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Diapause Initiation and Alterations in the Life Cycle of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) as Induced by Photoperiodic Conditions

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Abstract

Grapholita molesta (Busck) is one of the major pests of Rosaceae, causing significant damage to buds and fruits. In Southern Brazil, its population density is reduced during Rosaceae dormancy months. The present study evaluated the influence of different photoperiods (L:D) (10:14, 11:13, 12:12, 13:11, 14:10 and 16:8) at 25 ± 1ºC and 60 ± 10% RH on diapause induction of *G. molesta* eggs, larvae, prepupae, and pupae. The effects of the photoperiod on the life cycle of non-diapausing insects and on the second generation were also assessed. Prepupal diapause was observed only when eggs and neonates (≤ 12h-old larvae) were exposed to photophases from 10h to 14h long. Development of non-diapausing individuals and those from the second generation tended to be longer in photophases between 10h and 14h long.

Introduction

Grapholita molesta (Busck), the Oriental fruit moth (OFM), is a key pest of rosaceous plants, especially peach and apple trees in southern Brazil, causing significant damage to buds and fruits (Botton *et al* 2001, Monteiro & Hickel 2004). The presence of OFM in the field is reduced during peach tree dormancy months, from April to August, when buds are lignified and fruits are unavailable (Arioli *et al* 2005), but studies on the biology of *G. molesta* in such adverse conditions in Brazil are inexistent.

Survival strategies in unfavorable periods are important adaptations for some insect species, allowing them to synchronize their life cycles to periods of suitable conditions. Diapause is one of such strategies and is characterized by a general physiological mechanism allowing insects to survive seasonal adverse conditions, usually in a resistant stage. Insects in diapause may show changes such as accumulation of metabolic reserves, suppression of developmental and reproductive functions and reduced metabolic activity (Beck 1980, Tauber *et al* 1986). Diapause induction and termination in insects with genetic predisposition can be controlled by a number of stimuli, such as photoperiod, temperature, humidity and food availability (Andrewartha 1952, Denlinger 1986, Tauber *et al* 1986, Schowalter 2006).

Dickson (1949) observed full-fed larvae of *G. molesta* entering diapause when young larvae were exposed to a short photophase and low temperature. Photoperiod effects on the biology and the responsiveness of other development stages to the stimulus photoperiod provides to induce diapauses in this species remain unknown. Therefore, we aimed to determine the influence of different photoperiod regimes on the induction of diapause at the egg, larval, prepupal and pupal stages of *G. molesta*, as well as their effect on the life cycle of non-diapausing insects.

Material and Methods

A 5-y-old laboratory strain of *G. molesta* from Bento Gonçalves, Rio Grande do Sul, Brazil, continuously reared on an artificial diet (Arioli *et al* 2007) at controlled conditions (25 ± 1°C; 60 ± 10% RH; and a photophase of

16h) was used in our experiments.

Diapause induction was evaluated by exposing eggs (≤ 15h post-oviposition), larvae (neonates = ≤ 12h-old; and 6 d-old larvae), prepupae (less than 24h-old) and pupae (less than 24h-old) of *G. molesta* to different photoperiod regimes (L:D - 10:14; 12:12; 14:10 and 16:8) under controlled temperature (25 ± 1°C) and humidity (60 ± 10% RH). Eggs and larvae were also exposed to 11:13 and 13:11 (L:D) photoperiod regimes to evaluate their role on the induction of diapause.

Adults of *G. molesta* of an unknown age were transferred to a new cage at 6 p.m. where they remained until the next morning (8 a.m.). After that, the adults were removed and sections of the cages containing eggs were cut out and disinfected with a 15% sodium hypochlorite solution (2% of active chloride) and placed into a container with an artificial diet for larval eclosion. Pairs of larvae were transferred to 2.5 x 6 cm tubes containing the artificial diet upon their eclosion, and maintained at a 30 degree angle at controlled conditions (25 ± 1°C; 60 ± 10% RH; photophase of 16h), until their transference to one of the photoperiods tested. Once they were transferred to one of the photoperiods to be tested, they remained until the adult stage was reached.

Survival and development time were evaluated for all treatments. Diapause was determined by the prepupa failure to reach adult stage in a period 10 times greater (20 days) than that required for its development under control conditions (photoperiod of 16L:8D). The development of non-diapausing individuals of *G. molesta* was evaluated until adult emergence, when adults that emerged on the same day were paired for a maximum of 10 couples/treatment. Approximately 50 larvae from eggs deposited on the third day were kept in culture conditions until the adult stage. The time required for full development of the second generation was recorded.

The percentage of diapausing and emerged insects among treatments was analyzed using the Fisher's exact tests ($P < 0.05$). Mean values for the duration of the life cycle were compared using Kruskal-Wallis ($\alpha = 0.05$).

Results

The percentage of insects that entered diapause was significantly higher when eggs were exposed at 13h photofase ($P < 0.05$) (Table 1). For all treatments in which diapause was detected, adults were obtained only for those regimes with a 11h- or 14h-long photophase, but survival was lower than in the control group (Table 2). The total developmental time of non-diapausing *G. molesta* individuals was significantly higher in the 14h photophase (34.0 ± 1.56 days) than in the 16h one (21.1 ± 0.09 days) ($H = 67.64$; $df = 1$; $P < 0.0001$).

The percentage of diapause was higher by exposing neonate larvae (≤ 12h old) to 12h- or 13h-long photophases (Table 1), although survival was quite reduced (Table 2). The total developmental time (neonate-adult) of non-diapausing *G. molesta* was significantly shorter (18.1 ± 0.09 days) in the 16h photophase than in the others ($H = 204.88$; $df = 5$; $P < 0.05$).

Table 1 Percentage (mean ± SE) of diapausing prepupae of *Grapholita molesta* when eggs and neonates (≤ 12h-old) were exposed to different photoperiods (25 ± 1°C; 60 ± 10% RH).

Photoperiod (L:D)	% diapause	
	Eggs	Neonates
16:08	0 ± 0 a (130)	0 ± 0 a (130)
14:10	68.3 ± 3.75 b (211)	32.32 ± 4.07 c (215)
13:11	96.9 ± 2.32 d (130)	82.7 ± 6.44 d (130)
12:12	82.8 ± 2.93 c (122)	80.0 ± 1.26 d (125)
11:13	90.4 ± 5.14 c (128)	26.0 ± 6.62 c (150)
10:14	88.7 ± 2.91 c (150)	11.87 ± 4.29 b (276)

Values in the same column followed by the same letter are not significantly different by Fisher's Exact test ($P > 0.05$); Values between parentheses indicate number of observations.

Diapause was not observed when six d-old larvae were exposed. The percentage of emerged adults in the 14h photophase was higher if compared to the other treatments ($P < 0.05$) (Table 2). The lifetime (6 d-old larvae-adult) was significantly shorter in the 16L:08D (18.1 ± 0.09 days) and 10L:14D (18.3 ± 0.09 days) photoperiods ($H = 89.50$; $df = 3$; $P < 0.05$).

No diapause was observed when the prepupae were exposed to the different photoperiod regimes, and no difference in adult emergence was observed (Table 2). However, prepupae (1.9 ± 0.07 days) ($H = 69.48$; $df = 3$; $P = 0$) and pupae (7.1 ± 0.04 days) ($H= 144.83$; $df = 3$; $P = 0$) development time were extremely reduced at 16h photophase if compared to the remaining treatments.

The pupal stage was not sensitive to the length of the day and no diapause was observed. Similarly to what was observed for the prepupal stage, no effect on adult emergence was observed (Table 2). The pupae at 16h photophase developed faster (7.1 ± 0.04 days) than at the other conditions ($H = 132.03$; $gl = 3$; $P = 0$).

In general, average *G. molesta* lifetime (egg-adult) in the second generation was significantly longer in all photophases than in the control (Table 3).

Discussion

Eggs and neonate larvae of *G. molesta* exposed to 10h- to 14h-long photophases produced diapausing prepupae, extending the range of the day length previously reported to induce diapause in this species (photophase of 12h) (Dickson & Sanders 1945, Dickson 1949). Dickson (1949)

Table 2 Percentage (mean ± SE) of adult emergence of *Grapholita molesta* when eggs, neonates (≤ 12h-old), 6 days-old larvae, prepupae and pupae were exposed to different photoperiods (25 ± 1°C; 60 ± 10% RH).

Photoperiod (L:D)	% adults				
	Eggs	Neonates	6-day old larvae	Prepupae	Pupae
16:08	88.0 ± 11.98 a (130)	88.0 ± 11.98 a (130)	88.0 ± 11.98 a (130)	87.8 ± 2.41 a (130)	90.3 ± 0.98 a (130)
14:10	42.2 ± 12.50 b (211)	76.9 ± 2.33 ab (215)	96.3 ± 0.97 b (111)	91.6 ± 2.22 a (104)	94.3 ± 2.50 a (107)
13:11	- (130)	67.3 ± 9.26 b (130)	NEI	NEI	NEI
12:12	- (122)	62.7 ± 8.36 b (125)	75.1 ± 5.90 c (129)	87.3 ± 1.00 a (103)	84.7 ± 1.61 a (108)
11:13	10.0 ± 10.02 c (128)	75.8 ± 6.60 abc (150)	NEI	NEI	NEI
10:14	- (150)	83.7 ± 4.88 ac (276)	88.76 ± 3.08 a (122)	91.5 ± 2.18 a (112)	92.0 ± 2.99 a (109)

Values between parentheses indicate number of observations; NEI = non-evaluated induction; Values in the same column followed by the same letter are not significantly different by Fisher's Exact test (P > 0.05).

observed that the proportion of prepupae in dormancy was determined by the length of the day to which larvae of *G. molesta* were exposed. In total darkness or total light, only 1.9% and 0.3% of the insects entered diapause, respectively. As the length of the day was reduced, higher was the proportion of diapausing prepupae, with a maximum of 98.7% at 12h photophase. This author, however, did not report the effects of larval age on the larvae capacity to respond to the photophase stimulus, and we demonstrated that larvae older than five days are responseless to the photoperiod regime.

The high percentage of diapausing *G. molesta* during photophases of 11, 12, and 13h indicated that they could anticipate the arrival of environmental conditions unfavorable for development. Thus, when a photophase becomes critical (i.e., during winter), the insects have already entered diapause, ensuring their survival. According to Tauber *et al* (1986), the primary feature of diapause is its anticipatory nature. Environmental cues that signal future changes are perceived by insects, often much ahead the exposure of insects to the unfavorable conditions.

The fact that only eggs and neonate larvae of *G. molesta* responded to the photoperiod stimuli may be related to the habit of the insect. Eggs are laid on the leaves, buds, and fruits, where they are exposed to direct light. The same is observed in neonates until they penetrate in the shoots and/or fruits. After that, the effect of photoperiod ceases, which could explain why 6 d-old larvae were unable to respond to the stimulus that induces diapause in this species.

The insect endocrine system is influenced by environmental factors, such as photoperiod, temperature, food and moisture, which may affect chemical signals from

Table 3 Average lifetime (egg-adult) (mean ± SE) of second generation *Grapholita molesta* individuals, when the progeny was exposed as eggs, neonates (≤ 12h-old), 6 day-old larvae, prepupae, and pupae to different photophases (25 ± 1°C; 60 ± 10% RH).

Photoperiod (L:D)	Induction stage				
	Eggs	Neonates	6 day-old larvae	Pre-pupae	Pupae
16:08	21.5 ± 0.15 a[1]	21.5 ± 0.23 a	21.5 ± 0.23 a	21.5 ± 0.15 a	21.5 ± 0.15 a
14:10	22.9 ± 0.30 b	22.2 ± 0.22 ab	22.9 ± 0.22 b	23.3 ± 0.25 b	22.3 ± 0.14 b
13:11	AC[2]	20.8 ± 1.02 ab	NEI[3]	NEI	NEI
12:12	AC	AC	22.9 ± 0.21 b	24.3 ± 0.21 c	21.9 ± 0.13 ab
11:13	AC	24.6 ± 0.21 c	NEI	NEI	NEI
10:14	AC	23.0 ± 0.33 b	23.4 ± 0.27 b	22.9 ± 0.24 b	24.1 ± 0.15 c

[1]Values in the same column followed by the same letter are not significantly different by Kruskal-Wallis (P > 0.05); [2]AC = absence of couples; [3]NEI = induction was not evaluated

the brain, modifying the level of hormones that control insect development and diapause induction (Beck 1980, Tauber *et al* 1986, Huffaker & Gutierrez 1999 and Schowalter 2006). Diapause is regarded as an inactivation of the brain-prothoracic gland system. Under appropriate photoperiodic influence, the neurosecretory cells of the brain fail to release prothoracicotropic hormone, which in turn will not active the prothoracic glands to produce ecdysteroids, halting insect growth and development (Saunders 1982).

In Bento Gonçalves (29º 10' S) and Vacaria (28º 31' S), two important Rosaceae producing regions in the state of Rio Grande do Sul in southern Brazil, photophase can vary from 13.78-13.84h at the boreal summer solstice to 10.15-10.21h at the boreal winter solstice. Thus, we would expect to find diapausing individuals of *G. molesta* throughout the year. However, this insect is found in high numbers in orchards at the end of spring and summer, and its occurrence is considerably reduced in the winter months. These observations suggest that other factors, besides photoperiod, such as temperature, food, and genetic information, may be affecting or modifying the responsiveness of *G. molesta* to the stimulus provided the length of the day for diapauses induction. This hypothesis is supported by the observations of adults of *G. molesta* all year-round in the state of Rio Grande do Sul (Hickel *et al* 2003, Arioli *et al* 2005).

The larval stage of non-diapausing *G. molesta* was longer when neonates (≤ 12h-old) were exposed to photophases of 10, 11, 12, 13, and 14h-long, than under the control (16h), as observed to several other insects (Ali & Ewiess 1977, Mourão & Panizzi 2000, Chocorosqui & Panizzi 2003, Niva & Takeda 2003).

The developmental time of the second generation of *G. molesta* was generally longer when parents were exposed to a photophase shorter than 16h. These results may be related to hormone alterations which may increase the development time triggered by the action of photoperiod in insects that did not enter in diapause. However, no studies have documented hormonal alterations in non-diapausing insects as an influence of photoperiod, as well as its effect on the offspring.

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References

Ali M, Ewiess MA (1977) Photoperiodic and temperature effects on rate of development and diapause in the green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). Z Angew Entomol 84: 256-264.

Andrewartha HG (1952) Diapause in relation to the ecology of insects. Biol Rev 27: 50-107.

Arioli CJ, Carvalho GA, Botton M (2005) Flutuação populacional de *Grapholita molesta* (Busck) com armadilhas de feromônio sexual na cultura do pessegueiro em Bento Gonçalves, RS, Brazil. Ciência Rural 35: 1-5.

Arioli CJ, Molinari F, Botton M, Garcia MS (2007) Técnica de criação de *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) em laboratório utilizando dieta artificial para a produção de insetos visando estudos de comportamento e controle. EMBRAPA-CNPUV, 12p. (EMBRAPA-CNPUV, Boletim de Pesquisa 13).

Beck SD (1980) Insect photoperiodism. New York, Academic Press, 387p.

Botton M, Arioli CJ, Colletta VD (2001) Monitoramento da mariposa oriental *Grapholita molesta* (Busck, 1916) na cultura do pessegueiro. Bento Gonçalves, EMBRAPA-CNPUV Comunicado Técnico 38, 4p.

Chocorosqui VR, Panizzi AR (2003) Photoperiod influence on the biology and phenological characteristics of *Dichelops melacanthus* (Dallas, 1851) (Heteroptera: Pentatomidae). Braz J Biol 63: 655-664.

Denlinger DL (1986) Dormancy in tropical insects. Annu Rev Entomol 31: 239-264.

Dickson RC (1949) Factors governing the induction of diapause in the oriental fruit moth. Ann Entomol Soc Am 42: 511-537.

Dickson RC, Sanders E (1945) Factors inducing diapause in the oriental fruit moth. J Econ Entomol 38: 605-606.

Hickel ER, Hickel GR, Souza OFF, Vilela EF, Miramontes O (2003) Dinâmica populacional da mariposa oriental em pomares de pessegueiro e ameixeira. Pesq Agropec Bras 38: 325-337.

Huffaker CB, Gutierrez AP (1999) Ecological entomology. New York, John Wiley & Sons, 756p.

Monteiro LB, Hickel E (2004) Pragas de importância econômica em fruteiras de caroço. In Monteiro LB, May de Mio LL, Serrat BM, Motta AC, Cuquel FL (eds) Fruteiras de caroço: uma visão ecológica. Curitiba, UFPR, 309p.

Mourão APM, Panizzi AR (2000) Estágios ninfais fotossensíveis a indução da diapausa em *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae). An Soc Entomol Brasil 29: 219-225.

Niva CC, Takeda M (2003) Effects of photoperiod, temperature and melatonin on nymphal development, polyphenism and reproduction in *Halyomorpha halys* (Heteroptera: Pentatomidae). Zool Sci 20: 963-970.

Saunders DS (1982) Insect clocks. Oxford, Pergamon Press, 409p.

Schowalter TD (2006) Insect ecology: an ecosystem approach. San Diego, Academic Press, 572p.

Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. New York, Oxford University Press, 411p.