


AUTHOR QUERY FORM

 ELSEVIER	Journal: ASPEN Article Number: 157	Please e-mail or fax your responses and any corrections to: E-mail: corrections.essd@elsevier.spitech.com Fax: +1 61 9699 6721
---	---	--

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult <http://www.elsevier.com/artworkinstructions>.

We were unable to process your file(s) fully electronically and have proceeded by

☐ Scanning (parts of) your article
 ☐ Rekeying (parts of) your article
 ☐ Scanning the artwork

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1	Please check the asterisks in Table 2 legends. They are not used to mark any cell content within the table body.
Q2	Please check the asterisks in Table 3 legends. They are not used to mark any cell content within the table body.
Q3	Please check the pair of asterisks in Table 4 legend. It is not used to mark any cell content within the table body.
Q4	Please check the change: Tables 3A and 3B were renamed Tables 3 and 4.
Q5	The citation 'Sushil Kumar (2005)' (original) has been changed to 'Sushilkumar (2005)'. Please check if appropriate.
Q6	The citation 'Tauber et al., 1988b' (original) has been changed to 'Tauber et al., 1988'. Please check if appropriate.
Q7	The citation 'William et al., 2006' (original) has been changed to 'Williams et al., 2006'. Please check if appropriate.
Q8	Uncited reference: This section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section. Thank you.
Q9	Please provide publisher name here.

Thank you for your assistance.



Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Enhancing bio-suppression of *Parthenium hysterophorus* L.: Diapause in *Zygogramma bicolorata* Pallister and its manipulation through insulin-like peptides (ILPs)

R.K. Gupta^{a,*}, Salil Gupta^b, K. Bali^a, K. Srivastava^a

^a Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology-Jammu, Chatha, Jammu and Kashmir, India – 180009

^b Department of Horticulture, Jammu, India – 180002

ARTICLE INFO

Article history:

Received 19 March 2010

Revised 9 June 2010

Accepted 11 June 2010

Available online xxxx

Keywords:

Zygogramma bicolorata

Diapause

Parthenium hysterophorus

Biocontrol

Insulin-like peptides (ILPs)

ABSTRACT

In-depth investigations on diapause behaviour of *Z. bicolorata* revealed that the adults entered diapause at any time from August to December and that the number peaked (42.00%) during the last half of November. The percentage of adults entering diapause increased with a decrease in day length. Weight of diapausing adults was significantly higher than weight of non-diapausing adults. The percentage of adults undergoing diapause at 30 °C was significantly lower than those undergoing diapause at 15 and 25 °C. The percentage of adults burrowing increased with increasing moisture. In silty soil and soil with high organic matter, 46.7% and 49.2% of adults entered diapause, respectively, whereas in sandy soil, only 23.5% of adults entered diapause. When newly emerged beetles were exposed to 5 µg of human insulin 30/70, a significantly lower percentage of treated adults underwent diapause compared to untreated adults under both feeding and no feeding conditions. Insulin treatment also influenced the emergence period from diapause (93.92 ± 1.73 days), percent emergence (81 ± 1.54%) and fecundity/month (512.7 ± 25.38 eggs) of *Z. bicolorata* in treated adults as compared to untreated adults (109.05 ± 2.2, 74.00 ± 1.82 and 438.3 ± 19.33 eggs, respectively). However, there was no significant impact of insulin on adult longevity. These findings are of great utility in the biological suppression of *Parthenium* as it will enhance the effectiveness of this beetle through manipulation of diapause.

© 2010 Published by Elsevier B.V. on behalf of Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society.

Introduction

Biological control of *Parthenium hysterophorus* L. (Asteraceae) is the most cost-effective, environmentally safe and ecologically viable alternative management strategy. A biocontrol program of this weed was initiated in India in 1983 with the introduction of the leaf feeding beetle, *Zygogramma bicolorata* Pallister (Chrysomelidae: Coleoptera). The establishment of the beetle resulted in a significant reduction of *Parthenium* in localized areas. Further biocontrol programs were initiated in 1989 in Jammu and Kashmir and large scale releases were made in 1992 (Gupta et al., 2002). Since then, the beetle dispersed over an area of more than 9000 km² (Gupta, 2008) and weed suppression is evident in many parts of the state (Gupta et al., 2004).

Introduced insects do not always get to live in an environment that makes their optimal growth possible. They must adapt to and survive conditions that do not support continuous development. Diapause plays an important role in the life cycle of this beetle as an adaptive strategy to escape adverse seasonal conditions (Jayanth and Bali, 1993a). *Z. bicolorata* adults burrow into the soil and diapause within

chambers that they form approx. 1 to 3 cm beneath the surface. They enter diapause when conditions are unfavourable, especially in the regions where day length and temperature differ within a season (Tauber et al., 1986). The egg, larval and pupal stages do not enter diapause. Non-diapausing adults are capable of breeding under laboratory conditions during winter. Normally, this insect remains active between March and October and, after completely defoliating *Parthenium* plants in a particular area, migrates to new areas. As a result, by the end of the rainy season, very few adults are likely to be present in the area of initial defoliation. However, since diapause occurs throughout the breeding season, some diapausing adults are left behind at each location, which are ready to defoliate the growth of the weed during the following year. This eliminates the necessity of reintroducing the beetle to the same area if the weed density increases (Jayanth and Bali, 1993b). Even though defoliation by *Z. bicolorata* has a visible effect on *Parthenium* under field conditions, the timing of diapause onset, maintenance and termination may determine successful establishment of this beetle to new areas. When diapause is facultative rather than obligatory, insects can develop without interruption while conditions are favourable. In *Z. bicolorata* this phenomenon turns out to be both advantageous as well as disadvantageous. This is an advantage because it can exploit seasonal resources and bridge harsh winters, dry seasons or other

* Corresponding author.

E-mail address: rk Gupta@entomology.azh.ac.in (R.K. Gupta).

seasonally harsh conditions that would otherwise prove unfavourable for beetle survival. However, since it leads to arrest in development and suppressed metabolism, the diapaused beetle resumes their activity late in the season when the *Parthenium* has germinated and started flowering. The ability of this beetle to synchronize its period of activity to the availability of its plant host is likely to play a major role in its establishment and effectiveness as a biological control agent (Gupta et al., 2006). Therefore, manipulation of diapause may aid in improving the efficiency and efficacy of biological control so that it can terminate early in the season before pre-monsoon *Parthenium* starts flowering.

Several environmental and biological factors influence diapause behaviour of *Z. bicolorata* (Jayanth and Bali, 1993b), but it is difficult to manipulate them under natural conditions. However, it is possible to manipulate diapause using insulin-like peptides (ILP) (Arpagaus, 1987) or insect growth regulators (IGR) (Watanabe and Tanaka, 2000). Insects do not have a tissue that is specialized in carbohydrate homeostasis such as the pancreas. However, they do have a group of insulin-producing cells (IPC) that are located in the brain and constitute an endocrine organ for the regulation of growth and sugar metabolism (Geminard et al., 2006). After their release into open hemolymph circulation, the *Drosophila* insulin-like peptides reach their target tissues and elicit their biological functions. Insulin signaling mediates JH synthesis and is integral to diapause in the mosquito, *C. pipiens* (Sim and Denlinger, 2008). These insulin signaling pathways and FOXO (forkhead transcription factor) control key characters of diapause, including the metabolic switch to lipid storage, the halt in ovarian development, and enhanced overwintering survival. Under long day lengths, insulin signaling leads to the production of JH which prompts ovarian development, and to the suppression of FOXO which prevents the accumulation of fat stores. By contrast, under short day lengths, the insulin signaling pathway is shut down, which halts JH synthesis and releases the suppression of FOXO. Genes encoding both insulin-like peptides-1 and -5 were suppressed using RNA interference in mosquitoes that were programmed for nondiapause, and ovarian maturation (Sim and Denlinger, 2009). Knocking down insulin-like peptide-1 with RNAi in non-diapausing mosquitoes resulted in a cessation of ovarian development akin to diapause, and this arrest in development could be reversed with an application of JH. Considering all these observations, an in-depth investigation was carried out to understand diapause behaviour of *Z. bicolorata* under varied conditions for better biological suppression of *Parthenium*.

Materials and methods

Natural diapauses

Burrowing was used as a behaviour indicator for diapause. Studies on diapause in *Z. bicolorata* were carried out in diapause cages. A diapause cage consisted of a clear glass jar (15 cm diameter) with a 5 cm column of lightly moistened, air dried, field collected soil (clay loam) compressed with a wooden rod. The cage was covered on the open side with a white muslin cloth tied with rubber bands. One hundred adults were collected from the field every two weeks from 15th of June to 31st of December, 2007. They were fed for one week with *Parthenium* leaves (plants grown in small plastic pots) and then released into four diapause cages (25 adults in each) without food and monitored for one week. Adults which burrowed into the soil within one week were counted as being in diapause. Individual weights of diapausing adults were measured using an electronic balance (Avon weighing system Ltd.).

Diapause behaviour under controlled conditions

Rearing of beetle

Ten pairs of beetles (7 days old) were released in a glass jar of 30 cm diameter (A) containing a freshly cut, apical *Parthenium* stem

placed in a glass vial filled with water. The open mouth of the vial was covered with parafilm so that only leaves were available to adult beetles for feeding and egg laying. After 24 h, the beetles were discarded. They were allowed to lay eggs for 24 h only because we desired the cohorts of grubs of the same age. On day 4, the newly hatched grubs that emerged from the eggs were provided with a fresh vial with a freshly cut, apical *Parthenium* stem. The developing grubs were provided a fresh vial on alternate days until day 15 when the fully matured grubs dropped to the bottom of the jar and burrowed into the soil for pupation. Adults emerged on day 24, however the female started laying eggs on day 31 (7 days pre oviposition). So after every month, rearing cycle of test insect was maintained as described above. Since the diapause is influenced by the age of adults and about 50% diapause was obtained in 60 day old adults (Jayanth and Bali, 1993b), we selected adults of this age from our laboratory population in November. Each treatment consisted of 125 adults (5 replicates of 25 adults) in diapausing cages placed in biological oxygen demand incubators maintained at $70 \pm 5\%$ relative humidity and 10 h light period. Each cohort was observed for the extent of diapause.

Effect of temperature on diapause behaviour of *Z. bicolorata*

To study the effect of temperature, a diapausing cage was filled with clay loam soil compressed with a wooden rod, and the moisture level was adjusted to 15% w/v. For each treatment, five cages with 25 beetles each were then placed in BOD incubators maintained at 15, 25 or 30 °C and observed for percent diapause within one week.

Effect of soil type on diapause behaviour of *Z. bicolorata*

Beetles were released in diapausing cages containing clay loam soil, sandy soil, silty soil and soil containing >0.75% organic matter. The moisture level for each soil type was maintained at 15% w/v. Adults that burrowed were counted as being in diapause.

Effect of soil moisture on diapause behaviour of *Z. bicolorata*

To study the role of soil moisture on diapause behaviour of *Z. bicolorata*, beetles were released in diapausing cages filled with oven dried clay loam soil containing 5%, 10% or 25% soil moisture w/v and placed in BOD incubators as above. They were observed for one week for initiation for diapause and the number of adults burrowing was noted.

Effect of insulin treatment on diapause behaviour of *Z. bicolorata*

Two hundred adults were anesthetized (using CO₂) and were individually given an intra-hemocelic application of 5 µl of human insulin 30/70 (30% soluble insulin/70% Isophane insulin) on the ventral side of the abdomen. The injections were made through the side of the abdomen at the third abdominal inter segmental membrane with a Hamilton microsyringe 10 µl. Another 200 untreated adults (control group) received only carrier solution. Treated and control group (eight replicates of 25 adults each) were released into diapausing cages which were prefilled with clay loam soil. These insects were not provided any food during the experimental period. A similar set of experiment was conducted wherein the adults were provided with *Parthenium* leaves in diapausing cages. All the experimental cages were kept at 26 ± 2 °C, $70 \pm 5\%$ relative humidity with a 10 h light period. The numbers of adults burrowing per replicate were observed in each group for two weeks. For post-diapause studies, in experimental cages where diapausing adults had burrowed earlier, water was sprinkled to study the emergence. The first thirty adults were observed to determine the number of days taken to emerge from diapause. Ten pairs of post-diapause adults were selected from each group and reared individually on leaves of *Parthenium* for recording their longevity and fecundity.

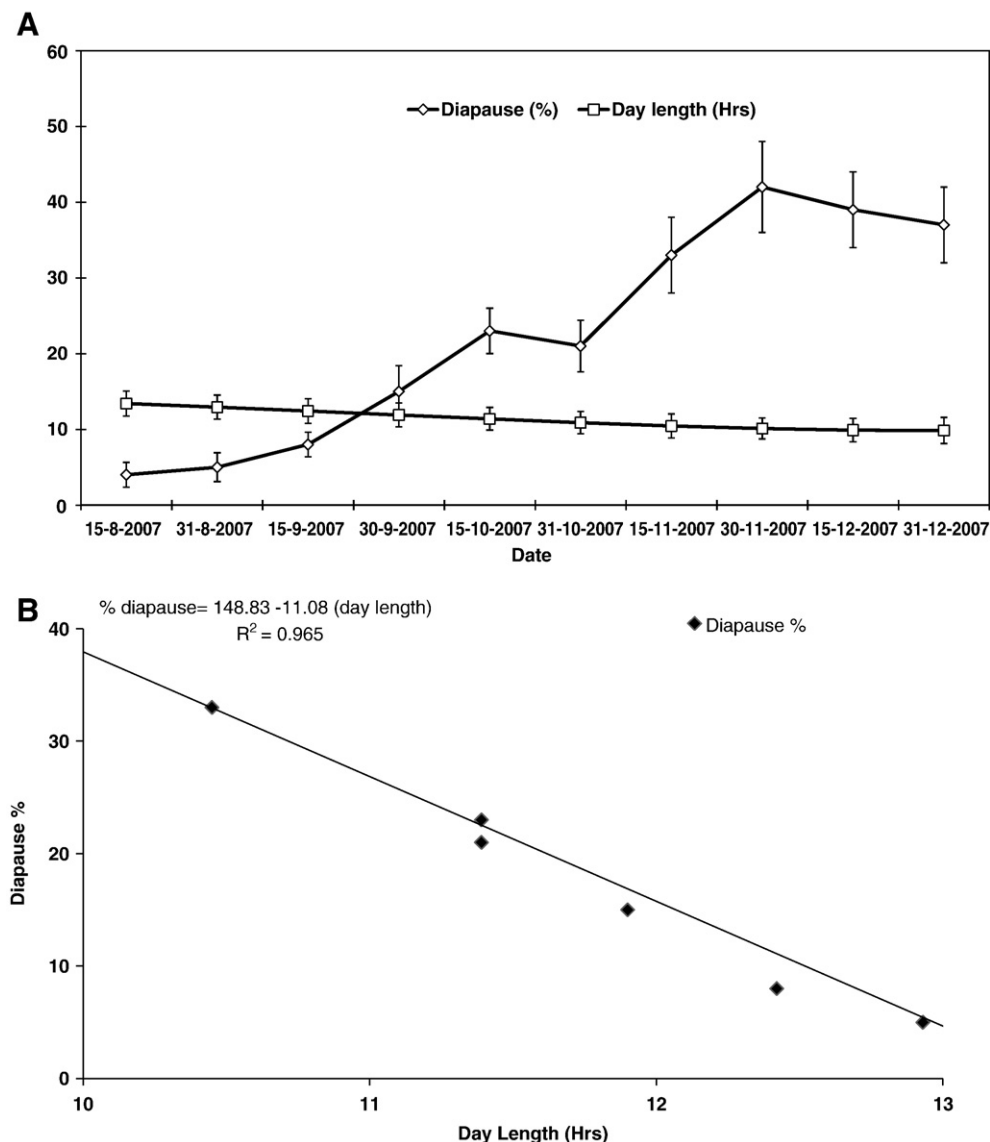


Fig. 1. A, Natural diapause in *Z. bicolorata* and its relationship to day length under Jammu condition. B, Regression plot showing influence of day length on diapause behaviour of beetle.

Data analysis

All data were checked for normal distribution. Data on natural diapause of beetle was subjected to correlation and regression analysis with meteorological data and day length. Laboratory diapause data were subjected to ANOVA followed by Tukey's post hoc test for comparison of means. All percentage data were subjected to arcsine square root transformation prior to ANOVA. The Mann-Whitney *U* test was used to test the significance of the mean weights of diapausing and non-diapausing beetles, as well as for the comparison of diapause in insulin treated and non-treated beetles. All statistical analyses were performed by SPSS 11.0.

Results

Z. bicolorata adults burrowed into chambers they formed 1 to 4 cm beneath the soil surface. None of the adults collected during June and July in Jammu entered diapause. However, diapause gradually increased from 4 to 37% from August to December (Fig. 1A). The percentage of adults entering diapause increased with time. From August to December when the day length became shorter, this

percentage went up and peaked at $42 \pm 6\%$ during the last half of November. The day length influenced the diapause behaviour in 96.6% of the cases (Fig. 1B) and can be predicted by a regression equation as $Y (\% \text{ diapause}) = 148.830 - 11.0893 \times (\text{day length})$ with a coefficient of determination (R^2) value of 96.6%. Moreover, day length, maximum temperature, minimum temperature and rainfall exhibited a significant negative correlation with percent diapause (Table 1) with correlation coefficients of 0.971, 0.797, 0.962 and 0.554, respectively. Mean weight of diapausing adults was significantly higher than those of non-diapausing adults in October ($z = -3.592$; $df = 9$), November ($z = -2.268$, $df = 9$) and December ($z = -3.556$, $df = 9$), 2007 (Fig. 2).

Table 1
Relationship between diapause behaviour of *Z. bicolorata* and abiotic factors.

	Max. temp. °C	Min. temp. °C	Rainfall (mm)	Mean relative humidity (%)	Day length (hours)
Diapause %	-0.797**	-0.962**	-0.554*	-0.734**	-0.971**

* Correlation is significant at 0.05 level.

** Correlation is significant at 0.01 level.

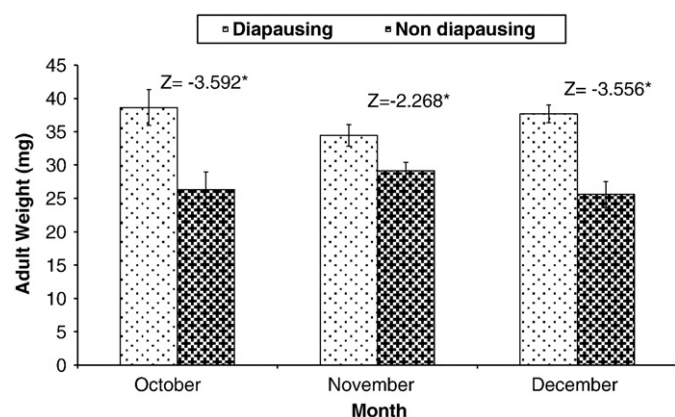


Fig. 2. Mean adult weight in diapausing and non-diapausing population of *Z. bicolorata* during peak period of diapause.

Diapause behaviour under controlled temperature

Temperature significantly influenced adult diapause ($F=7.355$; $df=14$, $P<0.05$). The percent diapause decreased significantly from 40.8 to 20.8% with the increase in temperature from 15 to 30 °C (Table 2).

Role of soil moisture and soil type on diapause behaviour of *Z. bicolorata*

Z. bicolorata adults attempted to burrow into dry soil but were not able to penetrate. However, when the soil surface was moistened, the beetle started burrowing in the soil. The percentage of adults entering diapause increased from 8.8 to 47.20 with the increasing moisture level from 5.0 to 25.0% [$F=22.78$, $P<0.05$] (Table 3). The highest diapause percentage (49.2%) was observed in soil with organic matter $>0.75\%$ and lowest diapause percentage (23.5%) was observed in sandy soil [$F=5.55$, $P<0.05$] (Table 4).

Effect of insulin treatment on diapause behaviour of *Z. bicolorata*

Topical application of 5 µg of human insulin 30/70 caused *Z. bicolorata* adults to undergo diapause at a significantly lower percentate (21%) than untreated adults (37.5%), when kept in diapausing cages without feeding ($z=-3.784$; $df=7$; $P<0.05$). Even when the diapausing beetles were allowed to feed on *Parthenium* in diapausing cages, the percent diapause was significantly lower (16.5%) in treated adults compared to untreated adults (29.0%), [$z=-3.804$, $df=7$, $P<0.05$] (Fig. 3). Further, insulin treatment significantly influenced emergence period from diapause, percent emergence and fecundity (per month) of *Z. bicolorata* which were 93.92 ± 1.73 , 81 ± 1.54 and 512.7 ± 25.38 , respectively, in treated adults compared to 109.05 ± 2.2 , 74 ± 1.82 and 438.3 ± 19.33 , respectively, in untreated adults. However, there was no significant impact of insulin on the longevity of adults.

Table 2
Effect of different temperatures on diapause behaviour of *Z. bicolorata*.

Temperature	No. recovered after 7 days	Per cent diapause
30 °C	125	20.8 ^a
25 °C	125	28.6 ^b
15 °C	123	40.8 ^c

*Mean within a column superscripted by different letters are significantly different ($P<0.05$, Tukey HSD^a).

**Calculated by averaging 5 replicates of 25 diapausing adults.

Table 3
Diapause behaviour of *Z. bicolorata* under variable soil moisture.

Per cent soil moisture	Per cent diapause
5	8.8 ^a
10	26.4 ^b
25	47.20 ^c

*Mean within a column superscripted by different letters are significantly different ($P<0.05$, Tukey HSD^a).

**Calculated by averaging 5 replicates of 25 diapausing adults.

Table 4
Diapause behaviour of *Z. bicolorata* in various soil types.

Type of soil	Per cent diapause
Sandy soil	23.5 ^a
Clay loam soil	41.3 ^{ab}
Silty (canal) soil	46.9 ^b
Soil with organic matter ($>0.75\%$)	49.2 ^b

Mean within a column superscripted by different letters are significantly different ($P<0.05$, Turkey HSD^a).

**Calculated by averaging 4 replicates of 25 diapausing adults.

Discussion

The present study has clarified the role of certain environmental and biological factors on the diapause behaviour of *Z. bicolorata*. However, diapause itself is not a well defined state, but a dynamic succession of developmental, behavioural and physiological events (Tauber et al., 1986). Diapause behaviour of *Z. bicolorata* is complex. Not all adults of *Z. bicolorata* enter diapause and it is unclear whether diapause is obligatory. At the same time, this, too, could not be ascertained what triggered diapause behaviour in *Z. bicolorata*, especially because abundant food was available and weather conditions were favourable from July to October which rules out the possibility of this being facultative diapause. Jayanth and Bali (1993b) also found that the surviving adults perished in the field by February, without reproducing. They may not qualify for inclusion under 'facultative' category either. Since none of the adults collected from the field during May, June and July entered diapause, the population during this period consisted mainly of adults that had emerged after diapause. Nevertheless, the F_1 generation was also present but was probably too young to enter diapause. These observations are similar to the findings made by Sushilkumar (2005). The adults entered diapause only once during its lifetime but newly emerged adults appeared to be the most sensitive stage that induced diapause in *Z. bicolorata* in response to reduced daylength. Vinogradova et al. (1996)

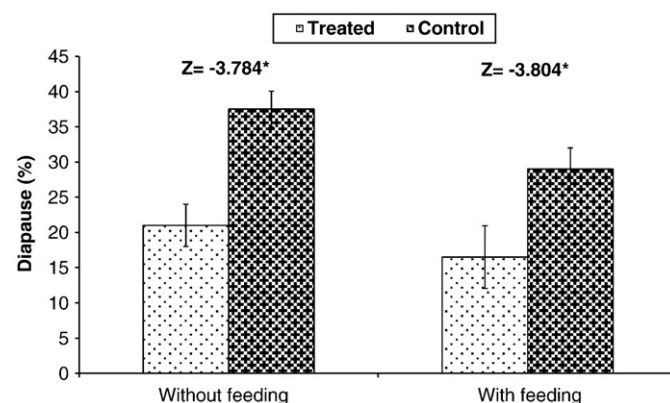


Fig. 3. Effect of insulin on diapause behaviour of *Z. bicolorata* under feeding and no feeding conditions.

reported similar findings for inducing diapause in *Zygogramma* *saturalis*. However, in the Colorado potato beetle, another related insect, pupae or new adults were the most vulnerable for diapause with a critical photoperiod of 15 h at 25°C (de Kort, 1990). The criterion of burrowing behaviour as an indicator of diapause was originally used and tested against *Z. bicolorata* by Jayanth and Bali (1993b) and in the Colorado potato beetle by Bech (1968), de Wilde (1969), and de Wilde and Hsiao (1981). Adults were capable of entering diapause after burying themselves 1 to 6 cm into the soil, which probably makes it a more efficient biological control agent because it eliminates the necessity of reintroducing the beetle to the area where it was originally introduced and also because it ensures proper colonization and dispersal of this beetle (Jayanth and Bali, 1993b). While the burrowing adults are able to consume the recurrent growth of the weed during the following year, they resume their activity late in the season when the *Parthenium* germinates and starts flowering. Our past studies reveal that even though defoliation by *Z. bicolorata* has a visible effect on *Parthenium* under field conditions, the timing of onset, maintenance and termination of diapause are important in the successful adoption of this beetle to new areas. Since *Parthenium* seeds persist in the soil for a long time with about 50% of the buried soil seed-bank remaining viable for up to 6 years (Navie et al., 1998). It continues to germinate throughout the year from the accumulated soil seed-bank and many flushes occur throughout the year. Five such major germinating flushes of *P. hysterophorus* have been observed in one year. Defoliation by *Z. bicolorata* had significant impact on plant height, plant density and flower production in flushes F_3 , F_4 and F_5 but it did not lead to any significant influence in flushes F_1 and F_2 . These two flushes exhibited longer periodicity, profuse branching, a longer flowering period and maximum flower production and contributed the majority to the existing seed soil bank in the area of study. However, the appearance of the beetle on these two flushes was delayed and asynchronous. Because these flushes contribute less to the soil seed-bank, the existing soil seed-bank is unlikely to be depleted in totality. Therefore, either the augmentative releases of laboratory reared beetles should be carried out during March–April every year on these flushes or beetle diapause should be manipulated to facilitate its early termination and to synchronize it with the early flushes, F_1 and F_2 .

The percentage of field collected adults burrowing into the soil increased from August to November in Jammu. This increase in diapause was attributed to decreased day length, decrease in temperature and senescing host-plant foliage, as reported in *L. decemlineata* by Hare (1990). The reduction in percentage of diapause observed during October could be due to the presence of many non-diapausing adults from previous generations and the dryness of the soil (rainfall during October was 0 mm and R.H. ranged from 59.5 to 64.5). These results substantiate earlier findings of Jayanth and Bali (1993a). As the percent diapause of *Z. bicolorata* increased from August to December, the light period decreased from 13.16 to 9.87 h, which negatively influenced diapause behaviour of *Z. bicolorata* in 96.6% of the insects. Tauber and Tauber (1976) observed that photoperiod plays an important role in the induction and termination of diapause in *Leptinotarsa decemlineata*, a very closely related beetle which entered diapause after exposure to a critically short photoperiod. This short day diapause is probably a complex of physiological regulatory mechanisms at the neuro-endocrinological and target tissue levels (Tauber et al., 1988). While inactivation of the corpora allata remained one feature of the diapause syndrome, changes in ecdysteroid concentration in the Colorado potato beetle in relation to day length were also observed and it was suggested that ecdysteroids might be involved in induction and termination of diapause (Briers and de Loof, 1981; Briers et al., 1982).

Mean weight of diapausing adults was significantly higher than those of non-diapausing adults during the peak period (October, November and December). This indicates that diapausing and non-

diapausing individuals can be differentiated on this criteria. This higher mean weight of diapausing adults might be due to the accumulation and breakdown of metabolic reserves, such as lipids and glycogen (Tauber et al., 1986; Danks, 1987; Adedokun and Denlinger, 1984), the deposition of extra layers of hydrocarbons for water proofing the cuticle (Yoder et al., 1992), or the synthesis of storage proteins (Chippendale, 1988). In Colorado potato beetles, food utilization during diapause was used primarily to increase nutritional reserves in the fat body and not for reproduction. Lipids started to accumulate in the fat body immediately after emergence, followed by proteins from day 6 onwards (Dortland and de Kort, 1978). The proteins, which accumulated first in the haemolymph and later in the fat body, have been called diapause proteins. Also, other blood proteins increased under short day, resulting in a concentration of total proteins 3 times higher in the haemolymph (de Kort, 1990).

In the present study, the experiment was planned with a temperature range of 15 to 30°C, as continuous exposure to 30°C or higher induced emergence in this pest within few days (Jayanth and Bali, 1993c). A lower percentage of adults underwent diapause as the temperature increased from 15 to 30. The significance of this inverse relationship between temperature and diapause can be utilized for our future studies on comparing diapause among intra-population or for identifying a non-diapausing strain through laboratory selection. Over the last ten years, there has been heavy defoliation by this beetle in moist areas near streams and canals. Therefore, it was necessary to ascertain whether this occurs due to soil moisture. Our findings clearly establish that soil moisture plays an important role during diapause initiation and emergence. It provides ideal physical conditions for successful burrowing and emergence from diapause chambers. Moreover, unseasonal rains during November and December may provide favourable conditions for burrowing. Jayanth and Bali (1993b) observed that *Z. bicolorata* adults could not penetrate dry soil for diapause and that they attempted burrowing but were unable to penetrate when the soil alone was moistened. Most of the adults collected during November, when the soil was dry, burrowed within 2 h of being given moist soil in the laboratory. In moist areas, as the proportion of adults undergoing diapause remains high, more adults emerged from the soil after termination of diapause. More defoliation was then observed. The physio-chemical attributes of soil, such as structure, texture, and water potential, also played an important role in diapause of *Z. bicolorata*. While information on this aspect in the target species is completely lacking, this phenomenon is well known in the Colorado potato beetle. Canal soil (silty) and soil with high organic matter (>0.75%) were most suitable for diapause. Lighter and loamy soil rich in organic substance with better aeration and no excessive water were most suitable for Colorado potato beetle (Kuusik et al., 2001; Hiisaar et al., 2006). Significant variation in percentage of adults entering diapause in beetles collected from different geographical populations suggests that the beetle is gradually adapting to adverse conditions in different locations. Therefore the scope for selection of non-diapausing strains of this beetle in the future cannot be ruled out. As cooler areas lead to increased diapause, the beetle is likely to have more impact in areas with moderate climate.

Insulin-like peptides (ILPs) exist in insects and are encoded by multigene families that are expressed in the brain and other tissues. Upon secretion, these peptides likely serve as hormones, neurotransmitters, and growth factors. Suppression of insulin signaling has been implicated in the induction of adult diapause in *D. melanogaster* (Williams et al., 2006; Tu et al., 2005) and is integral to diapause in the mosquito, *C. pipiens* (Sim and Denlinger, 2009). Our findings suggest that insulin plays an important role in the termination of diapause, along with various physiological and biochemical changes, without affecting longevity or fecundity of *Z. bicolorata*. Further, treated adults exhibited a low percentage of diapause, reduced time for emergence from diapause and increased percent emergence. The ability of

vertebrate insulin to break pupal diapause in *Pieris brassicae* is well documented. It triggers a reactivation of the neuroendocrine system leading to a neosynthesis of ecdysone beginning 6 days after treatment (Arpagaus, 1987). The involvement of ecdysteroids in the induction and termination of diapause is well established in adults of CPB (Briers and de Loof, 1981; Briers et al., 1982). Watanabe and Tanaka (2000) showed that topical application of pyriproxyfen to autumn *Aulacophora nigripennis* adults led to early termination of diapause, but also changed various physiological and biochemical traits such as chill tolerance and myo-inositol, lipid and glycogen metabolism. This suggests that most of the overwintering traits of this beetle may be under the control of JH which generally regulates adult reproductive diapause (Danks, 1987), which was also the case for the beetle (Watanabe and Tanaka, 1998). Cold-hardiness and lipid metabolism were often linked to the diapause in insects that have winter diapause (Denlinger, 1991). The potential role of insulin or ecdysteroids for enhancing the efficacy of *Zygogramma* beetle on *Parthenium* control cannot be ruled out and will be explored further.

Uncited reference

Wu & Brown, 2006

Acknowledgements

The authors are grateful to Dr. K. P. Jayanth for suggestions and guidance during the course of investigation and to the Director of Research, SKUAST-Jammu for support. Our sincere thanks are also due to the Department of Science and Technology, Government of Jammu and Kashmir and the National Center for Weed Research, Jabalpur for encouragement and co-operation.

References

- Adedokun, T.A., Denlinger, D.L., 1984. Cold-hardiness: a component of the diapause syndrome in pupae of the flesh flies, *Sarcophaga crassipalpis* and *S. bullata*. *Physiol. Entomol.* 9, 361–364.
- Arpagaus, M., 1987. Vertebrate insulin induces diapause termination in *Pieris brassicae*. *Dev. Gene Evol.* 196, 527–530.
- Beck, S., 1968. *Insect Photoperiodism*. Academic Press, New York, p. 288.
- Briers, T., de Loof, A., 1981. Moulting hormone activity in the adult Colorado potato beetle, *Leptinotarsa decemlineata* in relation to reproduction and diapause. *Int. J. Invertebr. Rep. Dev.* 3, 379–386.
- Briers, T., Peferoen, M., de Loof, A., 1982. Ecdysteroids and adult diapause in Colorado potato beetle, *Leptinotarsa decemlineata*. *Physiol. Entomol.* 7, 379–386.
- Chippendale, G.M., 1988. Role of proteins in insect diapause. In: Sehna, F. Azabza, Denlinger, D.L. Wroclaw (Eds.), *Endocrinological Frontiers in Physiological Ecology*. Pol.; Wroclaw Tech. Univ. Press, pp. 331–346.
- Danks, H.V., 1987. Insect dormancy: an ecological perspective. *Biological Survey of Canada (Terrestrial Arthropods)*, Ottawa, Ont, p. 439.
- de Kort, C.A.D., 1990. Thirty five years of diapause research with the Colorado potato beetle. *Entomol. Expt. Applic.* 56, 1–13.
- de Wilde, J., 1969. Diapause and seasonal synchronization in the adult Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Symposium of the Society of Experimental Biology. Dormancy and Survival*. Cambridge University Press, Cambridge, UK, pp. 263–284.
- de Wilde, J., Hsiao, T., 1981. Geographical diversity of the Colorado potato beetle and its infestation in Euracia. In: Lashomb, J.H., Casagrande, R., Ross, Hutchinson (Eds.), *Advances in Potato Pest Management*, pp. 37–72 (Stroudsburg, PA).

- Denlinger, D.L., 1991. Relationship between cold hardiness and diapause. In: Lee Jr., R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman & Hall, New York, pp. 174–198.
- Dortland, J.F., de Kort, C.A.D., 1978. Protein synthesis and storage in the fat body of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Insect. Biochem.* 8, 93–98.
- Geminard, C., Arquier, N., Layalle, S., Bourouis, M., Slaidina, M., Delanoue, R., Bjordal, M., Ohanna, M., Ma, M., Colombani, J., Leopold, P., 2006. Control of metabolism and growth through insulin-like peptides in *Drosophila*. *Diabetes* 155 (2), 55–58.
- Gupta, S., 2008. Diapause behaviour and impact assessment of *Zygogramma bicolorata* Pallister on *Parthenium hysterophorus* L. in Jammu. M. Sc. Thesis submitted to SKUST-Jammu. L. 54.
- Gupta, R.K., Bali, K., Khan, M.S., 2002. Biological war against congress grass in J&K. *Popular Sci.* 10, 28–33.
- Gupta, R.K., Bali, K., Khan, M.S., Monobull, Md., Bhagat, R.M., 2004. Predatory bugs of *Zygogramma bicolorata*: an exotic beetle or biological suppression of *Parthenium hysterophorus* L. *Curr. Sci.* 87, 1005–1010.
- Gupta, R.K., Bali, K., Monobull, Md., Bhagat, R.M., 2006. Biological suppression of *Parthenium hysterophorus* L. in J&K: a decade of introduction of an exotic beetle *Zygogramma bicolorata* Pallister. *Proceeding of Ist J&K Science Congress*, Feb 21–22, University of Jammu, J&K, India, pp. 215–216.
- Hare, J.D., 1990. Ecology and management of the Colorado potato beetle. *Annu. Rev. Entomol.* 35, 81–100.
- Hiiesaar, K., Metspalu, I., Joudu, J., Joger, K., 2006. Over-wintering of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in field conditions and factors affecting its population density in Estonia. *Agron. Res.* 4, 21–30.
- Jayanth, K.P., Bali, G., 1993a. Biological studies on *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), a biocontrol agent of *Parthenium hysterophorus* L. (Asteraceae). *J. Biol. Control* 7, 93–98.
- Jayanth, K.P., Bali, G., 1993b. Diapause behaviour of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae), a biocontrol agent of *Parthenium hysterophorus* in Bangalore, India. *Bull. Entomol. Res.* 83, 383–388.
- Jayanth, K.P., Bali, G., 1993c. Temperature tolerance of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) introduced for biological control of *Parthenium hysterophorus* (Asteraceae) in India. *J. Entomol. Res.* 17, 27–34.
- Kuusik, A., Hiiesaar, K., Metspalu, L., Hermann, P., 2001. The physiological characteristics of the perennial diapause in the Colorado potato beetles. *Trans. Esto. Agril. Univ.* 212, 161–166.
- Navie, S.C., Panetta, F.D., McFadyen, R.E., Adkins, S.W., 1998. Behaviour of buried and surface-lying seeds of *Parthenium* weed (*Parthenium hysterophorus* L.). *Weed Res.* 38, 338–341.
- Sim, C., Denlinger, D.L., 2008. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *Proc. Natl Acad. Sci. U. S. A.* 105, 6777–6781.
- Sim, C., Denlinger, D.L., 2009. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *Insect Mol. Biol.* 18 (3), 325–332.
- Sushilkumar, 2005. Biological control of *Parthenium* through *Zygogramma bicolorata*. NRCWS Technical Bulletin No. 5, p. 89.
- Tauber, M.J., Tauber, C.A., 1976. Insect serenity disperses maintenance termination and post diapauses development. *Annu. Rev. Entomol.* 21, 81–107.
- Tauber, M.J., Tauber, C.A., Masaki, S., 1986. *Seasonal Adaptations of Insects*. Oxford University Press, New York.
- Tauber, M.J., Tauber, C.A., Obrycki, J.J., Gollands, B., Wright, R.J., 1988. Voltinism and the induction of aestival diapause in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* 81, 748–754.
- Tu, M.P., Yin, C.M., Tatar, M., 2005. Mutations in insulin signaling pathway alter juvenile hormone synthesis in *Drosophila melanogaster*. *Gen. Comp. Endocrinol.* 142, 347–356.
- Vinogradova, E.A., Pantuykhov, G.A., Bratchikova, N.Y., 1996. Environmental control and reproductive diapause in the leaf beetle, *Zygogramma saturalis* F. (Coleoptera: Chrysomelidae). *Folia Biol.* 44, 67–72.
- Watanabe, M., Tanaka, K., 1998. Effect of juvenile hormone analogues on diapause termination and myo-inositol content in *Aulacophora nigripennis* adults (Coleoptera: Chrysomelidae). *Appl. Entomol. Zool.* 33, 259–262.
- Watanabe, M., Tanaka, K., 2000. Hormonal control of diapause and over wintering traits in a leaf beetle, *Aulacophora nigripennis*. *Physiol. Entomol.* 25, 337–345.
- Williams, K.D., Busto, M., Suster, M.L., So, A.K., Ben-Shahar, Y., Leever, S.J., Sokolowski, M.B., 2006. Natural variation in *Drosophila melanogaster* diapause due to the insulin-regulated PI3-kinase. *Proc. Natl. Acad. Sci. U. S. A.* 103 (43), 15911–15915.
- Wu, Q., Brown, M.R., 2006. Signaling and function of insulin-like peptides in insects. *Annu. Rev. Entomol.* 51, 1–24.
- Yoder, J.A., Denlinger, D.L., Dennis, M.W., Kolattukudy, P.E., 1992. Enhancement of diapausing flesh fly puparia with additional hydrocarbons and evidence for alkane biosynthesis by a decarbonylation mechanism. *Insect Biochem. Mol. Biol.* 22, 237–243.