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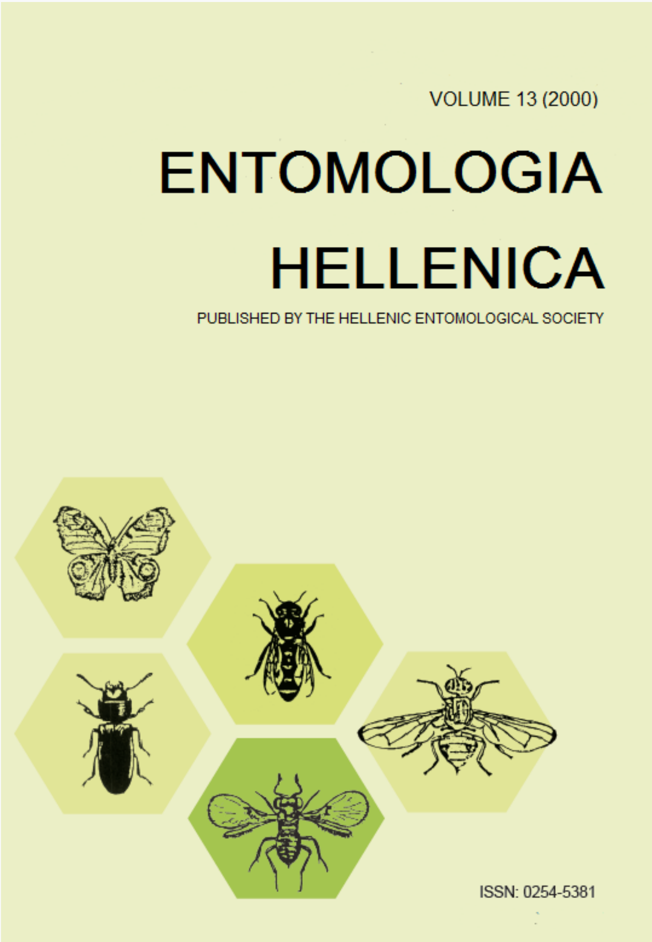
ENTOMOLOGIA HELLENICA

Vol 13 (2000)



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doi: [10.12681/eh.14037](https://doi.org/10.12681/eh.14037)

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To cite this article:

Haralambidis, C. G., & Tzanakakis, M. E. (2000). Time of Diapause Termination in the Pistachio Seed Wasp Eurytomα plotnikovi (Hymenoptera: Eurytomidae) ίη Northem Greece and Under Certain Photoperiods and Temperatures. *ENTOMOLOGIA HELLENICA*, *13*, 43–50. https://doi.org/10.12681/eh.14037

Time of Diapause Termination in the Pistachio Seed Wasp *Eurytoma plotnikovi* (Hymenoptera: Eurytomidae) in Northern Greece and Under Certain Photoperiods and Temperatures[1]

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ABSTRACT

Mummified pistachios containing fully grown diapause larvae of *Eurytoma plotnikovi* Nikol'skaya were collected from mid-November on from trees in coastal northern Greece and placed in a field cage. Every 5 weeks, fruits from the cage and from the trees were placed at 16L:8D h and 26°C. Pupation within 1 or 2 weeks showed that diapause was terminated between early April and early May.

Two years later, strarting in early September, mummified fruits from the same trees were collected at bimonthly intervals and subjected for a few to several months to combinations of certain temperatures and photoperiods, followed by different conbinations of temperatures and photoperiods, then to 16L:8D h and 25°C. In larvae of early September, 6 months at 6°C was the most effective treatment for diapause termination. Diapause was also terminated or almost so after: (1) 4 months at 25°C and long photophase followed by 5 months at 14° or 6°C, (2) 4 months at 20°C and a 12 h photophase followed by 3 months at 14° or 6°C, (3) 4 months at 14°C and short photophase followed by 3 months at 6°C, and (4) 4 or 6 months at 14°C and short photophase. The results are in line with previous work, showing that, in larvae picked in early autumn, low temperatures and short days for a few to several months followed by high temperatures and a long day favor an early and synchronous termination of diapause. The time of diapause termination being in mid-spring, long days seem to be a reasonable signal for diapause termination, as shown by previous work.

In larvae of early January, for a synchronous diapause termination four months at 20°C and a 12 h photophase followed by 3 months at 14°C and an 8 h photophase was the most effective treatment, without the need for a long-day final condition.

Introduction

Eurytoma plotnikovi Nikol'skaya (Hymenoptera: Eurytomidae) has been reported as a pest of pistachios in parts of the Middle East, such as Turcomania, Iran, Turkestan and Ghazaghistan (Nikol'skaya 1934, Davatchi 1956). It has long been the major seed-damaging wasp the junior author has encountered in northern Greece and, probably, it is the major species damaging pistachios also in central and southern Greece, of

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which Anagnostopoulos (1935) gave the morphological characteristics and the life history under the name *E. pistaciae* Anagnos. Despite its economic importance, data on the seasonal development of this insect are few (Anagnostopoulos, 1935; Davatchi, 1956; Melifronides and Zyngas, 1982). It is a univoltine species which develops inside the fruits of a number of plants of the genus *Pistacia*, feeding on the seed (Davatchi 1956). It overwinters as a fully grown larva inside the infested fruit, whether the latter remains on the tree or has fallen to the ground. Pupation takes place in spring and the adult emerges in late spring to early summer after boring a circular hole through the pericarp. In the Nicosia area of Cyprus the adults emerge in May (Melifronides and Zyngas, 1982), while in central continental Greece in late May to late June, according to Anagnostopoulos (1935). Using her ovipositor, the female inserts an egg on the inner surface of the endocarp. After having consumed most or all of the seed, the larva attains full growth before the end of summer and enters an autumnal-hibernal diapause within the fruit. In the Thessaloniki area of coastal northern Greece, larval growth is completed and diapause starts in mid to late summer. Laboratory work with larvae from that area showed that diapause development depends on both temperature and photoperiod, low temperature having a strong favorable effect on the earlier part, and long day on the later part of diapause (Tzanakakis et al. 1992). The time of year diapause in *E. plotnikovi* is terminated in nature has not been determined in any part of this insect's area of distribution. The present paper is the first report on the subject. The paper also gives the responses to certain photoperiod and temperature combinations of larvae collected from the field before, during and after winter.

Materials and Methods

Diapause termination in the field

On 15 November 1985, mummified fruits containing fully grown larvae were collected from a few pistachio trees, *Pistacia vera L.*, of the Aristotelian University Farm, in Micra, approximately 10 km to the south of the city of Thessaloniki. They were placed in a 30×30×30 cm wire-screen cage. The cage was fastened to the northern side of the trunk of a nearby olive tree, so that it was protected from direct sunlight. Every 5 weeks, 50 mummified fruits from the cage and 50 from the pistachio trees were taken to the laboratory. They were sprayed with dicofol to kill undesirable predatory mites and dried on a bench, at room temperature, for a few hours. Subsequently, they were placed in transparent cups of hard plastic, covered with white organdy, and placed at 16L:8D and 26±1°C. Preliminary observations had shown that this photoperiod and temperature condition was favorable for an early pupation of larvae which had completed their diapause. Seven days later, 50 fruits from the cage, marked Cage A, and 50 from the trees were cracked open and the stage of the insects recorded. After recording, the larvae or pupae were placed each in a perforated transparent gelatin capsule and returned to the same long-day/high-temperature condition. After another 7 days, the stage of the insects was recorded again. On that same, 14th, day the remaining 50 fruits from the field cage, marked Cage B, were cracked open and the stage of the insects also recorded. The purpose of these treatments was to compare on-tree with in-cage conditions on the one hand, and in-fruit with in-capsule conditions on the other.

The insect diapauses as a fully grown larva. Therefore, the criterion of diapause termination was pupation within 1 or 2 weeks at 16L:8D and 26°C. To detect possible differences in pupal development on the last day of observations, the pupae were arbitrarily classified as totally white, with red eyes, with black eyes, and with black body. For monthly temperatures and natural daylengths in the orchard area see Tzanakakis et al. (1992).

Diapause termination under laboratory conditions

In a second experiment, samples of 50 mummified fruits containing fully grown larvae were taken at bimonthly intervals from the same trees of the Aristotelian *University* Farm at Micra, starting on 5 September 1987. They were taken to the laboratory, placed in cups as above, and exposed for a few to several months to the combinations of photoperiods and temperatures seen in columns 2 and 3 of Table 3. Subsequently, all fruits were transferred to 16L:8D and 25°C and pupation recorded weekly for 3 weeks. As the insect was known to pupate in spring, the 16L:8D and 25°C condition was expected to favor postdiapause development. The shorter-photophase/lower-temperature combinations aimed at approximating autumn or spring and winter conditions, so as to see which seasonal conditions were more important for diapause completion on one hand, and on trying certain conditions that did not similate the natural conditions following the time of sampling the infested fruits. This was to the extent possible, because we had to use incubators already set for other experiments which were carried out concurrently with ours. Thus, with early-September larvae, treatment A aimed at offering the larvae 4 months of "summer" followed by 5 months of "autumn" and no "winter". We had to use 8h, 14°C instead of the desired 12h, 14°C. Treatment B aimed at offering 4 months of "summer" followed by 5 months of "winter" and no "autumn". We had to use 0h, 6°C instead of the desired 8h, 6°C. With early January larvae, treatment L was the same with treatment C applied to early-September larvae, the purpose being to compare the response of larvae in early with those in more advanced diapause development. Treatments M and N had the same purpose.

The shortest daylength plus twice the civil twilight in the latitude our larvae were taken from is in December 10h, as computed from Beck (1968). The effect of part or all of the twilight to our larvae inside the mummified pistachios is not known. Consequently, 8h represented a photophase shorter than the minimal in the area.

The present work preceded that by Tzanakakis et al. (1992), which was carried out with larvae taken two years later from the same trees. Therefore, the photoperiod and temperature combinations we used were not planned to complement that work. Unfortunately, the file containing the number of larvae in each treatment could not be located. In the first experiment, 66.3% of the fruits sampled contained healthy larvae. Therefore, it is assumed that each percentage in Table 3 was based on approximately 25-35 grown larvae.

Results

Diapause termination in the field

No pupation was recorded before the 9th of May. As seen in Table 1, over 90% of the larvae taken from the trees on 9 May had pupated within a week at 26°C and long day. A small percentage had reached the adult stage, suggesting that pupation of those individuals may have occurred on the trees before they were taken to the laboratory conditions. In fact, 11 fruits from the trees opened on 9 May contained only pupae, whereas 29 fruits from the cage contained only larvae. After another week in the laboratory, most insects had become adults. Therefore, in most larvae inside fruits that remained on the trees,

Table 1. Stages of *E. plotnikovi* after 1 and 2 weeks at 16L:8D and 26±1°C, in pistachios collected from the tree and from field cages in Micra, Thessaloniki (50 fruits per case).

Date of collection	From	Weeks at 16L:8D and 26°C	Number of live larvae	Percent of Pupae	Percent of Adults
4.IV.1986*	Tree	2	35	0	0
	Cage A	2	32	0	0
	Cage B	2	—	0	0
9.V.1986	Tree	1	34	85.3	5.9
	Tree	2	34	8.7	82.5
	Cage A	1	32	37.5	0
	Cage A	2	32	75.0	0
	Cage B	2	36	63.8	5.6
5.V.1986	Tree	0	26	23.1	0
	Tree	3	33	93.9	0

* The percent of pupae of previous dates, being zero, is not given in the table.

diapause was terminated between 4 April and 9 May. A small percentage of individuals remained in the larval stage after 2 weeks at 26°C and long day. Whether those individuals were in prolonged diapause, destined to continue as such for another year, is not known.

In fruits maintained in the field cage since mid-November, diapause was terminated later than in fruits on the trees. Even by the 14th day under the above laboratory condition only 64 to 75% of the larvae pupated. Subsequent work already published, with larvae from the same orchard, has shown that after larvae are exposed to short days and low temperature, as was the case with larvae of the present experiment, pupation takes place much sooner under long days than under short days (Tzanakakis et al. 1992). We do not know whether the delayed termination of diapause in the caged fruits was due to differences in photoperiod, in temperature, or in both from those on the trees. Not only the percentage of pupation was substantially lower as compared with those taken from the trees, but also the stage of pupal development, as recorded one week after they were taken to 26°C and long day (Table 2). All pupae from the cage were in the earliest, white stage, while 96.5% of pupae from the trees were in more advanced stages of development.

Diapause termination under laboratory conditions

In the orchard in question, the larvae of *E. plotnokovi* attain full growth in late July to early

Table 2. Color of pupae of *E. plotnikovi* collected on 9.V.1986 in Micra Thessaloniki (see Table 1).

From	Weeks at 16L:8D and 26°C	Percentage with: Body white	Eyes red	Eyes black	Body black	Number of pupae
Tree	1	3.6	21.5	46.5	28.5	28
Cage A	1	100.0	0	0	0	12
Cage A	2	12.5	29.5	25.0	33.3	24
Cage B	2	8.7	21.7	4.4	65.2	23

August. Therefore, our larvae inside fruits collected in early September must have spent approximately 4 to 5 weeks of diapause in nature, under late summer conditions of high temperatures and long day, before being subjected to the treatments seen in Table 3.

In fruits collected in early September (Table 3), all the treatments (A-I) allowed diapause development and completion in a high percentage of the larvae, and termination mostly in the third week at the final condition of long day and 25°C. The most effective treatment was I, consisting of 6 months at 6°C in the dark. By the end of this low-temperature period, diapause was complete or almost so, therefore, over 50% of the larvae pupated by the end of the 2nd week of the final long-day/high-temperature condition. Six or 4 months at 8h and 14°C (treatments G, H) were also among the most effective, showing that the mildly low temperature of 14°C was also effective for the completion of the earlier part of diapause. If 5 months of this condition followed 4 months of 16h, 25°C (A) or of 12h, 20°C (C), the percentage of diapause termination was slightly lower. If 5 or 3 months of 6°C in the dark followed 4 months of the same conditions (B, D), the percentage of diapause termination was slightly higher. The main conclusions from the above treatments are that 6°C and 14°C under short day are effective for the completion of the earlier part of diapause and that 4 months of higher temperatures applied before 3 months of low temperature periods did not add to the effect. Why treatment E was less effective than D is a question to be answered after further investigation. Larvae inside fruits collected in early November (K) responded to 8h, 14°C approximately as those collected in early September.

In fruits collected in early January, the larvae were in a more advanced stage of diapause development than the early September larvae. The former had experienced approximately 2 months of autumn and 2 months of winter conditions. In these, early-January larvae, 4 months at 6°C (O) were sufficient for the completion of the earlier diapause stage, whereas 3 months (P) were almost so. Four months at 8h, 14°C (N) were also sufficient, whereas 6 months (M) were even more so, with 75% of the larvae pupating within the first subsequent week at long day and high temperature. Four months at 12h, 20°C followed by 3

Table 3. Diapause termination during 3 weeks at 16L:8D and 25°C in larvae of *E. plotnikovi* in fruits collected in Micra, Thessaloniki from autumn 1987 to spring 1988, after being subjected to various protoperiod and temperature conditions. Fifty fruits per treatment.

Treatment	Maintained at	For months*	Percent diapause terminated at 16L:8D and 25°C on week 0	1	2	3
	Fruits collected on 5 September 1987					
A	16h, 25°C**	4				
	then 8h, 14°C	5	0	0	0	70
B	16h, 25°C	4				
	then 0h, 6°C	5	0	0	0	91
C	12h, 20°C	4				
	then 8h, 14°C	3	0	0	0	77
D	12h, 20°C	4				
	then 0h, 6°C	3	0	0	0	85
E	8h, 14°C	4				
	then 0h, 6°C	3	0	0	0	64
G	8h, 14°C	6	0	0	0	85
H	8h, 14°C	4	0	0	0	91
I	0h, 6°C	6	35	0	62	92
	Fruits collected on 5 November 1987					
K	8h, 14°C	4	0	0	3	100
	Fruits collected on 5 January 1988					
L	12h, 20°C	4				
	then 8h, 14°C	3	74	82	82	82
M	8h, 14°C	6	10	75	75	75
N	8h, 14°C	4	0	0	18***	85
O	0h, 6°C	4	0	0	6***	94
P	0h, 6°C	3	0	0	0	39
	Fruits collected on 5 March 1988					
Q	16h, 25°C	1	84	100		
R	0h, 6°C	3	0	0	0	79
	Fruits collected on 1 May 1988					
S	16h, 25°C	0	23			
	Fruits collected on 9 May 1988					
T	16h, 25°C	0	—	—	—	94

* Months refer to 4-week periods.

** Only the photophase is given in this column, to facilitate reading.

*** In these two cases the larvae were held for 11 days instead of 2 weeks.

months at 8h, 14°C (L) was the most effective of the six treatments, with 74% of the larvae pupating before they were transferred to the long-day/high-temperature final condition.

Larvae in fruits collected in early March had experienced the natural photoperiod and temperature conditions of autumn and winter, and should be expected to have satisfied their low-temperature requirements for the completion of the earlier diapause stage. Therefore, they should have needed only a long-day/high-temperature condition for a few weeks to terminate diapause. This in fact occurred, as shown in treatment Q. An additional 3 months at 6°C brought pupation only in the 3rd subsequent week at 16h, 25°C

In fruits collected in early May, the larvae must have had satisfied their photoperiod and temperature requirements, including most or all for the later diapause stage, and should be ready for pupation or already pupated at least in part. In fact, treatment S shows that on 1 May pupation in the field had started. In fruits collected a week later and kept under the long-day/high-temperature condition, most larvae had terminated their diapause by the end of the 3rd week, when the fruits were opened.

Discussion

Diapause termination in the field

In a number of insect species the estimation of emergence of adults of the overwintering generation and, therefore, the timing of the first insecticidal application of the season is based on adult emergence records from field cages. Our data point out the need for keeping field-caged infested pistachios in conditions close to those of the insect's natural habitat. To minimize the unavoidable error, one possibility would be to cage infested fruits as late as possible before the beginning of adult emergence from them on the trees. However, the percentage of infested fruits that will remain on the trees throughout winter –to be caged in early spring– is unpredictable, as unpredictable is the percentage of predation of the diapausing larvae especially in autumn and winter by field rodents, whether the fruits are on the trees or on the ground. Therefore, the development of a technique is desired to maintain infested fruits in the field safely, so that pupation and adult emergence occur very close to those in fruits remaining in the orchard on the trees or the ground.

In *E. plotnikovi* diapause is terminated in mid rather than in early spring. This brings pupation and adult emergence at a time of the year when the pistachio fruit is optimal to receive the egg and subsequently allow successful larval growth. Therefore, long days are a reasonable signal for diapause termination in this species, as already proven (Tzanakakis et al. 1992). In the area in question (latitude 40.5°N), the approximate daylength plus twice the civil twilight (calculated from Beck 1968) is for 1 April, 15 April and 1 May, 13hr 36, 14hr 09 and 14hr 54 min respectively. The occurrence of post-diapause quiescence is unlikely, because at the time diapause ends, some time in April or early May, ambient temperatures are above the threshold for development. Thus, pupation must soon follow the termination of larval diapause.

Pupation of overwintered larvae in central continental Greece is reported to occur in mid-May (Anagnostopoulos 1935), somewhat later than in our case of coastal northern Greece. In Iran, pupation was observed towards the end of April (Davatchi 1956). In central continental Greece, Anagnostopoulos (1935) reports that larvae develop in June-July and are fully grown in August-September. In 1989, larvae collected from the same trees used in the present experiment were all fully grown on 2 August. We do not know whether full larval growth was attained substantially earlier than that date.

Diapause termination in the laboratory

Tzanakakis et al. (1992) worked with larvae of this species in fruits taken in early August and late September 1989 from the same trees as ours. Our larvae of early September 1987 were in fruits taken from the trees a few weeks later and earlier respectively. As mentioned above, the present work preceded that by Tzanakakis et al. (1992). Therefore, our teatments did not aim at supplementing their work. Furthermore, we had to use photoperiod and temperature combinations set for other running experiments. The ultimate long-day/high-temperature condition of Tzanakakis et al. was 16L:8L and 19°C, while ours was 16L:8L and 25°C. Our records stopped at the end of the third week at the ultimate condition, while those of Tzanakakis et al. (1992) continued for 24 weeks. Our larvae were exposed to photoperiod and temperature conditions different from those of Tzanakakis et al. (1992). On the other hand, our larvae of early January and later, were at a much later stage of diapause development and their responses should be expected to be different.. Consequently, comparisons of results with those of Tzanakakis et al. (1992) are limited to only a few cases. Those investigators have shown that *E. plotnikovi* depends on both temperature and photoperiod for diapause development, low temperature having a strong favorable effect on the earlier part, and long day on the later part of diapause. They also estimated that the number of long-day cycles at 26°C needed for late September larvae to terminate the later diapause stage and pupate is approximately 14. The results with our early September larvae are in agreement with those of Tzanakakis et al. (1992) in that periods of low temperatures followed by a long photophase and 25°C resulted in an early and synchronous termination of diapause. They further showed that even the mildly low temperature of 14°C favored diapause development, although 6°C was more effective.

The results with our early September larvae may give the impression that in most treatments (A-H), the low-temperature periods brought the earlier diapause stage to completion and that more than 14 long-day cycles, most probably 15-21 at 25°C were needed for the completion of the later stage and, therefore, for diapause to be terminated. Yet, treatment I, as well as treatments L and M of our early January larvae, show that this is not the case. Most probably, treatments A-H of early September larvae and treatment K of early November larvae brought the earlier diapause stage close to completion but not to full completion, so that diapause termination in most larvae occurred between 14 and 21 days at long day and 25°C. The fact that in treatment M of early January larvae most larvae pupated within a week at the long-day/high-temperature final condition, and in treatment L even without this final period, is another interesting new finding of the present work, showing that much more remains to be done to find out the conditions under which the long-day/high-temperature treatment favors the completion of the later diapause stage. In treatment L diapause was terminated by the end of the 3rd month of the short photophase of 8h and the mildly-low temperature of 14°C. It is not known when diapause would have been terminated had the long-day/high-temperature condition been applied before the end of the 3 months.

In early January larvae the 8h, 14°C condition for 4 or 6 months (N, M) brought diapause closer to termination than in early September larvae (H, G), as expected. The combination of the milder temperatures in treatment L was what the early January larvae needed for diapause termination. The relatively high percentage of diapause termination in treatment L, even before the larvae were exposed to the long-day/high-temperature condition and the equally high percentage in treatment M after only one week at that final condition, seem to be in contrast with the results of Tzanakakis et al. (1992) who showed that a final long day was needed for an early and synchronous termination of diapause in larvae collected in early August. However, our early-january larvae had experienced diminishing and fluctuating field temperatures and a steadily diminishing photophase from August to January. Therefore, more experimental work is needed to clarify how favorable a long photophase is during late diapause development in *E. plotnikovi* and what are the critical photoperiods for a favorable effect on diapause completion and termination.

In fruits collected and opened on 1 May (S), approximately one fourth of the larvae had pupated. This is in agreement with the results of the first experiment (Table 1).

Information on the requirements for diapause completion and termination in other univoltine seed wasps is limited. One of them, *Megastigmus pistaciae* Walker (Torymidae), infests the cultivated pistachio tree in continental Greece, but usually at much lower population densities than *E. plotnikovi*. It is thought to be bivoltine (Anagnostopoulos, 1935; Rice and Michaelides, 1988), but convincing work on its seasonal development is lacking. In only 10 fully grown diapause larvae of this species found in infested pistachios in late September 1989 in the Aristotelian University of Thessaloniki Farm in Micra, diapause was terminated normally after a long enough period at low temperatures, followed by 26°C and long day, or at 19°C and either long or short day. (Tzanakakis et al. (1992).

Another univoltine seed wasp, *Eurytoma amygdali* Enderlein (Eurytomidae), infests almonds in a number of countries of southeastern Europe, the Middle East and fairly recently France, Armenia, Azerbaijan and Georgia (Zerova and Fursov, 1991 and references therein). In coastal northern Greece it exhibits an aestival-autumnal-early hibernal diapause which ends in mid-winter (Tzanakakis et al. 1991). Work by Tzanakakis and Veerman (1994) showed that ptotoperiod did not affect diapause completion. There are two morphologically distinct diapause stages with different temperature requirements for their completion. The first diapause stage was completed synchronously at temperatures between 16° and

19°C. The second stage required lower temperatures between 4° and 10°C.

In the U.K. *Megastigmus spermotrophus* Wachtl infests seeds of the Douglas fir, *Pseudotsuga* Carr. In Kent, England the larva grows in early spring to early summer (Hussey, 1955). Upon full growth it enters an aestival-autumnal-hibernal dormancy, part of which is diapause. At 25°C 53% of the larvae terminated diapause and gave adults in 26 days, after exposure for 12 weeks at 3.3°C. The photoperiod in Hussey's incubators is not mentioned, therefore, no conclusion can be reached on whether low temperature alone was sufficient for diapause completion and termination.

The adaptations of various insect species with an aestival-autumnal-hibernal diapause to their specific environments and consequently their response to laboratory combinations of photoperiod and temperature have been reviewed lucidly by Tauber and Tauber (1976), Tauber et al. (1986) and Danks (1987).

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KEY WORDS: *Eurytoma plotnikovi*, diapause termination, insect dormancy, pistachio seed wasp, Eurytomidae.

Χρόνος Περάτωσης της Διάπαυσης του Ευρυτόμου των Φιστικιών *Eurytoma pistaciae* (Hymenoptera: Eurytomidae) στη Βόρεια Ελλάδα και σε Ορισμένες Φωτοπεριόδους και Θερμοκρασίες

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ΠΕΡΙΛΗΨΗ

Από φιστικιές του Αγροκτήματος του Αριστοτέλειου Πανεπιστημίου στη Μίκρα, αφαιρέθηκαν τα μέσα Νοεμβρίου μουμιοποιημένοι καρποί που περιείχαν διαπαύουσες προνύμφες του *Eurytoma plotnikovi* Nikol'skaya και τοποθετήθηκαν σε κλωβό κάτω από την κόμη παρακείμενου αειθαλούς δέντρου. Ανά 5 εβδομάδες ως και τις αρχές Μαΐου, αφαιρούνταν 50 καρποί από τον κλωβό και 50 από όσους είχαν αφεθεί στα δέντρα και τοποθετούνταν στο εργαστήριο σε φωτοπερίοδο 16L:8D και 26°C. Μετά μία και 2 εβδομάδες γινόταν καταγραφή του ποσοστού νύμφωσης, ως κριτηρίου περάτωσης της διάπαυσης. Διαπιστώθηκε ότι η διάπαυση περατώθηκε μεταξύ αρχών Απριλίου και αρχών Μαΐου.

Δύο χρόνια αργότερα, συλλέχθηκαν μουμιοποιημένα φιστίκια από τα ίδια δέντρα κάθε 2 μήνες, από αρχές Σεπτεμβρίου ως αρχές Μαΐου. Διατηρήθηκαν στο εργαστήριο σε διάφορες φωτοπεριόδους και θερμοκρασίες και τελικά όλα τα φιστίκια σε 16L:8D και 25°C για 3 εβδομάδες για διαπίστωση του ποσοστού νύμφωσης. Σε προνύμφες των αρχών Σεπτεμβρίου, 6 μήνες σε 6°C ήταν η πιο αποτελεσματική για περάτωση της διάπαυσης από τις συνθήκες που δοκιμάστηκαν. Η διάπαυση περατώθηκε ή σχεδόν περατώθηκε μετά από (1) 4 μήνες σε 25°C και μακρά φωτόφαση και στη συνέχεια 5 μήνες σε 14° ή 6°C, (2) 4 μήνες σε 20°C και φωτόφαση 12 ωρών και στη συνέχεια 3 μήνες σε 14 ή 6°C, (3) 4 μήνες σε 14°C και βραχεία φωτόφαση και στη συνέχεια 3 μήνες σε 6°C, και (4) 4 ή 6 μήνες σε 14°C και βραχεία φωτόφαση. Σε προνύμφες των αρχών Ιανουαρίου, τέσσερις μήνες σε 20°C και φωτόφαση 12 ωρών και στη συνέχεια 3 μήνες σε 14°C και φωτόφαση 8 ωρών ήταν η πιο αποτελεσματική από τις συνθήκες που δοκιμάστηκαν. Τέσσερις ή 6 μήνες σε 14°C ήταν επίσης αποτελεσματική συνθήκη. Τέσσερις μήνες σε 6°C ήταν λιγότερο αποτελεσματική συνθήκη, αλλά επίσης επέτρεψε την περάτωση της διάπαυσης στο πλείστο των προνυμφών. Τα αποτελέσματα αυτά συμφωνούν με εκείνα προηγούμενης εργασίας και δείχνουν ότι σε προνύμφες που συλλέχθηκαν στις αρχές φθινοπώρου, χαμηλές θερμοκρασίες και βραχείες ημέρες για λίγους ως πολλούς μήνες ακολουθούμενες από υψηλές θερμοκρασίες και μακρά ημέρα, ευνοούν την ενωρίς και ομοιόμορφη περάτωση της διάπαυσης. Δεδομένου ότι η περάτωση της διάπαυσης στο έντομο αυτό συμβαίνει τα μέσα της άνοιξης, οι μακρές ημέρες φαίνεται να είναι ένα λογικό σήμα για την περάτωση της διάπαυσης, όπως αποδείχθηκε από προηγούμενη εργασία.

Σε προνύμφες που συλλέχθηκαν στις αρχές Ιανουαρίου, για ομοιόμορφη περάτωση της διάπαυσης ο πιο αποτελεσματικός συνδυασμός, χωρίς να χρειαστεί στο τέλος συνθήκη μακράς ημέρας, ήταν 4 μήνες σε 20°C και 12h φωτόφαση, ακολουθούμενη από 3 μήνες σε 14°C και 8h φωτόφαση.