Insect Science (2008) 15, 441−445, DOI 10.1111/j.1744-7917.2008.00231.x

Effect of pine foliage damage on the incidence of larval diapause in the pine caterpillar *Dendrolimus punctatus* (Lepidoptera: Lasiocampidae)

Li-Li Huang[1], Guo-Hong Wang[2], Zhong He[1] and Feng Ge[1]

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, and [2]Key Laboratory of Developmental Biology and Neurobiology, College of Life Sciences, Fujian Normal University, Fuzhou, China

Abstract The pine caterpillar *Dendrolimus punctatus* (Walker) with a larval facultative diapause is one of the most destructive insect pests of the pine tree *Pinus massoniana* in China. The larvae feeding on pine trees with different damage levels were studied to determine the induction of diapause under both laboratory and field conditions. Developmental duration of larvae before the third instar was the longest when fed with 75%−90% damaged needles, followed by 25%−40% damaged needles and intact pine needles, whereas mortalities did not differ among different treatments under the conditions of 25°C and critical photoperiod 13.5:10.5 L:D. At 25°C, no diapause was induced under 15:9 L:D, whereas 100% diapause occurred under 12:12 L:D regardless of the levels of needle damage. Incidences of larvae entering diapause when they were fed with intact, 25%−40% and 75%−90% damaged pine needles were 51.7%, 70.8% and 81% under 13.5:10.5 L:D, respectively. Similar results were obtained in the field experiment. Incidence of diapause was significantly different among the pine needle damage levels of pine trees when the photoperiod was close to the critical day length, indicating that the effect of host plants on diapause induction was dependent on the range of photoperiod. The content of amino acid and sugar decreased and tannin increased in pine needles after feeding by the pine caterpillars, suggesting that changed levels of nutrients in damaged needles or a particular substance emitted by damaged pine trees was perhaps involved in the diapause induction of the pine caterpillar.

Key words *Dendrolimus punctatus*, diapause induction, leaf damage, photoperiod, *Pinus massoniana*

Introduction

In temperate zones, facultative diapause is a strategy that allows insects to initiate an additional generation when conditions are favorable, or to enter diapause when they are not (Tauber *et al*., 1986). Photoperiod is a reliable cue for seasonality, and temperature and food quality modify the response of insects to photoperiod due to interaction between the photoperiod and two other cues for temperate zone species (Tauber *et al*., 1986; Danks, 1987). The induction of diapause of phytophagous insects is dependent on host plant species and parts of host plants they consume (Tauber *et al*., 1986; Danks, 1987; Steinberg *et al*., 1992; Hunter & McNeil, 1997; Tanzubil *et al*., 2000; Wang *et al*., 2006). However, whether the damage levels of host plants affect the induction of diapause or not is still unclear, although food resources have important influences on population dynamic in insects.

Pine caterpillar *Dendrolimus punctatus* (Walker)

Correspondence: Feng Ge, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Datun Lu, Chaoyang District, Beijing 100101, China. Tel: +86 10 64807123; fax: +86 10 64807099; email: gef@ioz.ac.cn

(Lepidoptera: Lasiocampidae) is one of the most destructive insect pests of the pine tree *Pinus massoniana*. The larvae (undergoing about 6−7 instars) seriously damage pine needles and periodically break out every 3−5 years in China (Chen, 1990; Ge *et al*., 1997). This species can complete 2−3 generations per year, and overwinters as third to fourth instars of the second and third generations in the region near 30°N in China (Chen *et al*., 1988; Li *et al*., 1994; Wang *et al*., 1995). Diapause is induced at larval stage under shorter photophase than 13.5 h, the critical day length around 25°C. The first and second instars are most sensitive to photoperiod (Li & Jia, 1991; Huang *et al*., 2005). Previous studies indicated that damage of pine trees can be caused by the first, second and third generation, and serious damage of the pine tree is related to the difference between the second and third generation of the pine caterpillar (Chen *et al*., 1988; Li *et al*., 1994; Wang *et al*., 1995; Wen & Wen, 2000). We propose that pine needles, especially the damage levels of pine needles, are also involved in the facultative larval diapause induction in this species, and this may be related to changed levels of nutrients in damaged needles or a particular substance emitted by damaged pine trees.

The objective of this study was to evaluate the effect of needle damage of pine trees on diapause induction in *D. punctatus* under different photoperiods.

Materials and methods

Insect

Populations of *D. punctatus* were collected from the Yudu County (28°58'N, 115°25'E), Jiangxi Province, China in 2003. The pine caterpillars were continuously reared on pine needles in climate chambers for several generations under conditions of 25°C and 16: 8 L: D (Huang *et al*., 2005).

Damage levels of pine trees

The damage levels of pine trees were defined as the average length of damaged pine needles divided by the average length of intact pine needles. Four hundred random-sampled needles from upper and lower parts of the crown from all four aspects were checked from each tree.

Sixty pine trees (approximately 2 m high) with well-developing canopies (not damaged) near Jiangxi Agricultural University (28°46' N, 115°50' E) were chosen for laboratory and field experiments. Different numbers of larvae were released on one pine tree at different times according to the occurrence of *D. punctatus* in the field deduced from a previous preliminary experiment. Each pine tree was infested with 200 (25%−40% damage level) or 400 (75%−90% damage level) fourth instar larvae according to the occurrence of the first generation. Each pine tree, including the control, was covered by gauze net after introducing pine caterpillar larvae. The larvae were removed when the damage level reached the above levels. The trees covered in gauze netting were used for laboratory and field experiments. Laboratory and field experiments were carried out at the same time (from July to October).

Induction of diapause under laboratory conditions

Three damage levels of pine trees and six trees for each damage level were used for rearing the pine caterpillars: A, intact pine needles (control); B, 25%−40% damaged pine needles (infested by the larvae of the first generation); C, 75%−90% damaged pine needles (infested by the larvae of the first generation).

After hatching, larvae of the pine caterpillar were fed with intact pine needles from the trees with different damage levels at 25°C combined with different photoperiods (15: 9 L: D [the diapause-averting photoperiod], 13.5: 10.5 L: D [the critical photoperiod] and 12: 12 L: D [the diapause-inducing photoperiod]). Intact pine needles from the trees with different damage levels were used because most of the larvae (> 90%) fed on this kind of newly developed intact pine needle in the field. The experiments were carried out in climate cabinets equipped with three fluorescent 30 W tubes controlled by an automatic timer. The light intensity was approximately 2.0 W/m^2, variation of temperatures was ± 1°C, and relative humidity was 70% ± 10%. Fifty newly hatched larvae were reared in one transparent plastic box (7.5 cm wide at base and 15.0 cm deep) and daily supplied with fresh pine needles until diapause determination. Each treatment was replicated three times.

Incidence of diapause under field conditions

Three treatments simulated the needle damages of pine trees under natural conditions in the field to evaluate the effect of needle damage on the incidence of diapause. Each group of larvae hatched on the same day was divided into three subgroups which were reared under different damage levels: A, the intact pine trees (control); B, 25%−40% damaged pine trees (infested by larvae of the first generation); C, 75%−90% damaged pine trees (infested by larvae of the first generation). According to the occurrence period of the second generation larvae of the pine caterpillar, newly hatched larvae were introduced on pine trees with different damage levels on July 15, July 25, August 3, August 9, August 15 and August 25, respectively. Two hundred newly hatched larvae were used for each tree in each test.

The larvae were set on branches in gauze cages, and were checked daily until diapause determination. Each treatment comprised of three trees (replicates).

Chemical analysis of pine foliage contents

Leaves (500 g/treatment) were collected from intact pine, 25%−40% damaged pine and 75%−90% damaged pine used in the laboratory experiment at the same time as larvae-releasing. The needle was cut with a razorblade about 10 cm from the top of every needle around the tree. The pine needles were desiccated at 70 °C and stored in a desiccator. Dried pine needles (200 mg) were extracted in 70% acetone. Acetone was removed by passing gaseous nitrogen over the supernatant fluid at 55 °C, filtered through a 0.45 μm nylon filter, and hemoglobin solution was used as the reaction protein. The astringency of the tissue was expressed as that relative to the standard quebracho tannin. Amino acid concentration of pine needles was analyzed with an ion-exchange amino acid analyzer (Beckman Model 7300, Beckman, Fullerton, CA, USA) using the ninhydrin method (Sandstrom *et al*., 2000). Sugar content was analyzed with a spectrophotometric glucose test after invertase and isomerase addition (Hoch *et al*., 2002). Tannin content was analyzed by the method of phosphomolybdic acid−sodium phosphomolybdate (Horner *et al*., 1987). Each test was replicated three times.

Diapause determination

The larvae entered diapause at the third to fourth instar, settled in the axil of pine needles and ceased feeding. The diapausing larvae were recognized by their small size and yellow body color (Li & Jia, 1991; Huang *et al*., 2005).

Statistical analysis

Using the SPSS (SPSS Inc., Chicago, IL, USA), the data were tested with one-way analysis of variance (ANOVA) and means were compared by Tukey's test at $P = 0.05$. The data were arcsine square-root-transformed prior to analysis.

Results

Developmental duration, mortality of larvae before the third instar

Developmental duration of larvae before the third instar differed significantly among the treatments fed with the pine needles with different damage levels under the conditions of 25 °C and 13.5: 10.5 L: D (Table 1). Developmental duration of larvae before the third instar was the longest when fed with 75%−90% damage needles (14.67 ± 0.7 days), followed by 25%−40% damage needles (13.3 ± 0.4 days) and intact pine needles (12.0 ± 0.5 days). However, there was no significant difference among mortalities of larvae before the third instar fed with pine needles with different damage levels under same conditions (Table 1).

Table 1 Effect of different damage levels of pine needles on the duration of development and mortality of larvae before the third instar in *Dendrolimus punctatus* at 25 °C and 13.5: 10.5 L: D.

Damage level of pine needles	Developmental duration (day)	Mortality (%)
Intact	12.0 ± 0.5 c	6.0 ± 3.2 a
25%−40%	13.3 ± 0.4 b	8.2 ± 5.0 a
75%−90%	14.6 ± 0.7 a	8.0 ± 3.3 a

The pine needles were collected from six trees at each damaged level. Values (mean ± SD) followed by different letters within a column are significantly different by Tukey's test at $P < 0.05$. Each test was replicated three times.

Induction of diapause under laboratory conditions

No larvae entered diapause under 25 °C and 15: 9 L: D, whereas almost all the larvae diapaused under 25 °C and 12: 12 L: D regardless of the damage levels of pine needles. Diapause inductions differed significantly among different damage levels of pine needles under intermediate light conditions (13.5: 10.5 L: D) ($F_{2,8} = 15.42$; $P = 0.003$). The incidence of diapause was the highest when fed with 75%−90% damaged needles, followed by 25%−40% damaged needles and intact pine needles (Fig. 1).

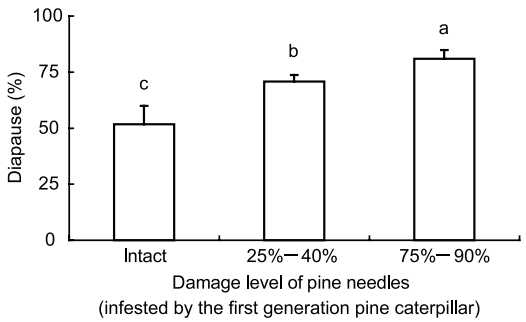

Fig. 1 Incidence of diapause of the larvae fed with pine needles with different damage levels in *Dendrolimus punctatus* at 25 °C and 13.5: 10.5 L: D. The pine needles were collected from six trees at each damage level. Each test was comprised of three replicates. Error bars indicate the SD. Bars with different letters are significantly different by Tukey's test ($P < 0.05$).

Incidence of diapause under field conditions

For the pine caterpillars released on pine trees in the field, the later the larvae hatched, the higher the incidence of diapause (Fig. 2). No larvae entered diapause hatching on July 15, whereas all larvae hatching on August 25 entered diapause. Diapause incidence was low in larvae hatching on July 25 ($F_{2,8} = 0.490$; $P = 0.635$) and high in larvae hatching on August 15 ($F_{2,8} = 0.510$; $P = 0.624$), thus the difference among different treatments was not significant. Incidences of diapause differed among the larvae hatching on August 3 ($F_{2,8} = 3.282$; $P = 0.109$), the incidence of diapause in the larvae feeding on the intact pine trees was significantly lower than in those feeding on the damaged pine trees. More than 50% larvae hatching on August 9 entered diapause, and diapause incidence increased significantly with the increased damage level ($F_{2,8} = 27.591$; $P = 0.001$), whereas the difference was not significant in the larvae feeding on the same damage-level needles.

Amino acid, sugar and tannin content in the pine needles

Amino acid, sugar and tannin contents were significantly different among the pine needles with different damage levels (Table 2). The tannin contents increased significantly with an increase of damage level ($F_{2,8} = 33.655$; $P = 0.001$), but reversed results were obtained for the total concentrations of amino acid ($F_{2,8} = 254.698$; $P < 0.001$) and sugar ($F_{2,8} = 74.968$; $P < 0.001$).

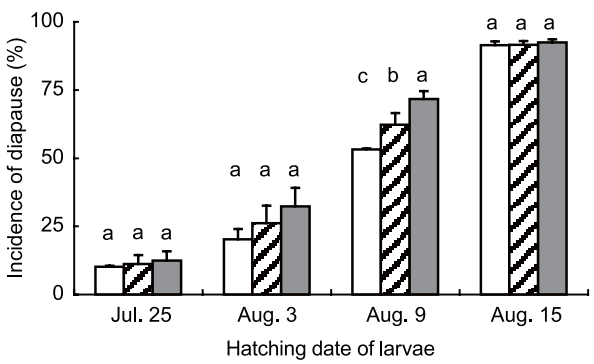

Fig. 2 Incidence of diapause of the larvae of *Dendrolimus punctatus* feeding on pine trees with different damage levels in the field in Nanchang during 2004. Each test was comprised of three replicates. Error bars indicate the SD. Bars with the same letter are not significantly different by Tukey's test ($P > 0.05$). □ the intact pine trees (control); ▨ 25%−40% damaged pine trees (infested by the first generation pine caterpillar); ■ 75%−90% damaged pine trees (infested by the first generation pine caterpillar).

Table 2 Contents of the amino acid, sugar and tannin in the needles of *Pinus massoniana* with different damage levels (2004, Nanchang).

Damage level of pine needles	Amino acid (mg/g)	Sugar (mg/g)	Tannins (mg/g)
Intact	84.90 ± 0.17 a	169.97 ± 0.26 a	31.35 ± 0.08 c
25%−40%	82.57 ± 0.50 b	159.30 ± 0.61 b	32.23 ± 0.03 b
75%−90%	75.31 ± 0.36 c	155.90 ± 0.79 c	33.75 ± 0.07 a

The pine needles were collected from six trees at each damaged level. Values (mean ± SD) followed by different letters within a column are significantly different by Tukey's test at $P < 0.05$. Each test was replicated three times.

Discussion

The laboratory experiment showed that all larvae entered diapause under short-day conditions, whereas no individuals entered diapause under long-day conditions regardless of the damage levels of the pine needles. The results thus indicated that mainly photoperiod determines the diapause induction in *D. punctatus* (Huang *et al.*, 2005). Diapause inductions differed significantly among different damage levels of pine needles only under the critical photoperiod in *D. punctatus* (Fig. 1). This was in agreement with a previous study, in which the effect of host-plant on diapause induction was expressed under the critical diapause-inducing condition because the host-plant was only a weak diapause-regulating factor (Wang *et al.*, 2006). The results indicated that the primary factor responsible for diapause induction is photoperiod and quality of the host-plant can work around the critical day length (13.5: 10.5 L: D) in this species.

Both laboratory and field investigations indicated that the damage levels of pine needles modified the diapause induction in *D. punctatus* under photoperiods near the critical photoperiod (the day length is ≈ 13 h 50 min to 13 h 20 min from July 25 to August 15 in Nanchang). Incidence of diapause increased with the increasing damage levels of the pine needles (Figs. 1,2). Previous results showed that changes in the nutritional elements of the pine needles caused by serious damage were detrimental for larval survival and development (Ge *et al.*, 1997) and the quality of the foliage consumed by the larvae could be one factor responsible for the variation in diapause induction in this species (He, 1995). The present study indicated that the duration of larval development before the third instar was affected by the damage level of the pine needles (Table 1), and the total concentrations of amino acid and sugar decreased and the tannin content increased significantly with an increase of damage level (Table 2). These results suggested that changed levels of nutrients in damaged

needles or a particular substance emitted by damaged pine trees was perhaps involved in the diapause induction of the pine caterpillar. Furthermore, the effect of modified needles from damaged pines on diapause induction may be related to the developmental duration of first and second instars, which leads to an increasing number of photoperiodic cycles that they experience.

Indeed, it is more likely that diapause induction is more strongly influenced by an interaction of food quality, photoperiod and temperature than by a single factor. Our results are consistent with most earlier studies (Tauber *et al*., 1986; Danks, 1987; Wang *et al*., 2006). These experimental results enable speculation on the incidence of diapause of the second generation of *D. punctatus* in the field. Serious damage to the pine trees in the course of the first and second generations may cause most larvae of the second generation to enter diapause; consequently, the population of the third generation will decrease. This was in agreement with previous investigations in the field (He, 1995; Wang *et al*., 1995; Wen & Wen, 2000). These response patterns might play an important role in the life history of the pine caterpillar. Larvae entered diapause in heavily damaged host plants near the critical day-length. Therefore, the production of the next generation might be avoided in this region due to lowered quality and quantity of food.

Acknowledgments

We thank Drs. Roland Mumm, Yutong Qiu and Ties Huigens for their critical reading and helpful comments on an earlier version of the manuscript. This research was supported in part by Key Program of National Natural Science Foundation of China (30330490) and Project from Natural Science Foundation of Fujian Province (2007J0305).

References

Chen, C.J., Wang, Z.X., Liu, G.Z., Gao, H., Tao, L., Chen, J.Y. and Wang, Z.N. (1988) Studies on introduction and utilization of the pine caterpillar *Dendrolimus spectabilis* CPV. *Forest Research*, 1, 14–24. (in Chinese with English abstract)

Chen, C.J. (1990) *Integrated Management of Pine Caterpillars in China*. China Forestry Publishing, Beijing. (in Chinese)

Danks, H.V. (1987) *Insect Dormancy*: *An Ecological Perspective*. Biological Survey of Canada (Terrestrial Arthropods), Ottawa. 439 pp.

Ge, F., Li, D.M., Qiu, Y.X. and Wang, G.H. (1997) Studies on the changes of some chemicals in damaged pine needles and their effects on population parameters of pine caterpillar. *Acta Entomologica Sinica*, 40, 337–342. (in Chinese)

He, Q.Q. (1995) Inquiry to law of differentiation in second and third generation of *Dendrolimus punctatus* Walker. *Journal of Zhejiang Forest Science and Technology*, 15(2), 38–41. (in Chinese)

Hoch, G., Popp, M. and Korner, Ch. (2002) Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos*, 98, 361–374.

Horner, J.D., Cates, R.G. and Gosz, J.R. (1987) Tannin, nitrogen, and cell wall composition of green vs. senescent Douglas-fir foliage. Within- and between-stand differences in stands of unequal density. *Oecologia*, 72, 515–519.

Huang, L.L., Xue, F.S., Wang, G.H., Han, R.D. and Ge, F. (2005) Photoperiodic response of diapause induction in the pine caterpillar *Dendrolimus punctatus*. *Entomologia Experimentalis et Applicata*, 117, 127–133.

Hunter, M.D. and McNeil, J.N. (1997) Host plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology*, 78, 977–986.

Li, Z.L. and Jia, F.Y. (1991) The photoperiodic reaction (PhPR) of the pine caterpillar: studies on the mechanism of the second generation differentiation. *Forest Research*, 4, 409–413. (in Chinese)

Li, Z.L., Jia, F.Y., Hou, W.W., He, Z., Wen, Z.Z. and Wen, X.S. (1994) Photoperiodical reaction of the pine caterpillar *Dendrolimus punctatus* (Walker). *Acta Entomologica Sinica*, 37, 31–37. (in Chinese)

Sandstrom, J., Telang, A. and Moran, N.A. (2000) Nutritional enhancement of host plants by aphids–a comparison of three aphid species on grasses. *Journal of Insect Physiology*, 46, 33–40.

Steinberg, S., Podoler, H., Applebaum, S.W. (1992) Diapause induction in the codling moth, *Cydia pomonella*: effect of larval diet. *Entomologia Experimentalis et Applicata*, 62, 269–275.

Tanzubil, P.B., Mensah, G.W. and McCaffery, A.R. (2000) Diapause initiation and incidence in the millet stem borer, *Coniesta ignefusalis* (Lepidoptera: Pyralidae): the role of the host plant. *Bulletin of Entomological Research*, 90, 365–371.

Tauber, M.J., Tauber, C.A. and Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York. 411 pp.

Wang, C., Wang, G., He, Q. and Chen, Y. (1995) A study on the generation differentiation of pine caterpillar *Dendrolimus punctatus (Lepidoptera:* Lasiocampidae). *Scientia Silvae Scinicae*, 31(6), 359–364 (in Chinese)

Wang, X.P., Ge, F. and Xue, F.S. (2006) Host plant mediation of diapause induction in the cabbage beetle, *Colaphellus bowringi* Baly (Coleoptera: Chrysomelidae). *Insect Science*, 13, 189–193.

Wen, Z.Z. and Wen, X.S. (2000) Trend of pine caterpillar (*Dendrolimus punctatus*) population dynamis: Forecasting the differentiation rates of the second and third generation by means if critical date of differentiation. *Forest Research*, 13, 447–450. (in Chinese)

Accepted May 21, 2008

