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Effects of Photoperiod and Temperature on the Summer Diapause of the Dark Grey Cutworm, *Agrotis tokionis* Butler

숫검은밤나방(*Agrotis tokionis* Butler)유충의 하면에 미치는 온도와 광주기의 영향

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ABSTRACT To elucidate the role of two environmental factors, temperature and photoperiod, in diapause induction and development of the dark grey cutworm, *Agrotis tokionis* B., field and laboratory experiments were implemented. As larvae entered diapause later, the diapause duration became shorter resulting in the synchronization of pupation of the test population. The summer diapause of this insect was assumed to be obligatory because every mature larvae had to experience summer dormant period at all experimental conditions of temperature and photoperiod. It was suggested that the diapause stage could be divided into two different phases in relation to temperature reaction. At the temperatures examined, the duration of diapause was shortened by higher temperature in the early phase, while it was shortened by lower temperature in the late phase. The diapause period was the shortest under short-day condition (LD 8 : 16)

KEY WORDS *Agrotis tokionis* Butler, summer diapause, temperature, photoperiod, developmental velocity, diapause induction, diapause termination

초　　록 숫검은밤나방(*Agrotis tokionis* Butler)유충 夏眠의 개시와 종료에 온도와 광주기가 어떠한 역할을 하는지 조사하였다. 자연조건에서 하면기간은 유충이 하면을 시작하는 시기가 늦을수록 짧아져서 조사집단내 용화시기는 일치하는 경향을 보였다. 실험한 모든 광주기 및 온도조건에서 유충의 발육속도에는 변화가 있었으나 하면개시에는 아무런 영향을 미치지 못하였기 때문에 이 곤충의 하면은 내인성으로 추측되었다. 그러나 하면의 종료는 이상의 두가지 환경조건의 변화에 영향을 받았다. 온도조건에 대한 반응에 따라 하면기간은 2시기로 나눌 수 있었는데 전기에는 평균온도가 높을수록 하면시간이 단축되었으나 후기에는 저온 일수록 짧아졌다. 또한 실험한 광주기에서는 단일조건 (LD 8 : 16)에서 하면기간이 가장 짧았다.

검 색 어 *Agritis tokionis* Butler, 숫검은밤나방, 하면발육, 온도, 광주기, 발육속도, 휴면유발, 휴면종료

The dark grey cutworm, *Agrotis tokionis* Butler, is a subterranean insect which gives serious damages to various plant seedlings by means of cutting the root or stem. Adult emergence occurs in late September and early October and after 3~4 days of preovipositional period, female lays eggs from which 1st instar larvae hatch out about two weeks later. They overwinter as 2nd or 3rd instar (Kim et al.). After overwintering they resume growth to develop to 7th instar and the mature larvae enter summer diapause from late May through early July. Upon termination of the diapause, the larvae

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pupate in late August and early September in tobacco fields in spring. This prompted the reports on the estimation of field density, control threshold, and chemical control of this insect (Kim et al. 1981b, Sohn et al. 1982). Surveilance, however, revealed that recent occurrence of this pest was reduced so much that it no longer has been a pest of economic importance especially in tobacco fields (Park et al. 1987). The most probable reason for this would be the change of its environment and extensive studies on the bionomics of this insect in relation to the environment should be done to understand the population fluctuation in field. The effect of temperature on larval development and the effect of P.E. film-mulching on the larval activity in tobacco field were reported by the authors(Kim et al. 1985, 1986). We found that the duration of summer diapause of this insect became shorter as the time of diapause initiation got later (data not presented). Consequently, it showed a clear peak in the sequence of pupation which was unlikely considering the diapause initiation period. This indicated that this insect had a certain regulation mechanism in diapause development to synchronize the population's life cycle. The results of the study carried out to find a possible role of photoperiod and temperature of the summer diapause of the dark grey cytworm are contained in this paper.

MATERIALS AND METHODS

Diapause induction

Newly hatched larvae were reared individually at different temperature and/or photoperiod to see their effect on diapause induction. Larvae were fed on Chinese cabbage. Three sets of experiment were done. Exp. 1) 24 lavae were reared under each 18-, 12- or 6-hr photophase at 25 ± 1℃, Exp. 2) Under field condition, larvae are faced to short photophase from the 1st instar until they resume growth in spring after which time the daylength grows longer until diapause initiation. In experiment 2, 40 larvae were reared at 25 ± 1℃ under each different alternating photoperiod regimes as the following.

Photoperiod(L : D)	
1 st-3rd instar	4th-7th instar
16 : 8	16 : 8
16 : 8	8 : 16
12 : 12	12 : 12
12 : 12	16 : 8
12 : 12	8 : 16

Exp. 3) Larvae reared at 25 ± 1℃ and 16 hr daylength until the 3rd molting were kept at low temperature (4℃)for 20, 40, or 60 days to simulate the effect of low temperature during the overwintering. Then they were reared at three different temperatures (20, 25, 30℃) and under short (8hr) or long (16hr) daylength. Number of insects used for each temperature-photophase combination were 22 to 28.

Diapause termination

Exp. 4) Larvae were collected from late May through early June in Suwon and Jeonju in 1982. Among them individuals initiated diapause in early June were selected and kept under the room temperature and natural photoperiod. From July 10, about 40 days after the diapause initiation, the larvae were treated with different constant (20 ± 1℃, 27 ± 1℃ 34 ± 1℃) or alternating temperatures (27 ± 1℃ and 20 ± 1℃, 34 ± 1℃ and 20 ± 1℃, 34 ± 1℃ and 27 ± 1℃). High temperature of the alternating condition was kept from 9 a.m. to 6 p.m. and the photophase during the treatment was 15 hrs (from 6 a.m. to 9 p.m.). Exp. 5) Larvae were collected in late May and they were kept individualiy under different photoperiods (LD : 16 : 8, 8 : 16, 0 : 24) and at different temperatures (20 ± 1℃, 27 ± 1℃, 34 ± 1℃) immediately after the diapause initiation.

The 7th instar larvae stopped feeding showing a characteristic wandering behavior were considered as they initiated the diapause.

Exp. 6) To see the effect of high temperature duration on diapause development of the insect, each group of 40 larvae initiated diapause less than 3 days before June 10 were kept at high temperature (34℃) for 15, 30, 45, or 60 days under dark condition. Then they were switched to low temperature(20℃, dark) condition until they terminated the diapause. Some larvae experienced the high temperature were transferred to wire cages (8 cm diam., 15 cm height) which were buried under the ground. After a certain period of field condition they were recovered and kept under the same low temperature condition described as above.

RESULTS

Effects of temperature and photoperiod on diapause induction

Photoperiod has generally been known to signal the initiaton, maintenance, and termination of insect diapause. It is a relatively noise-free seasonal cue that can be found in nature. Three sets of photoperiod were taken to estimate the effects of dayength on the diapause induction (Table 1). In every cases, all the larvae completed development to be mature initated diapause without exception. Meanwhile the velocity of larval development was faster under longer photophase than under shorter ones in total larval period or in each instar but more distinctly in later instars. This indicates that in relation to the fact that the daylength is getting longer for the overwintered larvae after the vernal equinox, the developmental velocity is accelerated as the larvae are being close to the final instar. Larval mortality was much higher under the short-day condition especially in the final (6 ~7th) instars, and it was assumed that the extreme short-day condition, compared with the natural condition, might have made the larvae lose their physiological balance.

The dark grey cutworm, after it hatched out in late October, soon meets winter season and experiences the lowest temperature and shortest daylength in its life time while the temperature gets warmer and the day gets longer from the 4th larval instar in spring. Thus larval period was divided into two periods during which different photoperiods were provided (Table 2). The larval period during 1st~3rd instar was not varying with the daylength possibly because it was too short, but the period during 4th-7th instar tended to become shorter under long-day condition and longer under short-day condition. The effect of photoperiod on the development of larvae seemed more notable in the insects experienced neutral photoperiod (12hr photophase) during the earlier instars so that the larval period of 4th~7th instar was shorter in the order of under long-, neutral-, and short-day condition. However, the difference in developmental period during 4th~7th instar between the groups which experienced different photophase during 1st~3rd instar indicates that the development of the larvae was influenced by photoperiod throughout the larval stage and that the photophase during the earlier instars affected the magnitude of the response to the photophase during the later instars. Nevertheless, no evidence that the alternating daylengths affected the rate of diapause initiation was obtained because all the larvae entered diapause no matter what the photoperiod was.

To simulate the effect(s) of cold temperature during winter and photoperiod and temperature

Table 1. Effect of daylength on the larval development and diapause induction of the dark grey cutworm, *Agrotis tokionis* Butler, at 25℃

Photoperiod L : D	Larval period (Days)	Diapause induction (%)
18 : 6	53.5 ± 6.0	100
12 : 12	77.2 ± 8.5	100
6 : 18	85.1 ± 14.2	100

Table 2. Effect of alternating daylength on the larval development and diapause induction of the dark grey cutworm, *Agrotis tokionis* Butler, at 25℃

Photoperiod(L : D)		Developmental period(days)		Diapause induction (%)
1st−3rd	4−7th	1st−3rd	4−7th	
12 : 12	16 : 8	17.9 ± 1.7	39.1 ± 2.8	100
12 : 12	12 : 12	16.1 ± 1.5	51.4 ± 8.7	100
12 : 12	8 : 16	16.9 ± 1.2	58.5 ± 7.2	100
16 : 8	16 : 8	15.5 ± 1.4	36.0 ± 3.6	100
16 : 8	8 : 16	17.2 ± 1.8	40.3 ± 5.8	100

Table 3. Effect of alternating rearing temperature on the larval development and diapause induction of the dark grey cutworm, *Agrotis tokionis* Butler

Temp. (℃)		Cold period (days)[a]	Developmental period (days)[b]		Diapause induction (%)
1st−3rd	4−7th		1st−3rd	4−7th	
25	30	0	13.3 ± 2.0	32.3 ± 3.5	100
		20	14.2 ± 2.4	31.2 ± 7.9	100
		40	13.4 ± 1.8	31.2 ± 6.9	100
		60	13.6 ± 1.7	37.7 ± 5.5	100
25	25	0	14.3 ± 2.4	32.4 ± 4.7	100
		20	13.3 ± 2.6	39.5 ± 6.0	100
		40	13.3 ± 1.6	32.9 ± 3.7	100
		60	13.3 ± 1.7	38.9 ± 5.6	100
25	20	0	14.2 ± 2.5	44.6 ± 3.4	100
		20	13.6 ± 1.5	47.3 ± 8.0	100
		40	14.0 ± 3.0	42.4 ± 3.1	100
		60	13.8 ± 2.1	47.7 ± 9.8	100

[a] Larvae were exposed to cold treatment (4℃, dark) immediately after the 3rd ecdysis.

[b] Larvae were reared under 16-hr photophase and the cold period was subtracted from the total larval period until the diapause initiation.

Table 4. Effects of temperature and photophase on the larval development and diapause induction of the dark grey cutworm, *Agrotis tokionis* Butler

Cold period (days)	Temp. & Photophase(L : D) 4−7th instar	Developmental period[a] (days)	Diapause induction(%)
20	25℃, 16 : 8	39.5 ± 6.0	100
	25℃, 8 : 16	45.5 ± 6.6	100
	20℃, 16 : 8	47.3 ± 8.0	100
	20℃, 8 : 16	53.2 ± 6.3	100
40	25℃, 16 : 8	32.9 ± 3.7	100
	25℃, 8 : 16	36.2 ± 2.6	100
	20℃, 16 : 8	42.4 ± 3.1	100
	20℃, 8 : 16	45.5 ± 5.8	100
60	25℃, 16 : 8	38.9 ± 5.6	100
	25℃, 8 : 16	35.5 ± 2.5	100
	20℃, 16 : 8	47.7 ± 9.8	100
	20℃, 8 : 16	47.2 ± 5.2	100

[a] Larvae had been reared at 25℃, 16hr daylength before the cold period which was subtracted from the total larval period until the diapause initiation.

Table 5. Effect of temperature on the diapause development of the dark grey cutworm, *Agrotis tokionis* Butler

Temperature(℃)[a]	Diapausing period(days)[b]	Rate of pupation(%)
20 (20)[c]	42.9 ± 5.1	65
27-20 (22.6)	45.1 ± 3.8	80
27 (27)	52.0 ± 4.2	40
34-20 (25.3)	50.8 ± 4.3	75
34-27 (29.6)	51.3 ± 5.4	60
34 (34)	53.0 ± 0.0	10
41-20	−	0
41-27	−	0
41	−	0

[a] The low temperature condition started from Jul. 15 was 15 hrs. a day.

[b] Diapausing period from Jul. 15 until pupation.

[c] Numbers in parentheses are daily mean temperature.

after the winter, experiments were done (Tables 3 and 4). Regardless of the cold period, larval period was shorter as the rearing temperature after the cold treatment was higher;31~38 days at 30℃, 32~39 days at 25℃, and 42~48 days at 20℃;and larvae completed development did all enter the diapause without being affected by the cold treatment immediately after the 3rd molting. The degree of cold treatment, however, influenced the development of the larvae. Larval periods of those exposed to 40 days of cold treatment were about the same as of the larvae which did not pass the cold period, but shorter than those of larvae exposed to 20 or 60 days of

Table 6. Effects of photoperiod and temperature on the diapause development of *Agrotis tokionis* Butler larvae

Photoperiod (L : D)	Temperature(℃)	Mortality during diapause(%)	Duration of diapause(days)
16 : 8	20	46.9	104.0 ± 10.6
	27	46.7	93.3 ± 6.9
	34	85.7	85.6 ± 2.9
8 : 16	20	30.3	84.8 ± 7.7
	27	17.6	73.7 ± 6.3
	34	100	–
0 : 24	20	32.0	91.6 ± 6.8
	27	31.2	80.8 ± 5.8
	Room temp.[a]	46.9	80.5 ± 6.2

[a] Daily mean temperature was 25~30℃.

Table 7. Effects of the pretreatment of the high timperature (34℃, dark) on the termination of diapause of *Agrotis tokionis* Butler

Duration at high timp.	Mortality during Diapause(%)	Duration of diapause (days)[a]
J. 10-J. 25	28.6	80.0 ± 6.1
J. 10-J. 10	14.3	74.8 ± 6.6
J. 10-J. 25	26.1	77.9 ± 9.9
J. 10-Aug. 10	75.0	78.3 ± 3.4

[a] Larvae were reared at 20℃, dark condition after the treatment.

cold treatment (Table 3). Similar results were obtained with treatments of different rearing temperature and photoperiod after the cold period (Table 4) except that larval period became shorter at higher temperature and under long-day condition. With all the treatments, the insects did not show that they were affected by the environmental factors in diapause induction.

Effects of temperature and photoperiod on the diapause termination

Effect of temperature during the dormant period on the diapause termination was examined (Table 5.) Alternating daily temperature was set to simulate the temperature of night and day-time. Pupation rate of the tested insects were about or greater than 60% at temperatures between 20~30℃ but the duration of summer diapause was apparently shorter at lower temperatures; 42.9 days at 20℃ and 51.3 days at 34~27℃ (29.6℃, mean). Larvae exposed to high temperature (34℃, constant) showed very high mortality and those which were exposed to extreme high temperature (41℃, constant or alternating) were all dead. Larvae did not seem to respond to alternating temperature but the diapause duration (D) was proportionally shortened with the decreased daily mean temperature (T); $D=29.9+0.73T$ ($r=0.88^{**}$). Because this experiment was done with the larvae about 40 days after the diapause initation, another experiment was done with the larvae from the time of diapause initiation (Table 6). In table 6, duration of diapause was obviously shortest under a short-day (8 hr photophase) condition followed by dark and long-day condition respectively. On the other hand, under each photoperiod, diapaue duration was shorter as the temperature increased. This was certainly contradictory to the result of preceeded experiment (Table 5). An assumption for this was that there might be two phases of diapause in which the insect responded to the temperature differently; in the early phase physiological activity in insect body can be resumed by a cue - in this case, a certain period of relatively high temperature - and the diapause development can be accelerated by relatively low tomperature in late phase. So larvae within 3 days after the diapause initiation were kept at high temperature (34℃, dark) or at field condition for a certain period and moved to a low temperature (20℃, dark) condition (Tables 7 and 8). The duration of diapause of the larvae exposed to low temperature from June 25 was about 80 days and that of the larvae from July 10 - until when the larvae had been at high tremperature for 30 days - was about 74.8 days, the shortest, but it showed a tendency to be extended in the insects exposed to the low temperature thereafter. This seemed to agree with the assumption. In another observation (Table 8), when larvae were recovered from field condition and exposed to low tomperature, the duration of summer diapause was similar to the result of Table 7 except the recovery of July 25 which is inexplicable. Insects recovered on August 25 had already pupated. The two phases, suggested here, were considered to be corresponding to the activation process and activated phase of diapause development (Mansingh 1971). Provided that no physiological development could be done at 4℃, subtracting the cold period (15, 30, 45, 61 days) from the total period of diapause would be the actual duration of the diapause (Table 9). Except for that of 15-day cold period, it was about 71-74 days depending on the cold period but looked not significantly different each other. This indicates that the high temperature involved in the activation might not be an absolute but a relative temperature that was high enough to serve as a cue, compared with the temperature the insect had experienced before the diapause development.

Table 8. Sequence of diapause termination in field condition

Duration in field	Diapause period (days)[a]
Jun. 10-Jun. 25	81.3 ± 6.3
Jun. 10-Jul. 10	75.5 ± 4.3
Jun. 10-Jul. 25	84.1 ± 6.8
Jun. 10-Aug. 10	79.7 ± 3.3
Jun. 10-Aug. 25	–[b]

[a] Larvae were reared at 20℃, dark condition after they were recovered from field.

[b] All of the insects pupated by Aug. 25.

Table 9. Effect of cold treatment (4℃, Dark) on the summer diapaus development[a]

Cold treatment	Diapause period (days)[b]
Jun. 10-Jun. 25	81.5 ± 4.6
Jun. 10-Jul. 10	73.8 ± 4.6
Jun. 10-Jul. 25	71.0 ± 6.7
Jun. 10-Aug. 10	72.4 ± 4.4

[a] Larvae were reared at 20℃, dark condition after cold treatment.

[b] Subtracted the cold period from the whole period of diapause.

DISCUSSION

Effects of temperature and photoperiod on diapause of dark grey cutworm were studied. The synchronization of growth, developent, reproduction, etc., with available resources is controlled by the timing of seasonal cycles (Tauber et al. 1986). Diapause is an essential component of insect phenology, upon which the timing of other events depends. Among the environmental factors Masaki (1980) suggested that generally related with diapause incidence, food or moisture was excluded from consideration for this insect which has a wide range of host plant and inhabits in the temperate zone with enough precipitation. Since photoperiod and temperature did not affect the diapause induction, summer diapause of this univoltine insect was thougt to be obligatory as in the case of the evergreen bagworm, *Thyridopteryx ephemeraeformis* (Haworth) (Morden & Waldbauer 1980).

Andrewartha (1952) described diapause as a stage in which a physiogenesis goes on in preparation for the active resumption of morphogenesis and proposed a term, 'diapause development'. And Danielvski (1961) stated that the diapause development is a 'reactivation process' after eliminating the physiological state which impeded development. Both temperature and photoperiod were investigated as factors involved in diapause development of the dark grey cutworm and relatively clear responses to those factors were observed. The diapause durtation of the insect was shortest under the short−day condition(8hr photophase) although the threshold photoperiod for diapause termination was not clarified. It is generally known that summer diapause shows a reverse relation to temperature compared with winter diapause in the induction of diapause. Thiele(1973) claims that the induction of obligatory diapause − parapause − is controlled by genetically but termination is controlled by the alteration of temperature or photoperiod and the diapause stage between induction phase and termination phase may be divided into two phases, refractory and activated phase (Mansingh 1971). In the summer diapause of adult *Listroderes costirostris obliquus* Klug, high temperature and long day activate the diapause development and then low temperature and short day mature the ovary (Matsumoto 1959, 1963). There seemed to be two phases of the summer diapause of this insect in respect with the response to temperature. At the temperatures exmined, the duration of diapause was shortened by higher temperrature in early phase and by relatively low temperature in late phase.

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