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Effects of photoperiod and temperature on the development of *Bonagota cranaodes*

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Abstract. The Brazilian apple leafroller, *Bonagota cranaodes* (Meyrick) (Lepidoptera: Tortricidae) was reared in the laboratory under a long-day (LD 14 : 10 h) and a short-day (LD 7 : 17 h) photoperiod at 22°C, and under two different temperatures (10-13ºC and 21-22ºC). The development time from larval to adult eclosion did not differ between the two photoperiods, but between the two temperature regimes. However, the larvae did not enter diapause, even at short day conditions and low temperatures. The number of adults obtained did not differ with temperature and light conditions. Field captures with pheromone traps showed that Brazilian apple leafroller occurs in apple orchards throughout the year and that population densities were lower in winter. Control measures should accordingly be taken during off-season.

Key words. Diapause, field trapping test, insect control, monitoring, sex pheromone.

Introduction

Insects in temperate *climate* zones are challenged to endure harsh temperature regimes and the absence of food resources during winter. They survive such unfavorable conditions in diapause. Some univoltine species undergo an obligatory, genetically fixed diapause. In other univoltine and all multivoltine species, the diapause is induced by external cues which indicate the end of the summer, such as decreasing day length or temperatures (Beck, 1980).

Control of orchard insects in temperate climate zones, such as Oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) and codling moth, aims at the non-diapausing life stages. Pheromone-based methods are obviously restricted to the flight period of adult moths, but even insecticide sprays can hardly be used to control overwintering larvae, which are protected by a hibernaculum and which are hidden in the soil or under tree bark.

In orchards in tropical and subtropical climate zones, control measures against native insects are not necessarily restricted to the periods when trees are in leaf, as native species may have access to native host plants providing food resources throughout the year.

We are currently developing a pheromone-based control method for Brazilian apple leafroller *Bonagota cranaodes* (Coracini *et al.*, 2001, 2003), which is an important pest of apple in Southern Brazil and Uruguay (Lorenzato, 1984). One important part of this program is to time the use of pheromones. We have therefore monitored the occurrence of adult moths in fruit orchards throughout the year and we have investigated whether *B. cranaodes* undergoes diapause.

Material and Methods

Insect rearing

B. cranaodes were obtained from a laboratory rearing at Embrapa, Vacaria, Brazil, where the insects are reared on a semiartificial agar-based diet (Mani *et al.*, 1978). Insects were reared from first instar larvae until adults under four conditions, involving two photoperiods, LD 7 : 17 h and LD 14 : 10 h, and two temperatures, 10-13ºC and 21-22ºC.

Development under different photoperiods

Two containers (1.5 L of diet) infested with 500 first instar larvae (first generation) were kept under a LD 7 : 17 h photoperiod. The adults eclosing from these containers were counted daily, and the adults were tranferred for mating and oviposition to cages which were kept in the same room. Four days after the last moth had emerged, the diet container was checked for remaining larvae and pupae. These were transferred to plastic

Petri dishes (9 x 3.5 mM) containing moistened filter paper. Eclosed adults were counted daily.

The larvae hatching from the oviposition cages (second generation) were placed in batches of 500 into containers with 1.5 L of agar diet. One of these container was kept under a LD 7 : 17 h photoperiod and the other one under a LD 14 : 10 h photoperiod, both at a constant temperature of 22°C. Adults were counted after eclosion, and the diet was checked for dead larvae.

Development under different temperatures

In this experiment 500 newly hatched larvae (first generation) were placed in groups of 25 larvae each into small plastic recipients with 75 g of agar diet. The recipients were kept inside two climatic chambers, one with the temperature of 10-13ºC and the other one with the temperature of 21-22ºC, both at a constant photoperiod of LD 7 : 17 h. It was used the same procedure for counting dead larvae/pupae and adults eclosion as described above. The adults were tranferred for mating and oviposition to cages which were kept in climatic chamber.

The larvae hatching from the oviposition cages (second generation) were placed in groups of 25 larvae each into small plastic recipients (75 g of agar diet), and kept inside the same climatic chamber as the first generation. It was counted dead larvae/pupae and adults eclosion.

Field trapping tests

Trap tests were done at Rubi Apple Orchard, Vacaria-RS, Brazil, from January to December, 2004. Tetra traps (Arn *et al.*, 1979) were baited with 10 µg of the optimized four-component sex pheromone blend (Coracini *et al.*, 2001), formulated on red rubber septa (Merck ABS, Dietikon, Switzerland). Chemical and isomeric purity of the compounds was >99.5%.

The traps (n = 10) were placed at ca. 1.7 m in apple trees. Traps were 5 m apart, and were arranged in random order in a line along tree rows. Traps were inspected once a week.

Statistical analysis

Prior to statistical analysis, data were checked for ANOVA assumptions and, if needed, transformed to avoid heterogeneity of variances. The number of days required for *B. cranaodes* adults to emerge and the number of adults obtained under different photoperiods, different temperatures, and different generations were compared using Fisher's test. Significance level was set to 0.05.

Results

Effects of daylength on B. cranaodes development

The development time from first instar larvae to eclosion of adult was very similar under the long- and short-day photoperiods. This shows that exposure to a short daylength did not induce *B. cranaodes* to enter diapause. The mean development time in these experiments ranged from 52 to 59 days, which compares to a development time of 53.4 days in the continuous lab-rearing under a LD 14 : 10 h photoperiod (n = 12). There was also no difference between the number of adults emerging under the two photoperiods (Table 1).

Observations of mating and oviposition behavior under long and short photoperiod did not indicate a difference between the treatments. Matings occurred within the first hour after onset of the dark period, and female oviposition behaviour was the same, under both photoperiods.

The most important mortality factor was migration of larvae out of the diet boxes. More larvae escaped during the second generation (Table 1).

Effects of temperature on B. cranaodes development

Larval development time from hatching until adult depended on temperature, but a similar number of adults emerged for both temperatures. There was no difference between the number of adults emerged for both generations and both temperatures (P < 0.02) (Table 2). However, it was needed about 43 days to obtain the first adult at 21 - 22ºC, and 160 days at 10 - 13ºC (P < 0.02). The results showed that low temperature did not induce *B. cranaodes* to enter diapause.

As during the previous experiment, the most important mortality factor was migration of larvae out of the rearing recipients.

Field trapping tests

Captures in pheromone traps show that *B. cranaodes* adults were present in the apple orchard all year around, even during the winter (Fig. 1). Rather high captures of *B. cranaodes* were recorded during the end of the peak growing season from February to April, when multiple insecticide sprays were applied to control *B. cranaodes* and *G. molesta* infestations.

The control level recommended for *B. cranaodes* is when weekly pheromone trap captures surpass 30 males/trap. However, in fall and winter, the grower sprayed insecticide when detected any increase on the adult population (June, July, and August) (Fig. 1). From September on started the frequent insectcide use due to the occurrence of *B. cranaodes*, *G. molesta*, and *Anastrepha fraterculus* (Diptera: Tephritidae).

This field test also showed that 10 µg lures baited with the optimized 4-component pheromone remained attractive over six months.

Discussion

According to our findings, short daylength and low temperature do not induce diapause in Brazilian apple leafroller *B. cranaodes*.

Diapause is the basic means by which insects and related arthropods in temperate zones cope with unfavorable environmental conditions (Tauber *et al.*, 1986). Diapause induction, maintenance, termination, and postdiapause development and growth are mainly regulated by abiotic factors such as photoperiod, temperature, and moisture. Several studies have illustrated the influence of photoperiod and temperature on diapause maintenance and termination (Boyne *et al.*, 1985; Ishirara & Shimada 1995).

Photoperiod is the major diapause-inducing environmental stimulus in most species. So far, it has been shown in a few species only that diapause induction is mediated by temperature (Tauber *et al.*, 1986; Danks, 1987). Photoperiod has been shown to effect the growth rate in other lepidopteran species, with larval growth being slower under shorter photoperiods (Danilevskii *et al.*, 1970; Goettel & Philogène, 1978). Beck (1980) suggests that these growth responses are correlated with the photoperiodic effect of diapause induction. In *B. cranaodes*, duration of larval development was the same for long and short-day conditions (Table 1).

Although the ecology of insect diapause has been extensively studied in insects, most of the available data concerns insects from temperate climate zones, where insects are subject to marked seasonal changes in photoperiod, temperature and availability of food resources. Diapause is usually induced by decreasing day length (Chippendale & Reddy, 1973; Goettel & Philogène, 1978). The situation is quite different in the Tropics, since there are only minor seasonal changes in daylength (Tanzubil *et al.*, 2000). Under such conditions, the key environmental factors influencing diapause are rainfall, temperature and food in conjunction with photoperiod (Adkisson *et al.*, 1963; Scheltes, 1978; Denlinger, 1986; Kfir, 1993). In many insect species from temperate climate zones, larval exposure to low temperatures is not necessary for diapause development. However, low temperatures that might have occurred during the larval development could have an impact on diapause development. Many of the photoperiodic responses are also temperature-dependent, with temperature affecting circadian entrainment, photoperiodic summation and aspects of general physiology involved in diapause induction (Veerman & Vaz Nunes, 1980). This was observed for example for the tortricidae species *Adoxophyes orana*, *Choristoneura fumiferana*, and *Endopiza viteana* (Han & Bauce, 1996; Tobin *et al.*, 2002; Milonas & Savopoulou-Soultani, 2004) and for the noctuidae specie *Sesamia nonagrinoides* (Fantinou *et al.*, 2003). For *B. cranaodes*, the interaction between short day and low temperature did not lead to diapause (Table 2). Under these conditions, *B. cranaodes* larvae slowed down the growth and development. It may be that the low temperature provides a shorter period suitable for feeding, which in turn reduces metabolic functions and retards the larval development.

Our field tests corroborate the results of the laboratory tests and confirm that *B. cranaodes* does not diapause. The adults were present all year around, despite the lower temperature and shorter day regime during winter. This highlights the potential of pheromone-based methods for control of *B. cranaodes* during off-season. Population densities are lowest during off-season and attempts should then be made to further reduce population densities before onset of the new apple growing period. Therefore, the use of mating disruption method for *B.cranaodes* is under development in Brazil.

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Table 1. Development of Brazilian apple leafroller *B. cranaodes* larvae under two different photoperiods.

Treatm.	Generation	Photoperiod (L/D)	N° larvae used[1]	N° dead insects	N° adults emerged	Development time (days)[2]
dark	1st	7/17 h	500	33	326a	51.9a
dark	1st	7/17 h	500	52	325a	52.3a
dark	2nd	7 /17 h	500	51	249a	58.9a
light	2nd	10/14 h	500	43	228a	51.8a

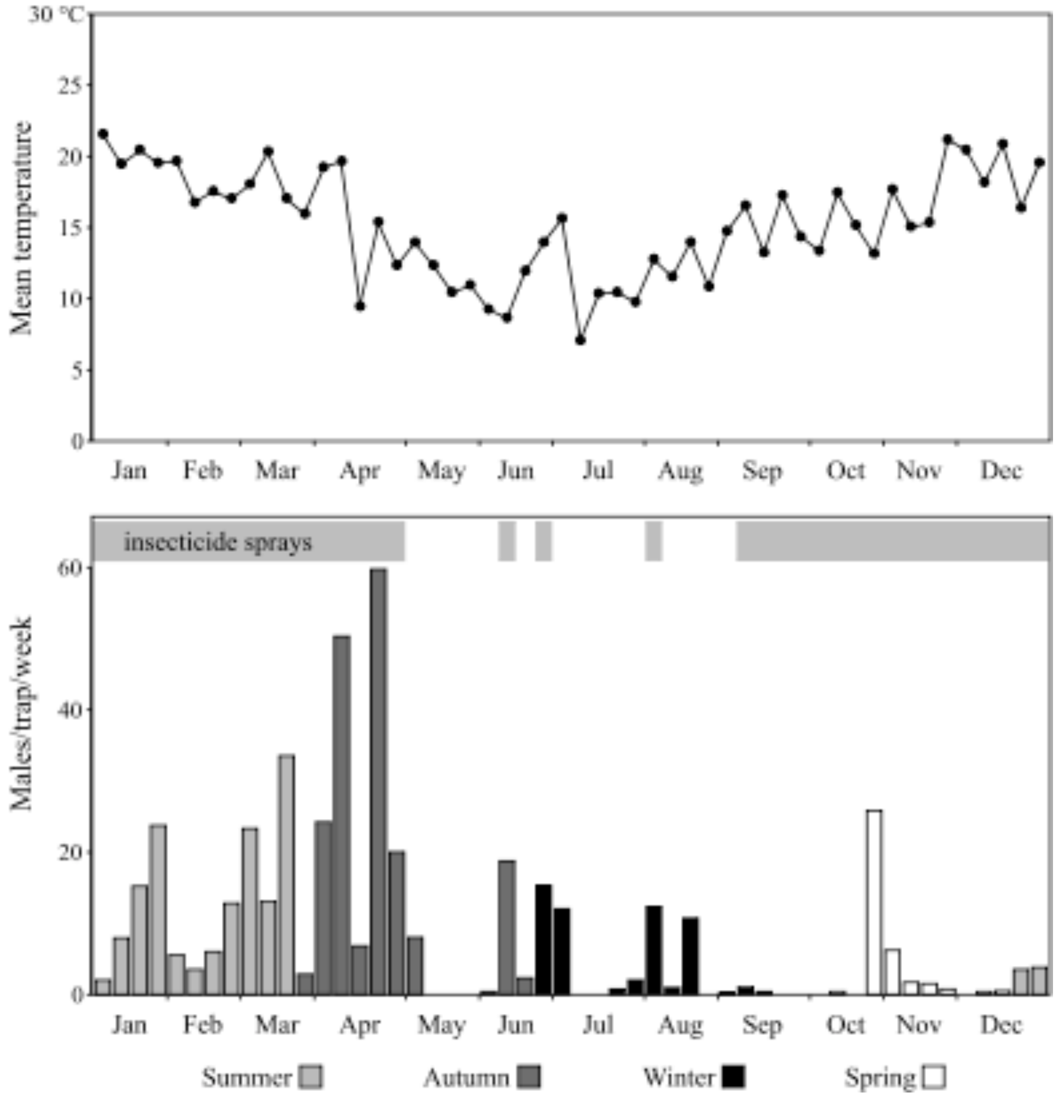
[1] All treatments began with recem-emerged larvae.
[2] Mean value for growth period from larvae to adult.
Within the same column and same generation, numbers followed by the same letter are not significantly different (Fisher test, $P > 0.05$).

Table 2. Development of Brazilian apple leafroller *B. cranaodes* larvae under two different temperatures.

Temp. (ºC)	Generation	Photoperiod (L/D)	N° larvae used[1]	N° dead insects	N° adults emerged	Development time (days)[2]
10-13	1st	7/17 h	500	45	237a	167.1a
10-13	2nd	7/17 h	500	47	241a	155.6a
21-22	1st	7 /17 h	500	34	273a	45.3a
21-22	2nd	7 /17 h	500	41	257a	42.8a

[1] All treatments began with recem-emerged larvae.
[2] Mean value for growth period from larvae to adult.
Within the same column and same temperature, numbers followed by the same letter are not significantly different from each other (Fisher test, $P > 0.05$).

Fig. 1. Weekly mean air temperature and trap catch of Brazilian apple leafroller *B. cranaodes* males in pheromone traps at Schio Orchard, Vacaria-RS, Brazil, from January to December 2004.



30 °C
25
20
15
10
5
0
Mean temperature
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
insecticide sprays
60
40
20
0
Males/trap/week
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Summer
Autumn
Winter
Spring