults, previously kept under different duration of chilling treatments, failed to oviposit. Termination of diapause in these beetles was even not achieved when five female adults were individually kept in each container under constant 15° and 20°C controlled conditions (16L: 8D) for the longevity experiment as described above. Thus no obvious trend could be derived except that only few beetles

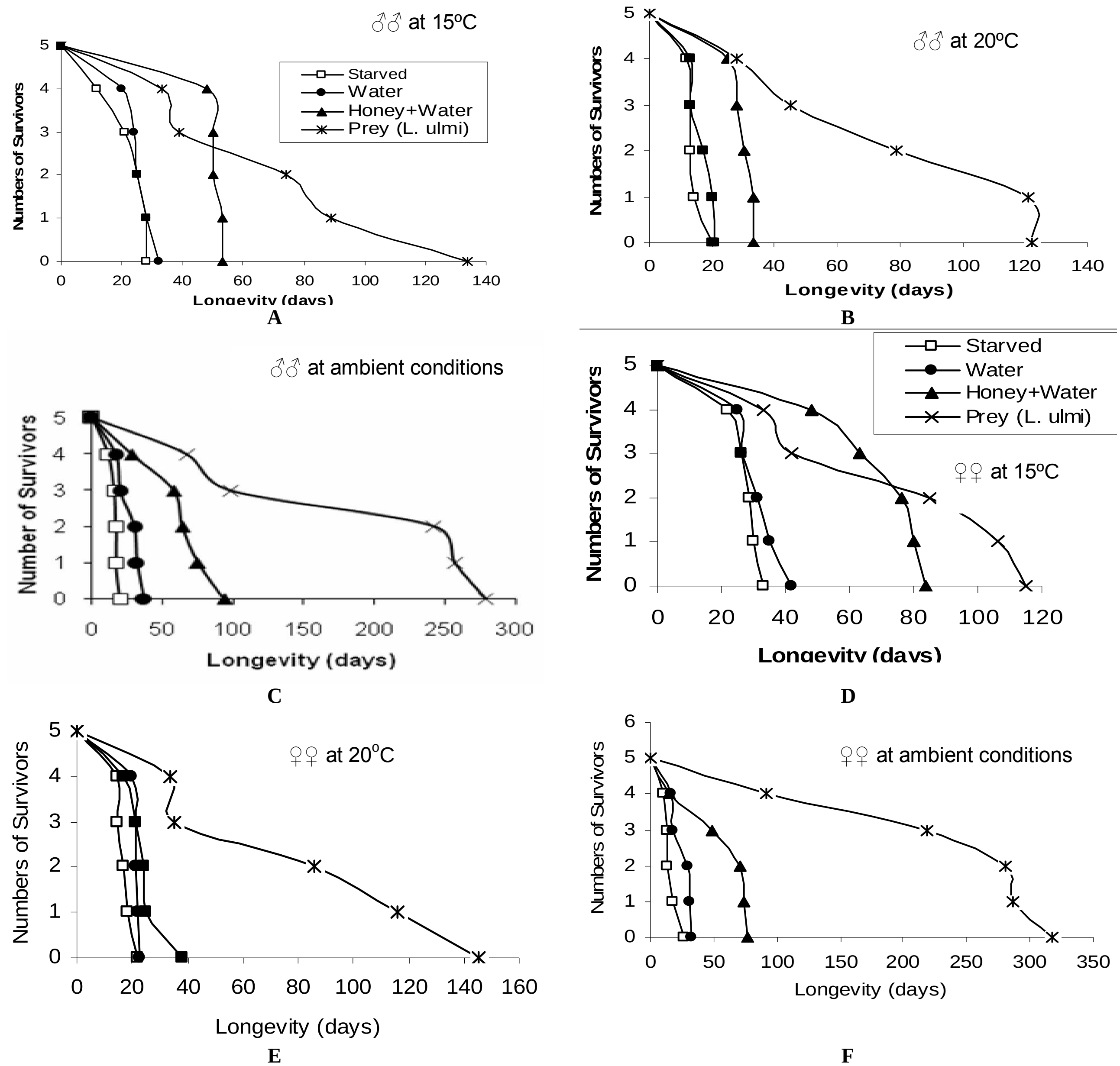

Fig. 1. Survivorship of adult *E. quadripustulatus* males (A, B, C) and females (D, E, F) at different temperatures.

were able to survive for longer time under low temperature (-5°C). The reason for this unsuccessful attempt at diapause termination in this species under present conditions is unclear.

DISCUSSION

The present data on longevity of laboratory studied beetles show that the survival of *E. quadripustulatus* varies significantly on the different diets. Survivals of adults without food for a period of two weeks suggest that the beetles are capable of surviving without prey in the field under variable environmental conditions. The liquid diets particularly honey solution extended the longevity of adults. This suggests that a liquid energy source (honey) is very important for survival of *E. quadripustulatus*. Hilal (1983), while studying the longevity of first instar larvae of coccinellid *C. septempunctata*, in addition to these views, concluded that the larvae can survive comparatively

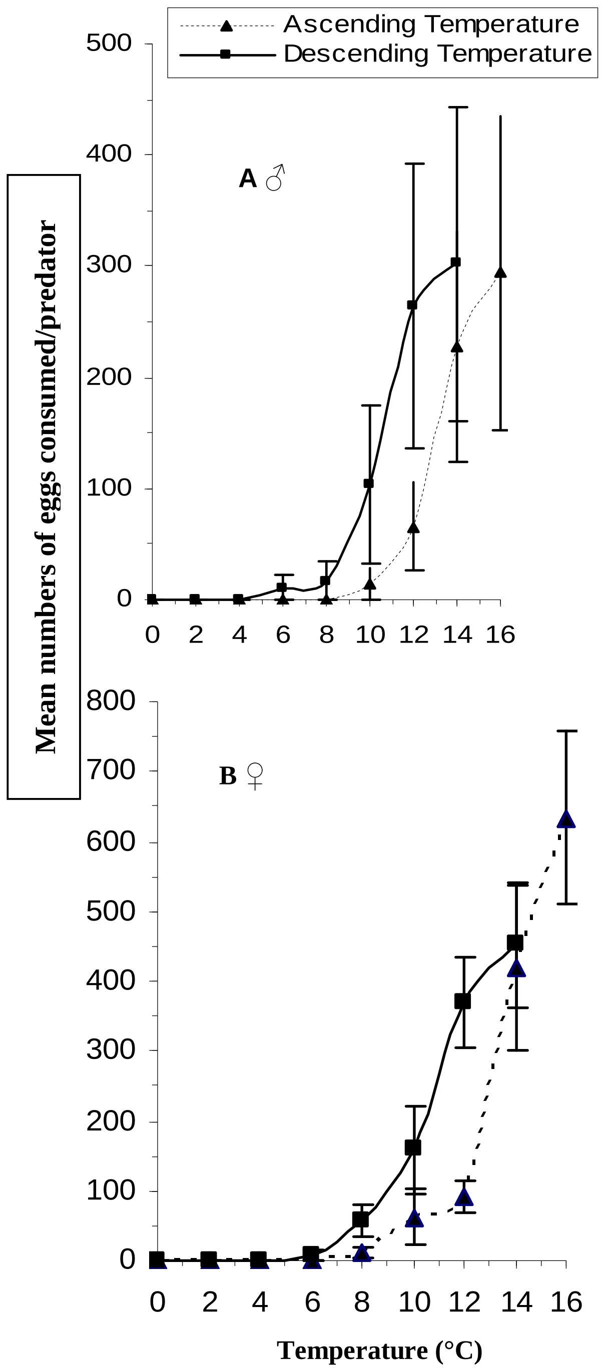

Fig. 2: Numbers of *L. ulmi* eggs eaten by adult *E. quadripustulatus* males (A), and females (B) under increasing and decreasing temperature regimes

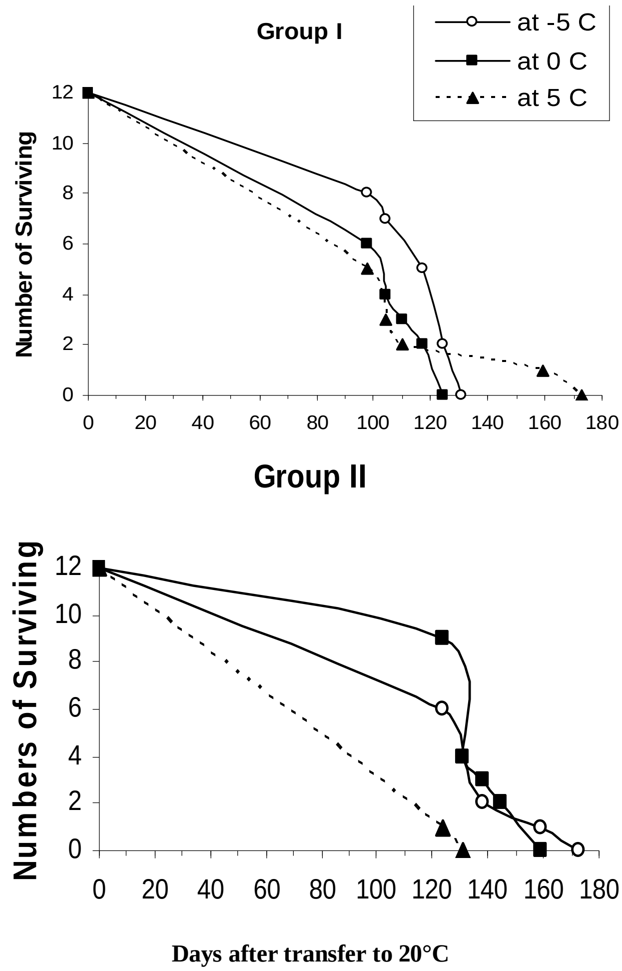

Fig. 3. Survivorship of female adults *E. quadripustulatus* exposed to 20°C before being stored at -5, 0 and 5°C for 90 days (Group I) and 120 days (Group II) in order to terminate diapause.

for longer time without natural prey if they have access to water or artificial honey dew. Similarly honey has been used as one of the important ingredients for the preparation of artificial diets (Smirnoff, 1958). Survival on natural prey proved to be the best food and high nutritional source compared to other studied diets for adults. Almost 50% of the adult beetles fed on natural prey lived for >5 months under studied temperatures. Both sexes survived for longer times on natural prey under ambient conditions than constant temperatures. Atlihan and Özgökçe (2002) found highest survival in *Exochomus nigromaculatus* at

25°C and lowest at 35°C. Present study suggests that beetle can survive alone on single prey (scale insect) in the field even when no other preys such as aphids are available. Results also revealed one of the important features for a successful predator is to live for long period on the prey species and also not feed on the plant in the prey absence (Al-Zyoud *et al*., 2006).

In addition to the above explanations, for the establishment of adult *E. quadripustulatus* in apple orchards from one continent to the other, during transportation/shipment, honey solution or infested spurs with scale insect (especially when gravid females are present) could be a good source of keeping the adult beetles alive.

The influence of altering environmental conditions, particularly temperature, on adult *E. quadripustulatus* was apparent and resulted in more food consumption than when previously kept under same conditions. This increase in feeding reflects adaptability of the species under varying field conditions. Thus the increase in feeding rate is apparently an important means of response to climate change. The evidence of variation in food consumption between both sexes emerged that male and female ate more food under the decreasing temperature regime than when it was increasing. This indicates that the change in environment did not adversely affect the performance of any particular sex while both the sexes showed positive response to feeding. The difference in feeding between both sexes is similar to the fact that generally females eat more than the males.

Despite the fact that these less active beetles were collected in early spring from the field conditions and kept under modified environments they showed the capability of adapting to changed environmental conditions. This indicates that *E. quadripustulatus*, if shifted to different environment (e.g; from colder regions to warmer areas), would probably response with behavioural change to acclimatize. Precht *et al*. (1973) cited that physiological adaptation is part of the ability of the organism to maintain homeostasis or stability under more extreme environmental conditions. Adaptation may enhance the ability to survive and to be favourable for the well being of the organism; however, such adaptation may or may not be favourable. Gawande (1966) found that alternation with low temperature (from 26-27°C to 10°C) stimulated the feeding rate in the Coccinellid *Chilomenes sexmaculata* F. larvae when exposed for longer time (8 and 16h) than shorter period (1 and 4h) for adults. Hattingh and Samways (1994) while studying the physiological and behavioural aspects of citrus scale predators *Chilocorus* spp., observed reduced feeding and oviposition rate under fluctuating temperatures (15-25°C) than constant temperatures (3-38°C). These authors from their own findings could not get the reflection of studied species to climatic adaptations except that the species survived under high temperatures. Under the present experimental conditions it is not clear whether this is behavioural or physiological response of *E. quadripustulatus* to feeding rate but excessive feeding under high compared to low temperatures reflects the potential adaptability of the subject species.

Collectively, present data suggest that in field conditions if there is any gradual change in environmental conditions, particularly increase or decrease in temperature, the beetles may be able to increase predation activities in a more pronounced fashion. As the beetle does not hibernate completely, predation activities could be commenced as and when changes take place because the availability of prey (particularly gravid female scales) would not be a hindrance for scale feeding species.

Results on diapause termination of female adults of *E. quadripustulatus* appeared as an unsuccessful attempt. Females kept in the cold from September 1994 until December 1994 and January 1995 then transferred to warm conditions, did not lay eggs even when exposed to warm conditions for 50 to 80 days. In a longevity experiment, when newly emerged females were kept under constant temperature of 15 and 20°C for a period of ±5 months, no egg was collected. This phenomenon is unclear under the present experimental conditions and thus no conclusion could be drawn except that few individuals succeeded to survive for longer times which were previously kept under low temperatures before transferring them to warm conditions.

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