

Pirimicarb, an aphid selective insecticide, adversely affects demographic parameters of the aphid predator *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)

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Abstract: Demographic toxicology is recommended for toxicity determination of the long term effects of a pesticide since it gives a more accurate and efficient measure of the effect of a pesticide. Thus, in the current study the sublethal effects of pirimicarb (carbamate insecticide) two concentrations of LC₃₀ and LC₁₀ were used against third instar larvae of *Hippodamia variegata* (Goeze) in order to determine the effects of the pesticide on demographic parameters of the predator under laboratory conditions. Results showed that pirimicarb did not affect individual life parameters such as development time of larva, pupa, adult longevity, female and male longevity, adult preoviposition period (APOP), and total preoviposition period (TPOP). However, population parameters such as intrinsic rate of increase (r), net reproductive rate (R_0), mean generation time (T), and finite rate of increase (λ) was affected by sublethal treatment. For example, intrinsic rate of increase (r) was 0.18 day⁻¹ in the controls but it was 0.13 and 0.14 day⁻¹ in the treated insects with LC₁₀ and LC₃₀ concentrations, respectively. Also, there were significant differences between mean generation time (T) of the treatments and the controls i.e. mean generation time of the controls was 29.03 days while mean generation time in the two treatments of LC₁₀ and LC₃₀ was 33.93 and 31.66 days, respectively. The finite rate of increase was also significantly affected by sublethal effects of the pesticide. The results showed that pirimicarb, even at low concentrations, has potential to adversely affect the predatory ladybird, therefore care should be taken when this insecticide is used in the Integrated Pest Management (IPM) program.

Key words: carbamate, ladybird, life table, sublethal concentration

Introduction

The predatory ladybird, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), was originally from Palearctic regions (Gordon 1987), but nowadays it is a well-known predator of aphids in many parts of the world (Franzmann 2002; Kontodimas and Stathas 2005). *Hippodamia* spp. has a great capacity for feeding on aphids, thus, it is an effective predator of aphids (Kontodimas and Stathas 2005).

Generally, aphids and other insect species are attacked by various insect or non-insect species which act as predators and parasitoids that suppress their populations. However, the control of these pests usually depends on chemical pesticides. Application of the pesticide in the IPM program could lead to problems of insect resistance, environmental and food contamination, and pest resurgences (Youn *et al.* 2003; Garrat and Kennedy 2006). Insecticides can also affect natural enemies by death (lethal effect) or acute toxicity or sublethal effects on biological attributes such as development and reproduction (Qi *et al.* 2001; Provost *et al.* 2003; Galvan *et al.* 2005). Therefore, it is necessary to evaluate the effects of insecticides on natural enemies as well as on the pest itself in order to

have a better understanding of the effects of the chemicals on the biological components of the system.

Some compatibility exists between chemical and biological control since the application of pesticides affects both pest and its natural enemy. In this context, the use of non-selective insecticides is not recommended since these compounds often lead to aphid resurgence because of their high fecundity rate (Borgemeister and Poehling 1989). Selective insecticides are needed for the control of the pest in order to avoid side effects on the pest's natural enemies in the Integrated Pest Management (IPM) programs. Regarding aphid control of the pesticides available, pirimicarb has been reported to be the most selective on aphids, and it has limited or no effect on natural aphid enemies such as parasitoides and lady beetles (Unal and Jespon 1991; Oakley *et al.* 1996; Rumpf *et al.* 1998; Jansen 2000; Rahmani *et al.* 2016).

Traditionally, acute toxicity of insecticides on beneficial insects has been considered to be a lethal dose. However, a lethal dose may not reflect the overall deleterious effects of an insecticide due to sublethal effects on the insect physiology and behavior (Desneux *et al.* 2007). So, by

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life table analysis, it is possible to understand the overall toxicity of pesticides on beneficial organisms more accurately (Kim *et al.* 2004). Side effects of several insecticides on pests and their natural enemies already have been evaluated (Stark *et al.* 2003; Stark *et al.* 2007; Desneux *et al.* 2007). However, studies regarding *H. variegata* have focused mainly on biological characteristics (Fan *et al.* 1995; An *et al.* 2000; Jafari 2011), functional responses (Fan and Zhao 1988; Feng *et al.* 2000; Pang *et al.* 2000), life tables (Lanzoni *et al.* 2004; Kontodimas and Stathas 2005), seasonal dynamics (Soleimani and Madadi 2015), the influence of temperature on its development (Michels and Bateman 1986; Michels and Flanders 1992) and only recently the effects of pesticides (Cong *et al.* 2008; Rahmani *et al.* 2013; Rahmani and Bandani 2013; Megha *et al.* 2015). So, the aim of the current study was to evaluate lethal and sublethal effects of pirimicarb (a carbamate insecticide) on *H. variegata*.

Materials and Methods

Insect rearing

A colony of *H. variegata* was obtained from the laboratory of Insect Ecology in the Department of Plant Protection, University of Tehran. This colony was maintained and reared on *Aphis fabae* Scopoli (Hemiptera: Aphididae) in a growth chamber at 27±1°C, 70±10% relative humidity (RH), and a photoperiod of 16 : 8 h (L : D) for several generations before being used for the assay (Atlihan and Chi 2008).

To maintain genetic variability, every six months a number of *H. variegata* adults were collected from the field and introduced to the stock colony. Aphids were reared on potted broad bean plants, *Faba vulgaris* (Fabaceae), at 22±1°C, 70±10% RH, and a photoperiod of 16 : 8 h (L : D).

Insecticide

The insecticide used in this experiment was pirimicarb (commercial formulation, Pirimor® WP 50%).

Laboratory bioassay

Toxicity of the insecticide was assessed on the third instar of *H. variegata* larvae, using the contact method. The larvae were obtained from 6-hours old cohort eggs.

After determining the concentration range based on preliminary experiments, 1 µl of each solution was applied on the beetle's dorsal abdomen using a micropipette. Beetles in the control groups were treated with acetone. To reduce locomotion activity during applications, the larvae were maintained at 4°C for 5 min prior to treatment. Six concentrations of the insecticide: 1,600; 2,512; 4,466; 7,943; 14,388 and 25,600 mg (a.i.) · l⁻¹ were prepared to treat the insects. For each concentration (treatment), 74 insects were used. Treated insects in groups of 4 or 5 individuals were put in Petri dishes (60 mm diam., 10 mm height) and sufficient *A. fabae* (i.e. the desirable number of aphids applied as preys during 24 h) were placed in the Petri dish in order to provide food for the beetles. Mortality was assessed 24 h after the treatment.

Effects of sublethal concentrations on biological parameters and life history data

Four cohorts of about 100 eggs (0–6-hours old) were selected from the lady beetle laboratory colony and placed into Petri dishes (90 mm diam.) based on Schneider *et al.* (2009). The experiment had four treatments including LC₁₀, LC₃₀ and two controls (acetone and no treatment). Each egg was considered as one replicate (Chi and Yang 2003; Schneider *et al.* 2009).

Eggs were kept in a growth chamber at 27±1°C, 65±10% RH, and a photoperiod of 16 : 8 h (L : D). The eggs were checked every 6 h and newly emerged larvae were transferred to new Petri dishes (60 mm diam.). Petri dishes were kept in the incubator and were supplied daily by enough *A. fabae* of all stages as food sources.

When larvae became the third instar (0–12-hours old), they were treated with the insecticide using topical application with two sublethal concentrations of the insecticide that was 652 and 1,522 mg (a.i.) · l⁻¹ for LC₁₀ and LC₃₀ respectively. Third instar larvae (L₃) were chosen because high natural mortality occurred when first and second instar larvae are used (Booth *et al.* 2007) and third instar larvae are the first instar with high voracity (Schneider *et al.* 2009). Larval mortality and development were checked every 12-hours until the adult stage. After the emergence of adults, males and females were paired and checked daily in order to record their survival and their oviposition. The experiments continued until the death of all the individuals.

The life table parameters including intrinsic rate of increase (r), net reproductive rate (R_0), mean generation time (T), gross reproductive rate (GRR), and finite rate of increase (λ) were estimated.

In addition to development time, age-stage specific survival rates (s_{xj}), age-stage specific fecundity (f_{xj}), life expectancy (e_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), age specific maternity ($l_x m_x$), reproductive value (v_{xj}), preoviposition period of female adult (APOP), and total preoviposition period of females counted from birth (TPOP) were calculated.

Data analyses

In the toxicity test, concentration-mortality regression for the larvae was evaluated using probit analysis (Polo-PC; LeOra Software 1997) in order to determine the LCs. Differences in toxicity were considered significant when 95% fiducial limit (FL) did not overlap (Adams *et al.* 1990).

Data on *H. variegata* life table parameters were analyzed according to the age-stage, two-sex life table theory (Chi and Liu 1985) and the method described by Chi (1988) using the computer program TWOSEX-MSChart (Chi 2012).

The population parameters of each cohort were estimated as follows:

– net reproductive rate (R_0):

$$R_0 = \sum l_x m_x$$

– intrinsic rate of increase (r):

$$1 = \sum e^{-r(x+1)} l_x m_x$$

– mean generation time (T):

$$T = \ln R_0 / r,$$

– gross reproductive rate (GRR):

$$GRR = \sum m_x$$

– finite rate of increase (λ):

$$\lambda = e^r.$$

where: $l_x m_x$ – age specific maternity; m_x – age specific fecundity

The intrinsic rate of increase was estimated using the iterative bisection method from the Euler-Lotka equation [$1 = \sum e^{-r(x+1)} l_x m_x$] with age indexed from 0 (Goodman 1982).

The age-stage life expectancy (e_{xj} , where x = age and j = stage) was calculated according to Chi and Su (2006).

Analysis of ANOVA (SAS PROC GLM) (SAS Institute Inc. 2003) and comparison of means using Fisher least significant difference (Duncan) were conducted for determining the differences in life history traits among *H. variegata* exposed and unexposed to chemicals (SAS Institute Inc. 2003). The significance level was $p < 0.05$.

The means and standard errors of the life table parameters were estimated using the bootstrap techniques (Efron and Tibshirani 1993) embedded in the TWSEX-MSChart (Chi 2012).

Survival, fecundity and reproductive value curves were constructed using SigmaPlot 11.0.

Results

Bioassays and determination of lethal concentrations

Concentration-response bioassay showed that LC_{10} , LC_{30} and LC_{50} values for the third instar larvae were 652.138 (21.41–1515.20), 1522.869 (294.50–2752.51) and 2740.073 (880.03–4615.21) mg (a.i.) · ml⁻¹, respectively (Table 1).

Effects of sublethal concentrations on biological parameters and life history data

Pirimicarb significantly affects all the population parameters tested except GRR (Table 2).

The effect of pirimicarb on the r was 0.18 day⁻¹ in the controls but it was 0.13 and 0.14 day⁻¹ in the treated insects with LC_{10} and LC_{30} doses, respectively (Table 2). There were no significant differences between two treatments i.e. LC_{10} and LC_{30} , but the two treatments were significantly different from the controls. The same trend was seen in the λ , with significant differences between controls and treatments. The finite rate of increase in controls was about 1.20 day⁻¹ while it was 1.14 for LC_{10} and 1.16 for LC_{30} . However, there were no significant differences between the two treatments (LC_{10} and LC_{30}). There was no significant difference ($p < 0.05$) in GRR (Table 2).

As shown in Table 3, none of the life history parameters were affected by LC_{10} and LC_{30} treatments. Duration of the third instar larvae lasted 2.19, 2.20 and 2.01 days in control, LC_{10} and LC_{30} treatments, respectively ($df = 164$, $F = 1.06$, $p = 0.36$). Duration of the fourth instar larvae was evaluated as 3.16, 3.60 and 3.61 days in control, LC_{10} and LC_{30} treatments, respectively ($df = 172$, $F = 2.96$, $p = 0.07$). Development time of pupation in the controls,

Table 1. Toxicity of pirimicarb on third instar larvae of *Hippodamia variegata* within 24 h

Insecticide	No. of subjects	Concentration [mg (a.i.) · l ⁻¹ (95% CI) ⁻¹]			Slope±SE	X ² (df)
		LC ₁₀	LC ₃₀	LC ₅₀		
Pirimicarb	518	652.138 (21.41–1515.20)	1522.869 (294.50–2752.51)	2740.073 (880.03–4615.21)	2.056±0.201	75.415(34)

Table 2. Effects of sublethal concentration of pirimicarb on population parameters (mean±SE) of *Hippodamia variegata*

Population parameter	Control	Solvent	LC ₁₀	LC ₃₀
Intrinsic rate of increase (r) [day ⁻¹]	0.18±0.007 a	0.18±0.008 a	0.13±0.012 b	0.14±0.010 b
Net reproductive rate (R_0) [offspring/individual]	232.49±39.44 a	222.51±42.90 a	101.62±30.43 b	117.42±30.72 b
Gross reproductive rate (GRR) [offspring/individual]	442.48±68.63	534.41±87.82	403.32±105.43	354.61±89.42
Mean generation time (T) [day]	29.03±0.49 b	29.13±0.72 b	33.93±1.62 a	31.66±1.03 ab
Finite rate of increase (λ) [day ⁻¹]	1.20±0.00 a	1.20±0.01 a	1.14±0.01 b	1.16±0.012 b

Means in the same row followed by the same letter are not significantly different ($p > 0.05$) using the Tukey-Kramer procedure

Table 3. Life history parameters (mean±SE) of *Hippodamia variegata* treated with sublethal concentration of pirimicarb

Developmental time [days]	Control	Solvent	LC ₁₀	LC ₃₀	p	F	df
Larvae 3	2.19±0.08	2.16±0.06	2.20±0.10	2.01±0.09	0.36	1.06	164
Larvae 4	3.16±0.07	3.68±0.27	3.60±0.107	3.61±0.107	0.074	2.96	172
Pupa	3.17±0.38	3.14±0.35	2.97±0.33	3.15±0.37	0.07	0.30	169
Adult all	62.810±2.43	57.510±2.75	64.107±3.71	61.026±4.04	0.47	0.84	169
Female	60.87±16.47	56.86±22.10	56.46±20.35	53.82±27.38	0.72	0.44	86
Male	64.90±20.57	58.52±14.07	70.73±16.97	66.85±21.60	0.29	1.26	79
APOP	3.53±0.15	6.70±1.96	7.23±3.18	5.53±2.29	0.45	0.89	86
TPOP	18.03±0.18	22.10±2.01	22.92±3.21	21.06±2.33	0.23	1.47	86
Fecundity [individual]	709.3±59.70	642.1±77.99	740.7±118.21	583.5±86.08	0.61	0.60	86

APOP – adult preoviposition period; TPOP – total preoviposition period

Means in a row followed by different letters are significantly different ($p < 0.05$) (Duncan)

LC₁₀ and LC₃₀ treatments, was 3.17, 2.97 and 3.15 days, respectively ($df = 169$, $F = 0.30$, $p = 0.07$). Female longevity in the controls was 60.87 days. This parameter in LC₁₀ and LC₃₀ treatments, was measured as 56.46 and 53.82 days, respectively ($df = 86$, $F = 0.44$, $p = 0.72$). Development time of males was longer than of females. Male longevity in the controls, LC₁₀ and LC₃₀ treatments, was 64.90, 70.73 and 66.85 days, respectively ($df = 79$, $F = 1.26$, $p = 0.29$). The length of the preoviposition period in the controls, LC₁₀ and LC₃₀ treatments, was 3.53, 7.23 and 5.53 days, respectively ($df = 86$, $F = 0.89$, $p = 0.45$). Fecundity of the females in the controls was 709.3 eggs. This parameter in LC₁₀ and LC₃₀ treatments, was 740.7 and 583.5 eggs, respectively ($df = 86$, $F = 0.60$, $p = 0.61$) (Table 3). Moreover, the biological parameters of the acetone treated individuals did not show any significant differences in comparison with the other treatments (Table 3).

In the relative number alive (s_{xj}), i.e. age-stage survival rate which gives the probability that a new born egg will survive to age x and stage j overlapping occurs between stages (Fig. 1). In both LC₁₀ and LC₃₀ treatments, the relative number of male and female adults during their life declined in comparison with control and acetone treated individuals. Thus, the amount of decline in LC₁₀ was more than in LC₃₀ treatment almost all stages i.e. the individuals treated with LC₁₀ were more affected than LC₃₀ (Fig. 1C and D). The adult longevity in LC₁₀ treatments in both females and males was shorter than adult longevity in LC₃₀.

Values for the l_x of total cohort, female f_{xj} , m_x of the total population and $l_x m_x$ are presented in Figure 2. Fecundity decreased with time although numerous peaks were observed. In controls, the survival rate was almost 63% at day 14. However, in two treatments including LC₁₀ and LC₃₀ the survival rate was 29 and 45%, respectively (Fig. 2C and D). Data extracted from Figure 2 showed

that in the controls the first peak f_{xj} , m_x and $l_x m_x$ at day 19 were 22.13, 11.64, and 7.21, respectively while these data for LC₁₀ at day 20 were 15.46, 7.17, and 2.11, respectively. These data for LC₃₀ were the same as the LC₁₀ treatment (Fig. 2D). Fecundity peaks were high in the controls at days 20–40 while those in LC₁₀ treatment were high around day 60.

The life expectancy curve (e_{xj}) shows the total time that an individual of age x and stage j is expected to live (Fig. 3). In the controls, when a female individual reached the adult stage, life expectancy increased to 60 days. This value for the female adults in acetone treatment was 58 days and in insecticide treatments in both concentrations, about 56 days.

The reproductive value (v_{xj}) is the expectation of future offspring of individuals of age x and stage j (Fisher 1930; Pianka 1994) (Fig. 4). If the preoviposition period is counted as time from birth to first reproduction in females (TPOP) (Amir-Maafi and Chi 2006) the mean TPOP for control treated females was 18.03 days and it was similar to their first peak of reproduction which was in day 18. However, TPOP in LC₁₀ and LC₃₀ treated individuals was 22.92 and 21.06 days, respectively ($df = 86$, $F = 1.47$, $p = 0.23$) while their first peak of reproduction was in day 19 and 20, respectively (Table 3).

Discussion

In this study, sublethal effects of pirimicarb (carbamate: 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate), a selective systemic insecticide with stomach and respiratory action against aphids was studied in order to determine its long term effects on population numbers, longevity, reproduction and other demographic parameters beneficial for the insect, the predatory ladybird (*H. variegata*). In fact, during integrated pest management

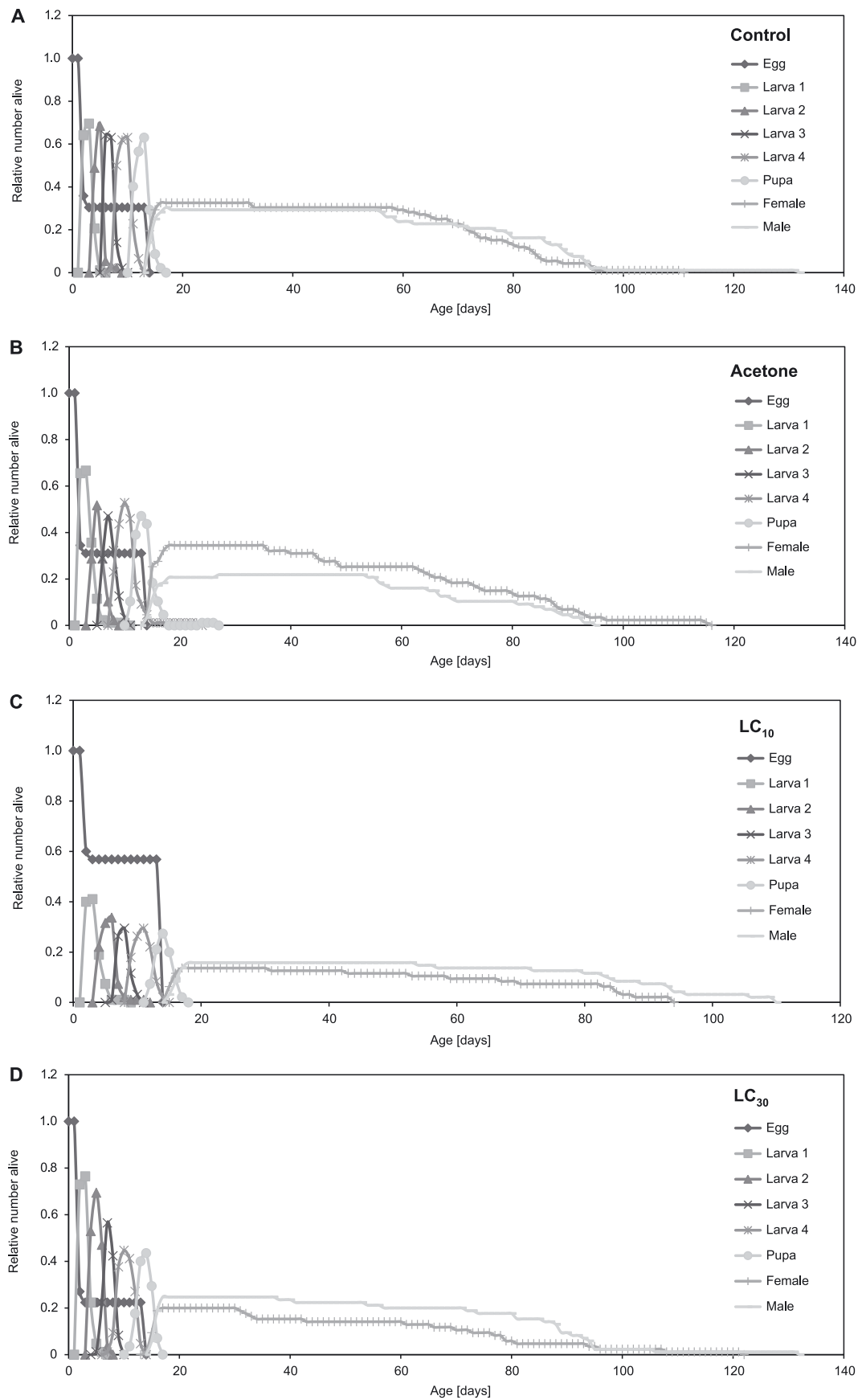


Fig. 1. Relative number alive in each age-stage group (s_{xj}) of *Hippodamia variegata* in: control (A), acetone treated (B), LC_{10} treated (C) and LC_{30} treated (D) individuals

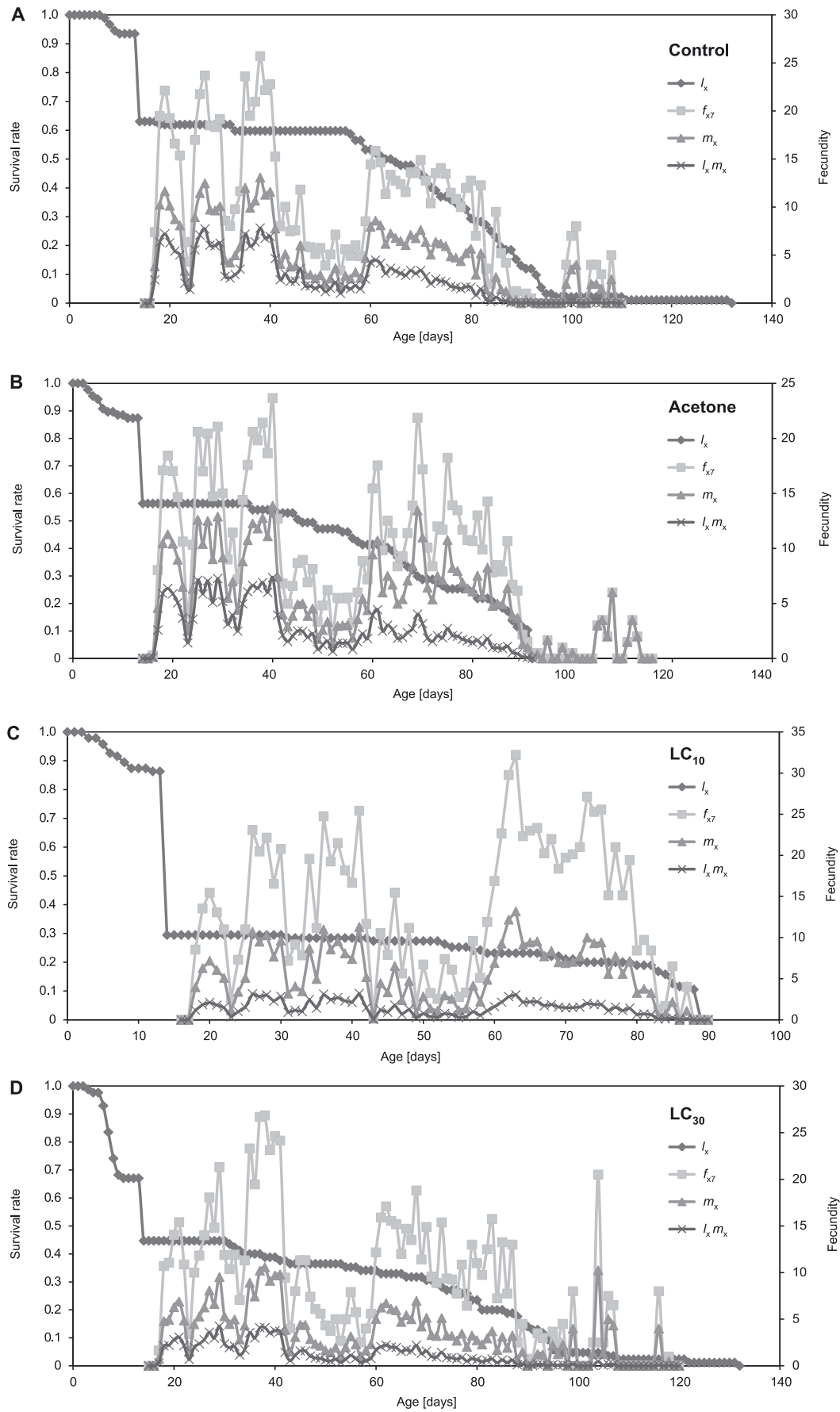


Fig. 2. Age-specific survival rate (l_x), female age-stage specific fecundity (f_{xT}), age-specific fecundity (m_x), and age specific maternity ($l_x m_x$) of *Hippodamia variegata* in: control (A), acetone treated (B), LC₁₀ treated (C) and LC₃₀ treated (D) individuals

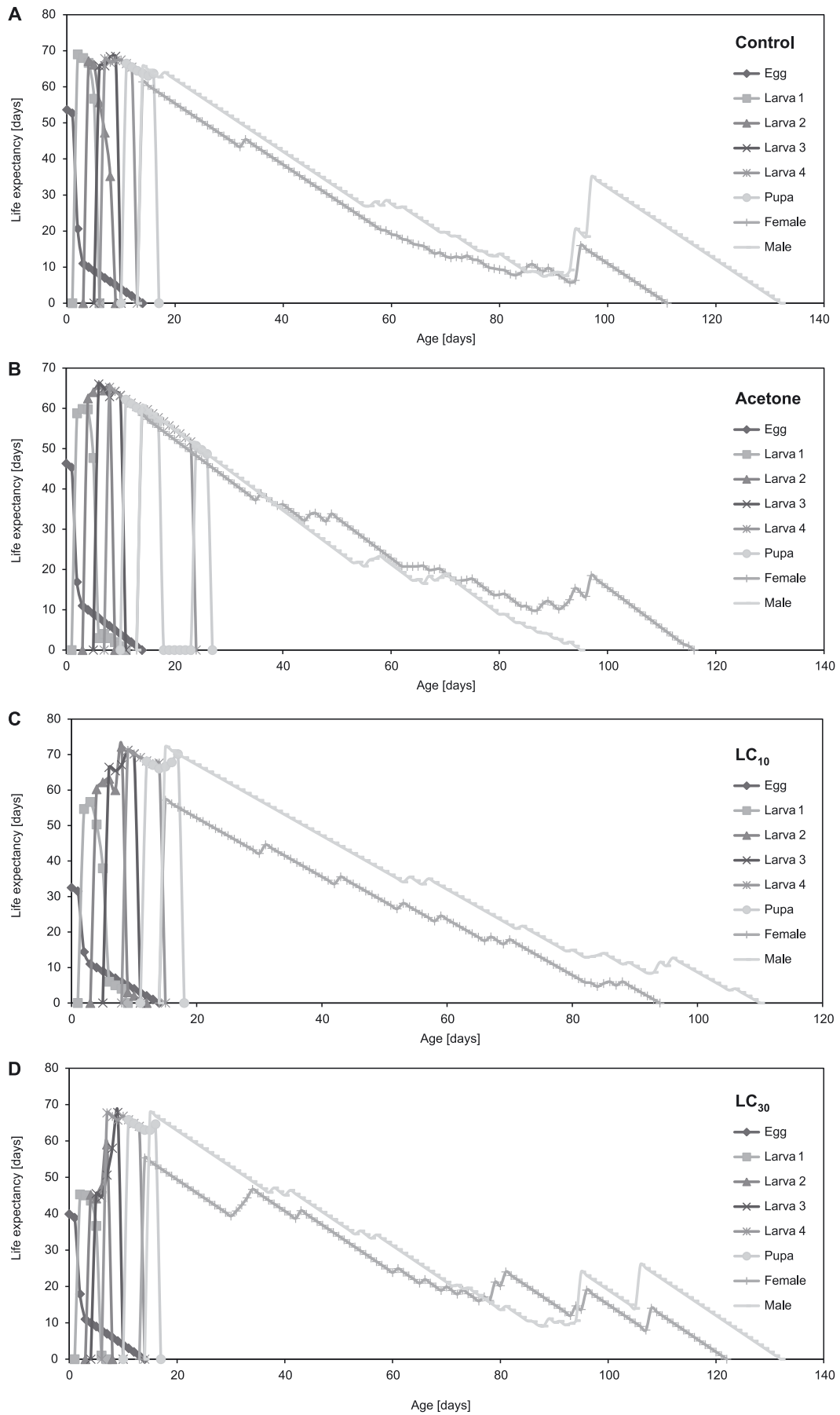


Fig. 3. Age-stage-specific life expectancy (e_{xj}) of *Hippodamia variegata* in: control (A) acetone treated (B), LC₁₀ treated (C) and LC₃₀ treated (D) individuals

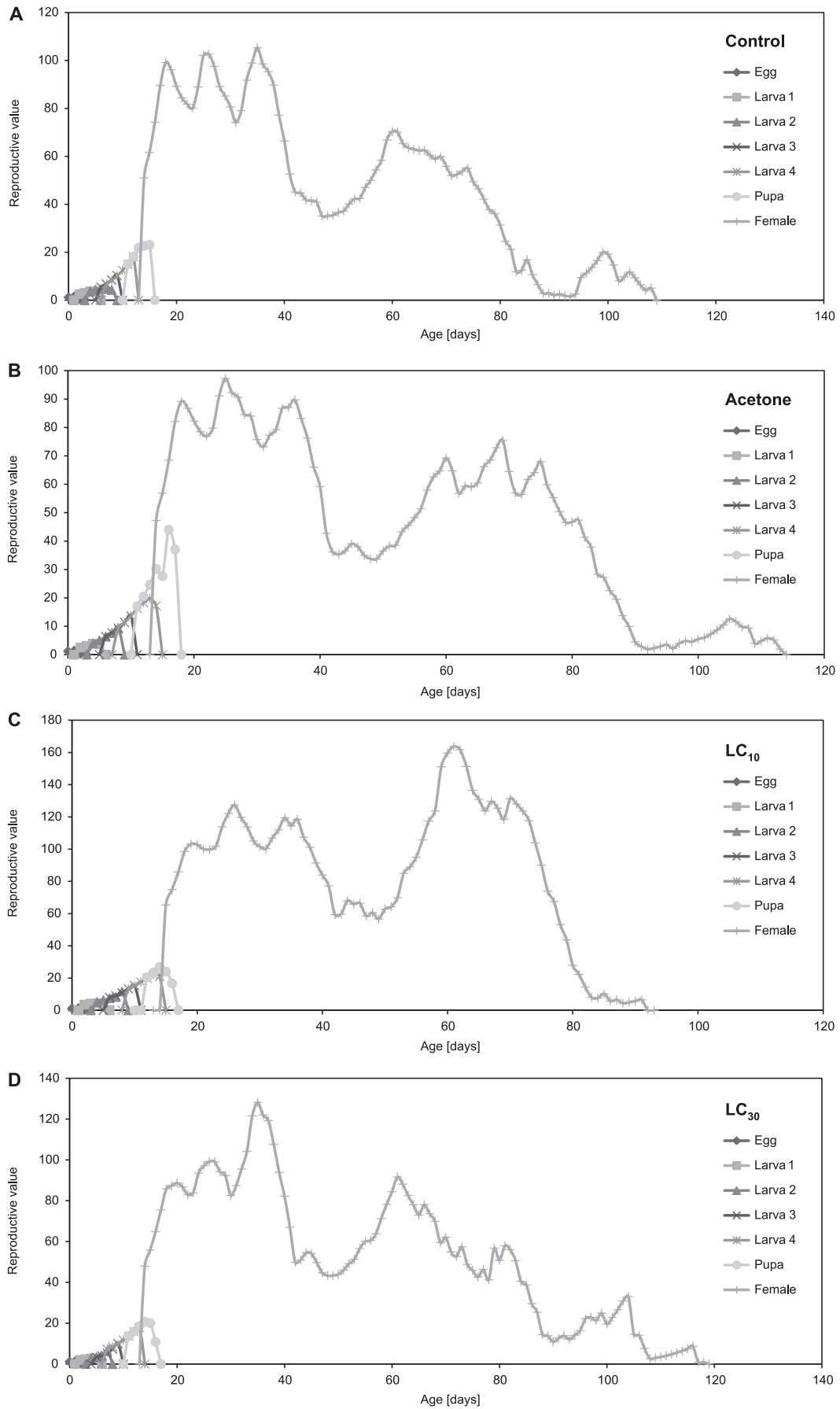


Fig. 4. Age-stage specific reproductive value (v_{xj}) of *Hippodamia variegata* in: control (A), acetone treated (B), LC₁₀ treated (C) and LC₃₀ treated (D) individuals

programs, selective pesticides with low adverse effects on predators and parasitoids will be recognized and used together with other control methods such as biological control. Although, the estimated LC_{50} against the *H. variegata* was much more than the recommended dose in the field against the aphid, this insecticide has been found not to be safe for beneficial insects (Sterk *et al.* 1999; Dimetry and Marei 1992; Jansen 2000; Kennedy *et al.* 2001; James 2003). In this study, the lethal dosage caused 50% mortality in the ladybird, and was almost 10 times higher than the dose recommended for field usage. Due to its selectivity for aphids, this insecticide should not have acute toxicity toward the predatory beetle. In congruous with our results when the direct spray technique was used, James (2003) also found that pirimicarb could not produce 100% mortality in *Stethorus punctum-picipes picipes* (Casey) (Coleoptera, Coccinellidae) at the concentration recommended for field usage [220 mg (a.i.) · l⁻¹]. Cornale *et al.* (1996) found that pirimicarb at 25% concentration of that recommended for the field was safe for beneficial insects while it effectively controlled aphids.

The results confirmed the hypothesis that pirimicarb, although categorised as a safe insecticide against beneficial insects, in low concentrations (sublethal effects of LC_{10} and LC_{30}) could affect population performance of the *H. variegata*. The greatest effect was seen on the r which was 0.18 day⁻¹ in the controls but it was 0.13 and 0.14 day⁻¹ in the insects treated with LC_{10} and LC_{30} concentrations, respectively. Interestingly, there was no significant difference between the two treatments, i.e. LC_{10} and LC_{30} .

Sublethal effects of pirimicarb, in addition to the r , affect the λ , R_0 , and T of the predatory ladybird (*H. variegata*). Pirimicarb at sublethal concentrations, decreases population growth and at the same time it increases generation time. Thus in the long run it affects the population structure of the insect (Southwood 1981; Price 1997).

However, pirimicarb sublethal concentrations (LC_{10} and LC_{30}) did not affect the development time of third and fourth instar larvae, pupa, and adult, APOP, TPOP, and fecundity. This shows that its effects on individual life parameters are negligible. The relative number alive (s_{xj}) declined in both treatments in comparison with the controls. Interestingly, the decline in LC_{10} was greater than LC_{30} treatment which shows that when the insects are exposed to lower concentrations of the insecticide it may not induce the insect detoxifying enzymes, thus the insecticide exerts its deleterious effect in the long run. The same explanation probably is true for the effect of LC_{10} treatment on adult longevity.

Values of l_x , f_{x7} , m_x and $l_x m_x$ are affected by sublethal effects of pirimicarb, and again survival rate and fecundity was affected more by LC_{10} than LC_{30} . So, the current results showed that the use of demographic toxicology data is needed for the determination of the long term effects of pesticides (Forbes and Calow 1999; Cole *et al.* 2010).

The results of this study agree with the findings of Cole *et al.* (2010) who tested the impact of six selective insecticides on three predatory insect species, Tasman's lacewing [*Micromus tasmaniae* (Walker)], the transverse ladybird (*Coccinella transversalis* Fab.) and the damsel

bug (*Nabis kinbergii* Reuter). They found that pirimicarb adversely affected reproduction of *C. transversalis* even though only minor effects were observed in short term assays. However, Cabral *et al.* (2008) showed that pirimicarb at the dose recommended by the manufacturers for the control of aphids has no significant effect on the survival of *Coccinella undecimpunctata* L. Nevertheless, there are other studies that indicate adverse effects of pirimicarb on beneficial insects including adults and larvae of coccinellids, *Chrysopa* sp. (Neuroptera: Chrysopidae), *Nabis* sp. (Hemiptera: Nabidae), *Orius* sp. (Hemiptera: Anthocoridae), *Aphidius smithi* Sharma and Subba Rao (Hymen: Aphidiidae), and *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Aphidiidae) (Summers *et al.* 1975; Umoru and Powell 2010).

The results in this study showed that pirimicarb, even at sublethal concentrations has the potential to adversely affect *H. variegata*. Therefore, more care should be taken when this insecticide is used in the IPM program for aphid control.

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