

Effects of constant and changing temperature conditions on diapause induction in *Helicoverpa armigera* (Lepidoptera: Noctuidae)

G.K. Mironidis* and M. Savopoulou-Soultani

Aristotle University of Thessaloniki, Faculty of Agriculture, Department of Plant Protection, Laboratory of Applied Zoology and Parasitology, 54124 Thessaloniki, Greece

Abstract

The effects of photoperiod and temperature on the induction and termination of facultative pupal diapause in *Helicoverpa armigera* (Lepidoptera: Noctuidae) were investigated under laboratory conditions. Exposing *H. armigera* larvae to both constant and fluctuating temperature regimes with a mean of 25°C and 20°C resulted in a type-III photoperiodic response curve of a short-long day insect. The long-day critical daylengths for diapause induction were ten hours and 12 hours at the constant temperatures of 25°C and 20°C, respectively. Higher incidences of diapause and higher values both for the longer and the shorter critical photoperiods for diapause induction were observed at fluctuating regimes compared with the corresponding constant ones. At alternating temperatures, the incidence of diapause ranged from 4.2% to 33.3% and was determined by the temperature amplitude of the thermoperiod and by the interaction of cryophase or thermophase with the photoperiod. *Helicoverpa armigera* larvae seem to respond to photoperiodic stimuli at temperatures >15°C and <30°C; all insects entered diapause at a constant temperature of 15°C, whereas none did so at a constant temperature of 30°C under all the photoperiodic regimes examined. Although chilling was not a prerequisite for diapause termination, exposure of diapausing pupae to chilling conditions significantly accelerated diapause development and the time of adult emergence. Therefore, temperature may be the primary factor controlling the termination of diapause in *H. armigera*.

Keywords: *Helicoverpa armigera*, diapause incidence, photoperiod, temperature

(Accepted 29 July 2011; First published online 6 September 2011)

Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a cosmopolitan pest occurring in all of the Old World, approximately between the northern and southern fortieth

parallels (Hardwick, 1965). This insect is a major pest of a wide range of plants, including field and horticultural crops in many parts of the world (Fitt, 1989; Zalucki *et al.*, 1986, 1994). The pest status of this species is derived, in part, from its four life history characteristics: polyphagy, high mobility, high fecundity, and a facultative diapause that enables the species to survive seasonally changing, unstable habitats (Fitt, 1989). In northern Greece, *H. armigera* completes three or four generations per year and causes annual damage, especially to cotton crops (Mironidis, 2009; Mironidis *et al.*, 2010).

*Author for correspondence

Fax: +302310998832

E-mail: geomironidis@gmail.com, matilda@agro.auth.gr

Diapause is a genetically determined stage of suppressed development, and its expression may be controlled by environmental factors such as photoperiod and temperature (Danilevsky, 1961; Beck, 1980; Tauber *et al.*, 1986; Danks, 1987). Insect diapause is a dynamic physiological process consisting of several successive, distinct phases, including induction, preparation, initiation, maintenance, termination and sometimes post-diapause quiescence, with each phase being modified by diverse environmental factors, as recently reviewed by Košťál (2006). Photoperiod is one of the major abiotic factors that determines and/or modifies the several developmental phases of insects' diapause (Danilevsky, 1961; Beck, 1980; Saunders, 1982; Tauber *et al.*, 1986; Danks, 1987), particularly in species in temperate regions. However, photoperiodic responses may be modified significantly by other environmental factors, such as moisture, temperature and thermoperiod (Danilevsky, 1961; Beck, 1983a, 1985; Tauber *et al.*, 1986; Danks, 1987). This modification is typically provoked through three mechanisms: first, by setting the upper and lower thermal limits between which insects respond to photoperiod stimuli; second, by altering critical photoperiods; and, third, by altering or cancelling the diapause-inducing effect of photoperiod or by affecting the depth or duration of diapause (Leather *et al.*, 1993).

Temperate populations of *H. armigera* exhibit diapause under both field and laboratory conditions (Qureshi *et al.*, 1999, 2000; Zhou *et al.*, 2000; Shimizu & Fujisaki 2002, 2006; Ge *et al.*, 2005; Shimizu *et al.*, 2006; Liu *et al.*, 2009, 2010; Mironidis *et al.*, 2010). Under outdoor conditions, the incidence of diapause of *H. armigera* rapidly increased over a short period of time in September (Zhou *et al.*, 2000; Shimizu *et al.*, 2006) and/or October (Mironidis *et al.*, 2010). This increase could be a result of the combined effect of the decreases in photoperiod and ambient temperature (Shimizu & Fujisaki, 2006; Mironidis *et al.*, 2010) during the late-instar larval and/or prepupal stages (Kurban *et al.*, 2005, 2007). A photoperiod ranging from 11.5 to 12.5 h combined with low or decreasing temperatures of 19–23°C are optimal for diapause induction in the major pest species of the genus *Heliothis* (Pearson, 1958; Hardwick, 1965; Wellso & Adkisson, 1966; Roach & Adkisson, 1970; Roome, 1979; Cullen & Browning, 1978; Hackett & Gatehouse, 1982; Henneberry *et al.*, 1993; Qureshi *et al.*, 1999, 2000; Shimizu & Fujisaki, 2002, 2006; Shimizu *et al.*, 2006). Likewise, pupae of *H. armigera* enter diapause in September and October in response to the declining temperatures and photoperiod in northern Greece (Mironidis *et al.*, 2010).

Most experimental studies of photoperiodism are conducted under constant temperature conditions, and the results obtained from such studies usually provide the basis for an analysis of phenology and seasonal development in the field. The results obtained from constant-temperature experiments are often not applicable directly to the field (Lamb, 1961) because the diurnal variation in temperature, i.e. the thermal optimum, in fluctuating temperatures, may differ from the constant temperature optimum (Cloudsley-Thompson, 1953). In nature, fluctuating temperature conditions may expose insects to heat stress (Mironidis & Savopoulou-Soultani, 2010). Relatively little work has been published investigating the effect of gradually changing temperatures in the real diurnal cyclic variation of field conditions on the induction of insect diapause (Chippendale *et al.*, 1976; Bradshaw, 1980; Volkovich & Blumental, 1997; Musolin *et al.*, 2001). However, the effect of thermoperiod, which subjects insects to two different phases

of temperature (thermophase-cryophase), has been well documented by several authors (Beck, 1983b, 1985, 1988; Brown & Phillips, 1991; Eizaguirre *et al.*, 1994; Vaz Nuñez, 1998; Fantinou & Kagkou, 2000; Fantinou *et al.*, 2003; Wang *et al.*, 2004, 2007; Fantinou & Chourdas, 2006; Mironidis & Savopoulou-Soultani, 2008). Therefore, it seems important to study the combined effects of light and temperature rhythms on the diapause biology of *H. armigera*. Furthermore, because this insect terminates diapause in response to temperature (Wilson *et al.*, 1979; Hackett & Gatehouse, 1982; Mironidis *et al.*, 2010), the diapause intensity estimated under constant laboratory conditions (Shimizu & Fujisaki, 2002) may not be applicable under natural conditions, where the combined effects of fluctuating temperature and photoperiod in diapause regulation may be different (Mironidis *et al.*, 2010).

The present paper describes laboratory experiments designed to study the effects of constant and changing temperature conditions on the photoperiodic induction of diapause in *H. armigera*. In addition, the effects of temperature and photoperiod on the termination of diapause were examined.

Materials and methods

Insects

A laboratory colony of *H. armigera* was established in September 2007 from more than 400 larvae collected from cotton fields in northern Greece (41°N, 023°E). The insects were reared on an artificial diet (Smith, 1966) and held at 25°C, 60–70% RH, under a photoperiod 16:8 h (L:D). The maintenance and handling of the colony has been previously described (Mironidis & Savopoulou-Soultani, 2008). The offspring of the F₅ generation were used for experiments to reduce the possible influence of the host plant sources. The experiments were conducted in controlled-environment chambers (Precision Scientific, General Electric, Louisville, KY, USA and GRW 1000SB CMP). The light intensity in the chambers was measured as 22.5 μEin m⁻²s⁻¹ (400–700 nm) by a quantum sensor Li 188 B (LICOR Inc.). The relative humidity was approximately 60–65%. The reported temperatures were accurate to within ±0.5°C.

Diapause induction in different temperature regimes

Newly hatched larvae (<24 h old) of *H. armigera* were used in all experiments of diapause induction. Three types of temperature regimes were used: constant, fluctuating and alternating.

Constant temperatures

To obtain the diapause response curves and to determine the critical photoperiod for diapause induction at different constant temperatures, the larvae were reared at 15, 20, 25 and 30°C under different photoperiodic regimes throughout their larval stage.

Fluctuating temperatures

The effect of progressive change (stepwise change every hour) in temperature with a mean value of 25°C or 20°C under different photoperiodic conditions (0:24, 4:20, 8:16, 10:14, 12:12, 13:11, 14:10, 16:8, 24:0 h (L:D); photophase started at 8 am

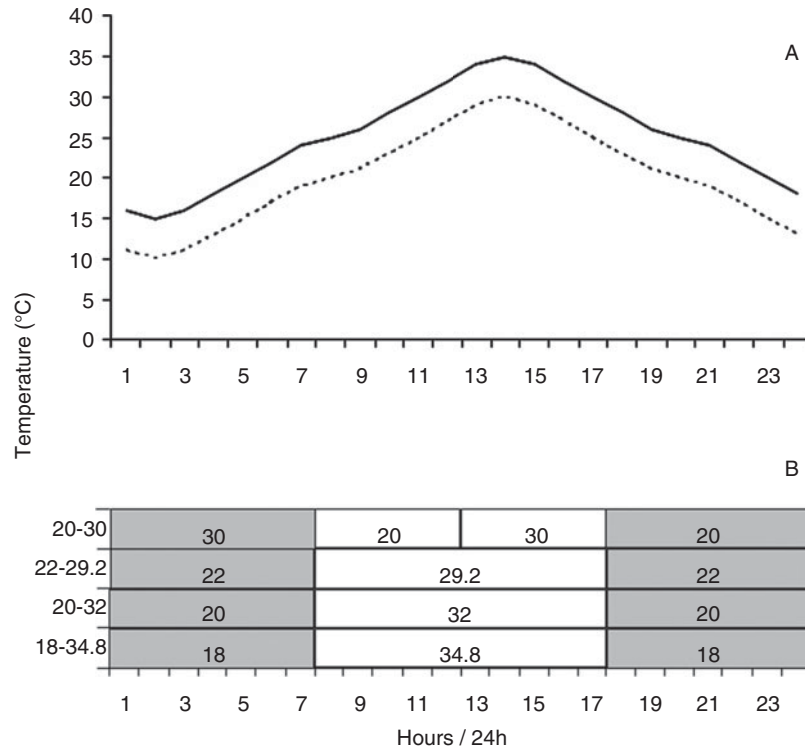


Fig. 1. (a) The hourly temperatures through the 24-h cycle of fluctuating regimes for the mean temperatures of 25°C and 20°C (—, 25°C; ---, 20°C). (b) Daily scheme of thermophase and cryophase of the alternating temperature regimes in combination with scotophase and photophase. Black bars, scotophase; open bars, photophase (■, dark; □, light).

every day) on diapause induction was examined. Figure 1a depicts the curves of hourly temperatures through the 24-h cycle of fluctuating regimes with mean temperatures of 25°C and 20°C. The transition from one temperature to another was essentially completed within approximately 1 min after switching.

Alternating temperatures

The effect of alternating temperatures of different amplitudes on diapause induction was determined by selecting four temperature cycles with thermophase:cryophase (T:C) temperatures, 18–34.8°C, 20–32°C, 22–29.2°C and 20–30°C, under constant photoperiodic conditions (10:14 h (L:D); photophase started at 8 am and finished at 6 pm every day). Each alternating temperature regime had a mean temperature of 25°C. The daily scheme of thermophase and cryophase of the alternating temperature regimes in combination with scotophase and photophase is depicted in fig. 1b. The transition from one temperature to another was essentially completed within approximately ten minutes after switching.

Newly hatched larvae (1st instar) were individually transferred from the colony to 50-ml plastic cups and were provisioned with an artificial diet. After ecdysis to the 4th instar, they were transferred to Petri dishes. Upon pupation, they were sexed based on the shape and size of the terminal segment (Reed, 1965). The pupal diapause status was determined using two criteria. The first was the retention of pigmented eye spots in the postgenal region (Shumakov & Yakhimovich, 1955). Immediately following larval-pupal

ecdysis, the eyespots are distinct, occurring in a straight line across the postgenal area. These disappear during the first five days after pupation at 25°C and during the first eight days at 20°C in non-diapausing pupae (Mironidis, 2009). The second criterion was the condition of the fat body in the pupal abdomen (Pearson, 1958). The fat body of newly formed pupae is composed of firm, rounded lobes and remains unchanged throughout diapause. Its histolysis coincides with movement of the eye spots and is, therefore, a valid supplementary criterion. Because the eye spots in some pupae can be very faint and are not discernible, it is useful to verify the condition of the fat body to determine if the pupae have entered diapause. Using the above criteria, 15 days after pupation, the pupae were checked under a stereoscope (M26, 40×; Leica, Wetzlar, Germany) for eyespot retention and for the condition of their liposome to separate diapausing from non-diapausing pupae.

Diapause development

To investigate the effect of chilling on diapause development, 20-day-old diapausing pupae of *H. armigera* were placed at 4°C for different lengths of time (ranging from 14 to 98 days) in continuous darkness. Diapausing pupae were obtained by rearing newly hatched larvae at 20°C and a short photoperiod of 8:16 h (L:D). After chilling, the pupae were exposed to 25°C or 20°C. One group of pupae was placed at a long-day photoperiod of 16:8 h (L:D), while the second group was placed at a short-day photoperiod of 8:16 h (L:D). They were then examined daily for adult emergence. Some diapausing

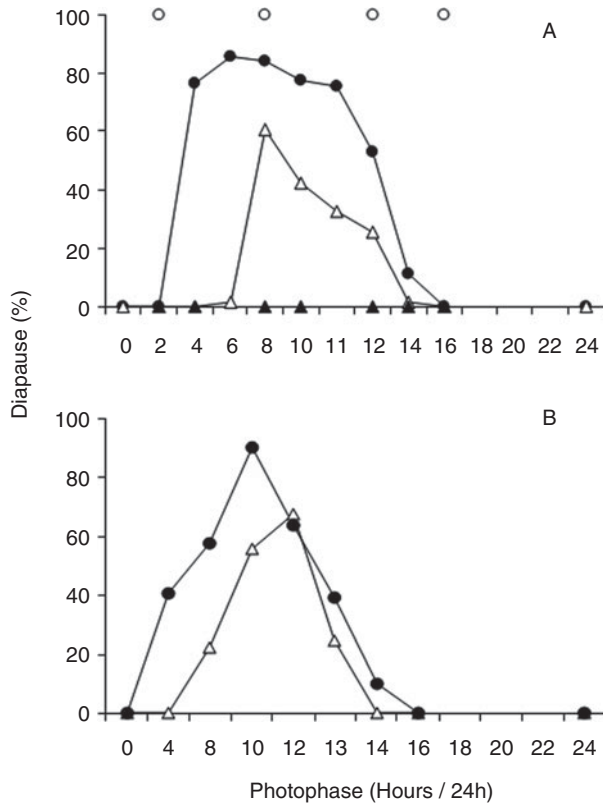


Fig. 2. The *H. armigera* diapause response curves to various photoperiodic regimes (a) at four constant temperatures (15, 20, 25 and 30°C) (○, 15°C; ●, 20°C; △, 25°C; ▲, 30°C) and (b) at two fluctuating temperatures with means of 20°C and 25°C (●, 20°C; △, 25°C).

pupae were kept at 25°C or 20°C without chilling as controls. Adult emergence was used as the criterion for the completion of diapause development (Hackett & Gatehouse, 1982; Kay, 1982; Qureshi *et al.*, 2000; Mironidis, 2009; Mironidis *et al.*, 2010).

Statistical analysis

The effect of the duration of exposure to chilling at 4°C on diapause termination of *H. armigera* pupae was determined by a one-way ANOVA. A logarithmic transformation $\log_{10}(x+1)$ of the data was used to avoid heterogeneity of variance: untransformed means are presented in the figures. The percentages were compared using the chi-squared test (Sokal & Rohlf, 1995). The *t*-test was used to compare the values of the means from two samples. The calculations were performed using the statistical package SPSS NLN, 14 (SPSS Inc., 2006).

Results

Diapause induction

The diapause response curves of *H. armigera*, reared from the 1st instar to the pupal stage under different photoperiodic regimes at four constant temperatures (15, 20, 25 and 30°C) and at two fluctuating temperatures with means of 20°C and

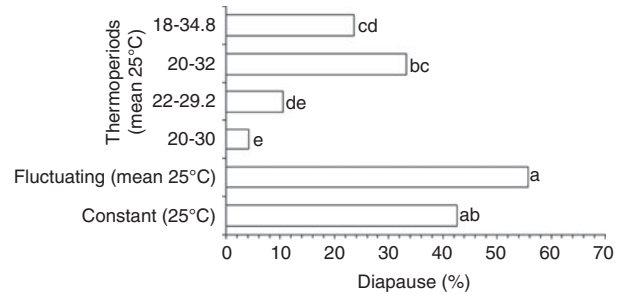


Fig. 3. The diapause incidence (percentage) in *H. armigera* larvae reared under different thermoperiods with a mean temperature of 25°C and at the corresponding alternating and constant temperatures under photoperiod 10:14h (L:D). The percentages followed by the same letter are not significantly different ($P < 0.05$, chi-squared test).

25°C, are presented in fig. 2. The incidence of diapause was under photoperiodic control at 25°C and 20°C both at constant and fluctuating temperatures, indicating that this insect displays a type-III-photoperiodic response curve of a short-long-day insect, analogous to the photoperiodic response curve suggested by Beck (1980).

At a constant temperature of 25°C, the shorter critical daylength was between 6 and 8 h, and the longer critical daylength was approximately 10 h, while photoperiods with a daylength longer than 14 h or shorter than 6 h resulted in non-diapause (fig. 2a). The fluctuating temperature with a mean of 25°C shifted the diapause response curve to the right in relation to the curve of the corresponding constant temperature; the shorter critical daylength was approximately 10 h, and the longer critical daylength was approximately 13 h, while the highest percentage of diapause (67.2%) was observed at a 12-h photoperiod, higher than that of the corresponding constant temperature (25.7%) (fig. 2b). All insects reared at 15°C entered diapause, whereas no diapause was induced at 30°C at any of the photoperiodic regimes examined (fig. 2a).

At a constant temperature of 20°C, no diapause occurred at photophases longer than 14 h or shorter than 4 h. The shorter critical daylength at a constant 20°C was between 2 and 4 h, and the longer critical daylength was 12 h, whereas the highest incidence of diapause (85.7%) was observed at a photoperiod of 6 h. At fluctuating temperatures with a mean of 20°C, the highest incidence of diapause (90.3%) was observed at a 10-h photoperiod, which was higher than that of the corresponding constant temperature (77.4%). Moreover, the two critical daylengths at fluctuating temperatures with a mean of 20°C were between 4 and 8 h and between 12 and 13 h, respectively.

The amplitude of the thermoperiod in the alternating temperature regimes with a mean of 25°C (fig. 1b) at a 10-h photoperiod had a significant effect on the diapause incidence of *H. armigera* ($\chi^2 = 24.9$; $df = 3$; $P < 0.001$). High incidences of diapause occurred when the larvae were subjected to thermoperiods in which the lower temperature coincided with the scotophase. Under the thermoperiods tested, the incidence of diapause decreased from 33.3% at a thermoperiod of 20–32°C to 23.5% at a thermoperiod of 18–34.8°C and to 10.5% at a thermoperiod of 22–29.2°C (fig. 3). However, when part of the thermophase coincided with the scotophase and part of the cryophase coincided with the photophase (20–30°C) (fig. 1b), the incidence of diapause was much

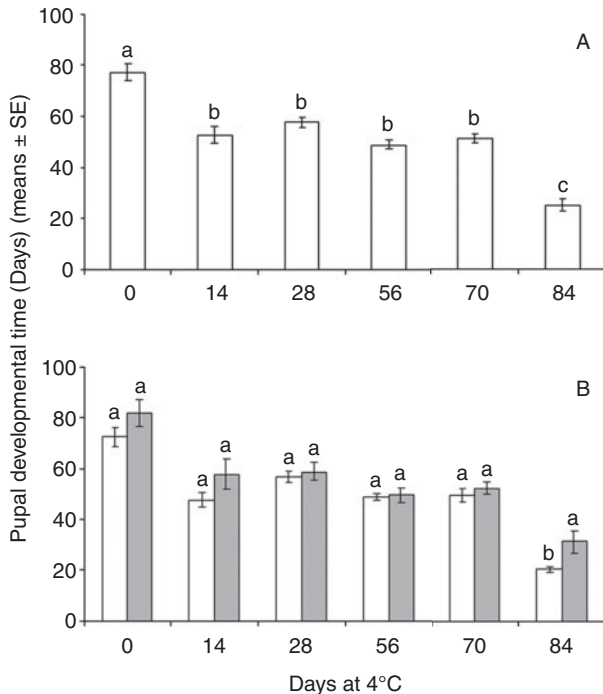


Fig. 4. The mean numbers of days required for adult emergence at 25°C in *H. armigera* pupae pre-chilled at 4°C for various lengths of time. (a) Total average irrespective of the photoperiodic condition at 25°C and (b) at a 16-h or 8-h photoperiod and 25°C. Different letters above the bars show significant differences in pupal developmental time: a, between the various times of exposure at 4°C ($P < 0.05$, Tukey HSD test); and b, between the two photoperiodic conditions tested after the same period of exposure ($P < 0.05$, *t*-test) (□, L:D: 16:8h; ■, L:D: 8:16h).

lower (4.2%) (fig. 3). The percentage of diapausing individuals in the alternating temperature regimes was significantly lower than that observed at the corresponding fluctuating (55.7%) or constant (42.6%) temperatures at the same photoperiod (10:14 (L:D) h) ($\chi^2 = 61.4$; $df = 5$; $P < 0.001$).

Diapause development

Although chilling at 4°C was not necessary for the completion of diapause development, it significantly decreased the time before adult emergence compared with unchilled controls at 25°C ($F = 56.6$; $df = 5, 158$; $P < 0.001$) (fig. 4a) and at 20°C ($F = 19.1$; $df = 7, 171$; $P < 0.001$) (fig. 5a). Pupae chilled for 14 days significantly reduced the time to adult emergence at 25°C compared with unchilled controls, but further reduction was not observed in this period until the chilling length reached 84 days (fig. 4a). When diapausing pupae were transferred at 20°C after their exposure at 4°C, chilling for 14, 28 and 42 days did not significantly differentiate the duration of the pupal stage, whereas pupae chilled for 70 and 98 days significantly decreased the time to adult emergence compared to the control (fig. 5a). In all treatments tested, the duration of the pupal stage of diapause individuals, irrespective of their exposure to chilling temperature, was shorter at a 16-h photoperiod than at an 8-h photoperiod, but the differences between the photoperiodic

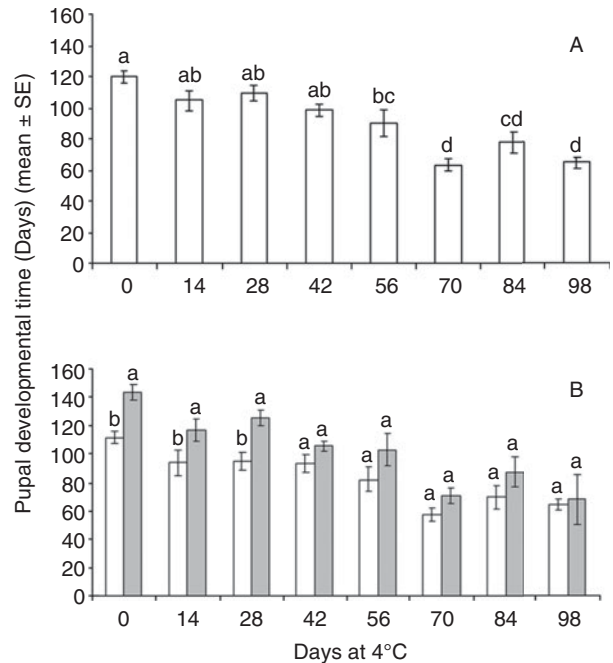


Fig. 5. The mean numbers of days required for adult emergence at 20°C in *H. armigera* pupae pre-chilled at 4°C for various lengths of time. (a) Total average irrespective of the photoperiodic condition at 20°C and (b) at a 16-h or 8-h photoperiod and 20°C. Different letters above the bars show significant differences in pupal developmental time: a, between the various times of exposure at 4°C ($P < 0.05$, Tukey HSD test); and b, between the two photoperiodic conditions tested after the same period of exposure ($P < 0.05$, *t*-test) (□, L:D: 16:8h; ■, L:D: 8:16h).

regimes were significant ($P < 0.05$, *t*-test) only in a few cases (figs 4 and 5b).

Discussion

In *H. armigera*, *H. zea* and probably other species, the incidence of diapause increases with increasing latitude (Fitt, 1989). In the tropics, populations of *H. armigera* breed continuously (Coaker, 1959) although a small proportion (2–4%) of pupae may enter diapause (Reed, 1965; Hackett & Gatehouse, 1982), whereas in subtropical-temperate regions, most individuals enter diapause before winter (Roome, 1979; Wilson *et al.*, 1979; Kay, 1982; Qureshi *et al.*, 1999, 2000; Zhou *et al.*, 2000; Mironidis *et al.*, 2010).

A population of *H. armigera* derived from northern Greece displayed a 'short-long day' diapause response curve (type III: Beck, 1980) in the laboratory. A type-III photoperiodic response shows two well-defined critical daylengths. Low temperatures tend to broaden the range of diapause induction (Beck, 1980). However, it has been reported that a 'long-day' (type I: Beck, 1980) photoperiodic response was observed at a constant 20°C for Japanese populations of *H. armigera* (Qureshi *et al.*, 1999, 2000; Shimizu & Fujisaki, 2002; Shimizu *et al.*, 2006). The difference in the results between the Greek (type III) and Japanese (type I) populations could be attributed to the fact that the Japanese researchers did not study photoperiods with photophases <8 or >18h. Moreover, our data are consistent with the findings of Roome (1979), who reported that a high

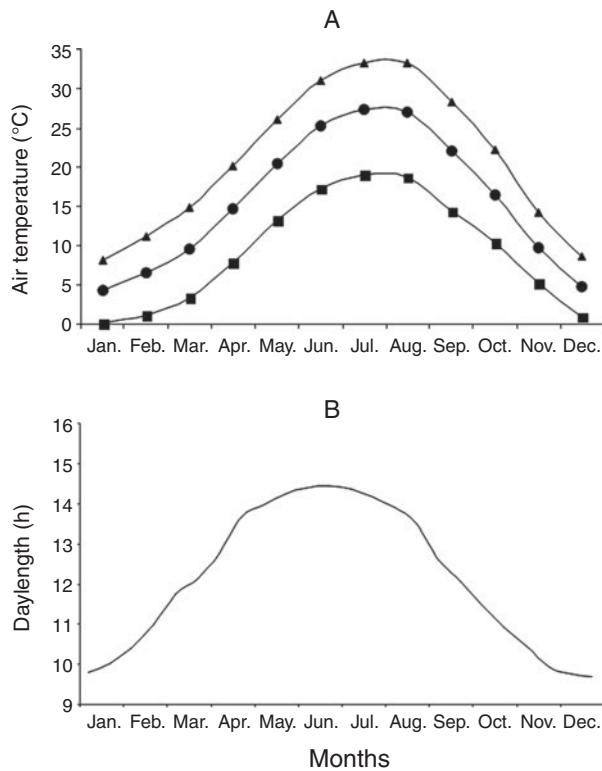


Fig. 6. (a) The mean monthly air temperature over 20 years (1989–2009) (—▲—, maximum temp.; —●—, mean temp.; —■—, minimum temp.) and (b) photoperiod throughout the year in northern Greece. The daylength was calculated as sunrise to sunset with a 0.5h addition as twilight (Hellenic National Meteorological Service) (—, Daylength).

incidence of diapause occurred in an *H. armigera* population of Botswana (South Africa) at a 6-h photoperiod and 18°C. The shorter critical daylength at constant temperatures 25°C and 20°C (6–8 and 2–4h, respectively) is usually not encountered by insects in their natural habitats during the growing season in Greece (fig. 6). The short-day portion of the type III diapause induction curve is of no apparent ecological significance, but it may provide information on the nature of the response mechanisms (Beck, 1980).

The photoperiodic response of diapause incidence is affected by temperature in all tested species of the genus *Heliothis* (Fitt, 1989). In the current study, we have shown that *H. armigera* larvae from northern Greece entered pupal diapause when the rearing temperature was low (15°C), whereas they failed to enter pupal diapause when the rearing temperature was high (>25°C), irrespective of the duration of daylength (fig. 2a). This result is consistent with the findings of other authors who studied the diapause response in other populations of *H. armigera* (Wilson *et al.*, 1979; Qureshi *et al.*, 1999; Jallow & Matsumura, 2001; Kurban *et al.*, 2007). Qureshi *et al.* (1999) reported that a *H. armigera* population from Okayama (western Japan) rarely entered diapause, even at a considerably short photoperiod of 8:16h (L:D) at 25°C. Qureshi *et al.* (1999) also reported that all individuals entered diapause at 15°C, even under a long photoperiod of 16:8h (L:D). Similarly, in *H. zea* (Pullen *et al.*, 1992), *H. punctigera* (Cullen & Browning, 1978; Browning, 1979) and *H. virescens*

(Henneberry *et al.*, 1993), it was found that higher percentages of pupal diapause were induced, irrespective of the photoperiodic conditions, when the larvae were reared at low temperatures. According to Kurban *et al.* (2007), temperature is an important diapause cue in *H. armigera* because it can modify the photoperiodic effect after the photoperiodic stimulus has been received by the sensitive stage (primary 5th larval instar: Kurban *et al.*, 2005).

The exposure of *H. armigera* larvae to fluctuating temperatures with a mean of 25°C or 20°C resulted in the same type of diapause response curve (type III: Beck 1980) as observed at constant temperatures. Furthermore, a higher incidence of diapause was observed at fluctuating conditions than at the corresponding constant temperatures (fig. 2). Similar results have also been documented by other researchers (Chippendale *et al.*, 1976; Bradshaw, 1980). This high incidence of diapause under changing conditions within the temperate zone may be a part of the climatic adaptation achieved by *H. armigera* in Greece. This is contrary to its subtropical populations, for which it has been demonstrated that they are not so sensitive to changes in photoperiod and temperature (Hackett & Gatehouse, 1982; Shimizu & Fujisaki, 2006). An adaptive significance of this feature is quite clear. It ensures high percentages of survival for *H. armigera* temperate-zone populations during the cold season because, as we have shown in a previous study, the non-diapausing individuals are maladapted for winter survival in northern Greece (Mironidis *et al.*, 2010). In the temperate zone, autumn is characterised by unpredictably decreasing temperatures and predictably shortening daylengths (fig. 6). Under these conditions, individuals of the northern Greece population enter diapause between mid-September and early November, when the prevailing photoperiod ranges from 10 to 12h and the mean air temperature declines from about 24°C to 15°C (fig. 6), while individuals not destined for diapause are subjected to high larval mortality (Mironidis *et al.*, 2010). In the present study, we have shown that under gradually changing temperature conditions, *H. armigera* exhibited a higher incidence of diapause when the photoperiod was 10h and 12h with fluctuating temperatures with a mean of 25°C (55.7 and 67.2%, respectively) and 20°C (90.3 and 63.8%, respectively). In contrast, it has been reported that fluctuating temperatures provoked the diapause-preventing effect in some insect species compared to constant ones (Volkovich & Blumental, 1997; Musolin *et al.*, 2001). It is worth mentioning that few studies exist that examine the effect of fluctuating temperatures (hourly changing-temperature conditions) on insect diapause, and none of them refers to *H. armigera*.

Thermoperiod is considered to be an important factor in diapause determination in a number of insects (Beck, 1983b; Tauber *et al.*, 1986; Brown & Phillips, 1991; Vaz Nuñez, 1998; Fantinou & Kagkou, 2000). On the contrary, some researchers suggested that diapause could be induced when the thermophase is in coincidence with either the scotophase or the photophase of the photoperiod (Eizaguirre *et al.*, 1994; Wang *et al.*, 2004, 2007), underlining the importance of thermoperiod *per se* in diapause induction and not the scotophase temperature. Our data indicated that diapause was induced at all alternating temperatures tested, but the incidence of diapause was considerably low compared with corresponding fluctuating and constant temperatures under the same photoperiod (fig. 3). The ratio between diapausing and non-diapausing individuals was determined by the temperature amplitude and by the interaction of cryophase or thermophase

with the photoperiod of the thermoperiod. Specifically, the higher percentages of diapause incidence were observed when thermophase was in coincidence with the photophase of the thermoperiod, whereas when part of the scotophase was accompanied by a relatively high temperature (treatment 20–30°C), it resulted in a reduced percentage of diapause (fig. 3), probably because of the strong modifying effect of the unnaturally high night temperature (Fantinou & Kagkou, 2000).

One dispute regarding the relationship between diapause completion and low temperature concerns whether chilling is actually required for diapause development and whether it accelerates diapause development. In some insects, a period of chilling is not necessary for the termination of diapause, and most diapausing individuals may be activated spontaneously after an increase in temperature or photoperiod or both (Tauber *et al.*, 1986; Danks, 1987). For example, the exposure of *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae) (Milonas & Savopoulou-Soultani, 2004) and *Sesamia nonagrioides* (Levebvre) (Lepidoptera: Noctuidae) (Fantinou *et al.*, 1998) to chilling conditions was not necessary for the termination of diapause. However, in many insect species, the exposure to lower temperatures is required for diapause development. Diapausing larvae of *Colpoctypeus florus* (Walker) (Hymenoptera: Eulophidae) cannot resume development unless they experience a minimum period at chilling temperatures; otherwise, the individuals will die (Milonas & Savopoulou-Soultani, 2000). However, the length of exposure to chilling temperatures for diapause development to be completed depends on the insect species (Danilevski, 1961; Tauber *et al.*, 1986). Even though an exposure to chilling conditions was not a prerequisite for the completion of diapause development in *H. armigera*, it significantly decreased the time to adult emergence after chilling (figs 4 and 5). This acceleration of diapause development appears to be accompanied by a gradual decrease in trehalose content and an increase in glucose content in *H. armigera* (Izumi *et al.*, 2005). Moreover, our data showed that diapausing pupae without exposure to chilling completed their pupal stage in about 80 days and 120 days at 25°C and 20°C, respectively, while even a minimum length of exposure (14 days at 4°C) accelerated the time of adult emergence compared with the results of unchilled controls (figs 4 and 5). These findings are similar to those of Izumi *et al.* (2005), who reported that diapausing pupae of *H. armigera* maintained at 20°C (without exposure to chilling) did not break diapause 90 days after pupation, and the trehalose and glucose contents were almost the same as those at day 0. The decrease in trehalose content and the increase in glucose content may be linked to the termination of diapause in *H. armigera* (Kurban *et al.*, 2008).

The time of diapause termination depends on its intensity, which is predetermined for each species as well as for each strain of a species (Tauber *et al.*, 1986). The cold exposure of diapausing individuals combined with subsequent temperature increase favours enhanced and synchronous reactivation (Milonas & Savopoulou-Soultani, 2000). For the Greek population of *H. armigera*, the diapause termination and the eclosion of adults in the spring are determined by the local soil temperature conditions, with the adult emergence being concentrated in a period of 4–6 weeks between late April and early June (Mironidis *et al.*, 2010). Consequently, temperature has a primary role in diapause termination of *H. armigera* under outdoor conditions (Mironidis *et al.*, 2010).

In the laboratory, long photoperiods tend to accelerate diapause development (figs 4 and 5b).

Diapause induction and termination is a complex phenomenon influenced by a multiplicity of stimuli, such as photoperiod and temperature (Tauber *et al.*, 1986; Danks, 1987). Our data showed that there was a significant interaction between temperature and photoperiod in terms of their effects on the induction and termination of diapause in *H. armigera* under laboratory conditions. However, the type of temperature regime (constant, fluctuating or alternating) may enhance or diminish the effects of photoperiod on the diapause induction of *H. armigera*. As diapause is the most important mechanism for winter survival of *H. armigera* in northern Greece (Mironidis *et al.*, 2010), knowing the factors controlling the induction and termination of diapause is essential for both understanding the temporal patterns of diapause incidence and forecasting of post-diapause populations in field; hence, it can significantly help the construction or improvement of phenology models.

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