UDC 595.75.: 591.543.42

Factorial Regulation of the Seasonal Cycle of the Stink Bug *Graphosoma lineatum* L. (Heteroptera, Pentatomidae). I. Temperature and Photoperiodic Responses*

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Abstract. The influence of constant temperatures on immature stages and period of maturation in *Graphosoma lineatum* from Belgorod, Russia, was studied. The developmental rate of eggs in the zone of constant temperatures between 14.4 and 31.0°C can be represented by the equation $y' = 1.615T - 24.08$ (y' - rate of development, %; T - temperature, °C). For nymphs it has the form $y' = 0.47T - 8.14$ in the zone between 23.8 and 28.3 °C: for female maturation $y' = 2.74T-64.16$ in the zone between 24.4 and 28.2 °C. The theoretical threshold of development for eggs was 14.9 °C, for nymphs 17.3 °C, for female maturation 23.4 °C, the total degree-day requirement of the whole generation was evaluated as 325. The population from the forest-steppe zone has facultative imaginal diapause controlled by qualitative photoperiodic reaction (PhPR) of the long-day type. It was studied at two constant temperatures (24 and 28 °C). The critical day lengths were 17 h 15 min at 24 °C and 15 h 45 min at 28 °C. The quantitative PhPR was demonstrated (43.8 days at photophase 17 hrs. and 48.8 days (+11%) at photophase 19 hrs). At 28 °C this effect was not observed.

Keywords: *Graphsoma lineatum*; photoperiod; lower development threshold; sum of effective temperatures.

The seasonal cycle of most insects from temperate latitudes consists of a succession of physiological conditions: active development and rest. The diversity of rest forms found in insects, its various ontogenetic and seasonal restrictions in combination with periods of activity of various duration create an enormous diversity of phenological representations, each of which is strictly synchronized with local climatic conditions.

A leading role in the complex of factors regulating seasonal development of insects belongs to temperature and the photoperiod (Danilevskiy, 1961; Saunders, 1976; etc.).

Various aspects of photoperiodism and thermoperiodism are examined in numerous articles and reviews (Danilevskiy, 1961; Beck, 1968, 1983; Saunders, 1976; Zaslavskiy, 1984; Tauber et al., 1986; etc.), although the life cycles in different taxonomical groups are far from uniformly studied. In particular, undoubtedly, among those poorly studied with respect to seasonal adaptations is the order of true bugs (Heteroptera). Among the representatives of this very large order of insects (world fauna numbers more than 40,000 species) only a few can be considered more or less studied, but these few species are either agricultural pests or interesting as potential agents of biological control of pest populations. This, in turn, defies the practical nature of such investigations.

This article is a continuation of a series of works on the experimental study of the regulation of the seasonal cycles in the Heteroptera of the forest-steppe zone (Saulich et al., 1993; Numata et al., 1993; Volkovich and Saulich, 1994; Saulich and Volkovich, 1994; etc.).

As the subject of the study we selected the Italian stink bug, *Graphosoma lineatum* L. (= *f. italicum* Müll.), a species widely distributed in the Palearctic and typical of the central zone of Russia. The number of generations per season throughout its range varies with geographical latitude. We know that in southern Sweden (57°N) the population of this species is univoltine (Larsson, 1989), but in southern France (Toulouse, 43.5°N) the species produces two generations per year (Nguyen Ban, 1964). Arnol'di (1948) describes the development of this species in two generations also in Crimea. This is evidence of factorial regulation of seasonal development in this species. Elucidation of the ecological and physiological mechanism controlling the seasonal cycle of the Italian stink bug is the objective of this work.

MATERIAL AND METHODS

The investigations were performed in the Vorskla Forest Nature Reserve (Belgorod Prov., forest-steppe zone, 50°N) over two summers: in 1992 temperature-related development of the embryonic stage of *G. lineatum* was studied (unpublished data of O. V. Markin)[1]; in 1994, the temperature-related larval and preovipositional development and the photoperiodic response were studied.

In the experiments in 1992, adults collected in nature were maintained at 26° on a long photoperiod. Freshly deposited eggs were collected for experiments 4 times per day. Eggs in the experiment were kept in plastic Petri dishes, with 30-60 in each, at a constant temperature. In each dish with eggs we placed a test tube with water in order to maintain proper humidity throughout the experiment. Humidity was an average 90% and was not a limiting factor even at high temperature. Hatching of larvae was recorded 5 times per day.

In the experiments in 1994, adult insects collected in nature were maintained in glass vessels (0.8 liters) at 28° on a 18:6 photoperiod, with 3-5 pairs in each. Twice during the day food was changed—inflorescences and fruits of Umbelliferae and leaves of burdock, on which the females willingly deposited eggs. Deposited eggs were kept under the same conditions in Petri dishes with a moist cotton pad. Hatched larvae, 20-25, were used in experiments. They were maintained in Petri dishes 100 mm in diameter, the lids of which were provided with 50-mm diameter openings for aeration covered with silk bolting cloth. Food included dry seeds of dill and coriander; later, fresh umbels were added (flowers and fruits) of dill (*Anethum graveolens*), hemlock (*Conium maculatum*), wild parsnip (*Pastinaca sylvestris*), cow parsley (*Anthriscus sylvestris*), and other umbelliferans. Food was changed every other day. Adolescent larvae were divided into groups of 10 and then 4-6. Winged bugs were paired off (♀ and ♂); they were kept under the same conditions as the larvae.

Dates of alatation of each individual and the appearance of the first oviposition were recorded. If the female did not deposit eggs in the first 20-25 days after wings appeared, then at the end of this time they were dissected, and, based on the condition of the gonads and fat body, a conclusion was made regarding its physiological condition: activity or diapause. Females with undeveloped gonads (states I and II, after Hodek, 1971) and a well-defined fat body were considered to be diapausing.

*Originally published in Entomologicheskoye Obozreniye, Vol. 74, No. 4, 1995, pp. 736-743.

[1]The authors use this opportunity to thank O. V. Markin for kindly providing us with his data on embryological development of the subject.

ISSN0013-8738/96/0009-0084

Table 1

Characteristics of embryonic development of *Graphosoma lineatum* L. at different temperatures (data of O. V. Markin)

Variant	Mean temperature during the period of embryonic development, °C	Duration of development, days	Rate of development, %	TET_1, degree-days
1	17.35	24.8	4.03	60.8
2	19.2	14.8	6.77	63.6
3	23.6	6.9	14.47	60.1
4	23.75	7.8	12.9	68.6
5	26.1	5.5	18.18	61.6
6	26.4	5.0	20.0	57.5
7	30.0	4.0	24.75	61.0
8	30.95	4.0	25.0	64.2

Table 2

Characteristics of larval development of *Graphosoma lineatum* L. at different temperatures and on different photoperiods

Variant	Mean temperature during larval development, °C	Day length, hr	Duration of development, $M \pm m_M$	Rate of development, %	TET_2, degree-days
1	23.8	16	27.9 ± 0.36	3.59	181.4
2	24.55	18	31.0 ± 0.22	3.23	226.3
3	25.1	17	27.1 ± 0.21	3.70	211.4
4	25.1	18	27.9 ± 0.44	3.59	217.6
5	25.3	19	32.1 ± 0.87	3.12	256.8
6	27.4	17	20.9 ± 0.24	4.78	211.1
7	27.5	19	19.6 ± 0.19	5.11	199.9
8	28.1	16	19.5 ± 0.33	5.13	210.6
9	28.1	18	20.1 ± 0.23	4.98	217.1
10	28.3	15	19.4 ± 0.36	5.15	213.4

Experiments were conducted in photothermal chambers with programmed control (Braun and Goryshin, 1978). Deviation in temperature from the set level did not exceed ± 1.0°. Humidity in the chambers was not specially regulated and fluctuated from 50 to 70%. In the experiments in 1994, we used a photoperiod of from 15 to 19 hours. Illumination in the chambers was 180-250 lx (we used DS-20 luminescent lamps).

RESULTS AND DISCUSSION

1. Effect of Constant Temperature on Duration of Embryonic Development

The role of temperature in regulating the duration of embryonic development was investigated in

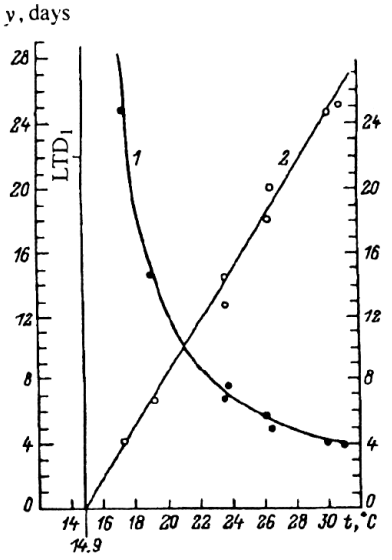

Fig. 1. Effect of constant temperature on duration of embryonic development of *Graphosoma lineatum* L. Hyperbola (*1*) - duration of development (left ordinate - days); reciprocal of the hyperbola (2) - rate of development (right ordinate - %). Abscissa - temperature. Black circles - mean duration of development by variant; clear circles - mean rate of development by variant. LTD_1 vertical - theoretical lower threshold of embryonic development.

the range from 17.35 to 30.95° (Table 1). The results of the experimental data are described by two equations:

$$y = 100/1.615T - 24.08 \quad (1)$$

and

$$y' = 1.615T - 24.08, \quad (2)$$

where y is the duration of embryonic development, days; T is the mean temperature during the period of embryonic development, °C; and y' is the rate of embryonic development ($y' = 100/y$), %.

Figure 1 contains data characterizing embryonic development in the different versions of the experiment. The rate of development line (y') intersects the abscissa, thus designating the lower temperature threshold of embryonic development (LTD_1). It is 14.9°, which is almost 2° higher than the LTD reported in the literature for the Toulouse population of *G. lineatum* (Nguyen Ban, 1964). Unfortunately, the technical conditions of those experiments make us doubt the accuracy of their results.

Using the theoretical LTD, we can determine the total effective temperature (TET) required to complete embryonic development based on the formula:

$$TET_1 = y \times (T - LTD_1), \quad (3)$$

where TET_1 is the total effective temperature for embryonic development, degree-days; y is duration of development, days; T is the mean temperature used in the variant of the experiment, °C, and LTD_1 is the LTD of embryonic development.

Data on each experiment variant are found in Table 1. On average the TET_1 was 62 degree-days and varied without any definite pattern from 57.5 (-7.2%) to 68.6 (+10.6%) degree-days.

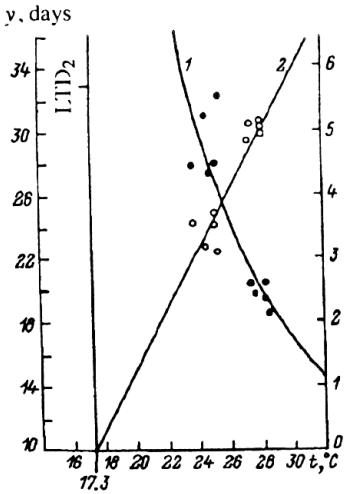

Fig. 2. Effect of constant temperature on duration of larval development of *Graphosoma lineatum* L. Hyperbola (*1*) - duration of development (left ordinate - days); reciprocal of the hyperbola (*2*) - rate of development (right ordinate - %). Abscissa - temperature, °C. Black circles - mean duration of development by variant; clear circles - mean rate of development by variant. LTD_2 vertical - theoretical LTD for larvae.

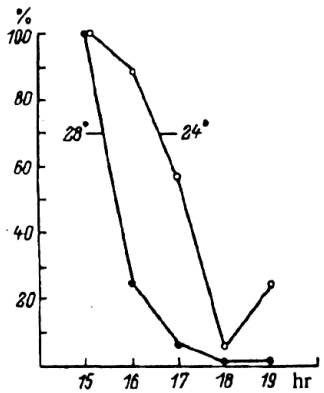

Fig. 3. Photoperiodic response of *Graphosoma lineatum* L. at different temperatures. Abscissa - photoperiod, hr; ordinate - proportion of diapausing females, %.

2. Effect of Constant Temperature on Duration of Larval Development

The experiments conducted in 1994 with larvae and winged bugs differed somewhat in their design from the experiments done by O. V. Markin in 1992. Thus, the range of investigated temperatures was narrower - from 23.8 to 28.3° (Table 2).

The obtained data were statistically tested (Zaytsev, 1990). In Tables 2 and 3, the Duration of Development field is the arithmetic mean of values (M) and its single error (m_M). The t-test calculated for each variant shows that the arithmetic mean of duration of development is reliable even at the 99.9% confidence level unless otherwise noted.

We know that in the Heteroptera the quantitative photoperiod response may be expressed in the form of acceleration or slowing of larval development (Saunders, 1983; Kiritani, 1985; Hori, 1986, 1987; Saulich et al., 1993). This was taken into account and is discussed below.

The results of the experimental data on duration of larval development are described by the equation:

Table 3

Characteristics of the preovipositional period of *Graphosoma lineatum* L. at different temperatures and on different photoperiods

Variant	Mean temperature during the maturation period, °C	Day length	Duration of maturation days, $M \pm m_M$	Ratio of maturation, %	TET_3, degree-days
1	24.4	16	17.5 ± 1.50	5.71	17.5
3	24.7	17	16.7 ± 0.84	5.98	21.7
2	25.05	18	13.5 ± 0.71	7.41	21.6
5	25.4	19	16.7 ± 2.90	5.99	33.4
7	27.0	19	11.1 ± 0.40	8.98	40.0
6	27.4	17	11.8 ± 0.39	8.51	47.2
8	27.9	16	10.5 ± 0.34	9.52	47.2
9	28.2	18	10.0 ± 0.91	10.0	48.0

Note. In variant No. 1, M is reliable at $P = 90\%$, in all other cases at $P = 99.9\%$.

$$y = 100/0.47T - 8.14, \quad (4)$$

and the rate of larval development may be described by the equation:

$$y' = 0.47T - 8.14, \quad (5)$$

where y is the duration of larval development, days; T is the mean temperature during larval development, °C; y' is the rate of larval development ($y' = 100/y$), %. Figure 2 contains a graph of these data.

The LTD for larvae (LTD_2) is 17.3°. It also is somewhat higher (1.3°) than the threshold obtained in experiments with the Toulouse population (Nguyen Ban, 1964).

Based on a formula analogous to formula (3) we also calculated for each variant the TET_2 (for larval development). On average it is 215 degree-days, varying without any definite pattern from 181.4 (-15.6%) to 256.8 (+19.4%) degree-days.

3. Effect of Constant Temperature on Duration of Maturation of Females

The duration of the preovipositional period, like the duration of development of the preimaginal stages, depends on temperature. The corresponding data are contained in Table 3. In this table variants of the experiments are arranged in order of increasing mean temperature, and their numbers correspond to the numbers of the variants of the experiments with larvae, from which the adults were obtained. Changes in order had two causes: 1) slight fluctuations in mean temperature (up to ± 0.5°); 2) absence of active females in the variant with a 15:9 photoperiod.

The experimental data on duration and rate of preovipositional development are described by the equations:

$$y = 100/2.74T - 64.16 \quad (6)$$

and

$$y' = 2.74T - 64.16, \quad (7)$$

where y is the duration of the preovipositional period, days; T is the mean temperature during this period, °C; and y' is rate of maturation of females ($y' = 100/y$), %.

The hyperbola and its reciprocal, which correspond to these equations, in form resemble the graphs in Fig. 2, and are not provided here. The theoretical LTD for maturation of females (LTD_3) is 23.4°. Examining the value of TET_3 (for maturation) we note that with growth the value of the TET_3 increases, although it is believed that this parameter should be constant, at least in the optimal temperature range. Rubtsov (1938) recorded an increase in the TET approaching areas of thermal depression and hard coldness. Danilevskiy (1947) associated with this "regulatory activity of the organism directed toward resisting unfavorable conditions." If this is correct then in the imaginal stage in *G. lineatum* the range of optimal temperatures is extremely narrow and runs from 23.5° (LTD_3) to 27-28°, which seems unlikely. Possibly we are seeing a mathematical effect: with the small TET, close to the LTD each additional day and degree has a greater specific impact than with a larger TET. At this time there is no other explanation for the increase in the TET_3 with temperature; in the temperature range of 27.0-28.2° (variants 6-9) the mean TET_3 is 45.6 degree-days.

4. Qualitative Photoperiodic Response

In heterodynamic insect species the qualitative photoperiodic response (PhPR) controls the onset of one of two alternative physiological conditions: active development or diapause. *G. lineatum* is characterized by a reproductive diapause: adult insects, appearing at the end of summer, feed and enter diapause, overwinter and, only at the end of spring of the following year after feeding, mate and deposit eggs. The qualitative PhPR regulating facultative diapause has been investigated in the Toulouse (Nguyen Ban, 1964) and Voronezh and Krasnodar (Gusev and Popov, 1968; Popov, 1971) populations of *G. lineatum*. The objective of our investigation was to determine the parameters of the PhPR of the Belgorod population of the species. As mentioned above, the insects were reared on constant photoperiods with day length of 15, 16, 17, 18 and 19 hr at two temperatures (24 and 28°). The PhPR curves are found in Fig. 3.

As we see, the PhPR of *G. lineatum* is of the long-day type. Diapause in all bugs is induced when the photoperiod is 15 hrs. The material of Popov (1971) makes it possible to postulate that under this temperature regime diapause will invariably occur in all individuals on shorter photoperiods.

The main parameter characterizing the ecological role of the PhPR is the critical photoperiod — the length of the day at which 50% of individuals diapause. As in many other Heteroptera, for example *Pyrrhocoris apterus* (Saulich et al., 1993), the critical photoperiod is subject to temperature variability. An increase in temperature of 4° (from 24 to 28°) causes a decrease in the critical photoperiod (or threshold) of 1.5 hr (from 17 h 15 min to 15 h 45 min).

5. Quantitative Effects of Photoperiodism

We know that the PhPR may be expressed not only qualitatively but also quantitatively. Among the Heteroptera this has been found, for example, in *Pyrrhocoris apterus* (Saunders, 1983; Saulich et al., 1993; Numata et al., 1993), *Carbula humerigera* (Kiritani, 1985), *Palomena angulosa* (Hori, 1986, 1987), etc. In experiments with *G. lineatum* these effects were weakly expressed, although at $T = 24°$ the summed duration of larval development and maturation of females regularly increased with an increase in the photoperiod from 17 to 19 hr (starting with the 16-hr photoperiod the same tendency

was expressed in the value of the TET needed for larval development and maturation of females). The increase in duration of development was 11%, and of the TET 46%. However, at $T = 28°$ we did not find this tendency.

In *Pyrrhocoris apterus* and *Carbula humerigera* the delay in development was greatest at prethreshold values; with further increase in the photoperiod the times of development decreased, returning to those values that occurred under the short-day regimes. In our experiments, at $T = 24°$ duration of development was greatest on the longest investigated photoperiod (19 hours).

CONCLUSION

Our experiments made it possible to identify the temperature norms for embryonic, larval, and preovipositional development and the main parameters of the PhPR of the Belgorod population of *G. lineatum*.

The total effective temperature required for the development of one generation is roughly 325 degree-days. Temperature conditions in Belgorod Prov. for insects with LTD = 12-13° are estimated at 700-800 degree-days (Agroklimaticheskiye Resursy, 1972). Thus, the appearance in hot years of a second generation of *G. lineatum* is not limited by the thermal resources of the forest-steppe zone; however, phenological observations and communications in the literature (Korinek, 1939; Puchkov, 1961) do not confirm this version. Thorough discussion of this question and analysis of factorial regulation of the seasonal cycle will be the subject of a separate study.

This study received financial support of the Competitive Grant Center for Research in the Area of Problems of the Forest Complex.

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