UvA-DARE (Digital Academic Repository)

Effect of temperature on the termination of diapause in the univoltine almond seed wasp *Eurytoma amygdali*.

Tzanakakis, M.E.; Veerman, A.

DOI

[10.1111/j.1570-7458.1994.tb01756.x](https://doi.org/10.1111/j.1570-7458.1994.tb01756.x)

Publication date

1994

Published in

Entomologia Experimentalis et Applicata

[Link to publication](#)

Citation for published version (APA):

Tzanakakis, M. E., & Veerman, A. (1994). Effect of temperature on the termination of diapause in the univoltine almond seed wasp *Eurytoma amygdali*. *Entomologia Experimentalis et Applicata*, 70, 27-39. <https://doi.org/10.1111/j.1570-7458.1994.tb01756.x>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Effect of temperature on the termination of diapause in the univoltine almond seed wasp *Eurytoma amygdali*

M. E. Tzanakakis¹ & A. Veerman

Department of Pure and Applied Ecology, University of Amsterdam, Kruislaan 302, 1098 SM Amsterdam, The Netherlands ¹ Current address: Laboratory of Applied Zoology & Parasitology, University of Thessaloniki, 540 06, Greece

Accepted: June 3, 1993

Key words: almond seed wasp, *Eurytoma amygdali*, diapause termination, insect dormancy, temperature, prolonged diapause, photoperiod, Hymenoptera

Abstract

Diapausing larvae of *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae) were collected in early August and late September. They were subjected to various photoperiod and temperature regimens for up to 20 weeks, then kept at L16:D8 and 19 °C for another 14 to 26 weeks for diapause to be terminated and pupation to take place.

Photoperiod did not affect diapause completion. It was confirmed that the two morphologically distinct diapause stages have different temperature requirements for their completion. The first diapause stage was completed synchronously at temperatures between 16 and 19 °C. A higher temperature of 26 °C delayed diapause development. The second stage required lower temperatures between 4 and 10 °C. Spontaneous termination of diapause was observed at constant 19 °C.

When applied to the first diapause stage for 20 weeks, low temperatures made the larvae refractory to subsequent intermediate temperatures. The first stage was thus maintained until a higher temperature of 26 °C made the larvae regain their ability to respond to the intermediate temperatures and complete this stage. Larvae grown in Retsou almonds had a higher diapause intensity than larvae grown in Truoto almonds.

The results suggest that, in nature, the high temperatures of late summer and early autumn are likely to maintain the first diapause stage. Subsequently, the less warm temperatures of autumn allow the completion of the first stage by late autumn, and the low temperatures of late autumn and of winter allow the completion of the second diapause stage by mid winter.

Introduction

The almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae), has been reported as a pest of almonds in a number of countries of southeastern Europe and of the

Middle East and recently in France, Armenia, Azerbaijan and Georgia (Zerova & Fursov, 1991 and references therein). It is a univoltine species, with part of the population completing its life cycle in two years or, according to certain authors, in three or even four years because of prolonged

diapause. The egg is deposited within the seed. The larva feeds on the embryo, and upon completion of its growth sometime in midsummer, it enters diapause within the usually intact seed integument. For references on the biology and seasonal development see Zerova and Fursov (1991) and Tzanakakis *et al.* (1991).

In Israel, Plaut (1972) found diapause terminated in January. He observed that during the early part of diapause the larva is of dull grey colour, and during the later part it is white. This conspicuous change in colour is caused by massive defecation, which in Israel took place mainly in November.

In the area of Thessaloniki of coastal northern Greece, Tzanakakis *et al.* (1991) found the first diapause stage to be completed some time between late September and late November, and diapause to end some time between late December and late January. In the laboratory, the first stage was completed and terminated in over 50% of the larvae within 8 to 12 weeks at a thermoperiod of 19 ± 1 °C and a long or a short photophase, while the second stage required considerably lower temperatures. Therefore, the two morphologically distinct diapause stages have different thermal optima for their completion and/or termination.

The work reported in the present paper aimed at determining in detail the photoperiod and temperature requirements for the completion and termination of each of the two diapause stages of *E. amygdali*.

Materials and methods

Larvae of early August. Almonds containing diapause larvae were collected on 1 August 1989 from an orchard near the coast, 10 km from the city of Thessaloniki. The almonds were of two varieties: Retsou, a local soft-shell variety, and Truoito, a hard-shell variety. They were brought to the laboratory, surface-treated momentarily with hot water to kill undesirable arthropods, allowed to dry at room temperature and flown to Amsterdam. They were placed in shallow

20 × 15 × 3 cm trays of semitransparent plastic inside incubators with temperature and photoperiod control. To minimize differences in illumination and temperature, there were only two layers of almonds in each tray which were mixed once a week and the trays were moved around once a week. Light from neon tubes was, at the level of the almonds or of the exposed larvae, from 700 to 1200 lux.

Those early-August larvae had completed growth and were at the beginning of their first diapause stage (Pittara & Katsoyannos, 1985; Tzanakakis *et al.*, 1991). They were kept for a total of four weeks at high temperatures with long days, namely one week at room temperature in Thessaloniki where daily temperatures varied between 20 and 30 °C, then for three weeks at L16:D8 and 26 °C in Amsterdam. The purpose of those four weeks at high temperatures was to allow for a possible intensification of diapause, and to offer the larvae high temperatures similar to those they are exposed to in nature (August mean monthly 24.8 °C), before subjecting them to conditions similar to those in autumn and winter. Subsequently, the almonds were exposed for up to 20 weeks to various photoperiod and temperature regimens applied in a sequence and for periods similar to those of late summer, autumn and winter in nature. Upon completion of each 20-week treatment, the almonds were split open to record the stage (grey or white) of the insects inside and the larvae were placed individually in 1.5 × 5 cm cylindrical glass vials. Subsequently, they were transferred to L16:D8 and 19 °C which was chosen as the reference condition (R) and their stage (grey larva, white larva, or pupa) was recorded at weekly intervals for 14 or more weeks. A condition similar to R was known to allow post-diapause development in this insect and was used as a reference condition in recent similar work with larvae from the same area (Tzanakakis *et al.*, 1991).

In the treatment marked 'changing' (treatment S, Table 1) the photophase was kept at 14 h in week 1, decreased to 13, 12, 11 and 10 h at the end of weeks 3, 5, 8 and 12, respectively, and then increased again to 11, 12, 13, 14 and 16 at the end

Table 1. Termination of the first and second diapause stages at R (L16:D8 and 19 °C) in larvae of *E. amygdali* from almonds of two varieties, collected in early August and subjected inside the fruits for 20 weeks to various photoperiod and temperature regimens

Photoperiod (L:D) and temperature (°C) during weeks					Number of insects	Almond variety	Percentage terminating		No. of weeks from 20th week to termination of second stage	
<div><div></div><div></div><div></div><div></div><div></div><div></div></div> <div>048121620</div>							First stage	Second stage	50%	Range
A	16:8				30	Re	Oa ¹	Oa	—	—
	10°				30	Tr	3a	3a	—	2–2
B	10:14				45	Re	Oa	Oa	—	—
	10°				36	Tr	Oa	Oa	—	—
C	10:14				27	Re	Oa	Oa	—	—
	4°				23	Tr	9a	Oa	—	—
D	10:14				42	Re	67bc	64b	1	0–1
	16°:6°				31	Tr	84b	84b	0	0–1
E	10:14	10:14			29	Re	55c	55b	2	1–2
	19°	16°:6°			24	Tr	96bf	88b	1	0–3
G	10:14	10:14	10:14	10:14	31	Re	71bc	71b	2	1–6
	19°	16°:6°	19°	16°:6°	27	Tr	85b	78bd	2	0–2
H	10:14	10:14			30	Re	63bc	53b	2	1–2
	19°	10°			24	Tr	88b	75bd	2	1–2
I	10:14	10:14			33	Re	79bc	76b	2	1–3
	19°	4°			24	Tr	88b	79bd	2	2–3
K	12:12	10:14	10:14		27	Re	59bc	37c	—	1–2
	19°	16°:6°	10°		25	Tr	88b	88b	1	1–2
L	12:12	10:14	10:14		28	Re	79bc	64b	1	0–2
	19°	10°	16°:6°		26	Tr	88b	88b	1	1–3

Table 1. Continued

Photoperiod (L:D) and temperature (°C) during weeks					Number of insects	Almond variety	Percentage terminating		No. of weeks from 20th week to termination of second stage
First stage	Second stage	50%	Range
M	12:12	10:14	10:14	R	37	Re	62cd	62b	2	1–3
	19°	16°6°	4°		31	Tr	87b	84b	2	1–2
N	16:8			R	46	Re	72bc	Oa	–	–
	26°				32	Tr	87b	Oa	–	–
R	16:8 (R)				36	Re	83b	14e	–	2–13
	19°				31	Tr	90b	55cd	12	0–14
S	Changing				45	Re	82b	Oa	–	–
	19°				32	Tr	91b	34c	–	0–14 ²
T	10:14			R	44	Re	80bd	9de	–	1–13
	19°				38	Tr	95bf	42c	–	0–14
U	16:8 (R)	10:14	R	42	Re	71bc	12de	–	0–14	
	19°	19°		42	Tr	100f	43c	–	0–13	

¹ Within each column and almond variety (Re or Tr), percentages followed by the same letter do not differ significantly at the 0.05 level.

² No records were kept beyond the 14th week post-treatment (34th week from start of experiment).

of weeks 20, 24, 26, 29 and 31, respectively. This was to offer a photoperiod approaching the natural one from late August through early April.

In the five treatments seen in Table 2, the larvae were taken out of the fruits at the start, thus being exposed in vials throughout the 34 weeks of the experiment. Their responses were compared with those of larvae which were inside the fruits during the first 20 weeks.

The change of the larvae from grey to white was our criterion of the completion of the first, grey, diapause stage. Pupation was used as the crite-

riion for diapause termination, i.e. for the completion of the second, white, diapause stage. Our conclusions concerning the effectiveness of each treatment were based on the time needed for 50% of the larvae to change to the next stage and on the cumulative percentage of larvae that had pupated by the end of the experiment.

Previous work (Tzanakakis *et al.*, 1991) showed that larvae with their diapause terminated, pupate within a week at L16:D8 and 19 ± 1 °C. Consequently, we consider that pupation within a week at R (L16:D8 and 19 °C)

Table 2. Termination of the first and second diapause stages at R (L16:D8 and 19 °C) in larvae of *E. amygdali* which were subjected in glass vials from the beginning of the experiment to treatments B, D, R, S and T seen in Table 1. Fifteen larvae from each almond variety per treatment

Treatment	Almond variety	Percentage terminating		No. of weeks to termination of			
		First stage	Second stage	First stage		Second stage	
				50%	Range	50%	Range
Be	Re	Oa ¹	Oa				
	Tr	Oa	Oa				
De	Re	53bd	47b	18	6–18	19	18–20
	Tr	93ce	93c	9	7–18	18	17–18
Re	Re	93c	Oa	4	4–8		
	Tr	93ce	7a	4	4–4		33–33
Se	Re	93c	Oa	4	4–4		
	Tr	100e	Oa	4	4–16		
Te	Re	67cd	7a	5	5–12		33–33
	Tr	87cde	67bc	4	4–4	25	23–32

¹ Within each column and almond variety (Re or Tr), percentages followed by the same letter do not differ significantly at the 0.05 level.

means that diapause was brought to full completion. Accordingly, pupation within the second week as showing that diapause was almost complete, and pupation after the second week as showing that diapause was not fully completed during the treatment that preceded R.

Larvae of late September. Almonds were collected on 25 September 1989, from an orchard 4 km to the south of the Micra one. They were of the variety Retsou only. After they were surface-treated in the same way as the early-August almonds, they were split open and the seeds, containing the diapausing larvae, were flown to Amsterdam. The larvae were then taken out and placed individually in glass vials. They were subsequently kept for five days at L17:D7 and 25 °C. Each treatment included larvae of all weight categories in equal proportions with any other treatment.

The purpose of the experiment with late-September larvae was to test the effect of the various treatments on larvae in early second-stage diapause. This is why in a number of treatments the larvae were first subjected to L10:D14 and

19 °C for up to 4 weeks. At the end of each week, those larvae changing to white were transferred to the next, second condition seen in Table 3. Thus, the larvae received the second photoperiod and temperature condition within a few days after they entered the second diapause stage. The treatments were of shorter duration than those of early-August larvae, so as not to extend the treatments much beyond the time diapause is terminated in nature in the area. Further details are given in the Results section. Percentages of larvae completing either diapause stage were compared at the 0.05 level using the chi-square test.

Results

Larvae of early August

In the larvae of early August which were inside the almonds during the various 20-week treatments, there was no evidence of any photoperiodic effect on the completion of either diapause stage. Therefore, for the sake of brevity, the photoperiod will often not be mentioned in the

Table 3. Diapause termination in larvae of *E. amygdali* collected from Retsou almonds late in September, 1989, near Thessaloniki and subjected, soon after completion of the first diapause stage, to various photoperiod and temperature regimens. Larvae were exposed in vials from the start of the experiment

Photoperiod (L:D) and temperature (°C) during weeks					Number of insects	Percentage terminating diapause ¹	No. of weeks to diapause termination				
<div><div></div><div></div><div></div><div></div><div></div></div> <div>0481216</div>							Post-treatment ²		From start		
							50%	Range	50%	Range	
A	10:14 19°	10:14 16°:6°	10:14 10°	10:14 4°	R	32	100a ³	2	1–12	18	17–28
B	10:14 19°	10:14 10°	10:14 16°:6°	10:14 4°	R	29	97a	2	2–7	18	18–23
C	10:14 19°	10:14 10°		R		30	60b	4	2–26	16	14–38
D	10:14 19°	10:14 10°		16:8 26°		34	50b	2	1–2	14	13–14
E	10:14 19°	10:14 10°	10:14 4°	R		29	24c		2–12		14–24
G	10:14 19°	10:14 10°	R			33	18c		22–32		30–40
H	10:14 19°	10:14 10°	10:14 19°			30	10c		9–32		17–40
I	10:14 19°		10:14 10°	R		22	23c		2–28		14–40
K	16:8 26°	16:8 19°	16:8 10°	R		31	3d		27–27		39–39
L	0:24 19°			R		31	19c		14–27		26–39

Table 3. Continued

Photoperiod (L:D) and temperature (°C) during weeks			Number of insects	Percentage terminating diapause ¹	No. of weeks to diapause termination				
<div><div></div><div>0</div><div>4</div><div>8</div><div>12</div><div>16</div></div>					Post-treatment ²		From start		
					50%	Range	50%	Range	
M	10:14 19°		R	30	17cd		8–21		20–33
R	16:8 (R) 19°		R	24	17cd		18–28 ²		30–40
N	17:7 25°		R	30	20c		20–28		32–40
O	16:8 26°		R	30	7cd		25–26		37–38
P	10:14 19°	16:8 26°	R	24	8cd		16–24		28–36
Q	16:8 19°	16:8 26°	R	25	8cd		26–27		38–39
CC	10:14 19°	10:14 10°	R	43 ⁴	72e	3	1–6	15	15–18
S	10:14 19°	10:14 10°	10:14 19°	38 ⁴	66be	2	2–9	14	14–21

¹ I.e. pupating by end of 40th week.

² At 19 °C and a long (R) or a short day, or at 26 °C and a long day.

³ Percentages followed by the same letter do not differ significantly at the 0.05 level.

⁴ In this treatment the larvae were inside the fruits during the 1st 12 weeks.

next of this section. It should be remembered that four weeks of long day and high temperature (26 °C) preceded the treatments seen in Tables 1 and 2.

It is seen in Table 1 that neither the first nor the second diapause stage were completed after 20 weeks at 10 °C or 4 °C (treatments A, B, C). In all the other treatments seen in Table 1 the first

diapause stage was completed in 50% of the larvae within the 20 weeks of the treatments. Unpublished data with only 10 larvae per treatment, but also treatments Re, Se and Te of Table 2, showed that, at continuous 19 °C, the first diapause stage in 50% of the larvae was completed by the end of the 4th or 5th week.

After the first diapause stage was completed at 19 °C, further exposure to low temperatures was needed (H, I) for the second diapause stage to be completed synchronously. Without the low temperature (R to U), the completion of the second diapause stage was delayed and spread over many weeks. Furthermore, it occurred in 14% or less of Retsou larvae and 34–55% of Truoito larvae. The need for low temperatures for the synchronous completion of the second diapause stage is also shown in treatments E, G, K, L and M.

A thermoperiod of 16°:6 °C under short photophase (Table 1, D), allowed the completion of both diapause stages within the 20 weeks of this treatment in Truoito larvae, while an additional week at 19 °C was needed for Retsou larvae (see also Table 2, De). Obviously, it was the 16 °C thermophase that allowed diapause development during the first diapause stage, and the 6 °C cryophase (and possibly also the 16 °C thermophase) that did so during the second stage.

When we collected our experimental almonds in early August, we also found 16 mummified Retsou ones which had remained on the trees from the previous year. They contained 16 prolonged-diapause larvae in their first diapause stage. Those larvae, exposed singly in glass vials, were kept at L10:D14 and 19 °C. Over 50% of them completed the first diapause stage by the end of the 4th week, and all but one by the end of the 6th week. This shows that prolonged-diapause larvae, after having spent one year in their first diapause stage in nature, responded to 19 °C in much the same way as did normal-diapause larvae in the year in which they reached full larval growth.

In August larvae from Truoito almonds the completion of either diapause stage occurred earlier and in higher percentages than in larvae from Retsou almonds (Table 1). This suggests

that diapause intensity was higher in Retsou larvae. However, not all differences were significant at the 0.05 level. For example, they were significant for both diapause stages in treatments E, H and T, but not in D and I, and they were significant for the second but not the first stage in treatment R.

In general, exposed larvae (Table 2) responded to treatments B, D and T seen in Table 1 in much the same way as did larvae which remained inside the fruits during the first 20 weeks. On the other hand, most larvae from Truoito almonds subjected to treatments Re and Se did not complete diapause, whereas larvae inside the fruits (Table 1, Re, Se) did to a substantial percentage.

Larvae of late September

The larvae were only from Retsou almonds, collected in late September. The collection date was planned so as to have the larvae been exposed to natural conditions for approximately two months more than the larvae of early August. Therefore, after having experienced in nature the whole month of August with its fairly high temperatures, the larvae, while still on the trees, had three-and-a-half weeks of shorter days and milder temperatures during September. Most treatments included an initial period of up to four weeks at L10:D14 and 19 °C to allow the larvae to complete the first diapause stage. Therefore, most diapause development during the first diapause stage took place under natural conditions and the later part of it at 19 °C. Thus, the photoperiod and temperature regimens were applied to the early second diapause stage. The larvae were in vials throughout the experimental period.

It is seen in Table 3 that 12 weeks of low temperatures (treatments A, B) allowed diapause completion in a high percentage of the larval population and termination was early and synchronous, 50% of the larvae pupating in 2 weeks at R (L16:D8, 19 °C). Exposure to 10 °C for only 8 weeks (C) was less effective, the difference being significant, while 4 weeks at 10 °C followed by 4 weeks at 4 °C (E) were even less effective. Likewise, only 4 weeks at 10 °C (G, H, I, K) resulted

in a relatively low percentage of diapause completion.

In larvae which were inside the almonds during the first 12 weeks (CC), the response was virtually the same as when the larvae were exposed in vials (C). In the only other group which was also inside the almonds during the first 12 weeks (S), a post-treatment short-day photoperiod did not substantially affect the response of the larvae as compared to the long-day photoperiod (CC).

Treatment K was the most unfavourable for diapause termination of all, with only one larva pupating 27 weeks post-treatment at R.

In treatments that did not include low temperatures (L to R), the percentage of diapause completion did not differ significantly from that of treatments including only 4 weeks at 10 °C (G, H).

In general, the results of the experiment with larvae of late September corroborate those with larvae of early August in that the requirements for the synchronous completion of the second diapause stage were similar, consisting of a sufficiently long period of low temperatures.

Effect of intermittent low and high temperatures.

The purpose was to see the effect of repeated short exposures to unfavourable low and high temperatures on the completion of the later part of the first diapause stage. It is seen in Table 4 that the temperatures tested did not affect the ability of the larvae to complete that stage. As to the time needed for completion, exposure for one day per week to 10 °C or three days to 26 °C delayed the time to 50% pupation by one week, while exposure for only one day per week to 26 °C

caused no measurable delay. Under continuous 26 °C, the completion of the first diapause stage was considerably delayed.

Effects on prolonged-diapause larvae. In our experimental larvae of early August we can distinguish three categories which did not terminate diapause during the 34 weeks of the experiment. In the first category, all or almost all the larvae of certain treatments remained in the first diapause stage because of untimely long exposures to low temperatures (Table 1, A, B, C). The larvae in this artificially-induced prolonged diapause were further subjected for 8 weeks to L16:D8 and 26 °C, i.e. to a summerlike condition, which was suspected to be a prerequisite for the completion of the first diapause stage, if the larvae were subsequently transferred to R. Indeed (Table 5) the first diapause stage was completed within four weeks at R in a high percentage of Retsou larvae and in a lower but still high percentage in Truoito larvae. The difference between Retsou and Truoito larvae was significant only in treatment A.

The second category includes a small percentage of larvae in each treatment, which remained in the first diapause stage because of high diapause intensity although the conditions were favourable for the completion of that stage in most other larvae. Those larvae underwent treatments D to N, S and U seen in Table 1. The larvae were also subjected for 8 weeks to long day and 26 °C then transferred to R. By the end of the 4th week at R the first diapause stage was completed in 67% of 119 Retsou larvae and in 87% of 38 Truoito ones. Therefore, in larvae with an

Table 4. Completion of the first diapause stage in larvae of *E. amygdali* taken out of Retsou almonds collected in Micra in late September 1989, and kept in vials at 19 °C, with intermittent short periods of 10° and 26 °C. Twenty five to 26 larvae per treatment

Photoperiod (L:D) and temperature (°C)	Percent completing first stage	No. of weeks to completion of first stage		
		50%	80%	Range
R (16:8, 19°), continuously	92	2	2	1-5
R, with 1 day per week at 10:14 and 10°	88	3	4	1-12
R, with 1 day per week at 16:8 and 26°	100	2	2	1-5
R, with 3 days per week at 16:8 and 26°	100	3	4	1-13
16:8 and 26° continuously	88	12	13	3-13

Table 5. Termination of the first diapause stage by the end of the 4th week at R (L16:D8 and 19 °C) after 8 weeks at L16:D8 and 26 °C in larvae of *E. amygdali* which had not terminated this stage during the first 34 weeks of treatments A, B and C seen in Table 1

Treatment during first 34 weeks	Almond variety	Total no. of larvae	Percentage terminating first diapause stage
A	Re	30	87a ¹
	Tr	28	64bcd
B	Re	43	84ad
	Tr	35	69bcd
C	Re	23	91ac
	Tr	14	64bcd

¹ Percentages followed by the same letter do not differ significantly at the 0.05 level.

intense diapause, an exposure to long day and high temperature for 8 weeks allowed the subsequent early and synchronous completion of the first diapause stage at R.

In the third category are larvae which completed the first but not the second diapause stage by the end of the first 34 weeks, obviously because they were not exposed to low temperatures. In this group are included most larvae of treatments N and U of Table 1. Those larvae were further subjected for 8 weeks to L10:D14 and 10 °C, then transferred to R. It is seen in Table 6 that a substantial percentage of these larvae terminated the second diapause stage by the end of the 4th week at R. Therefore, 10 °C for 8 weeks

resulted in an early and synchronous diapause termination in a substantial percentage of the larvae. It is noteworthy that the percentage of diapause termination was significantly higher in treatment N which involved an initial 20-week exposure to 26 °C than in U which involved an equal exposure to 19 °C. The percentage of diapause termination in Truoito larvae was higher than in Retsou ones, but not significantly.

Larval mortality during the experimental period was generally low. In early-August larvae it was zero to 11% during the 14 weeks post-treatment in all but one treatment seen in Table 1, and 13 to 20% during the 34 weeks of the experiment in most exposed larvae (Table 2). Exceptions were treatment C (Table 1) in which mortality was 37% for Retsou and 26% for Truoito larvae, and treatment Be (Table 2) of exposed larvae in which the total mortality was 33% for Retsou and 80% for Truoito larvae. This mortality occurred mostly six weeks after the end of the 20-week low-temperature period. In late-September larvae (Table 3), larval mortality during the 34 weeks of the experiment varied with the treatment from zero to 9% and was 4% for the total of larvae.

Discussion

Under the laboratory conditions we examined, diapause termination was of two types: (1) One that occurred within the first few weeks after the larvae were transferred to 19 °C and long day

Table 6. Termination of diapause at R (16:8 and 19 °C), after 8 weeks at L10:D14 and 10 °C of second-diapause-stage larvae of *E. amygdali* which had not terminated this stage during the first 34 weeks of two treatments seen in Table 1

Treatment during first 34 weeks	Almond variety	No. of larvae	Cumulative percentage of larvae terminating second diapause stage at R by the end of weeks		
			2nd	3rd	4th
N	Re	33	24	45	61ad ¹
	Tr	27	59	70	81bd
U	Re	26	23	31	31ce
	Tr	24	27	46	50ae

¹ Percentages followed by the same letter are not significantly different at the 0.05 level.

(R). This termination was early and synchronous and could be considered (and will be referred to) as 'normal', because it has the attributes of diapause terminated in nature. (2) One that was much delayed and that covered a broad span of time. This is the type considered as 'spontaneous' (Beck, 1968; Tauber *et al.*, 1986; Danks, 1987).

Our work produced no data suggesting that photoperiod affected diapause completion in larvae which remained in their natural habitat, i.e. inside mummified almonds. This is not surprising in view of the fact that, even in direct sunlight, no measurable light was transmitted through the pericarp of infested almonds of the Retsou or Truoto varieties, even with such a sensitive light meter as Model 500-1, EG & G, able to measure 1×10^{-4} lux. However, the single case of photoperiodic response which was observed under unnatural conditions of illumination in exposed Truoto but not Retsou larvae of early August subjected to treatment C (Table 2) suggests further work to elucidate this matter. Had the present work been done only with Retsou larvae and only with larvae inside the almonds, this case of photoperiodic effect would have been missed.

Temperature requirements for completion. The first diapause stage was completed normally at temperatures between 16 and 19 °C. Both these temperatures are above the threshold for post-diapause development. A fairly high temperature of 26 °C maintained the first diapause stage for long periods.

Even three-day-per-week intermissions of 26 °C delayed diapause development in late-September larvae which were near the end of the first diapause stage. Therefore, the high to fairly high temperatures of August and part of September are likely to maintain the first diapause stage in nature. Subsequently, the milder temperatures of part of September, most of October and part of November justify the completion of the first diapause stage by the end of November.

For its normal completion, the second diapause stage requires low temperatures such as 10° or 4 °C, which are below the threshold for postdiapause development. Exposures for 8 weeks or

longer to 10 °C were needed for normal completion of the second diapause stage, i.e. for an early and synchronous termination of diapause and pupation. Therefore, additional evidence is provided to recent work (Tzanakakis *et al.*, 1991) that in *E. amygdali* the two morphologically distinct diapause stages have different requirements for their completion. More than one diapause stage with different photoperiod and/or temperature requirements for their completion have been found in a number of insects and mites (for references see Lees, 1955; Danilevskii, 1965; Beck, 1968, 1980; Braune, 1973; Tauber, & Tauber, 1976; Saunders, 1982; Danks, 1987, 1991; Hodek, & Hodkova, 1988).

In several insects that require low temperatures for normal diapause completion, high temperatures must precede the low ones (Danks, 1987). In *E. amygdali* too, 10° or 4 °C to be effective must be preceded by 19 °C. This is because the first diapause stage cannot be completed at these low temperatures.

Spontaneous termination of diapause was detected under constant 19 °C, after long exposures which are not encountered in nature in the region.

Storage temperatures for larvae. Behavioural, physiological, toxicological and other studies with *E. amygdali* may require the timely supply of adults at selected times of the year. This can be done by collecting mummified almonds containing diapausing larvae and keeping them at suitable temperatures. The present work, in conjunction with recent work with larvae from the same location (Tzanakakis *et al.*, 1991) suggests the following: For almonds collected in late July or in August, storage at low temperatures such as 0° to 10 °C should be avoided. Such almonds should better be stored at intermediate temperatures of 16° to 20 °C till most larvae complete the first diapause stage and become white. This may take a few to several weeks, depending on diapause intensity, which may in turn depend on the year and on the almond variety. Subsequently, the larvae can be stored at low temperatures (4° to 10 °C) for periods of eight weeks or longer. This will bring diapause to an end and, as these tem-

peratures are below the threshold for postdiapause morphogenesis, the larvae will remain in a state of postdiapause quiescence for as long as needed. When the quiescent larvae are transferred to higher temperatures such as 19 to 26 °C, most of them should pupate within two weeks and give adults in another two to three weeks at 19 °C and in less time at 26 °C.

To avoid storing the almonds from mid summer, we can collect them from the trees in mid to late November. After splitting a dozen or so open to verify that most larvae are in the second, white, diapause stage, the almonds can be stored at the above low temperatures for eight weeks or longer, then handled as above. If collected from the trees in late January, the larvae will be in postdiapause quiescence and the almonds can be stored directly at low temperatures. However, the later the infested almonds are collected, the greater the risk of losing the larvae because of predation. Within the last two weeks of July the senior author observed over 40% of infested almonds in an orchard eroded by field rodents which fed on the diapausing larvae.

Differences in diapause intensity between larvae from different almond varieties. Most treatments showed a more intense diapause in larvae of early August from Retsou almonds than in larvae from Truoto almonds, although the differences were not significant in all treatments. It is likely that the differences are due to different nutritional quality of the seeds of the two almond varieties, but experimental evidence for this is lacking. Differences due to different food quality during the pre-diapause period are known to modify the photoperiod and temperature responses, including the intensity of diapause, in a number of insect species (for references see Tauber *et al.*, 1986; Danks, 1987).

Prolonged diapause. Prolonged diapause is the one that lasts more than a year in the field (Tauber *et al.*, 1986), or the one that extends over more than one adverse season (Danks, 1987). In *E. amygdali* prolonged diapause in nature is passed in the first (grey) stage. In our experimen-

tal larvae of early August the percentages in prolonged diapause after most treatments involving a period of intermediate then of low temperatures were not much different from those recorded in the field in the same location in 1981 and 1983 (Tzanakakis *et al.*, 1991). When our early-August larvae were subjected first to low temperatures for 20 weeks, they were unable to complete the first diapause stage in the following 14 weeks at R. Further exposure for 8 weeks to 26 °C restored their ability to complete this stage at 19 °C early and synchronously. If a similar response to low then to high temperatures occurs also in nature, it leads to an explanation of the course of prolonged diapause in the field: Larvae that do not complete the first diapause stage in autumn because of high diapause intensity, experience for many weeks the low temperatures of late autumn, winter and early spring. As a result they remain in that stage during spring and summer because their thermal thresholds are lowered. The high temperatures of summer raise their thresholds, i.e. they restore the ability of the larvae to respond to temperature in the same manner as the larvae grown in that season which have entered diapause in that summer. Thus, the larvae in prolonged diapause complete the first diapause stage in the autumn of the year(s) that follow the one they grew.

The environmental factors governing diapause completion in the laboratory have been studied in few univoltine insects possessing an autumnal-hibernal diapause as fully grown larvae. Among them a congeneric species of seed wasp, *E. plotnikovi* Nikol'skaya, from the same locality as our experimental *E. amygdali* has been studied under comparable conditions during the same period (Tzanakakis *et al.*, 1992) to allow comparisons. *E. plotnikovi* infests pistachio seeds in much the same way as *E. amygdali* infests almond seeds. It is also univoltine and undergoes an autumnal-hibernal diapause as a fully grown larva in mummified fruits of its host tree. Yet, there is a substantial difference between the two species in the time diapause is terminated in nature. In the Micra area, *E. plotnikovi* terminates its diapause approximately three months later than

E. amygdali does. *E. plotnikovi* also has at least two diapause stages with different requirements for completion, but its response differs from that of *E. amygdali* in that photoperiod does play an important role. In *E. plotnikovi* sufficiently long periods at low temperature were needed for the completion of the earlier part of diapause and long days for the completion of the later part of diapause. Therefore, the two congeneric seed wasps, infesting their host plants in a similar way in the same locality of northern Greece, regulate their seasonal development with different mechanisms for diapause termination; one depending only or mostly on temperature, and the other on both photoperiod and temperature. One explanation for this difference is that the almond fruit has a much thicker pericarp than the pistachio fruit, no measurable light being transmitted through its pericarp to reach the larva. Therefore, the almond seed wasp had to evolve a mechanism not relying on light.

The responses of *E. amygdali* provide one more example in support of the view expressed by a number of authors (Beck, 1980; Danks, 1987) that attempts at classifying diapause into categories are precarious.

Acknowledgements

This research was carried out during a sabbatical leave of the senior author from the University of Thessaloniki. The senior author is indebted to Prof. M. W. Sabelis for permission and encouragement to use the facilities of the Department under his direction. Many thanks are due to Mr. R. L. Veenendaal for recording pupation during the latter part of the work and for repairing and adjusting equipment and to Dr. D. S. Koveos for the statistical analysis of the data. Also to Messrs. N. A. Kouloussis and C. Kafetzis for collecting and shelling the late-September almonds, to Mr. A. Hadjicostas for allowing the collection of those almonds from his orchard, and to Miss M. Pantzaropoulou for typing the tables.

References

- Beck, S. D., 1968. Insect Photoperiodism. Academic Press, New York & London.
- Beck, S. D., 1980. Insect Photoperiodism. 2nd edn. Academic Press, London & New York.
- Braune, H. J., 1973. The role of temperature in controlling obligatory diapause. In: Wieser (ed.), Effects of Temperature on Ectothermic Organisms. Springer-Verlag, Berlin, Heidelberg, New York, pp. 233–238.
- Danilevskii, A. S., 1965. Photoperiodism and Seasonal Development of Insects. Oliver & Boyd, Edinburgh & London (English translation).
- Danks, H. V., 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Monograph Series No. 1, Ottawa.
- Danks, H. V., 1991. Life cycle pathways and the analysis of complex life cycles in insects. Can. Entomol. 123: 23–40.
- Hodek, I. & M. Hodkova, 1988. Multiple role of temperature during insect diapause: a review. Entomol. exp. appl. 49: 153–165.
- Lees, A. D., 1955. The Physiology of Diapause in Arthropods. Cambridge University Press, London.
- Pittara, I. S. & B. I. Katsoyannos, 1985. Male attraction to virgin females in the almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae). Entomologia Hellenica 3: 43–46.
- Plaut, H. N., 1972. On the biology of the immature stages of the almond wasp, *Eurytoma amygdali* End. (Hym., Eurytomidae) in Israel. Bull. ent. Res. 61: 681–687.
- Saunders, D. S., 1982. Insect Clocks, 2nd ed., Pergamon Press, Oxford.
- Tauber, M. J. & C. A. Tauber, 1976. Insect seasonality: diapause maintenance, termination, and post diapause development. Annu. Rev. Entomol. 21: 81–107.
- Tauber, M. J., C. A. Tauber & S. Masaki, 1986. Seasonal Adaptations of Insects. Oxford Univ. Press, New York, Oxford.
- Tzanakakis, M. E., E. J. Karakassis, G. Tsaklidis, E. Ch. Karabina, I. Ch. Argalavini & I. G. Arabatzis, 1991. Diapause termination in the almond seed wasp, *Eurytoma amygdali* Enderlein (Hym., Eurytomidae), in northern Greece and under certain photoperiods and temperatures. J. Appl. Entomol. 111: 86–98.
- Tzanakakis, M. E., R. L. Veenendaal & A. Veerman, 1992. Effects of photoperiod and temperature on the termination of diapause in the univoltine seed wasp *Eurytoma plotnikovi*. Physiol. Entomol. 17: 176–182.
- Zerova, M. D. & V. N. Fursov, 1991. The palaearctic species of *Eurytoma* (Hymenoptera: Eurytomidae) developing in stone fruits (Rosaceae: Prunoideae).