

NN 8201

no 369

C

VOLTINISM AND ITS DETERMINATION IN SOME
BEETLES OF CRUCIFEROUS CROPS

G. W. ANKERSMIT

NN08201.369

BIBLIOTHEEK
DER
BOUW- en HOGESCHOOL
WAGENINGEN

VOLTINISM AND ITS DETERMINATION IN SOME BEETLES OF CRUCIFEROUS CROPS

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSMVOOGEL,
HOOGLEERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 26 JUNI 1964 TE 16 UUR

DOOR

G. W. ANKERSMIT



H. VEENMAN & ZONEN N.V. - WAGENINGEN - 1964

STELLINGEN

I

De thans door een verschil in fenologie in stand gehouden reproductieve isolatie tussen het voorjaars- en het herfstras van *Ceuthorrhynchus pleurostigma* Marsh. heeft waarschijnlijk een geografische scheiding als oorzaak.

Dit proefschrift

II

De opvatting van BONNEMAISON en JOURDHEUIL, dat de diapauze van *Psylliodes chrysocephala* L. alléén te wijten is aan hoge temperatuur, droogte en jong blad als voedsel tijdens het vroeg imaginale stadium, is onjuist.

L. BONNEMAISON et P. JOURDHEUIL, Ann. Epiphyt. Série C, 5, 345-524, 1954.

III

De methode van afwisselend gebruik van insecticiden of acariciden van verschillend type ter voorkoming of vertraging van het optreden van resistente rassen dient te worden verworpen.

Tuinbouwgid 1964

R. L. BEARD: Selection of Physiologic Strains of *Oncopeltus* and its Relation to Insecticide Resistance. Bull. Conn. Agric. Exp. Sta. no. 611, 1957.

IV

Een harmonische bestrijding van plagen is in de eerste plaats noodzakelijk ter voorkoming van resistentie tegen chemische bestrijdingsmiddelen en het optreden van nieuwe plagen en pas in de laatste plaats ter bescherming van de consument tegen toxische stoffen.

V

In verband met besmettingsgevaar van de omgeving is kunstmatige veldinfectie met gele roest toelaatbaar bij tarwe maar niet bij gerst.

VI

De in de fruitteelt bij de praktijk aangetroffen neiging om de stikstofbemesting bij een grasstroken-cultuur merendeels op de zwart gehouden stroken uit te strooien moet met het oog op de vochtvoorziening van het gewas als verwerpe-lijk worden beschouwd.

VII

Gezien het toenemend aantal intermediaire vormen tussen persistent en non-persistent virus kan men deze terminologie beter laten vervallen en in de nieuwe termen de weg die het overgedragen virus in de vector volgt tot uitdrukking brengen.

J. S. KENNEDY, M. F. DAY and V. F. EASTOP. A conspectus of aphids as vectors of plant viruses. Commonw. Inst. Ent. London, 1962.

VIII

Het is noodzakelijk bij het bepalen van aantalsfluctuaties bij insekten de nadruk te leggen op de eitellingen.

VOORWOORD

Bij het voltooien van dit proefschrift wil ik in de eerste plaats U, Hooggeleerde DE WILDE, Hooggeachte Promotor, bedanken voor Uw adviezen tijdens het onderzoek en Uw zeer waardevolle kritiek bij het samenstellen van het proefschrift. De Hoogleraren en Lectoren van de Landbouwhogeschool wil ik gaarne mijn dank betuigen voor hun bijdragen aan mijn wetenschappelijke vorming. Met een gevoel van grote dankbaarheid wil ik hier mijn vroegere leermeester, wijlen de Hooggeleerde W. K. J. ROEPKE, gedenken, wiens heldere colleges in belangrijke mate mijn interesse voor de entomologie hebben doen ontwaken. Veel dank ben ik verschuldigd aan de leden van de wetenschappelijke staf en het overige personeel van het Laboratorium voor Entomologie voor geboden hulp. In het bijzonder wil ik hier Drs. W. COMPANJEN voor zijn bijstand bij het histologische deel van het onderzoek en Dr. Ir. G. B. STAAL voor het maken van de microfoto's, bedanken. Met veel waardering voor hun hulp wil ik hier ook noemen, Ir. S. C. BHARGAVA en Mejuffrouw H. KRAMER, voor hun zorg bij sommige kweekproeven en de Heren M. P. VAN DER SCHELDE en E. J. J. VERHAAF voor het vervaardigen van de tekeningen.

Voorts wil ik gaarne nog bedanken Dr. M. R. HONER en de Heer N. G. UILENBURG voor de correctie van de Engelse tekst en Mevrouw G. DE WILDE-VAN BUUL voor het nakijken van de Nederlandse samenvatting. Dank ben ik ook verschuldigd aan Mejuffrouw M. C. ALBERS, Mevrouw D. J. TIEBBES-ONDERSTAL en Mejuffrouw A. C. VAN DER VLIET voor het typen van het manuscript.

CONTENTS

1.	INTRODUCTION	1
1.1.	Types of annual cycles in insects	1
2.	ANNUAL CYCLE OF <i>Ceuthorrhynchus assimilis</i> PAYK.	3
2.1.	Literature	3
2.2.	Field observations	3
2.3.	Laboratory observations	8
2.3.1.	Methods	8
2.3.2.	Influence of photoperiod and temperature during the adult stage on the occurrence of diapause	10
	a) Effect on phototaxis	10
	b) Effect on geotaxis	10
	c) Effect on feeding activity	11
	d) Effect on hiding behaviour	11
	e) Effect on oöcyte growth	13
	f) Spermiogenesis	14
2.3.3.	Effect of the photoperiod during the larval stage on the occurrence of diapause in the adult	15
2.3.4.	The termination of diapause	15
2.3.5.	Histological study of the ovaries	16
2.4.	Seasonal synchronism between the physiological state of the host plant and the life cycle of <i>C. assimilis</i>	18
3.	ANNUAL CYCLE OF <i>Ceuthorrhynchus pleurostigma</i> MARSHAM	20
3.1.	Literature	20
3.2.	Field observations	21
3.3.	Laboratory observations	22
3.3.1.	Laboratory observations on the spring race	22
	A. Methods	22
	B. Influence of some photoperiods during the adult stage	22
3.3.2.	Laboratory observations on the annual cycle of the autumn or summer race	23
	A. Methods	23
	B. Influence of photoperiod and temperature during the adult stage on the annual cycle	24
	a) Effect on phototaxis	24
	b) Effect on geotaxis	25
	c) Effect on feeding activity	25
	d) Effect on hiding behaviour	28
	e) Effect on oöcyte growth	28
	C. The effect of food on oöcyte growth	32

D.	The effect of a change from short to long photoperiod during the adult stage on oöcyte growth	33
E.	Photoperiodic responses in weevils from Antibes (43° 35' N.L., Southern France)	34
F.	Influence of the photoperiod during the larval stage on the occurrence of diapause in the adult	35
G.	The termination of diapause	36
H.	Histological studies on the ovaries	37
I.	Seasonal synchronism between the host plant and the annual cycle of <i>C. pleurostigma</i>	38
3.4.	Hybridization of the two races	39
3.5.	Discussion and conclusion	40
4.	ANNUAL CYCLE OF <i>Psylliodes chrysocephala</i> L.	41
4.1.	Literature	41
4.2.	Field observations	42
4.3.	Laboratory observations	42
4.3.1.	Methods	42
4.3.2.	Effect of photoperiod and temperature on the annual cycle	43
	A. Effect of photoperiod and temperature on feeding activity	43
	B. Effect of photoperiod and temperature on the development of the ovarioles	46
4.4.	Discussion and conclusion	47
5.	DISCUSSION	48
	Summary	51
	Acknowledgements	54
	Samenvatting	55
	References	58

This thesis will also be published as Mededelingen van de Landbouwhogeschool Wageningen 64-8 (1964) [Communication No. 64-8 (1964) of the State Agricultural University Wageningen]

1. INTRODUCTION

The annual cycle of animals has to be adjusted to their seasonal environment. Many species are warned for adverse seasonal conditions by using environmental factors, correlated with season, as 'token' stimuli. The main stimuli so far known emerge from the photoperiod, temperature, food, and other animals. The information obtained from these 'tokens' serves to adjust the physiological condition of the animal in such a way as to assure a maximum survival during the period when the adverse conditions prevail. The 'tokens' operate on a developmental stage characteristic for the species. This may be the egg, larva, pupa or adult. The responsive stage is not necessarily the same as the sensitive stage. The response may be migration, seasonal polymorphism or diapause. Diapause prevents the species from being active when the environmental conditions are unfavourable and moreover synchronizes post hibernation or -aestivation emergence (ANDREWARTHA 1952, LEES 1955, DE WILDE 1962).

A study of the precise conditions under which diapause is induced and terminated provides us with a better knowledge of the possibilities of survival for the species in different parts of its areal. In connection with a previous study on the life history and control of *Ceuthorrhynchus assimilis* Payk. (ANKERSMIT 1956), we have investigated the factors determining the adult diapause in this insect. The results were compared with those obtained with *C. pleurostigma* Marsh. and *Psylliodes chrysocephala* L. both of which have a different type of annual cycle.

1.1. TYPES OF ANNUAL CYCLES IN INSECTS

The adjustment of the annual cycle of an insect to its environment involves that this cycle must vary with the geographic region in which the insect occurs (DANILYEVSKY 1957, MASAKI 1961). Within one species we may find homodynamic as well as heterodynamic development. *Philosamia cynthia* (Drury) (MASAKI 1961) has a homodynamic development in Assam, is multivoltine with a facultative diapause in southern Japan and strictly univoltine in Nagasaki (37° Northern Latitude). Also *Tetranychus urticae* Koch. is known to be partly homodynamic in its southern races and heterodynamic with a facultative diapause in the northern ones (BONDARENKO 1958). MASAKI (1961) points out that the heterodynamic multivoltine cycle is an intermediate step between the more primitive homodynamic multivoltine towards an obligatory univoltine cycle. In some cases the number of years needed for the completion of one cycle becomes even more than one. For example, *Dendrolimus pini* L. is bivoltine near the Adriatic coast (MELIS 1940), univoltine in Central- and Western Europe and semivoltine in Northern Russia (ERMOLAJEW and WASILJEV 1935).

Insects with a heterodynamic multivoltine cycle always have a facultative diapause induced by external factors. In the heterodynamic univoltine cycles

diapause is often also facultative, but in some cases, diapause seems to be obligatory. In the latter case no external factors are known so far which can prevent diapause. Within one species these two types of *univoltinism* may occur. In the Leningrad strain of *Acronycta rumicis* L. 70–60% of the population reacts to long experimental photoperiods by avoiding diapause but the remaining 30–40% obviously enters hibernation, (DANILYEVSKY 1960), yet the field population is univoltine in this region. GEISPITZ (1953) also mentions some cases of univoltine Lepidoptera which can be partially prevented from entering diapause by rearing at a special, rather narrow range of photoperiods. The species concerned (*Dasychira pudibunda* L., *Stilpnotia salicis* L. and *Euproctis chrysorrhoea* L.) are at least partially bivoltine in the southern parts of their distribution area (SORAUER 1952, ANONYMUS 1958). Also in the univoltine race of *Bombyx mori* L. we find a gradual change from multivoltine to univoltine races. In the latter the diapause-inducing range of temperatures is extended to such low values that diapause becomes obligatory (LEES 1955).

It would be of great interest to find out which conditions might prevent diapause in insects with an obligate diapause throughout their distribution area. These insects are often restricted in the extension of their voltinism by the limited period of the year during which suitable food is available. This occurs with insects living on buds, flowers, and growing seeds. To this group belong many weevils, especially *Ceuthorrhynchus* spp., some of which were investigated.

2. ANNUAL CYCLE OF *CEUTHORRHYNCHUS ASSIMILIS* PAYK.

2.1. LITERATURE

Ceuthorrhynchus assimilis, a serious pest of *Brassica* seed crops, is a much-studied insect. The species occurs throughout Europe, Algeria, Morocco and in several parts of North America (JUNK-SCHENKLING 1930, BONNEMAISON 1957).

There has been some confusion about the number of generations of this species. TASCHENBERG (1879) and DOSSE (in SORAUER, 1954) mention two generations but this is not confirmed by any of the extensive investigations on this insect in Germany by HEYMONS (1922), SPEYER (1923) and VON WEISZ (1940), in France by BONNEMAISON (1957), in California by CARLSON, LANGE and SCIARONI (1951) and in the Netherlands by ANKERSMIT (1956).

The life history of the weevil may be summarized as follows. The insect hibernates in the adult stage in the litter under hedges, shrubs and trees, emerges in spring and deposits its eggs, after a feeding period of one or two weeks, into the seed pods of several *Brassica* spp. The larvae feed on the seeds and are usually full-grown in June (in California some individuals already in May). They pupate in the soil in an earthen cell. The new generation appears in the course of July, in California in May. The young generation soon migrates to its winterquarters.

2.2. FIELD OBSERVATIONS

In order to establish the voltinism of the weevil in the field, flights were registered with the aid of yellow Moericke traps. The catches in these traps give a good impression of the activity of the weevils throughout the season. Some of the results are given in figs. 1a-h.

We can notice two major flight periods, one in spring and one in summer. A secondary peak, sometimes found in June, is caused by the growing attractiveness of the trap as the surface of yellow flowers (e.g. winter rape) diminishes. Moreover temperature is usually more favourable for flight activity in June than in May.

During the first flight period the weevils leave their hibernation sites and search for a suitable host plant. The spring flight may last several months. In 1953 the first weevils were noted leaving their hibernation sites on the 25th of March. In that year the last were found in the end of May.

This long period of emergence in spring may be caused by:

1. Differences in the thickness of the litter above the weevils in their winter-quarters influencing temperature during diapause and postdiapause.
2. Differences in their reactions to these temperatures.
3. Differences in the intensity of diapause.

Figs. 1a-h. Catches of *C. assimilis* with yellow Moericke traps near Wageningen from 1953-1956. (All numbers increased with one).

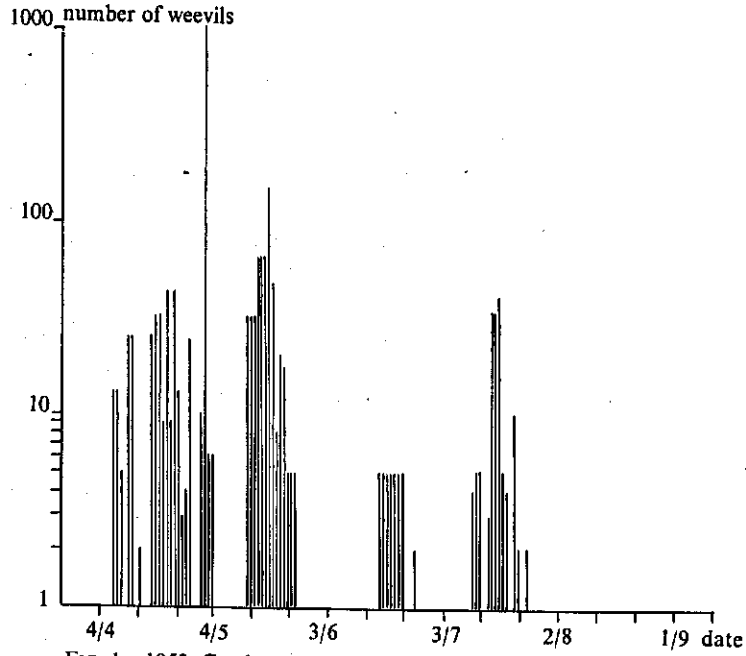


FIG. 1a. 1953. Catches near hibernation site

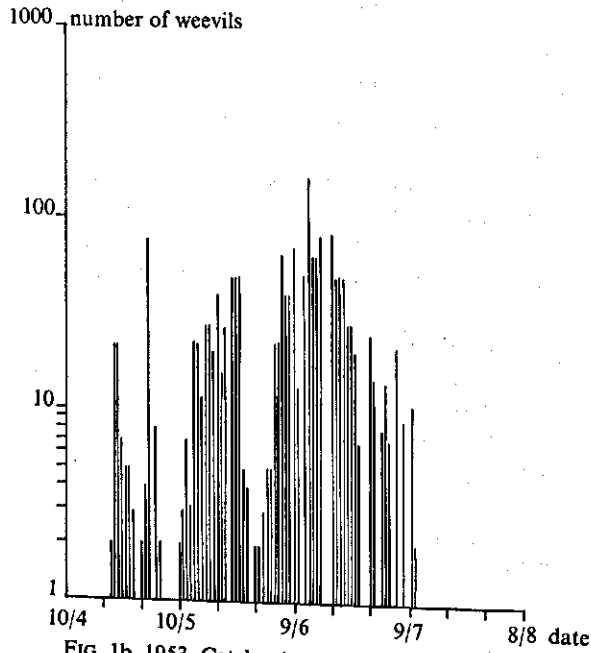


FIG. 1b. 1953. Catches in a rape field

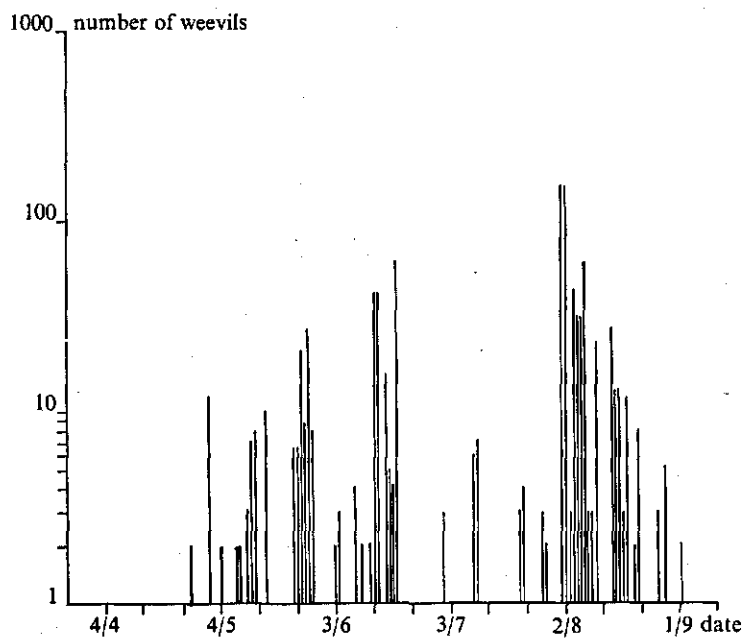


FIG. 1c. 1954. Catches near a rape field

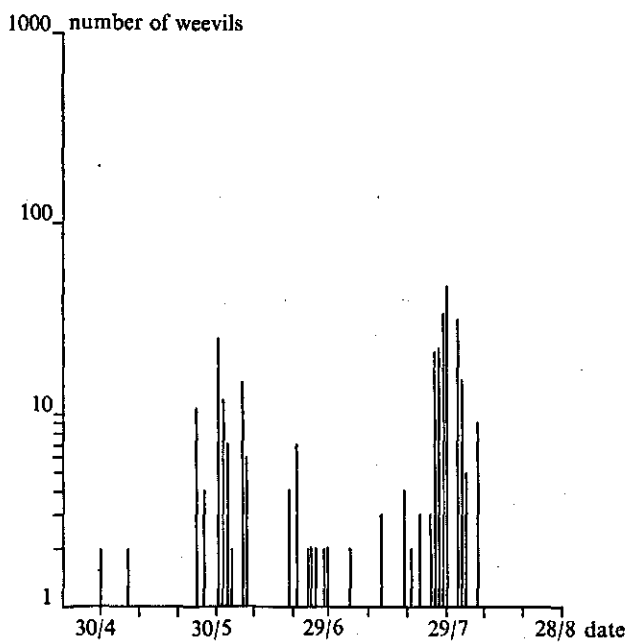


FIG. 1d. 1955. Catches north of a rape field

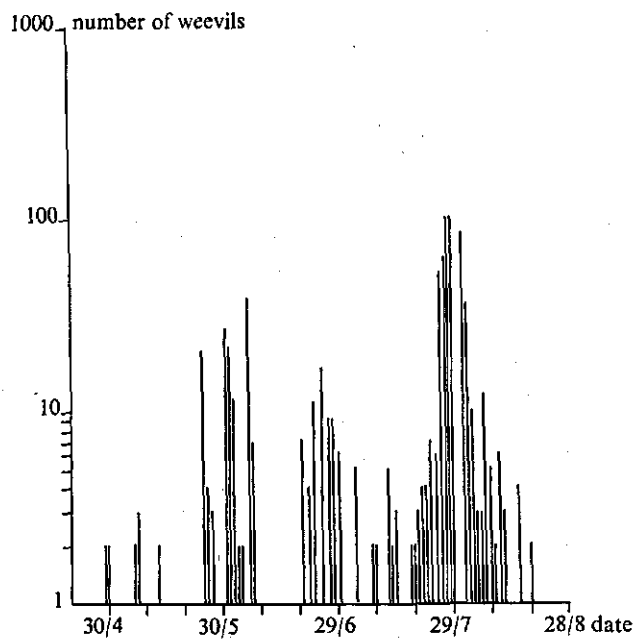


FIG. 1e. 1955. Catches south of a rape field

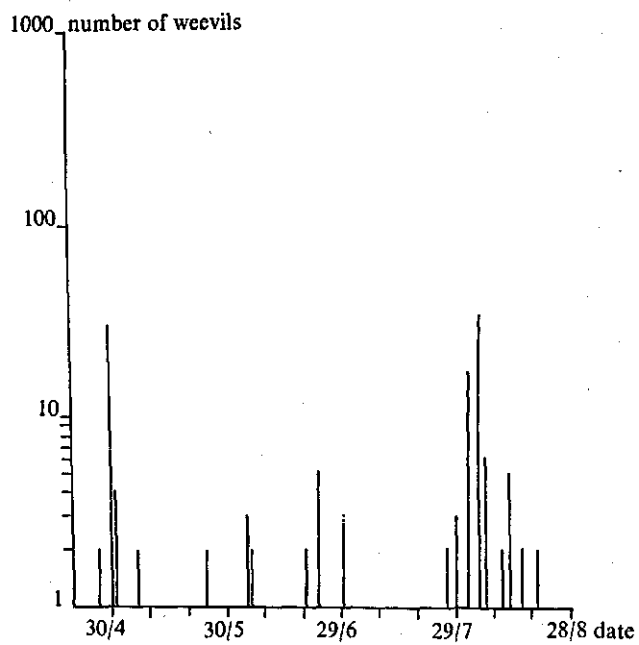


FIG. 1f. Catches on a place where rape had been grown the previous year

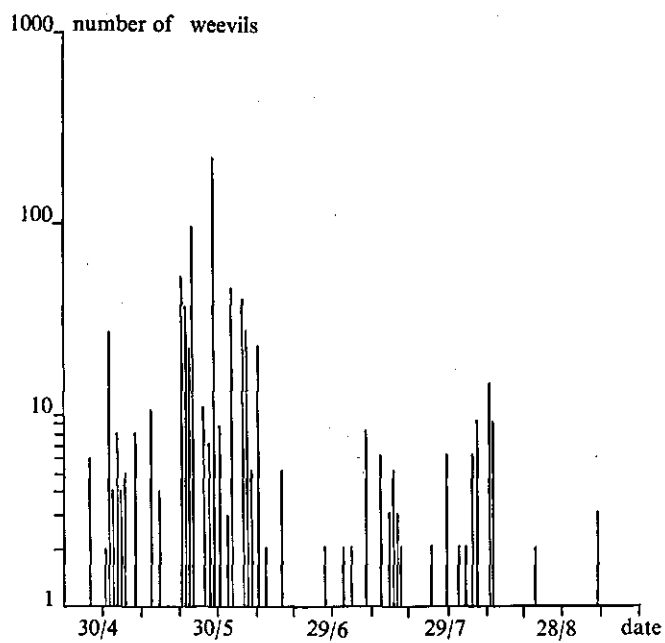


FIG. 1g. 1956. Catches north of a rape field

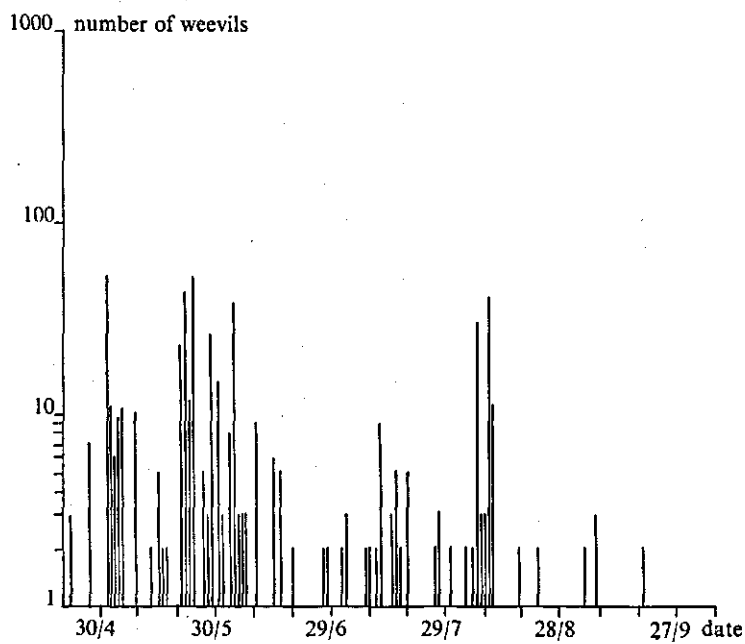


FIG. 1h. 1956. Catches south of rape field

During the second flight period the weevils search for winterquarters. They usually disappear in the course of August. Only in the very cold summer of 1956, active weevils were still numerous in September and a few were even observed as late as November 17th.

No developing oöcytes were found in the ovaries of the new generation. Apparently it enters diapause without producing eggs. Only occasionally were some weevils with growing oöcytes found in September. It was difficult to establish with certainty to which generation these weevils belonged. The intactness of their scales indicated that they might belong to the new generation. It was found in rearing experiments that in some weevils diapause is easily broken, such weevils already producing eggs in October, and it is therefore possible that the weevils observed in the field belonged to the new generation.

The possibilities of survival for the second generation are limited as only a few pods for egg deposition are available and the temperature in the autumn soon becomes too low to enable further development. Flight activity would be hampered by the low temperatures. Emerging young weevils possibly could not find a suitable hibernation site.

We may therefore conclude that *Ceuthorrhynchus assimilis* in the Netherlands, just as in other countries, has only one generation per year.

2.3. LABORATORY OBSERVATIONS

The purpose of these observations was:

1. To check the field observations by following prediapause and diapause in insects of known age.
2. To influence the occurrence of diapause by changing the environmental conditions. Photoperiod and temperature were chosen as such.

2.3.1. Methods

Pods of *Brassica napus* L. containing almost full grown larvae were collected in the field. These pods were placed on soil in cages or glass jars. The full grown larvae leave the pods and pupate in the soil from where the emerging weevils can be collected easily. The weevils were subsequently transferred to glass jars and were reared on daily renewed pods of *Brassica napus*. A layer of cellulose wool was placed on the bottom of the jars, which provided the weevils with a hiding place in which they could be detected easily. Peat litter was used in the first experiments but the examination of this material was time consuming and also disturbed the weevils during the regular countings which were necessary to establish the percentage diapausing weevils. The weevils were kept at the photoperiods and temperatures mentioned in the tables. Fluorescent white 40 watt tubes were used as a light source at distances from 20 cm - 100 cm. The photoperiods needed were obtained by means of automatic timeswitch.

Among the possible criteria for studying the development of the weevils, behaviour and ovarian activity stand out. As in the Colorado beetle (DE WILDE

1959) the syndrome of diapause involves changes in behaviour and a standstill in reproductive activity. In *C. assimilis* during prediapause the weevil leaves the food plant and descends into the soil. This behaviour may again be described in terms of the tactic components phototaxis, geotaxis and thigmotaxis. A decrease in feeding activity is also characteristic of diapause.

Phototaxis and geotaxis were studied in an apparatus designed by DE WILDE (figure 2). This consists of a plexiglass tray, internal dimensions $37.5 \times 7.2 \times 7.2$ cm, placed in a closed metal box. This box could be brought into any desired slope by means of an adjusting screw. The inclination was measured by means of a protractor and a waterlevel. On both sides the metal box could be opened by a slide. Light from a lamp could then penetrate into the box and the reaction of the weevils placed at the beginning of the experiment in the centre of the box could be followed. The weevils were protected against radiant heat by a water-cooling system.

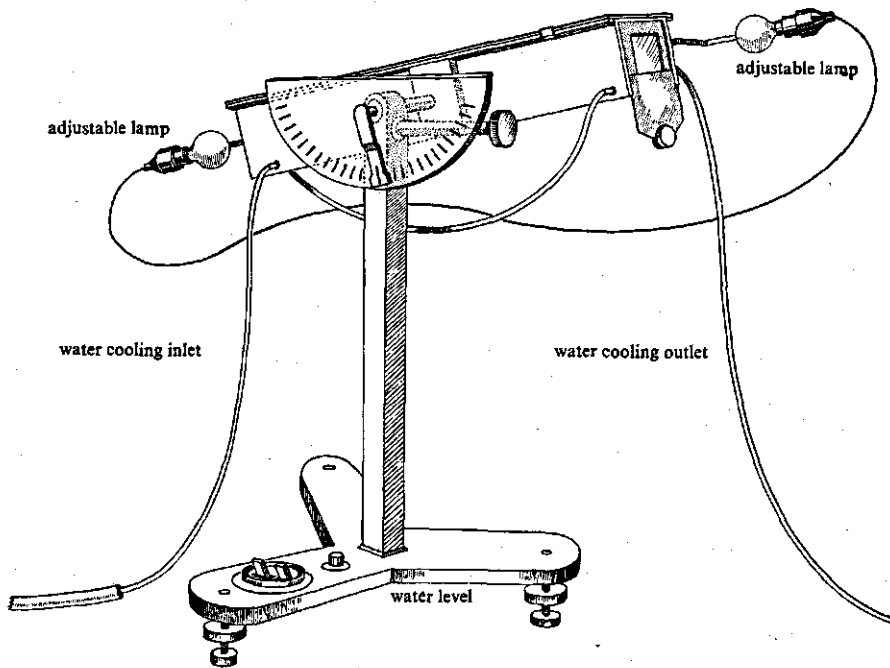


FIG. 2. Apparatus for testing photo- and geotactic responses.

A satisfactory method for the measurement of thigmotaxis was not found. Some indications were obtained by studying the hiding behaviour of the weevils. The exact determination of nutritive activity in *C. assimilis* is difficult as the weevils feed on the seeds inside the pods, yet some estimation can be made from the number of punctures in the walls of the pods. The other major characteristic of diapause is the standstill in oöcyte growth. This is a very clear feature which is easily determined as growing oöcytes in the ovarioles are clearly visible.

2.3.2. Influence of photoperiod and temperature during the adult stage on the occurrence of diapause

a. Effect on phototaxis

The phototaxis of weevils reared separately at 25°C and a photoperiod of 10 hours, 20 hours, and the normal daylength and temperature of July, August and September was tested. Each group contained 20 weevils. The test lasted 3 minutes, each weevil being tested twice. The results are given in table 1.

TABLE 1. Percentage weevils (*C. assimilis*) with a positive phototaxis when reared under different photoperiods.

Photoperiod and temperature	Age of the weevils in days								
	1	3	6	8	12	15	18	28	49
Room conditions of July-Sept.	57	89	97	92	89	73	60	60	64
20 hours 25°C	76	97	94	100	93	47	60	30	50
10 hours 25°C	70	97	100	94	94	93	66	65	53

Null hypothesis: no difference in phototaxis between the age groups 3–12 days and 15–49 days.

Room conditions $d = 27.5$ $t_6 = 7.6$ $P 0.01-0.001$

20 hours 25°C $d = 49.3$ $t_6 = 21.4$ $P < 0.001$

10 hours 25°C $d = 32.0$ $t_6 = 3.4$ $P 0.02-0.01$

In this experiment a transition from a positive to an indifferent phototaxis was found about 15 days after the emergence of the weevils.

In a second experiment 5 weevils were taken at random from rearing jars and tested for 5 minutes. Each test was repeated 4 times with the same weevils by alternating the illuminated side of the apparatus. The result is given in table 2.

In none of these experiments was a clear negative phototaxis observed, so that photoperiod does not have any influence on the phototaxis of the weevils. It seems, however, that at high temperatures phototaxis is less positive than at lower. At the low temperature of 10°C phototaxis is always strongly positive which may indicate that the prediapause is not yet finished. In spring, phototaxis is also highly positive (with egg-laying weevils 90%).

b. Effect on geotaxis

In some experiments the geotaxis was studied with the apparatus described in 2.3.1. The summarized figures are given in table 3. Egg laying weevils do not show a clear difference between the number of positive and negative choices ($t_3 = 0.18$). In the diapausing weevils a somewhat stronger positive geotaxis can be observed but here too, owing to the variable results in the low number of observations the difference is not significant ($t_2 = 2.3$).

TABLE 2. Percentage 40–55 days old weevils (*C. assimilis*) with a positive phototaxis. The weevils were reared at the photoperiods and temperatures mentioned in the table.

Photoperiod temperature	Percentage pos. weevils
10 hours 10°	90
17°	63
20°	52
15 hours 10°	80
17°	76
20°	78
20 hours 10°	85
17°	62
20°	72

TABLE 3. Reaction of egg laying and diapausing weevils (*C. assimilis*) on gravitation. Each weevil was tested several times.

Condition of the weevils	Number of weevils	Geotactic response expressed in percentages		
		Positive	Negative	No reaction
Egg laying	16	39	36	25
Diapausing (160–180 days old)	15	54	20	26

c. Effect on feeding activity

Though the exact quantity of food consumed by the weevils was not determined, some estimate of their feeding activity can be given. The weevils feed intensively during the first week after emerging from the pupa. After this, feeding activity diminishes, but 30 days old weevils are still feeding. No effect of the photoperiod could be found on this aspect of behaviour, only the temperature being of some importance. At 25°C food intake diminished 4–6 days after emergence and at 17°C 6–11 days after emergence.

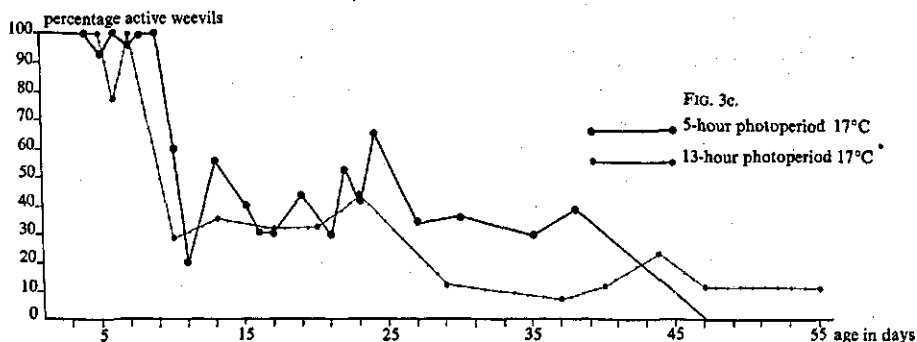
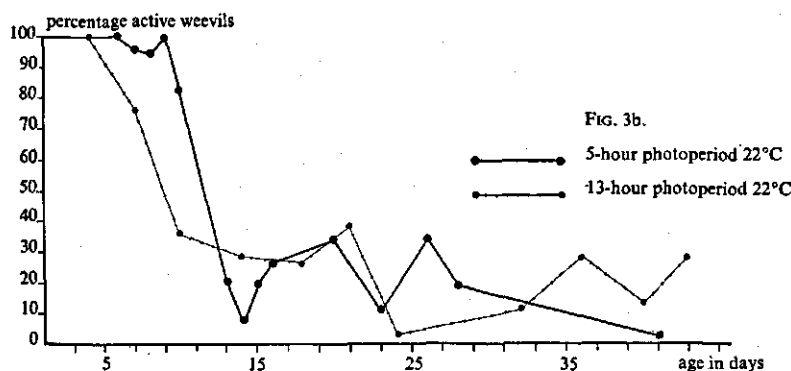
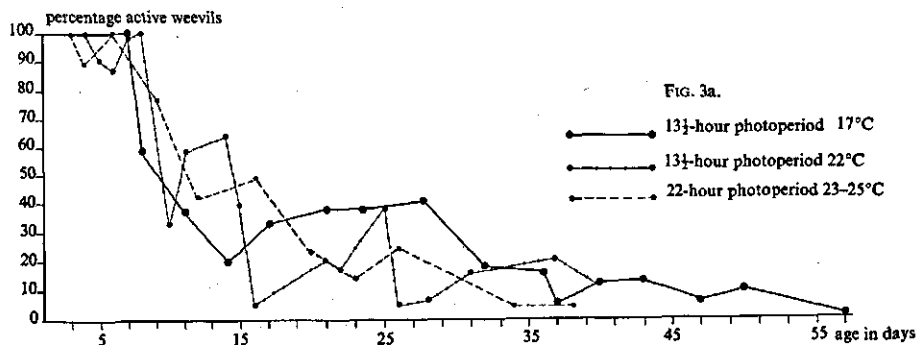
d. Effect on hiding behaviour

This was determined by counting the number of weevils found on the food and those hiding in peat litter in the bottom of the rearing jars or cellulose wool as described in 2.3.1. The results of these counts are given in figure 3a, b, c and d. The number of insects used in these experiments varied from 44–100 in the beginning of the observations to at least 30 in the end.

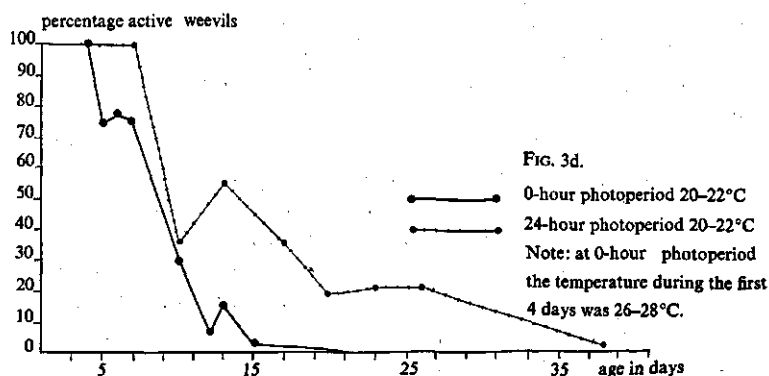
The conclusions from these observations are the following:

1. There is a period of general activity a few days after emergence.
2. This activity decreases between the fourth and tenth day. A point with only 50% active weevils was usually reached after 8–10 days.
3. There is no visible effect of temperature on the duration of this prediapauses in the region of 17–25°C.

FIGS. 3a-d. Percentage active weevils (*C. assimilis*) during a period of 1-2 months after emergence from the pupae when reared under different photoperiods and temperatures.



4. There is no influence of photoperiod except with 0 hours light. In that case all the weevils were in their hiding place within 20 days. A possible explanation for this phenomenon might be the still rather strong positive phototaxis of weevils of this age which may oppose their tendency to hide. The decrease in positive phototaxis is usually observed in about 14 days old weevils although hiding starts earlier. This means that the change in habitat is not merely the consequence of a change in phototaxis.



e. Effect on oöcyte growth

As diapause has to be regarded as an arrest of development which enables the insect to start reproduction at the moment when its host plant is again available in a suitable stage, this aspect of diapause is of utmost importance.

The ovaries of *C. assimilis* consist of two pairs of ovarioles located at both sides of the alimentary canal. Growing oöcytes pass through the vitellarium and are, when developed into eggs, collected in the eggchamber until oviposition. Ovarian activity is clearly visible after dissection of the weevil by an elongation and thickening of the ovarioles and the formation of chains of growing oöcytes.

BONNEMAISON (1957) investigated the growth of the ovarioles of this insect by measuring their length. He found a total length of 400-600 μ in the newly emerged weevils in July. The length increased throughout August to 650-800 μ . In spring the length was still 800 μ . These figures are approximately the same as were found by the measurement of the ovarioles of weevils collected in the field near Wageningen. The results are given in table 4.

These figures show an increase after emergence from the pupa during the

TABLE 4. Results of measurement of the length of the ovarioles of *C. assimilis* at various moments of the year 1953-1954.

Month of collection	Length in μ	Diameter of the germarium in μ	Number of weevils
July (young)	500	47	10
August	500	47	13
September	750	63	3
October	630	60	3
November	670	67	5
December	650	58	8
January	920	83	1
April (soil)	480	50	2
April (flying)	1170	117	2
May	1420	110	24
June	2430	178	17

prediapause period. During autumn and winter the dimensions remain almost the same but after emergence from the winter quarters the length of the ovaries increases markedly as a result of the formation of chains of oöcytes in the vitellarium.

Samples of weevils reared under the conditions mentioned in table 5 were regularly collected, killed with ethylacetate and dissected in Levy's fluid. The ovarioles were then transferred in Bouin (modification Dubosq-Brasil) and later stored in alcohol 70%. Measurements on the ovarioles of these weevils did not show any signs of growth within 2 months. They were all of the same size as in weevils taken from the field in autumn. From these results we may conclude that photoperiod and temperature during the adult stage are not likely to influence the growth of the ovarioles.

TABLE 5. Experimental conditions and numbers of insects used in testing oöcyte development in *C. assimilis*. None of the weevils showed oöcytes in the descending stage.

Photoperiod in hours per 24 hours cycle	Temperature in degrees centigrade	Number of weevils
0	room temperature ($\pm 20^\circ$)	50
5	17	73
5	22	50
10	10	18
10	17	27
10	20	36
10	25	20
13	17	66
13	22	74
13½	17	112
13½	22	108
14	room temperature ($\pm 20^\circ$)	4
15	10	50
15	17	54
15	20	59
20	10	15
20	17	24
20	20	24
20	25	20
22	25-30	45
24	room temperature ($\pm 20^\circ$)	84

f. Spermiogenesis

Copulating weevils can often be observed about one or two weeks after they arrive in the field. Females do not mate earlier though the males try to mate already during prediapause. Yet SPEYER (1923) states that he could not find functioning testes in the males during the autumn. He did not find developed sperm before January in weevils collected in a hibernation quarter. Some observations were made to check this statement but the results did not agree with those of Speyer. All 7 investigated 1½ month old males contained testes with well-developed, mobile sperm.

2.3.3. Effect of the photoperiod during the larval stage on the occurrence of diapause in the adult.

MÜLLER (1957), with the cicadellid *Stenocranus minutus* F., prevented diapause when he reared the larvae under short day conditions and the adults under a long photoperiod. He suggested that by such an alternation of short and long day diapause might be prevented in other univoltine insects. STRÜBING (1960), however, doubted the complete univoltinism of *S. minutus*.

In order to test this supposition the following experiment was made. Egg-laying weevils were collected in May in the field and caged with winter rape plants for 2 days. The plants were then considered as infested, replaced by others, and reared under short day conditions of 9 hours light by placing daily in the evening a metal cylinder over the plants and removing it in the morning. These short day conditions proved rather unfavourable for the plant. The larvae were therefore transferred after some days to young cut pods of winter rape and reared in glass jars under short day conditions. As the pods soon deteriorated, the larvae were transferred every other day to new young pods. In this way the larvae throughout their development were given young growing seeds only. Under normal growing conditions they will feed at the end of the larval stage on the ripening seeds in the pods. The emerging weevils were reared under the conditions mentioned in table 6. In all these cases, however, the weevils entered normally into diapause. It follows that the result of this experiment is negative.

TABLE 6. Rearing conditions of weevils (*C. assimilis*) used in the experiment on the effect of alternation of photoperiod in larval and adult stage.

Photoperiod in hours per 24 hours	Temperature in degrees centigrade	Number of weevils	Number entering diapause
13	25	20	20
	18.5	16	16
20	25	17	17
	18.5	7	7

2.3.4. The termination of diapause

As is known from the studies of various workers on *C. assimilis* (SPEYER 1923, RISBEC 1952, ANKERSMIT 1956, BONNEMAISON 1957), the weevils appear on fine spring days when the temperature rises above 15°C. The males leave their hibernation quarters first (RISBEC 1952, ANKERSMIT 1956). Though the weevils become active in spring only, most of them finished the diapause much earlier in the season. This can be shown by collecting them in the field and rearing in the laboratory as is shown in table 7. These results show that diapause is terminated in many specimens by the end of autumn. Yet some weevils were apparently still in diapause as they could not be induced to produce eggs by transferring them to a higher temperature. This was even the case with weevils collected on the 26th of March in a hibernation site. Some of

TABLE 7. Duration of diapause in *C. assimilis* under normal field conditions (1956-'57)

Date of collection	Rearing temp.	Number of days reared	Particulars	Number of weevils
27 September	25°C	2	active	20
27 September	25°C	7	much feeding	
27 September	25°C	22	no oöcytes	
27 September	25°C	52	no oöcytes	10
21 November	20°C	9	active	
21 November	20°C	17	try to mate	
21 November	20°C	24	2 eggs laid	10
5 December	20°C	28	eggs laid	
27-29 December	20°C	1	active	20
27-29 December	20°C	3	much feeding	
27-29 December	20°C	10	much feeding	
27-29 December	20°C	20	2 eggs	
27-29 December	20°C	21	3 eggs	
27-29 December	20°C	22	9 eggs	
27-29 December	20°C	23	10 eggs	
27-29 December	20°C	24	20 eggs	
			some weevils still hiding	

them were not yet active on the 20th of April though they were reared during this period at 20°C. On the other hand it is possible to observe egg laying in rearing experiments as early as October, as is shown in table 8; it shows the effect of a temperature treatment on beetles that have entered diapause in July. Rearing for a longer time at temperatures of 20-25°C was not possible owing to the high mortality which ensued. Oöcyte growth remained slow at temperatures of 17° or 10°C, since during more than 3 months almost no development was observed.

TABLE 8. Effect of temperature on growth of oöcytes of *C. assimilis*.

Temperature	Age in days	With oöcytes	Number
20°C	80-100	2	41
17°C	80-100	1	56
10°C	80-100	1 (small oöcytes)	42

2.3.5. Histological study of the ovaries

In addition to the morphological study, the histological structure of the ovaries was examined. In this way we were able to observe early stages of ovarian activity which could not be seen by mere morphological investigation. Moreover, the activities of the different ovarian tissues could be studied separately.

Methods

Weevils killed in ethylacetate vapour were dissected in Levy's fluid. Their ovaries were transferred to Bouin's fluid (modification Dubosq Brasil) and after 1 day stored in alcohol 70%. Paraffine slides were made of 5 microns thickness. Prior to embedding in paraffine the ovaries were stained with 5% eosine during 5 minutes. This made it possible to find the embedded ovaries back in the paraffine. The sections were finally stained with haematoxiline and eosine.

Histology of the ovarioles

The ovarium of *C. assimilis* is of the telotrophic type. The main body of its ovarioles is formed by the germarium containing oöcytes and trophic elements and the vitellarium where the main oöcyte growth takes place. As in other insects the whole is suspended by a terminal filament to the dorsal part of the thorax. The ovarioles are covered by an endothelial sheath, the nuclei of which are visible as elongated dots along the margin of the ovarioles. Inside this sheath according to LENKOWA (1949) and SCHLOTTMAN and BONHAG (1956) an inner envelope is found. The nuclei of this envelope are only locally visible.

The germarium. The germarium forming the anterior part of the ovariole consists of primary oöcytes, trophocytes and prefollicular tissue. Oöcyte differentiation apparently is completed during the very early adult stage. A mitotic figure was observed in the germarium of a one day old female only.

The trophocytes are concentrated in the anterior part of the germarium. Their nuclei grow considerably during the maturation of the ovarioles, their diameter increasing from about $3\ \mu$ to $15\text{--}20\ \mu$ (see plate 1 and 4). This increase is most obvious in the posterior part of the trophic tissue. When counting the number of nuclei on a transverse line in an undeveloped ovariole we find about the same number of nuclei as in a germarium of an egg-producing ovariole. The mature ovariole is about twice as thick as the immature ovariole. The main cause of this is the growth of the trophocyte nuclei.

The trophocytes are separated from the vitellarium by the prefollicular tissue and by oöcytes of various size but before the stage of descending. In one case nutritive cords were noticed in the lower part of the germarium (plate 2). It concerned a weevil kept for 4 months after the prediapause period at 10°C , which was afterwards transferred to 20°C for 18 days, when it was dissected. The oöcytes in this weevil, as the others of this group, did not show any clearly visible growth.

The prefollicular tissue serves in the formation of the follicular epithelium, which surrounds the growing oöcytes. This means that considerable numbers of cells are needed for the building up of this epithelium so that one might expect a rather intense mitotic activity in this tissue. Such activity was observed by SCHLOTTMAN and BONHAG (1956) in *Tenebrio molitor*. No mitotic figures were found in the prefollicular tissue of *C. assimilis*. This means that probably the prefollicular cells are already present in full number at an early stage.

The vitellarium. The vitellarium lies at the posterior part of the ovariole between the germarium and the egg-chamber. Its tissue consists of follicular epithelium and oöcytes. At first the follicular epithelium can be seen as minute cells gathering around the young oöcytes. Later these cells expand enormously when yolk formation has started (plate 5).

In mature ovarioles the number of follicles varies from 6 to 10. The end of the vitellarium is formed by an epithelial plug separating it from the egg-chamber (plate 3). In diapausing females we can only find oöcytes prior to the stage of yolk formation. After the termination of diapause we did find ovarioles containing growing oöcytes, accumulating yolk and proceeding their growth till the egg stage. Ovisorption was not found.

BONNEMAISON (1957) noticed ovisorption in post diapause weevils as a result of a preceding cold period in spring. This suggests that ovisorption occurs in this weevil if environmental conditions are unfavourable. He did not mention any diapause behaviour in these weevils.

DE WILDE, DUINTJER and MOOK (1959) found ovisorption in the Colorado beetle in the diapausing fraction of beetles reared under long day conditions at 25°C. These conditions prevent diapause in about 70% of this beetle. They also noticed ovisorption in the ovaries of beetles transferred from 'long day' to 'short day' conditions.

The physiological explanation valid in both cases is probably the induction of 'pseudo allatectomy' (JOHANSSON 1958, DE WILDE and DE BOER 1961). In *C. assimilis* we have a different situation as compared with the Colorado beetle. All weevils enter diapause under all conditions tested, and the weevils are not sensitive to photoperiod.

2.4. SEASONAL SYNCHRONISM BETWEEN THE PHYSIOLOGICAL STATE OF THE HOST PLANT AND THE LIFE CYCLE OF *C. assimilis*

The main host plants of *C. assimilis* are several *Brassica* spp. (e.g. *B. oleracea* L., *B. napus* L., *B. rapa* L.). All these host plants have their main flowering period in May and June. After these months the number of pods in the field diminishes rapidly. This flowering period is timed in two different ways.

Firstly, the seed may germinate in summer, but the plants do not flower in the same year as they need chilling before entering the vegetative phase. STOKES and VERKERK (1951) working with Brussels sprouts found flowering only if a cold treatment of 6-9 weeks was given to plants that were grown for at least 11 weeks in a warm greenhouse. The percentage of flowering plants increased when the cold treatment was given when the plants were in a more advanced stage, it became 100% with plants grown for 20 weeks in the greenhouse before chilling. This means that these plants will flower once a year only. As seeds may germinate just after maturation young plants will start growing at the beginning of August. They enter the sensitive phase for cold treatment in the second half of October and enter the generative phase in the course of December. This is more or less in agreement with the annual cycle of *C. assi-*

millis. As was shown in table 7 a number of the weevils collected in hibernation sites during December produce eggs when transferred to a higher temperature and fed. Those collected earlier do not. The diapause stage of the weevil and the vegetative phase of the plant are terminated at about the same time.

Secondly the plants need a long photoperiod for flowering. This case does not occur frequently in Brassicas but it is found e.g. in summer varieties of rape seed. Under our conditions these plants do not survive in winter. They must be sown in spring and then produce flowers only somewhat later than the winter varieties of rape seed.

To adapt itself to this annual cycle of the plant *C. assimilis* must produce eggs during spring only. This is assured by the univoltine cycle. When the new generation of *C. assimilis* emerges (usually in July) the quantity of pods is already low and continues to diminish throughout August. Such a situation is most unfavourable for the multiplication of the weevil. Being univoltine with an early break of diapause has therefore a high survival value for *C. assimilis*.

3. ANNUAL CYCLE OF *CEUTHORRHYNCHUS PLEUROSTIGMA* MARSHAM

3.1. LITERATURE

C. pleurostigma, a pest of several cruciferous plants, according to Junk-Schenkling (1930) occurs throughout Europe, in Siberia, and in Algeria. The study of ISAAC (1923) has revealed the occurrence of two races in this insect, one laying its eggs in the autumn and one ovipositing in spring. The spring race has as its main host plant *Sinapis arvensis* L. and, locally, cabbage species.

The two races were also observed by KAUFMANN (1923) in Germany, who noticed the occurrence of two distinct phenological forms, one ovipositing in autumn and the other in spring. As he sometimes found weevils during winter he thought it possible that hybridization would occur in some cases, giving rise to intermediate forms.

SCHEIDING (1956) confirmed ISAAC's findings but considered it likely that the time of year in which the eggs are laid determines the number of generations per year. In her opinion some early-laid eggs could give a second generation. This second generation was indeed produced in the laboratory. It was not established in the field.

VAN 'T SANT et al. (1961) observed both races in the Netherlands. The spring race was found only locally on cabbage, the autumn race occurred in many parts of the country. He studied the phenology of the weevil (1955, 1956, 1957, 1958) in the Netherlands. According to his observations the spring race emerges from the hibernation sites in the second half of May. The adults of the new generation appear after the middle of August. They do not lay eggs and disappear in their hibernation quarters under dead leaves and in grass. In the phenologically early year 1955 the weevils emerged from the hibernation quarters already in the second half of April and the adults of the new generation in the second half of July. The adults of the autumn or summer race appear according to VAN 'T SANT in the course of June. They oviposit in August and September. The ensuing generation hibernates in the larval stage.

The autumn race also occurs in Sweden (LAMPA 1894). The spring race is not mentioned. DE PIETRI-TONELLI (1950) noticed the autumn race in Italy. The spring race was not observed there either. DANON (1953) found both races in some localities of Yugoslavia (Varazdin) and in others (Zagreb) only the autumn race.

It seems that the autumn race has been noticed more generally than the spring race. This may be due to the economic importance of the autumn race. The spring race usually occurs on *Sinapis arvensis* and many authors have perhaps overlooked its presence. None of the investigators could observe any morphological differences between the two races. SCHEIDING (1956) was able to cross the two races but gave no details on the behaviour of the offspring and its fertility. The life history of both races is similar. Eggs are laid in the

root collar region and on the roots inside the plant tissue. According to DEUBERT (1955) the female produces some teratogenic substance, which enters the plant with the egg. The larva lives inside the ensuing gall, when full-grown it pupates in an earthen cell in the soil. After emergence the weevils feed on the leaves.

3.2. FIELD OBSERVATIONS

The phenology of *C. pleurostigma* can be followed to a large extent by checking the emergence holes in the galls and observing the formation of new tumors. The adults are rather inconspicuous and may be easily overlooked. They often crawl on the soil and can be found near the stembase during the oviposition period. Weevils in their prediapause phase are more often found on the leaves of the plant. They can be trapped in yellow Moericke traps but the numbers we observed were always rather low, except in one case in the north of the province of Groningen, where large numbers were caught.

The first galls of the autumn race were found by the end of September. The first emergence holes occurred at the end of November though the majority of larvae remained in the galls and did not pupate till next spring. Soil temperatures are usually too low to allow of any activity up till the end of March. The weevils emerged from the pupae during June. After a short feeding period the weevils entered diapause.

In one series of observations weevils emerged between the 20th and the 24th of June and stopped feeding to a large extent by the beginning of July. Their activity remained low up till the middle of August. Small oöcytes were observed in the ovaries on 13th August.

In the Netherlands the spring race mainly lives on *Sinapis arvensis*. It was found in rather numerous quantities on this weed near Wageningen, in the Rhine Delta and in the extreme south of the Netherlands. As VAN 'T SANT et al. (1961) remark it is of rather common occurrence on cabbage in a locality near Kampen (IJssel Delta).

Galls of the spring race were found throughout June. At the end of this month the first emergence holes appear but up to the end of August some plants with galls and larvae may be found inside. They enter diapause and hide under leaves and shrubs until next spring.

According to VAN 'T SANT et al. (1961) effective control of the weevil can be obtained by treatment with aldrin, chlordane, and parathion. These treatments are also effective against the cabbage root fly (*Chortophila brassicae* Bché). Their frequent application in spring against this fly has been the cause of a strong decline in the infestation by the spring race of *C. pleurostigma* on cabbage in the Netherlands.

It is clear that the two races have a different annual cycle resulting in a chronological separation of the adults. The exact nature of the responses underlying these differences can be studied only in laboratory experiments under controlled conditions.

3.3. LABORATORY OBSERVATIONS

3.3.1. Laboratory observations on the spring race

A. Methods

Galls of *C. pleurostigma* from *Sinapis arvensis* near Wageningen or from cabbage plants grown in a region near Kampen (IJssel Delta) were collected in the field. The galls were transferred to glass jars partly filled with soil and kept at room temperature. The weevils, emerging about three weeks later, were collected daily. They were reared in glass jars partly filled with cellulose wool and received rapeseed leaves as food. This material is readily accepted and available throughout the year except during severe winters. The observations included a study of behaviour, food consumption and ovarian development. The appearance of diapause behaviour can be followed by counting the number of active and hiding weevils. Ovarian development was studied by dissection as described in 2.3.2.e.

B. Influence of some photoperiods during the adult stage

Weevils were reared at photoperiods of 13 and 20 hours at 17°C. In each experiment 50–60 weevils were used.

Effect on behaviour. The results of the counts are given in figure 4. They clearly show that there is no difference between the two treatments. Hiding is more complete when compared with the results from the observations with *C. assimilis*. Nine days after emergence almost all weevils are hiding. A small number of weevils (8) was reared at 5 hours photoperiod and 17°C but they gave the same response. Also weevils reared at 13 hours and 21°C were soon hiding (7 days after emerging fig. 5). The latter remained inactive during about 75 days. Then they became active again and were feeding intensively.

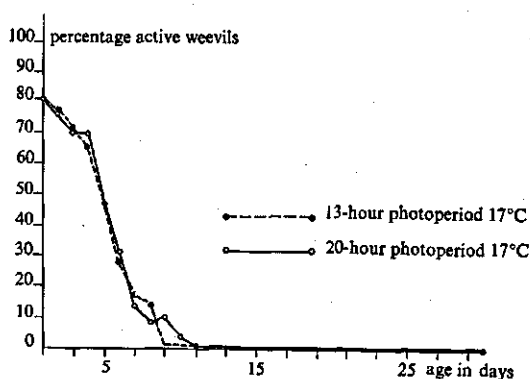


FIG. 4. Percentage active weevils (spring race *C. pleurostigma*) during one month, after emergence from the pupae when reared under photoperiods of 20 and 13 hours at 17°C.

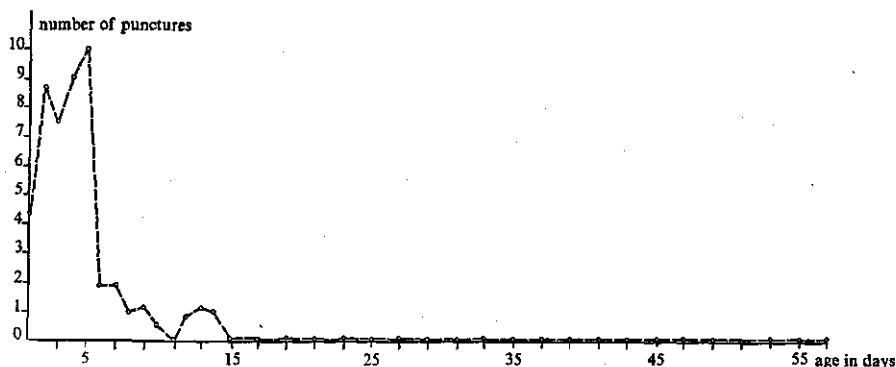


FIG. 5. Number of punctures made by adult *C. pleurostigma* (spring race) per weevil per day, when reared under a 13-hour photoperiod and 21 °C.

Development of the ovaries. The ovaries of the weevils reared at 5, 13 and 20 hours photoperiod and 17 °C were examined after 30 days. In none of them any signs of oöcyte growth could be observed. As was found with *C. assimilis* only small oöcytes are present but their growth seems to be slow and none of them were in the stage of yolk formation.

3.3.2. *Laboratory observations on the annual cycle of the autumn or summer race*

A. Methods

The rearing methods were largely the same as with the spring race. However this race can be more easily obtained, since the galls of this weevil are present throughout the winter. The first galls can be collected by the end of November. These galls produce under room conditions (about 20 °C) weevils in the second half of December. Galls on kale were chosen as being the best available source. The only problem in rearing the weevils from these galls is the high percentage of parasitism by *Diospilus oleraceus* Hal.

As with *C. assimilis*, photo- and geotaxis, hiding behaviour, feeding activity and the development of the ovaries under the conditions of several photoperiods and temperatures during the adult stage were studied.

Photo- and geotaxis were investigated with the apparatus described in 2.3.1. Feeding activity can be registered by daily counting the number of punctures made by the weevils. As ISAAC (1923) describes the weevils bite a hole into the leaf and scoop out the inner succulent tissues, leaving the outerwall of the lower and upper epidermis intact. These cavities have a diameter of approximately 0.5 mm. Their maximum size is determined by the length of the rostrum of the weevil. The errors in this estimation of feeding activity are firstly, possible differences in thickness and food quality of the leaves and secondly difficulties in counting the number of punctures when the cavities coalesce. Photoperiods were obtained as described for *C. assimilis* in 2.3.1.

B. Influence of photoperiod and temperature during the adult stage on the annual cycle

The effect of photoperiods of 0, 5, 10, 12, 13, 14, 16, 16½, 18 en 24 hours at temperatures of usually 17 and 21°C during the adult stage was investigated. The effect of temperature was more extensively studied at the photoperiods of 13, 16 and 20 hours.

a. The effect on phototaxis. The phototactic behaviour was compared between weevils reared at a long photoperiod (18 hours) and a high temperature (25–30°C) and weevils reared at a short photoperiod and a lower temperature (13 hours 20–25°C). For each observation 15 weevils were used. They were tested in groups of 5 weevils. The results are given in figure 6. They show no difference in reaction. We observed in both groups a decrease in the percentage of positive choice after one or two weeks. This decrease takes place somewhat earlier in the weevils reared under long day and higher temperatures than in those reared under short photoperiod and lower temperatures. This period with a rather negative phototaxis lasts for about 2–3 weeks. At the end of this period the phototactic response of both groups is again at the same level as at the beginning. No significant differences in this phototactic response could be established between the two groups ($D = 0.4 \pm 2.1$).

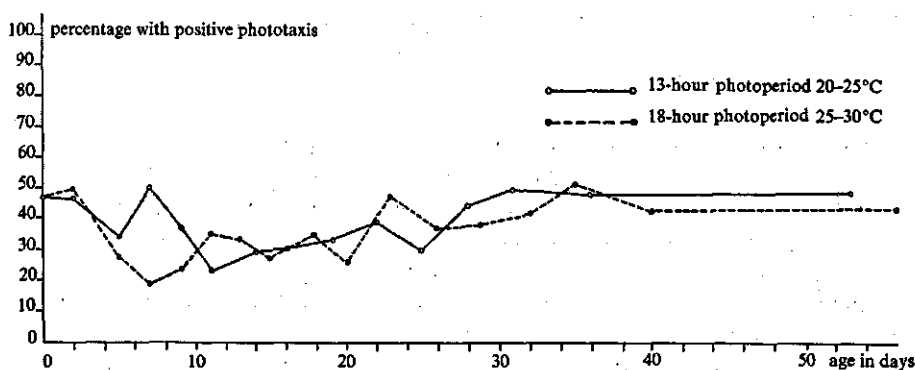


FIG. 6. Phototactic response of *C. pleurostigma* (autumn race) when reared under an 18-hour photoperiod and 25–30°C, and a 13-hour photoperiod and 20–25°C, respectively.

As may be expected phototaxis is never strongly positive. The normal place where these weevils occur is near the soil or on the underside of the leaves. The ovipositing weevils also did not make more than 50% of positive choices. The period with a more pronounced negative phototaxis coincides more or less with the later (3.3.2. B.c.) described period of decline in feeding activity. It can be noted that the rise in positive phototactic response also occurs with weevils reared at the long photoperiod (later described as diapause inducing). We can conclude that the phototactic response cannot be used as an indicator of diapause behaviour in this species.

b. The effect on geotaxis. With the same weevils used in the experiment on phototaxis described above, the geotactic response was studied. The apparatus described in chapter 2.3.1 was used. In all experiments the slope of the bottom of the tray was 45° . The results of these experiments expressed as the percentage of negative choices are given in fig. 7. It seems that geotaxis is somewhat more negative with weevils reared at a short photoperiod than with those reared at a long photoperiod ($D = 5.6 \pm 2.0$). In most observations geotaxis is neither strongly positive nor negative.

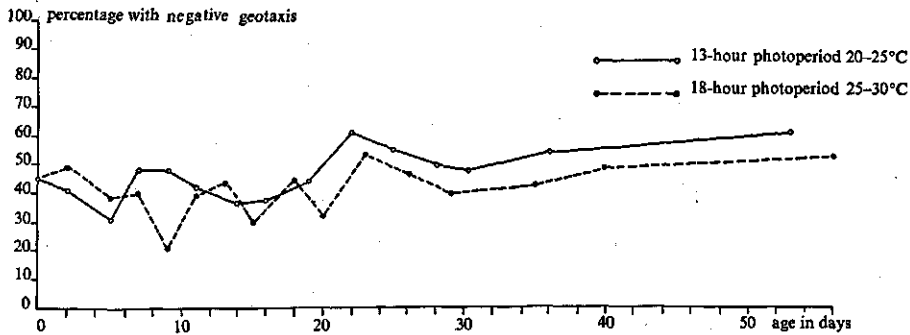
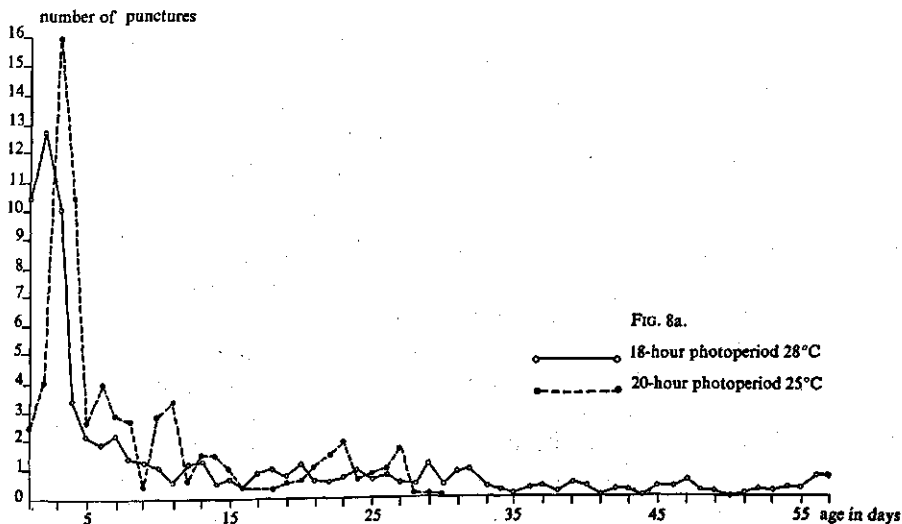
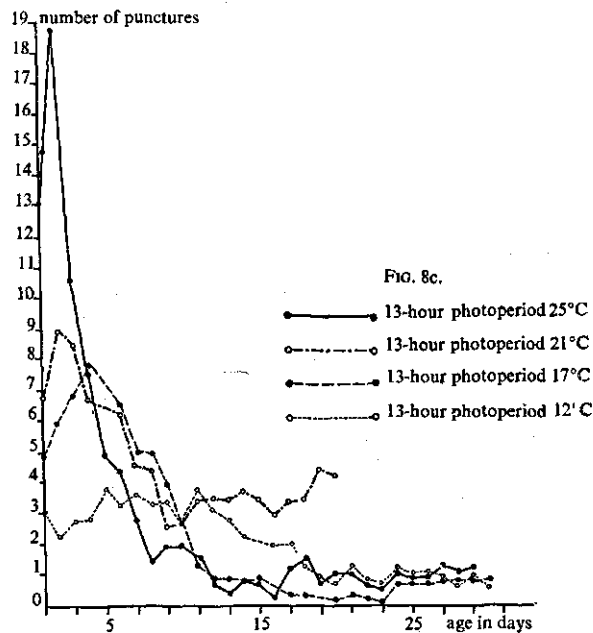
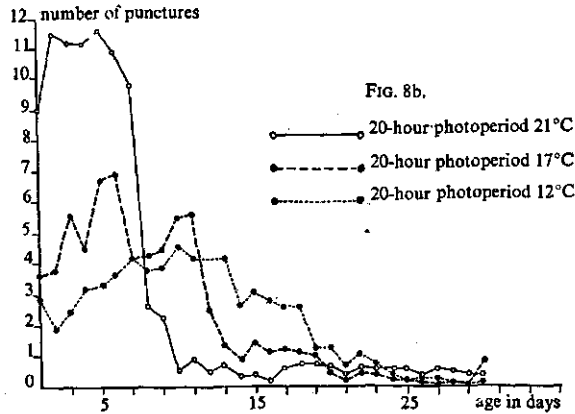


FIG. 7. Geotactic response of *C. pleurostigma* (autumn race) when reared at an 18-hour photoperiod and 25-30°C, and a 13-hour photoperiod and 20-25°C, respectively.

c. The effect on feeding activity. Feeding activity was evaluated by daily counts of the number of punctures made in the leaves under the conditions mentioned in the figures 8a, b and c. As with *C. assimilis* and the spring race of *C. pleurostigma*, a period of intensive feeding followed

FIGS. 8a-c. Number of punctures made by adult *C. pleurostigma* (autumn race) per weevil per day when reared under photoperiods of 20 hours.





by a steep decline to a rather low level is apparent. Contrary to the behaviour of the spring race feeding activity is not completely arrested but continues on a very low level with the insects reared at a photoperiod of 20 hours. With a photoperiod of 13 hours we also observed a decrease, but the rate of puncturing remained higher. With the weevils reared at 21°C the decline was much smaller. This shows that the activity continues in weevils reared at a photoperiod of 13 hours and not with those reared with a photoperiod of 20 hours.

Temperature also influences feeding behaviour. The period with intensive feeding clearly depends on the temperature as is shown in table 9. The period

with a high food consumption increased strongly with decreasing temperature. It seems to last somewhat longer when a long photoperiod is applied. The quantity of food eaten per day rises with temperature as is shown in table 10. At the photoperiod of 13 hours the correlation between the two variables is highly positive ($r = 0.99$, $t = 9.26$, at 2 degrees of freedom).

TABLE 9. Number of days after emergence of *C. pleurostigma* (autumn race) from the pupa with intensive food intake (more than 2 punctures per day per weevil).

Temperature	Photoperiod	
	13 hours	20 hours
28°C	-	6 (18 hours)
25°C	7	10
21°C	+	9
17°C	10	12
12°C	17	18

+) Food intake did not fall below the two-hole level.

TABLE 10. Number of punctures made by (*C. pleurostigma* autumn race), per day during the period of intensive feeding after emerging from the pupa.

Temperature	Photoperiod	
	13 hours	20 hours
28°C	-	7.1 (18 hours)
25°C	8.9	5.1
21°C	6.1	8.9
17°C	5.5	4.8
12°C	2.9	3.3

At the long photoperiod the relation is more obscure ($r = +0.59$, $t = 1.26$, at 3 degrees of freedom, which is insignificant). One can also study the total food consumption as is shown in table 11. This shows a positive correlation between total food intake and temperature ($r = 0.95$, $t = 43.3$, at 2 degrees of freedom, which is highly significant) for the short photoperiod, but an insignificant negative correlation ($r = 0.41$, $t = 0.78$, at 3 degrees of freedom) for the long photoperiod.

TABLE 11. Total food consumption expressed in the number of punctures made by *C. pleurostigma* (autumn race) during the period of intensive feeding.

Temperature	Photoperiod	
	13 hours	20 hours
28°C	-	42.8 (18 hours)
25°C	62.0	51.1
21°C	54.8	79.8
17°C	55.3	57.6
12°C	48.0	59.9

On evaluating these data one must realize, that both the rate of pre-diapause development and the rate of feeding are a function of temperature. The increase in the rate of feeding with temperature may be considered as a normal reaction, as activity increases with temperature and the rate of food intake also rises. Total food consumption, however, is a function of feeding rate and time. In the short day weevils feeding is continued and therefore only the rate is limiting. In long day beetles, however, the duration of pre-diapause sets the limit of food intake. This duration is shortened with higher temperatures and as a consequence total food intake is not increased.

d. *Effect on hiding behaviour.* In some experiments the number of insects found on the plant was compared with the number hiding in the cellulose wool on the bottom of the rearing jars. This number is very high with newly emerged weevils but it decreases rapidly. In rearing experiments only 3% of the insects was found on the plant at 13 hours and 17°C after 10 days. Then the number increased gradually, to reach about 33% (in 206 insects) after 50 days. When we make daily counts of the insects the number tends to be higher, as is shown in figure 12. In this graph the activity of weevils reared at 20 hours photoperiod and at a temperature of 17°C is compared with that of weevils transferred from these conditions to a short photoperiod (13 hours 17°C). The weevils continuously reared during 53 days at a long photoperiod show a rather low activity after the initial activity period. Weevils after 15 days transferred from a long day to a short day at first showed a lower activity than those kept constantly under long day conditions but their activity increased gradually and was higher about two weeks later (figure 12). In the second experiment weevils were transferred to a short photoperiod after 40 days under long day conditions. In this case a similar behaviour was noted. The transferred weevils began to show a lower activity (expressed as percentage non-hiding weevils) which increased rather rapidly after some days. These weevils then had well-growing oöcytes in their ovarioles. Owing to circumstances this experiment had to be stopped prematurely.

Summarizing the results of our observations on the behaviour of *C. pleurostigma* we can say that feeding activity and to some extent also hiding behaviour provided us with the clearest indications concerning the activity of the insects. The higher food consumption of insects, reared under short day conditions as compared with those reared under long day conditions coincided with a smaller number of hiding weevils. The phototactic and geotactic response showed no or only minor differences, respectively, between the insects reared at a long or at a short photoperiod. Obviously a long day induces a diapause behaviour in the autumn race of *C. pleurostigma* while a short day has the opposite effect.

e. *Effect on oöcyte growth.* The influence of photoperiod and temperature on *C. pleurostigma* can also be followed by investigating the development of their ovaries, the same considerations as for *C. assimilis* being valid (see 2.3.2.e).

We may distinguish 3 stages in development:

1. Ovarioles without visible, growing oöcytes (figure 9).
2. Ovarioles with clearly visible oöcytes in the vitellarium.
3. Ovarioles with eggs in the egg chamber and oöcytes in the vitellarium (figure 10).

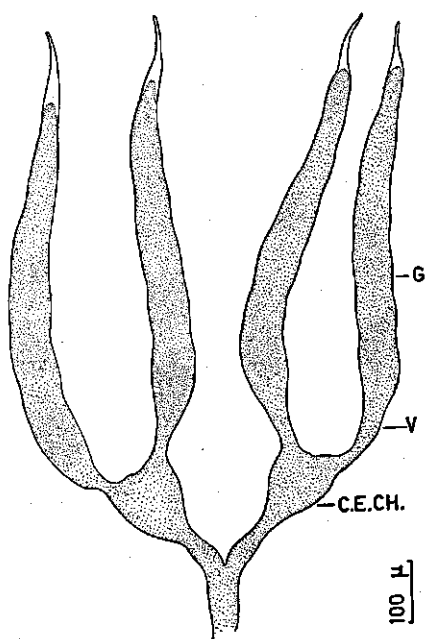


FIG. 9. Ovary of diapausing *C. pleurostigma* (autumn race).

G. Germarium V. Vitellarium
C.E.Ch. Common egg chamber

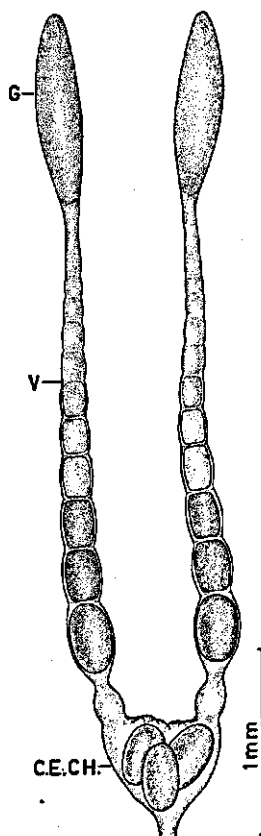


FIG. 10. Part of the ovary of ovipositing *C. pleurostigma* (autumn race). G. Germarium V. Vitellarium C.E.Ch. Common egg chamber

In later experiments the first stage was split up into two classes viz. those without visible oöcytes and those with very small oöcytes only. The results presented in the tables 12 and 13 show that photoperiods shorter than $16\frac{1}{2}$ hours are more or less favourable for follicular activity resulting in visible oöcytes. Longer photoperiods induce an arrest of activity. Photoperiods between 10 and 14 hours are optimal. Shorter photoperiods obviously are also less favourable for oöcyte growth.

Photoperiodic response curves have been constructed for several species of insects (e.g. LEES 1955). In most cases they show an opposite reaction as compared with our weevils, most insects sensitive to photoperiod being long day insects. They also show a change in response to daylength from diapause

TABLE 12. Influence of photoperiod and temperature on the development of the ovaries of *C. pleurostigma* (autumn race). Collected near Wageningen (51° 57' Northern Latitude.)

Photoperiod and temperature in degrees Centigrade	Age of the weevils in days	Total	Number of ♀♀		
			with eggs	with growing oöcytes	without development
0 hrs ²⁾ roomtemp. ¹⁾	24	22	7	11	4
5 " 17°	24	100	6	22	72
5 " 17°	36	10	9	1	0
10 " 17°	24	8	5	2	1
10 " 22°	24	12	5	6	1
13 " 12°	90	43	5	3	35
13 " 17°	24	40	4	12	24
13 " 17°	26	12	2	5	5
13 " 17°	30	37	6	7	24
13 " 17°	33	10	1	3	6
13 " 17°	65	3	3	0	0
13 " 17°	71	5	5	0	0
13 " 21°	16-20	24	15	7	2
13 " 22°	24	16	13	2	1
13 " 24°	42-45	14	1	4	9
13 " 24°	47-54	33	3	7	23
13 " 25°	30	36	13	13	10
15 " 21°	24	28	2	9	17
16 " 17°	24	12	1	0	11
16 " 22°	24	8	1	4	3
16½ " 21°	34	16	0	4	12
18 " room ¹⁾	24	44	0	1	43
18 " "	60	15	0	0	15
18 " "	62	1	0	0	1
18 " "	63	5	0	1	4
18 " "	75	7	0	2	5
18 " "	77	2	0	0	2
18 " "	79	4	0	0	4
18 " "	94	6	0	1	5
18 " "	115	10	4	1	5
20 " 17°	24	28	0	0	28
20 " 22°	24	41	0	0	41
20 " 25°	32	8	0	0	8
24 " room ¹⁾	24	26	0	0	26

¹⁾ The room temperature mainly varied between 20 and 22°C.

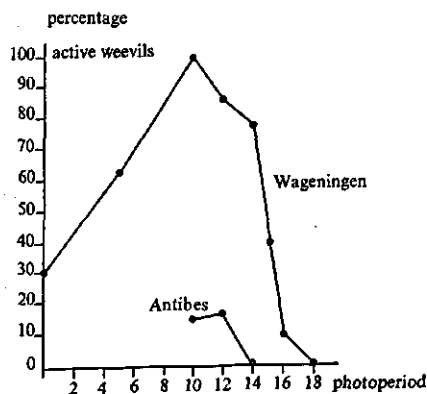
²⁾ Photoperiod approximately 5 minutes, this period being necessary for changing the food of the insects.

to non-diapause in a rather narrow range of photoperiods, comprising the intermediate or critical photoperiod. In *C. pleurostigma* this critical photoperiod is found between 14 and 16 hours (figure 11).

TABLE 13. Effect of photoperiod on oöcyte growth in *C. pleurostigma* (autumn race) at 21°C after 24 days. Weevils collected near Wageningen.

Photoperiod	Percentage of ♀♀			
	with eggs	with clearly visible oöcytes	with very small oöcytes	without visible development
0 hours (\pm 5 min.)	2	28	45	25
5 hours	8	55	17	20
10 hours	60	40	0	0
12 hours	15	70	0	13
14 hours	13	64	0	22
16 hours	0	10	62	28

FIG. 11. The influence of photoperiod at 21°C on oögenesis in two groups of *C. pleurostigma* (autumn race), respectively, collected near Wageningen 51°57' N.L. and Antibes 43°35' N.L.



The figures in table 12 also show that weevils can produce eggs provided that they are kept at a long photoperiod for a sufficiently long time. Eggs were found in 3–4 months old weevils reared at 18 hours (20–22°C) and in 95 days old weevils kept at 20 hours (17°C).

Temperature is also of great importance in photoperiodic response. With a photoperiod of 13 hours and a temperature of 21°C the first eggs were deposited 16 days after the emergence of the adult weevil from the pupa. At 24°C already a rather high number of weevils showed no visible oöcyte growth. When we studied the effect of temperature at a photoperiod of 16 hours we noticed (table 14) the highest number of weevils with some development at 21°C; however, indications were found that development advanced somewhat further in a small number of weevils at 17°C. At 25, 29 and 12°C the development of the ovarioles was clearly inferior.

The observations show that *C. pleurostigma* is a short day insect with a critical photoperiod between 14 and 16 hours and an optimal temperature for reproduction of about 21°C.

TABLE 14. Effect of temperature on oöcyte growth in *C. pleurostigma* (autumn race) (photoperiod 16 hours). Weevils 24 days old.

Temperature	Number of ♀♀			
	with eggs	with clearly visible oöcytes	with very small oöcytes	without visible development
29°C	0	0	0	49
25°C	0	0	5	31
21°C	0	0	29	13
17°C	1	2	11	25
12°C	0	0	4	36

C. The effect of food on oöcyte growth

During the rearing experiments it was observed that food grown in the greenhouse was less suitable for the weevils reared for egg production, since oöcyte growth with this type of food was generally negligible. One might also expect an effect of the age of the leaves on the development of the ovaries. Such effects are frequently found in other Arthropods. For instance, LEES (1953) observed in *Metatetranychus ulmi* Koch. a high percentage of winter females when the mites lived on senescent apple leaves, even if photoperiod and temperature were favourable for preventing diapause.

ADKISSON (1961) showed that in the pink bollworm *Pectinophora gossypiella* (Saunders) the highest percentage of diapause occurred with diets containing the highest oil content, although this effect was only present with short photoperiods. The quality of food also influences egg production in many insects. ANKERSMIT (1952) found for the soya bean leafbeetle (*Phaedonia inclusa* [Stål]) egg productions of up to 877 eggs when fed on young soya bean leaves, as compared with a maximum of 325 when no particular attention was paid to the age of leaves given to the beetles, except that they were in a good condition. A similar difference was observed by GRISON (1952), in the Colorado beetle *Leptinotarsa decemlineata* Say.

Experiments were also started to obtain some data on the effect of leaf age in the nutrition of the beetle with respect to oöcyte growth. The first experiment was carried out with leaves collected in the field. The weevils were reared under room conditions in March at a photoperiod of approximately 12-13 hours, and a temperature of approx. 20°C. The results are given in table 15. This experiment indicates that young leaves give no better oöcyte growth than old leaves. The experiment was repeated with young and old leaves from greenhouse plants, the photoperiod corresponding with natural daylength in December and January (approx. 8-9 hours) and a room temperature of approx. 20°C. The results are shown in table 15. These figures indicate that oögenesis was very much retarded with this type of food. The young leaves seemed to give a slightly better result.

From the foregoing experiments we may conclude that there is no important difference in the effect of old or young leaves with regard to oöcyte growth and that plants raised in a greenhouse are less suitable for a food.

TABLE 15. Growth of oöcytes in *C. pleurostigma* (autumn race) reared with old and young leaves, 24 days after emergence from the pupae.

	Old leaves			Young leaves		
	Number of weevils			Number of weevils		
	with eggs	with growing oöcytes	without visible oöcytes	with eggs	with growing oöcytes	without visible oöcytes
Leaves taken from the field	10	4	0	6	7	3
Leaves taken from a green house crop	0	4	32	3	1	19

D. The effect of a change from short to long photoperiod during the adult stage on oöcyte growth

Later (3.3.2.G) it will be shown that a decrease in photoperiod in summer induces the termination of diapause in the weevil. Now it was considered interesting to see whether or not an increase in the photoperiod from 13 hours to 20 hours would affect oöcyte growth. For this purpose weevils were reared during 5, 9 and 13 days at 13 hours light and 17°C and then transferred to 20 hours light and 17°C. Their ovaries were investigated when they were 24 days old. The result is given in table 16. This experiment indicates an increase in oöcyte growth in proportion to the length of their stay at a short photoperiod (5 days – 0%; 9 days – 23%; 13 days – 45%; 24 days – 40%). The effect of short day is not stopped by the transfer to long day conditions, as about the same percentage of weevils with eggs or growing oöcytes was found in weevils reared continuously at 13 hours 17°C and those transferred to long day conditions during 11 days. When the change in daylength occurred earlier the percentage of weevils with oöcyte growth diminished and no oöcyte growth was visible in weevils transferred after 5 days from a short photoperiod to a long one.

TABLE 16. Effect of increase in photoperiod on oöcyte growth in *C. pleurostigma* (autumn race).

Rearing conditions (temp. 17°C)	Number of ♀♀		
	with eggs	with clearly visible oöcytes	without visible development
5 days 13 hours	0	0	19
19 days 20 hours			
9 days 13 hours	1	4	17
15 days 20 hours			
13 days 13 hours	6	4	12
11 days 20 hours			
9 days 13 hours	0	0	16
24 days 13 hours	4	12	24

An explanation for these phenomena may be:

- a. The presence of a sensitive period in the life cycle of the weevil at the age of 5 to 13 days.
- b. A delayed effect of the induction of oöcyte growth by a short day treatment. This effect is stronger as the short day treatment lasts longer.

E. Photoperiodic responses in weevils from Antibes (43° 35' N.L., Southern France)

It is well known that geographic races of an insect species occurring at different latitudes may show different photoperiodic responses (DANILYEVSKY 1960). The response curve is well adapted to the local daylength and climatic conditions. Differences may especially be found in the position of the critical photoperiod. It was therefore thought interesting to study the response to photoperiod of a southern strain of *C. pleurostigma*.

Weevils obtained from Antibes (43° 35' N.L.) as mature larvae were reared and tested simultaneously with those of the experiment mentioned in table 13. The results of the investigation of the ovary development in the French weevils is given in table 17 and figure 11. They render likely a shift in response. At photoperiods of 10 and 12 hours some oöcyte growth can be observed but there is no growth at 14, 15 or 16 hours. The critical photoperiod lies between 12 and 14 hours. In the Dutch strain (collected at 51° 57' N.L.) the critical photoperiod is found between 14 and 16 hours.

TABLE 17. Effect of photoperiod at 21°C on *C. pleurostigma* (autumn race) obtained as larvae from Antibes.

Photoperiod	Number of ♀♀			
	with eggs	with clearly visible oöcytes	with small oöcytes	without visible development
10 hours	2	3	5	23
12 hours	2	2	4	15
14 hours	0	0	3	18
15 hours	0	0	0	20
16 hours	0	0	0	28

This means a shift of the critical photoperiod of at least two hours. DE WILDE (1962) mentioned a decrease in critical photoperiod of 1 hour per 3° latitude as generally found in the response of geographic races. As in our case the latitudes from which the weevils were obtained differed 8° 22', a shift of somewhat less than 3 hours should be expected. Our figures do not make it possible, however, to check this value more precisely, since no photoperiodic responses at 13 hours are known.

The second difference in photoperiodic response between the two geographic races is the low number of French weevils responding to daylength. This means that a high percentage of the population is univoltine with an obligate

diapause in summer. It would be interesting to test weevils obtained from even more southern latitudes. It may be possible that in such beetles the photoperiodic response disappears completely. In that case we could distinguish three races of *C. pleurostigma*, viz., one race ovipositing in spring without photoperiodic response (the spring race); one ovipositing in the autumn, with photoperiodic response (our autumn race), and one ovipositing in the autumn without photoperiodic response (a southern race). The phenological significance of these findings is that *C. pleurostigma* is probably univoltine in Southern France. Only a few field observations are known from this region. During a visit to Antibes by the end of October no full-grown larvae were found. Considering the temperatures in this area during October which are still high, — causing a rapid development of the larvae — oviposition does not take place long before the end of September. On the other hand only full-grown larvae were obtained from cabbage plants sent to Wageningen from Antibes on 15th March. This makes it likely that no new generation can emerge before the beginning of April, when the natural daylength has passed the critical photoperiod in weevils of this region. PIETRI TONELLI (1950) in Northern Italy, and IACOPINI (1957) in Toscane and SANTANIELLO (1938) in Southern Italy found only one generation per year.

F. Influence of the photoperiod during the larval stage on the occurrence of diapause in the adult

From the foregoing experiments we may conclude that photoperiod has a pronounced effect on the reproductive activity and feeding behaviour during the adult stage. In these experiments we always used insects collected as mature larvae in the field during autumn and winter. This means that larval development took place in the autumn under short day conditions (photoperiod decreasing from 14–8½ hours). It may be interesting to ascertain whether or not the photoperiodic response of the adults remained the same when their larval development took place under the long day conditions corresponding with the season in which the larvae of the spring race thrive (natural daylength increasing from 16 to 18 hours).

To this purpose weevils reared at 13 hours photoperiod were given cabbage plants in the beginning of May for egg deposition (natural daylength is then nearly 16 hours). The adults of the new generation emerged in July. They were tested for their response to photoperiod. The results are given in table 18. They

TABLE 18. Effect of photoperiod on *C. pleurostigma* (autumn race), reared from May till August.

Photoperiod and temperature	Number of ♀♀			Age of the weevils
	with eggs	with growing oöcytes	without visible development	
13 hours 17°C	5	2	2	30 days
20 hours 17°C	0	0	5	30 days

show that also under these conditions a normal response to short and long day treatments is found. The difference between the spring race and the autumn race should not be attributed to a difference in the conditions under which the larvae are living.

G. The termination of diapause

In the previous chapters it was shown that weevils grown under long day conditions show phenomena characteristic of an imaginal diapause. Their feeding activity diminishes, they hide near or in the soil while oöcyte growth is arrested. This diapause may be called aestivation since it occurs in summer. It is broken before the onset of autumn and we may ask ourselves what kind of mechanism ensures the insects to appear in late summer.

The diapause in winter is thought to be terminated under the influence of a rather low temperature and in some cases temperature curves showing the optimal temperature for diapause development have been constructed. These optimal temperatures are generally found near thresholds of development in the active stage. The low temperatures do not occur in summer and, though a temperature-dependent aestivation-diapause development is likely, some other mechanism would be needed to ensure a timely appearance of the insects. Any delay would be a serious setback since they would then fail to be present in a reproductive stage during the still favourable months of September and October.

In order to find out whether or not photoperiod plays a role in the termination of aestivation in *C. pleurostigma*, diapausing weevils of known age and reared under long day conditions were transferred to short day. The results are given in table 19. They show that diapause was rapidly broken while oöcyte growth was started by short photoperiods. As is shown in figure 12 the activity of these weevils increased, too.

TABLE 19 Influence of a change in photoperiod on the oöcyte growth in *C. pleurostigma* (autumn race).

Number of days at		Number of ♀♀		
20 hours 17°C	13 hours 17° C	with eggs	with growing oöcytes	without visible oöcytes
15	24-28	8	7	0
40	0	0	1	11
41	9	0	8	2
50	0	0	2	8
95	0	4	8	0

The question now arises how a perception of the photoperiod by the diapausing weevils is possible. From our studies on feeding activity and hiding behaviour we know that diapause in *C. pleurostigma* does not mean a com-

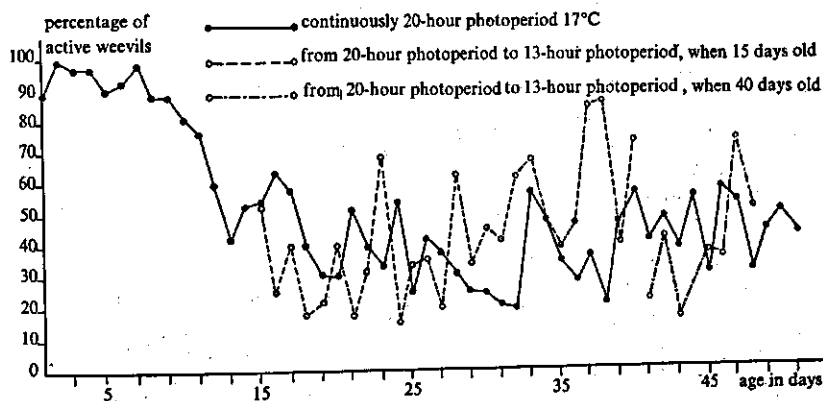


FIG. 12. Influence of a transfer of *C. pleurostigma* (autumn race) from a long photoperiod to a short one on the percentage of active weevils. The insects were examined daily.

plete cessation of all activities throughout the diapause period. We always notice a slight feeding activity. It is possible that a perception of the photoperiod occurs during these active moments. We can also imagine that sufficient light penetrates into the crevices in the soil and under the leaves where the weevils hide, to act as a 'token'.

H. Histological studies on the ovaries

The ovarioles of *C. pleurostigma* resemble those of *C. assimilis*. For the general description we refer to chapter 2.3.5. In the young ovariole the trophocyte nuclei are still small. As in *C. assimilis* the size of their nuclei increases as maturation proceeds. In the ovarioles of young, one week old weevils reared under a 10-hour photoperiod at 18°C, trophocyte nuclei do not measure more than 5–6 μ in diameter. In aestivating 24 day old weevils their size increases up to 10 μ (fig. 13), while they reach a diameter of 15–20 μ in egg producing females (fig. 14). In diapausing weevils the diameter of the ovarioles increases from almost 70 μ to about 120 μ at oviposition. No indications of an increase in number of trophocytes were found when comparing weevils 10 days after emergence with those at oviposition.

In ovarioles of 24 day old diapausing females young oöcytes can be found remaining very small (fig. 13) and never entering the descending stage. The number of follicles per ovariole in ovipositing weevils usually amounts to about 10. No ovisorption was found in diapausing or in active weevils.

The ovaries of the spring and the autumn race resemble each other closely. Only the size of the trophocyte nuclei in diapausing spring race weevils was often somewhat larger than in diapausing autumn race weevils (10–15 μ as compared with 10 μ). Oöcyte growth also proceeded a little further in some diapausing spring race weevils than in the diapausing autumn race weevils of about the same age.

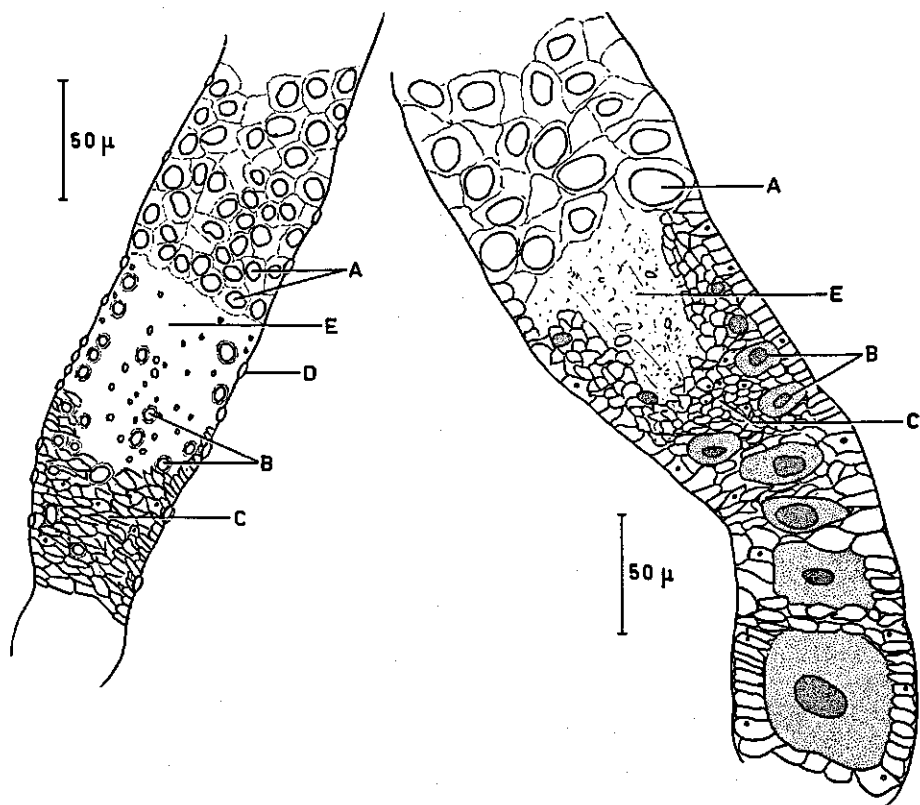


FIG. 13. Part of the germarium of a diapausing *C. pleurostigma* (autumn race). Reared during 24 days under a 24-hour photoperiod at 20°C.

A Trophocyte nuclei B primary oocyte C Prefollicular tissue D Nucleus endo-
thelial sheath. E Trophic core.

FIG. 14. Part of the germarium of *C. pleurostigma* (autumn race). Reared for 14 days under a 10-hour photoperiod at 17°C. Growth of oocytes started.

A Trophocyte nuclei B Primary oocytes C Prefollicular tissue E Trophic core.

I. Seasonal synchronism between the host plant and the annual cycle of *C. pleurostigma*

A rather remarkable feature of the insect is that in various locations within its areal it specialises on different food plants. VAN 'T SANT et al. (1961) observed that in the northern parts of the Netherlands (province of Friesland), *C. pleurostigma* occurs mainly on swedes and not or rarely on cabbage. Near Wageningen the insect is quite common on various cabbage varieties. In the latter locality it has been also found once on winterrape seed which is common host in other places. The spring race was not observed on cabbage near Wageningen; however, in this region it is found rather common on *Sinapis arvensis*. Near Kampen (IJssel Delta) the spring race is common on cabbage but not on *Sinapis arvensis*. Larvae of *C. pleurostigma* need young, well-growing

plants for their development. Gall formation proceeds most rapidly on these plants. If the growth of the galls is retarded by poor growing conditions, development of the larvae is also retarded, as was often observed in the rearings of *C. pleurostigma* after transplanting infested stock.

Cabbage has its origin in the Mediterranean countries. As already mentioned under 2.4, young, well-growing cabbage or winterrape plants are available in the autumn under natural conditions. Therefore this is the most favourable time for multiplication of the autumn race. In the Netherlands the spring race occurs, mainly on *Sinapis arvensis*. This plant has its juvenile stage in spring. So the spring race is well adapted to the cycle of its host plant. *S. arvensis* occurs in many parts of Europe, Northern Africa, South Western Asia and Siberia. It has been imported in North-America, South-Africa and New Zealand (HEGL, 1906). A second generation of the spring race on *Sinapis arvensis* would find mainly rather old plants which do not form an optimal substratum for the development of galls, so that a possible second generation would have to live on another host plant. However, in this insect, diapause soon ensues, lasting until November-December, when conditions for migration from a hibernation site to the food plants are unfavourable. The possibilities for a second generation of the spring race are therefore very small. Locally the spring race occurs on *Brassica* spp. These weevils apparently need not change their host plant although the diapause and climate largely prevent the occurrence of a second generation. It seems that we may consider the two races of *C. pleurostigma* as well adapted to the two different growth periods of their two host plants, the autumn race being adapted to host plants which have their vegetation period in the autumn, and the spring race to host plants which grow mainly in the spring.

3.4. HYBRIDIZATION OF THE TWO RACES

No morphological differences can be found between the two races of *C. pleurostigma* (personal communication from the Dutch coleopterist P. VAN DER WIEL, 1958). In other countries too, for instance in Germany (SCHEIDING, 1956) and England (ISAAC, 1923), the two races are accepted as one species. If this is true the two races must be crossable and the hybrids must be fertile. So far as could be found in the literature, the fertility of the hybrids has never been tested, though SCHEIDING (1956) mentions an experiment in which the races were crossed.

Females of the autumn race were isolated just after emerging from the pupa. They were crossed with males from the spring race and their offspring was reared on kale in cages. The emerging adults were reared at 13 hours and 21°C in plastic boxes, with a young rape plant as food and host plant for egg deposition. Food consumption was also followed. The observations showed that eggs were deposited 40–60 days after emergence. From these eggs a new generation was reared. This indicates that both races virtually belong to one species.

3.5. DISCUSSION AND CONCLUSION

From the foregoing observations we may conclude that the aestivation of the autumn race of *C. pleurostigma* is caused by the long photoperiods in summer. The termination of this diapause is induced by the shortening of the photoperiod below the critical level of about 16 hours during the summer. The aestivation is characterised by a decrease in feeding activity and a cessation of oöcyte growth.

The two races of *C. pleurostigma* differ in their response to photoperiod. The autumn race lays its eggs under conditions of short photoperiods, the spring race only after the termination of diapause. This race has an obligate diapause which cannot be prevented by rearing the weevils under short day conditions. A consequence of these two different annual cycles is a high degree of reproductive isolation between the two races. Both are in a reproductive phase of their life cycle in different periods of the year, the spring race during spring and the autumn race in the autumn. Meetings between insects of both races when one is not in a reproductive phase will not result in hybridization. Such meetings may occur in June and by the end of August.

Reproductive isolation is one of the characteristics of a species. Between two different races of a species this isolation is incomplete or of a recent date. In our case both possibilities have some probability.

Incomplete isolation may arise from meetings between spring race weevils with the autumn race in spring or by the end of August. In the first case we have hibernated autumn race weevils meeting the post-diapause spring race; in the second case post-diapause autumn race weevils may meet a few long-living spring race weevils emerged from hibernation in the previous spring. The chance that such meetings occur seems small but it should not be ruled out completely.

About the second possibility also we can only make some assumptions. It seems most likely that the two races were formed originally as geographic races. The autumn race should be considered as from Mediterranean origin, living on cabbage, and the spring race as adapted to more northern regions, living on *Sinapis arvensis*. With the introduction of cabbage in our region (probably some 2000 years ago) both races have come to be living in the same area. Their existence as races depends now on their reproductive isolation in time.

4. ANNUAL CYCLE OF *PSYLLIODES CHRYSOCEPHALA* L.

It was found in *C. pleurostigma* (autumn race) that the autumnal activity is a consequence of photoperiodic induction. Aestivation is induced by the long photoperiod in early summer and oöcyte development by the shorter photoperiod in August and September. It was considered useful to study another insect species with a similar annual cycle and to investigate whether this cycle is governed by the same factors.

4.1. LITERATURE

According to JUNK-SCHENKLING (1939-1940) *P. chrysocephala* occurs in Europe with South Scandinavia and Finland as its most northern regions. It furthermore occurs in the Caucasus, Asia Minor, Syria, and in Siberia. Extensive studies of this insect were made in France by BONNEMAISON and JOURDHEUIL (1954); in Germany by BÖRNER, BLUNCK, SPEYER and DAMPF (1921), KAUFMANN (1941a and b) and MEUCHE (1944). These studies have shown that the eggs are laid in the soil near the plants. The emerging larvae tunnel in the midrib of the leaves and in a later stage in the stem. They pupate in the soil. Two oviposition periods are generally noticed; one in the autumn and one in spring. According to BÖRNER et al. (1921) and BONNEMAISON and JOURDHEUIL (1954) these two oviposition periods are covered by one generation in which the egg-laying activity is split up into two periods separated by a winter. The latter authors gave experimental evidence that the beetles can survive the winter and start oviposition again in spring.

According to KAUFMANN (1940) two generations per year may occur in North Western Germany. However, he gives no extensive proof to support this statement. He only mentions that most larvae are full-grown by the end of November, that pupation takes place in early spring and that the new generation emerges in March and April. Eggs are laid in early spring. As pupal development takes about 30-35 days at 10°C (KAUFMANN 1941a) and the threshold of development for the pupa lies probably not below 5°C, any pupal growth during winter seems unlikely. The temperature of the upper soil in which *Psylliodes* pupates is generally below 5°C during winter in Western Europe. Even in France BONNEMAISON and JOURDHEUIL (1954) did not find two generations per year.

A third probability would be the existence of two phenological races. According to HORSFELD (1963) working in North Western Germany, some beetles do not oviposit in autumn but lay their eggs in spring. He gives no exact experimental evidence for this assumption.

VEENENBOS (1953) states that in the Netherlands egg laying takes place in autumn, but he does not mention egg deposition in spring.

According to KAUFMANN (1941b) the egg and the adult stage are best adapted to the severity of winter. He observed an extremely high mortality among the

larvae after cold winters. This means that in regions with a continental climate reproduction mainly occurs in spring. A cold winter will diminish the chances of survival of the larvae produced in autumn. The reverse is likely under maritime conditions. Probably therefore *P. chrysocephala* is only of minor importance in Russia.

The adults emerge in June (BONNEMAISON and JOURDHEUIL 1954) entering an aestivation diapause after some days of intensive feeding. They hide under litter and in humid places. The inception of diapause is attributed to climate factors as high temperatures and drought. With the onset of colder and more humid weather in late summer and early autumn the beetles again start their activity. This explanation is certainly not satisfactory for regions with a maritime climate, most summers being cool and moist. In the Netherlands the average temperature of June is 16.5°C and of September 15.5°C; humidity will be somewhat higher in September. BONNEMAISON and JOURDHEUIL also studied the effect of photoperiod on the adult stage but they concluded from their observations that this factor did not have any effect on the inception of diapause.

4.2. FIELD OBSERVATIONS

Only a few observations were made in the field. Large cone cages were placed at several spots in a rape field while the emerging beetles were counted. In 1960 the first insects appeared on June 3rd, the last on June 17th. The spring of 1960 was rather warm; in normal years the beetles may appear somewhat later.

Infested plants collected in the beginning of November and kept at 25°C produce beetles as early as 8th December. This is in accordance with the experiences with *C. pleurostigma* (autumn race). The phenology of both species differs only in minor respects.

4.3. LABORATORY OBSERVATIONS

4.3.1. Methods

Plants collected in the field, the first group in the beginning of November, were brought in a greenhouse and grown at 25°C. After about one month most of the larvae were full-grown and started pupating in the soil. The plants were then removed and cages were placed on this soil to collect the emerging beetles. They were collected daily and reared in tubes on rape leaves under conditions to be mentioned later. When food is being changed the beetles have to be anaesthetized with carbon dioxide in order to prevent them from escaping. Such escapes would probably influence the results to a large extent, since the most active beetles have the greatest capacity for jumping. This activity also made observations on photo- and geotaxis (by means of the available apparatus) rather difficult. Such observations were therefore omitted.

Food consumption can be easily studied since *Psylliodes* makes rather regular feeding holes in the leaves and the number of these holes can be counted daily. All feeding holes except the very small ones were counted.

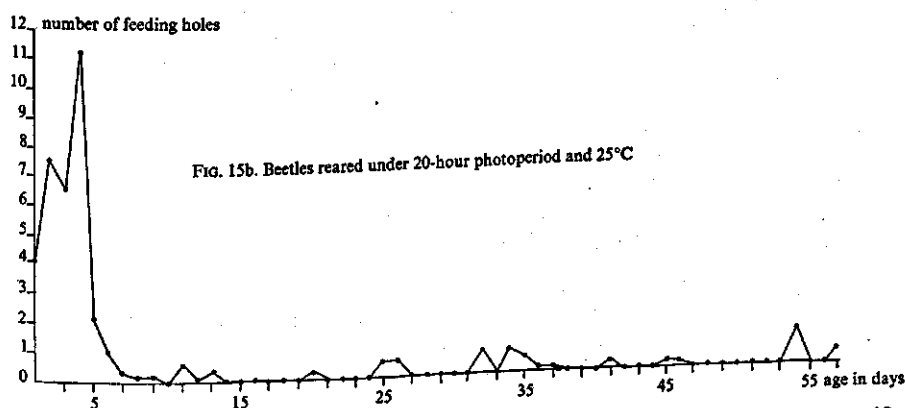
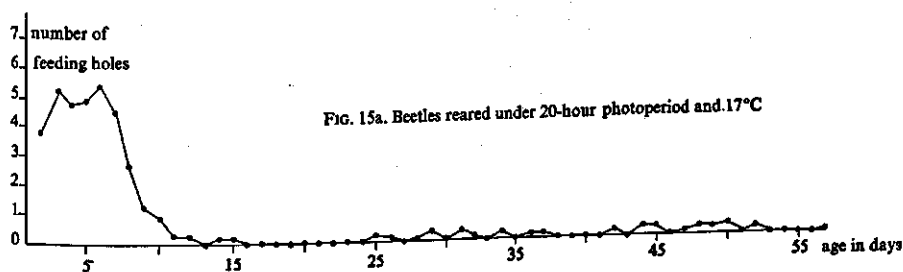
4.3.2. Effect of photoperiod and temperature on the annual cycle

For reasons mentioned in 4. the effect of photoperiod and temperature on the inception of diapause in *P. chrysocephala* was investigated. The activity of the beetles was followed by studying food consumption and oögenesis

A. Effect of photoperiod and temperature on feeding activity

Feeding activity during the adult stage is given in the figures 15 a-f. We notice first a period of high food consumption followed by a period of rest. During this period food intake does not normally rise above the level of 0.5 hole per beetle per day. A slightly higher activity can be established sometimes for a few days but then feeding diminishes again for several days. If a photoperiod of 13 hours is applied, food consumption starts again. With a photoperiod of 20 hours this increase is smaller and greatly retarded. These effects are to some extent influenced by temperature, as is shown in the tables 20 and 21. These figures show that the duration of the period of high feeding activity after emergence from the pupa decreases with increasing temperature. This effect is absent in beetles reared at 28°C, but here the decline in food consumption was slow during the last days of the prediapause period.

FIGS. 15a-f. Number of feeding holes made by adult *P. chrysocephala* per beetle per day.



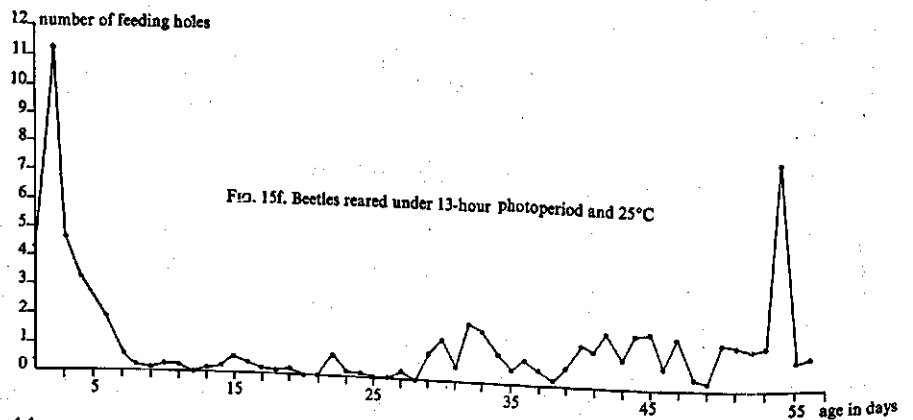
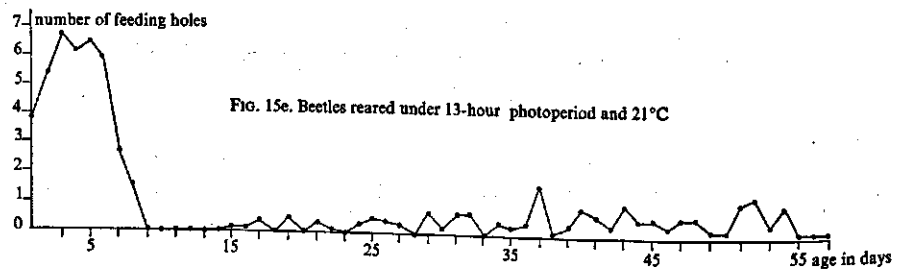
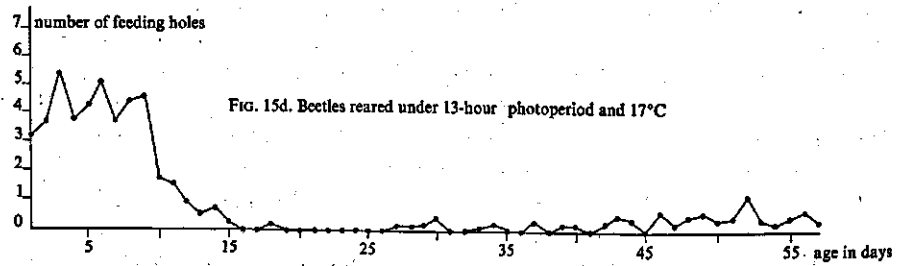
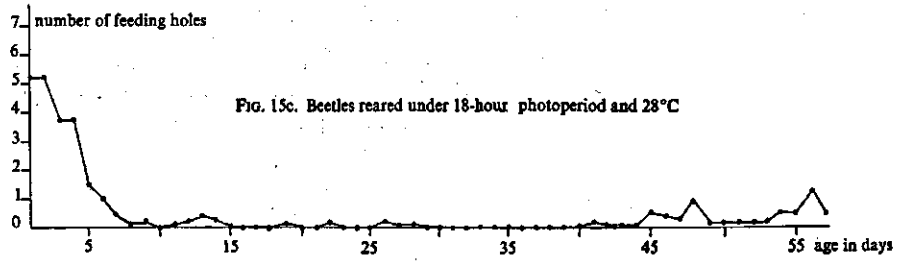


TABLE 20. Number of days directly after emergence of *P. chrysocephala* from the pupae with a food consumption causing more than 0.5 holes per beetle and per day.

Temperature in °C	Number of days at photoperiods of	
	20 hours	13 hours
17	10	14
21	—	9
25	6	6
28	7 ¹⁾	—

¹⁾ In this experiment an 18-hour photoperiod was applied.

TABLE 21. Number of days with a food consumption by *P. chrysocephala* resulting in less than 0.5 holes per beetle and per day.

Temperature in °C	Number of days at photoperiods of	
	20 hours	13 hours
17	79	37
21	—	25
25	57	23
28	49	—

The effect of temperature on the length of the period of low food consumption is shown in table 21. Here we see under short day conditions a decrease in the length of the period with low activity with increasing temperature. The period of rest lasts longer under the conditions of 20 hours photoperiod than under those of 13 hours. A temperature of 25°C seems to be most favourable since under these circumstances food consumption almost never stopped completely. On the other hand we can also assume that food reserves are sooner exhausted under high temperatures than under low ones. This behaviour resembles that of *C. pleurostigma*. In both insects a resting period can be observed which is broken under short day conditions. Long day conditions on the other hand serve to prolong the resting phase.

As in *C. pleurostigma*, we can also make an estimate of the quantity consumed at the various temperatures. This is given in table 22. The results show that the total food intake decreases with increasing temperature. In the number of feeding holes made per day an optimum is found, probably near 21°C. With the insects reared under long day conditions this optimum could not be established. We see a decrease in feeding activity per day at the extreme temperature of 28°C. These figures are fairly similar to those obtained with the autumn race of *C. pleurostigma* when reared under long day conditions. In this insect we found some indication (though not significant) of a decrease in total food consumption under long day conditions at the higher temperatures. In *P. chrysocephala* this decrease is more distinct and occurs both under long

TABLE 22. Total food consumption and food intake per day expressed in the number of holes in the leaves made by *P. chrysocephala* during the period of intensive feeding shortly after emergence from the pupae.

Temperature in °C	Photoperiod in hours	Number of holes per beetle	
		per day	total
17°	13	3.1	43.9
18.5°	13	3.1	39.7
21°	13	4.9	38.9
25°	13	4.2	29.5
17°	20	3.7	37.5 ¹⁾
25°	20	5.4	32.5
28°	18	3.0	20.9

¹⁾ As no counts were made on the first day, a correction was applied.

and short day conditions. This is caused by a decrease in the rate of feeding at the highest temperatures and an increase in the rate of pre-diapause development with rising temperatures.

B. Effect of photoperiod and temperature on the development of the ovarioles

The ovaries of *P. chrysocephala* were studied throughout the pre-diapause and the pre-oviposition period. The results are given in table 23. These figures show that photoperiods of 20 hours prevent oögenesis. Only under the conditions of 13 hours photoperiod growing oöcytes and eggs are found in the ovaries of dissected beetles. However, the pre-oviposition period lasts much longer than in *C. pleurostigma*. This probably means that we did not rear the insects under the most favourable conditions to prevent diapause.

TABLE 23. Effect of the photoperiod and temperature on the development of the ovaries of *P. chrysocephala*.

Photoperiod and temperature in °C	Age of the beetles in days	Number of beetles			Total
		eggs	with growing oöcytes	without development	
13 hours 17°	42	0	0	3	3
	64-80	1	5	0	6
13 hours 21°	37	0	1	0	1
	50-61	0	8	5	13
13 hours 25°	41	0	1	1	2
	56-60	3	1	0	4
18 hours 28°	61	0	0	6	6
20 hours 17°	76-80	0	1	10	11
	115	0	0	1	1
20 hours 25°	56-61	0	0	6	6

When we compare these results with the figures on food consumption we see that feeding activity started to increase again shortly before oöcyte growth became apparent. Because of the limited number of observations it is not possible to check the effect of temperature on oögenesis. Here no indications were found that a high temperature is detrimental.

4.4. DISCUSSION AND CONCLUSION

The above mentioned experiments show a clear positive influence of a short photoperiod of 13 hours on oögenesis and feeding activity. *P. chrysocephala* must be considered as a short day insect.

BONNEMAISON and JOURDHEUIL (1954) did not arrive at the same conclusion though they studied the influence of photoperiod on oviposition and feeding behaviour rather intensively. In one of their experiments (p. 414) they reared the insects under natural daylength (16–12 hours), 16 hours light and constant illumination, respectively, (other photoperiods were also tested but no results were obtained because of high mortality). No eggs were found under constant illumination while those reared at 16 hours produced eggs after 90 days and those reared under the natural daylength (16–12 hours), after 71–74 days. These figures confirm our results since constant illumination must be considered as long day.

BONNEMAISON and JOURDHEUIL found a rather rapid oviposition in their rearings during the winter season. Under a natural daylength, increasing from about 10–13 hours, the first eggs were laid after 42–64 days. During summer with a natural daylength decreasing from 16 to 13 hours the first eggs were found after 71–96 days. With the very short photoperiod of 8½ hours egg deposition was again retarded until 84–94 days after emergence from the pupa.

BONNEMAISON and JOURDHEUIL explained their results by assuming that a temperature below 18°C, a relative humidity above 80% and young leaves as food are the main factors responsible for diapause. They concluded that these factors could reduce the duration of diapause from 70–96 days to 38–45 days. Our conclusion from their figures is that photoperiod, too, has a marked effect on oögenesis and feeding activity and that the data obtained confirm the results of our experiments.

5. DISCUSSION

Insect species with a univoltine cycle throughout their areal are rather frequently found among Coleoptera, as is shown in table 24 (which is by no means complete). It is often found in species with a specialized feeding habit, larvae or adult being dependent on the presence of a particular kind of food which is available only during a short period of the year. Many of these species deposit their eggs in spring and the ensuing adults emerge in summer. They enter diapause long before the onset of unfavourable weather and emerge again in spring. Some of these species have a reversed cycle. They deposit their eggs in the autumn and the adults emerge in late spring or early summer, aestivate, and become active by the end of the summer. It seems likely that many more short day insects will be detected among this group.

It is remarkable that in genera comprising mainly univoltine species with a winter diapause we often find a few species with aestivation diapause. This occurs e.g. in the genera *Ceuthorrhynchus*, *Anthonomus*, *Apion*, *Phytonomus*, *Sitona*, *Listroderes*, *Psylliodes* and *Entomoscelis*, and in the crickets *Scapsipedus aspersus* and *Acheta pennsylvanica* (Burmeister), (MASAKI 1961). In some species races are known which have their main oviposition period in autumn or in spring. This change of the seasonal cycle within a species occurs also in other insects. HARPAZ (1961) mentions *Hylemyia antiqua* Meigen which in Europe and in North America has a diapause in winter but in Israel in summer. This phenological behaviour synchronises the insect with its host plants of the genus *Allium*. The same was found in the aphidivorous syrphid fly *Epistrophe balteata* De Geer (HARPAZ 1961). *Sitona lineatus* aestivating in Portugal and Israel and hibernating in northern countries is another example (Personal communication from J. C. SILVA DIAS [1960] and H. N. PLAUT [1963]). In these cases the two races are found in geographically different places. In *C. pleurostigma* this difference does not occur. According to MASAKI (1961) the cabbage moth *Mamestra brassicae* also shows a high diversity in the incidence and intensity of its diapause. These moths emerge earlier in the south as might be expected from the hibernating pupae; however, in the second flight the reverse occurs. Here the southern strains show a progressively increasing aestivation diapause which delays their appearance while the northern strains do not have an aestivation at all.

In the above cases we find several examples of forms within one species showing either winterdiapause or aestivation diapause probably as a result of selection by local environmental conditions. Aestivation occurs mainly in regions with warm winters and dry hot summers. These are the conditions which are common in the Mediterranean area. Yet also in Western Europe with its cool summers insects with the habit of aestivation are found.

One may imagine that some of these have originally immigrated from the Mediterranean area like the insects living on cabbage e.g. *C. pleurostigma*; however, also autochthonous development of the aestivating type of annual cycle in northern regions, seems plausible. The cycle in this case is mainly the

TABLE 24. Some genera with species having a similar annual cycle as *C. assimilis* and *C. pleurostigma*.

	Diapause in		Number of generations
	summer	winter	per year
<i>Anthonomus</i>	*		1
<i>ornatus</i> Blanch	*		1
<i>pedicularius</i> L.	*		1
<i>pyri</i> (cinctus) Kollar	*		1
<i>pomorum</i> L.		*	1
<i>rectirostris</i> L.		*	1
<i>rubi</i> Hbst.		*	1
<i>signatus</i> Say.		*	1
<i>spilotus</i> Redt.		*	1
<i>varians</i> Payk.		*	1
<i>vestitus</i> Boh.			p ¹⁾
<i>Ceuthorrhynchus</i>			
<i>pleurostigma</i> Marsh.	*		1
<i>pictitarsis</i> Gyll.	*		1 or 2
<i>leprieuri</i> Ch. Bris.	*		1
<i>assimilis</i> Payk.		*	1
<i>napi</i> Gyll.		*	1
<i>pleurostigma</i> Marsh.		*	1
<i>quadridens</i> Panz.		*	1
<i>rapae</i> Gyll.		*	1
<i>sareptanus</i> Schultze		*	1
<i>scrobicollis</i> Neresh. and Wagn.		*	1
<i>syrites</i> Germ.		*	1
<i>Phytonomus</i>			
<i>punctatus</i> F.	*		1
<i>meles</i> F.		*	1
<i>murinus</i> F.		*	1
<i>transsylvanicus</i> Petri		*	1
<i>variabilis</i> Herbst.		*	1
<i>Sitona</i>			
<i>flavescens</i> Mrsh.	*		1
<i>lineatus</i> L.	*		1
<i>hispidulus</i> F.		*	1
<i>lineatus</i> L.		*	1
<i>Listroderes costirostris</i>			
<i>ab. obliquus</i> Gyll.	*		1
<i>Psylliodes</i>			
<i>chrysocephala</i> L.	*	*	1
<i>affinis</i> Payk.		*	1
<i>attenuata</i> Koch.			1
<i>Entomoscelis adonidis</i> Pall.	*		1

¹⁾ Polyvoltine

result of an adaption of the insect to the annual rhythm of its host plant.

Sometimes we notice both an autumnal and a spring egg-laying period in one and the same generation. This is found in *P. chrysocephala*, *Phytonomus* and probably also *Sitona flavescens* (FOLSOM 1909, and GROSSHEIM 1928). When the reproductive periods of the offspring derived from these two egg-laying periods becomes more and more separated, races or species may develop. In *P. chrysocephala* the two groups are probably not separated, in *C. pleurostigma* the separation is more complete, though we can still consider them as one species. It is possible that the two related species *Anthonomus pomorum* and *Anthonomus pyri* have developed in this way.

Besides the differences in intensity and occurrence of diapause we noticed in the French strain of *C. pleurostigma* also a difference in the critical photoperiod. It is clear that the critical photoperiod plays a decisive role in the survival of the species in a region. If weevils of Dutch origin are transferred to Southern France, they may not survive because they emerge too early in summer; probably they do not even enter diapause at all when temperature is not too high. Only a higher temperature may protect them from becoming active in spring when the natural growing season of cabbage is completed. However, as the larvae are already full-grown by the end of November, an emergence of the Dutch weevils in April or early May is likely. This is also their time of appearance in Italy (PIETRI-TONELLI 1950). In that time of year the temperature is not yet too high (an average of 21°C being most favourable!) to prevent activity. Under natural conditions their host plants in that period of the year are not present in an adequate developmental stage for maintenance of the species. Therefore, immigrants will not be able to oviposit.

In the opposite case, viz. a transfer of beetles from Mediterranean France to the Netherlands, it is possible that they may start egg laying only after the beginning of October since at this time in Holland the natural daylength is less than 12 hours. As we found an intense diapause in a considerable percentage of weevils from the French population, it seems possible that some of them would remain in diapause and become active in spring. On the other hand, when some of these would be active in the autumn, an exchange of genes with the Dutch population could be possible. This exchange will not take place between Dutch insects transferred to Southern France. So here we have a possible case of a one-sided reproductive isolation between two populations. Yet mixing may occur through populations of intermediate regions. However, in this case the hybrids must obtain a lower critical photoperiod than the original northern strain.

In long day insects the reverse may be true. Long day insects from the south introduced into the north have a smaller chance of survival than those brought from the north to the south, since in the latter case they have to enter diapause too early or to produce only one generation per year (MASAKI 1961).

SUMMARY

1. *Introduction.* The influence of some token stimuli (photoperiod, temperature, and food) on the annual cycle was studied in a univoltine weevil with an oviposition period in spring (*Ceuthorrhynchus assimilis* Payk.) and a univoltine weevil with two races having their oviposition periods in spring and autumn respectively (*C. pleurostigma* Marsh.). Some additional information was obtained on another coleopteron with its main oviposition period in autumn (*Psylliodes chrysocephala* L.).

2. *Annual cycle of C. assimilis.* This univoltine species hibernates in the adult stage and deposits its eggs in spring in the pods of *Brassica* spp. The adults emerge in July from the pupae and soon enter diapause. With the aid of yellow Moericke traps a spring flight from the hibernation sites and a summer flight of the young adults towards these places can be registered. Only in exceptional cases, the new generation lays eggs in the same year. The possibilities of survival of such a second generation were considered negligible.

The influence of photoperiod and temperature on the inception of diapause in the adult stage was studied. As the syndrome of diapause in the adult involves changes in behaviour and a standstill in ovarian activity, these points were particularly followed.

In studies on several behaviour components (phototaxis, geotaxis, feeding activity and hiding) it was found that positive phototaxis in newly emerged weevils declined in about two weeks from 90% to less than 60% under photoperiods of 20 hours, 10 hours and natural daylength of summer. The geotactic response did not differ between ovipositing and diapausing weevils. Feeding activity as well as the percentage of non-hiding weevils declined sharply in about 6–11 days after emergence from the pupa. No effect of photoperiod on these aspects of behaviour was found. As hiding starts earlier than the decline in positive phototaxis, the change in habitat made by weevils entering diapause is not merely a consequence of a change in phototaxis.

Studies on the ovaries of *C. assimilis* reared under different photoperiods ranging from 0–24 hours per day at several temperatures did not reveal any growth of oöcytes. All weevils first enter diapause. The testes of diapausing males were found to contain well developed mobile sperm.

The application of a short photoperiod in the larval stage did not have any effect on the diapause behaviour of the adult. In some weevils diapause is terminated by the end of November; in most weevils it is completed by the end of December, but until the end of March diapausing weevils can still be found in hibernation sites, the majority then being in a stage of quiescence.

The ovaries of *C. assimilis* are of the telotrophic type. Oöcyte differentiation is probably completed in the very early adult stage. During maturation of the ovarium the trophocyte nuclei increase in diameter from about 3 μ to 15–20 μ . In diapausing females no oöcytes in the descending stage were noticed.

The annual cycle of *C. assimilis* is well synchronized with that of its host plants, most of the latter needing chilling during the vegetative phase and entering the generative phase in the course of December at about the same time at which diapause is completed in most weevils.

3. *Annual cycle of C. pleurostigma*. In this insect two races occur, one ovipositing in autumn (autumn race) and one in spring (spring race). The first aestivates and the second has a winter diapause. Both races often occur in the same region. Eggs are deposited in the root collar region or on the roots of several *Brassica* spp. (e.g. *Brassica napus*, *B. oleracea*, *B. rapa*). The spring race is also frequently found on *Sinapis arvensis*. The larvae live in a gall. The weevils feed on the leaves.

Studies of the inception of diapause in the spring race revealed that its occurrence is not influenced by photoperiods of 5, 13 and 20 hours applied during the adult stage. All weevils entered diapause after an initial period of high activity. No oöcyte growth was noted in their ovaries within 30 days.

In the autumn race, on the other hand, diapause is clearly influenced by photoperiod and temperature. Studies were made of several behaviour components (phototaxis, geotaxis, feeding activity and hiding). Weevils reared under 18 hours or 13 hours daylength showed no or only minor differences in phototactic and geotactic responses. Feeding activity remained higher in weevils reared under a 13-hour photoperiod than in those reared under 20 hours daylength. The percentage of hiding weevils did not differ very much. Yet a distinct increase in number can be observed when weevils are transferred from a 20-hour to a 13-hour photoperiod. In the insects reared under short day conditions a negative correlation was found between the duration of the initial feeding period and temperature and a positive correlation between the quantity of food eaten per day during this period and temperature. Under long day conditions the latter correlation did not exist. This difference is attributed to pre-diapause behaviour in the last group.

Photoperiods shorter than 16½ hours induce follicular activity, longer photoperiods result in arrest of oögenesis. The critical photoperiod lies between 14 and 16 hours. The optimal temperature is about 21°C, though also at 17°C the rate of development was often high. The age of the leaves had no distinct effect on ovarian activity, but the tender leaves of plants grown in a greenhouse were less suitable as food. Indications were found of a delayed effect of the short photoperiod on oöcyte growth or of the existence of a sensitive phase beginning in about 5 day old weevils.

In a geographic race collected in southern France (43° 35' N.L.) the critical photoperiod is between 12 and 14 hours light. In this race 10- and 12-hour photoperiods induce only 15 and 17% activity as compared with 100 and 85% in weevils collected near Wageningen (51° 57' N.L.). This makes plausible a partial obligatory univoltine cycle in the southern parts of the areal. Diapause was broken by a short day treatment, long day prolongs diapause.

In histological studies oöcytes in the descending stage were found in 24 day-old weevils reared under a 13-hour photoperiod. Trophocyte nuclei increase

in size from 5–6 μ in the early adult stage to 10 μ in the diapausing females, and 15–20 μ in the ovipositing weevils.

Both races are well synchronized with the annual cycles of their host plants, the autumn race being adapted to the natural vegetative growth period of cabbage and the spring race to that of *Sinapis arvensis*. The cultivation of cabbage throughout the growing season has obviously prolonged the period of the year in which host plants are available. Both races have not yet adapted themselves to these new possibilities, although the spring race occurs locally on cabbage.

The two races were crossed and the offspring proved fertile. In the hybrids diapause lasted about 40–50 days under a 13-hour photoperiod at 21°C, as compared with 75–80 days in the spring race under these conditions. Apparently the two races of *C. pleurostigma* provide an example of the maintenance of originally geographic races in which reproductive isolation is now affected by differences in the season of their activity as a consequence of differences in photoperiodic response.

4. *Annual cycle of Psylliodes chrysocephala*. In this species two oviposition periods are known, one in the autumn and one in spring. In continental regions the latter is the most important for reproduction. Eggs are laid in the soil; the larvae bore in the midrib and stem of *Brassica* spp. The adults emerge in June. They aestivate and become active again by the end of summer.

In laboratory experiments it was found that the duration of pre-diapause diminishes with increasing temperature. During this period feeding rate increases and total food consumption decreases with higher temperatures, both under long day (a 20-hour photoperiod) and under short day (a 13-hour photoperiod) conditions. Contrary to the case in *C. pleurostigma*, under both conditions a period of diapause is found which is terminated within 2–3 months under short day conditions, but not under long day conditions. This becomes evident in a higher feeding rate and a growth of oöcytes.

5. *Conclusion*. Several examples of species with an autumnal activity can be found among Coleoptera. They mainly belong to genera where univoltinism predominates. In some species races are known which have their main oviposition period in autumn or in spring. They are generally geographically separated. In a few cases, probably as a result of a recent change in the areal of the foodplant, these races occur in the same geographic region, their maintenance as a race now depending on their reproductive isolation in time.

It seems likely that short day insects like *C. pleurostigma* will not be able to maintain themselves when they are transferred from a high latitude to a distinctly lower one. As their critical photoperiod is too high, they will fail to enter aestivation diapause in late spring in these regions. Under natural conditions their host plants in that period of the year are not present in an adequate developmental stage to allow maintenance of the species. On the other hand, weevils from a lower latitude transferred to a higher one will only start their activity later than the autochthonous population in autumn. The reverse may be true for long day insects.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Prof. Dr. J. DE WILDE for his advice in the course of the work and for reviewing the manuscript. He also feels indebted to Mr. W. COMPANJEN for his help in the histological part of the study and to Dr. G. B. STAAL for the microphotography. Special thanks are also due to Dr. E. BILIOTTI and Mr. R. HAM of the 'Station de Zoologie agricole et de lutte biologique' at Antibes, France, for collecting and sending the 'Antibes strain' of *C. pleurostigma*. The author is indebted to Ir. S. C. BHARGAVA and Miss H. KRAMER for their care in some of the rearing experiments. He also wishes to thank Mr. M. P. VAN DER SCHELDE and Mr. E. J. J. VERHAAF for drawing the figures. Thanks are also due to Dr. M. R. HONER and Mr. N. G. UILENBURG for correction of the English text and to Mrs. G. DE WILDE-VAN BUUL for correction of the Dutch summary.

SAMENVATTING

1. *Inleiding.* De invloed van enige signaalfactoren (fotoperiode, temperatuur en voedsel) op de jaarcyclus is onderzocht bij een univoltine snuitkever met een ovipositieperiode in het voorjaar (*Ceuthorrhynchus assimilis* Payk.) en bij een univoltine snuitkever (*C. pleurostigma* Marsh.) waarbij twee rassen voorkomen, die respectievelijk hun ovipositieperiode in voorjaar en herfst hebben. Enige aanvullende gegevens werden verkregen over de jaarcyclus van een derde keversoort (*Psylliodes chrysocephala* L.), die zijn belangrijkste ovipositieperiode eveneens in de herfst heeft.

2. *Jaarcyclus van C. assimilis.* Deze univoltine soort overwintert als imago en legt haar eieren in het voorjaar in de hauwen van koolzaad en andere *Brassica* spp. De imagines komen in juli uit de pop en gaan spoedig in diapauze. Met gele vangbakken zijn de voorjaarsvluchten uit de winterkwartieren naar de koolzaadvelden en de zomervluchten van het veld naar de winterverblijfplaats geregistreerd. Alleen in uitzonderingsgevallen worden door enkele exemplaren van de nieuwe generatie in hetzelfde jaar nog eieren gelegd. De overlevingskansen van een hieruit voortkomende 2e generatie zijn vrijwel nihil.

De invloed van fotoperiode en temperatuur op het intreden van de imaginale diapauze zijn in het laboratorium onderzocht. Daar het syndroom van de imaginale diapauze veranderingen in gedrag en stilstand in de ontwikkeling van de ovariolen inhoudt, zijn deze aspecten in het bijzonder bestudeerd.

Uit de resultaten van het onderzoek naar enkele gedragscomponenten (fototaxis, geotaxis, voedselopname en schuilgedrag) bleek, dat de positieve fototaxis van pas uit de pop gekomen kevers na 2 weken bij alle onderzochte fotoperioden (20 uur, 10 uur en de natuurlijke daglengten van juli-augustus) daalt van ongeveer 90% naar minder dan 60%. De geotactische reactie verschilde niet tussen leggende en diapauze-kevers. De voedselopname per dag en het aantal niet verscholen kevers nam sterk af, 6–11 dagen na de ontpopping. Een effect van de fotoperiode op deze aspecten van het gedrag kon niet worden vastgesteld. Daar het verschuilen van de kevers eerder begint dan de afnemering van de positieve fototactische reactie, dient men de verandering van habitat van de kevers, die in diapauze gaan, niet uitsluitend te beschouwen als een consequentie van een verandering in de fototactische reactie.

Uit het onderzoek van de ovariolen bleek, dat geen van de fotoperioden (variërend van 0–24 uur) en geen van de hierbij gebruikte temperaturen enige ontwikkeling van de oöcyten tot in het afdalende stadium veroorzaakten. Alle kevers gaan eerst in diapauze. De testes van pas in diapauze zijnde ♂♂ bleken goed ontwikkeld, beweeglijk sperma te bevatten.

Het kweken van de larven onder een korte fotoperiode en van de hieruit voortkomende imagines onder een lange fotoperiode had evenmin invloed op het intreden van de diapauze. Bij sommige kevers is de diapauze tegen het einde van november al afgelopen, bij de meesten is dit eind december het geval, maar tot einde maart werden nog kevers in diapauze in hun winterkwar-

tier aangetroffen. Bij het merendeel van de dieren in de winterkwartieren is de diapauze dan verbroken.

De ovariën van *C. assimilis* zijn van het telotrophe type. De differentiatie van de oöcyten is vermoedelijk reeds voltooid in de zeer jonge imagines. Bij de rijping van het ovarium neemt de diameter van de kernen der trophocyten toe van ongeveer 3 μ tot 15–20 μ .

De jaarcyclus van *C. assimilis* is goed aangepast aan die van zijn waardplanten. De meeste *Brassica* spp. hebben een koude-behoefte gedurende een periode van de vegetatieve phase. Zij komen in de generatieve phase in de loop van december, dus bij het beëindigen van de diapauze van *C. assimilis*.

3. *Jaarcyclus van Ceuthorrhynchus pleurostigma*. Van deze soort zijn twee rassen bekend; een voorjaarsras, dat in het voorjaar zijn ovipositieperiode en in de winter een diapauze heeft en een herfstas, dat de ovipositieperiode in de herfst heeft en de diapauze in de zomer. Beide rassen komen in dezelfde gebieden voor. De eieren worden door het herfstas gelegd in de wortelhals en in de wortels van *Brassica* soorten (*Brassica napus* L., *B. oleracea* L., *B. rapa* L.). Het voorjaarsras legt haar eieren vooral in *Sinapis arvensis* L. en soms ook in kool. De larven leven in de ter plaatse gevormde gal. De kevers vreten aan de bladeren.

Bij het voorjaarsras bleek de fotoperiode tijdens het imaginale stadium geen invloed te hebben op het optreden van diapauze. Alle kevers gingen bij fotoperioden van 5, 13 en 20 uur bij een temperatuur van 17°C in diapauze, na enige tijd zeer actief te zijn geweest. Geen groei van oöcyten kon worden opgemerkt in ♀♀ van 30 dagen.

In het herfstas bleken daarentegen de fotoperiode en de temperatuur een duidelijk effect te hebben. Uit het onderzoek naar de gedragscomponenten, fototaxis, geotaxis, voedselopname en schuilgedrag, bleek dat de beide eerstgenoemden niet sterk verschilden tussen dieren in diapauze en de actieve dieren. Evenals bij *C. assimilis* werd gedurende de eerste tijd na het uitkomen uit de pop een periode van hoge voedselopname vastgesteld. Bij dieren, gekweekt bij lange dag (20 uur fotoperiode), trad na enkele dagen een sterke daling in de vreetactiviteit op; bij dieren gekweekt onder korte dag (13 uur fotoperiode) was dit in veel mindere mate het geval.

Tussen de duur van de periode met grote voedselopname en de temperatuur werd een negatieve correlatie gevonden, terwijl er een positieve correlatie bleek te bestaan tussen de hoeveelheid per dag opgenomen voedsel en de temperatuur gedurende deze periode. De laatste correlatie was wel aanwezig bij omstandigheden van korte dag, maar niet bij die van lange dag. Het percentage zich schuilhoudende kevers verschilde niet sterk tussen de dieren, die bij lange en korte dag gekweekt werden. Wel werd bij het overbrengen van de dieren van een lange dag naar een korte een toename van het aantal actieve kevers waargenomen.

Fotoperioden korter dan 16½ uur induceren activiteit van het follikelepitheel, langere fotoperioden veroorzaken een stilstand in de oögenese. De kritieke fotoperiode ligt tussen 14 en 16 uur. De optimale temperatuur is onge-

veer 21°C, hoewel ook bij 17°C nog een sterke voortgang van de oögenese werd waargenomen. De physiologische ouderdom van het blad van de voedselplant had geen duidelijke invloed op de activiteit van de ovariën. Het tere loof van kasplanten bleek minder geschikt te zijn als voedsel.

Er werden aanwijzingen gevonden van een nawerking van de korte fotoperiode na overbrenging naar een lange dag op de oögenese, of van het bestaan van een gevoelige periode, aanvangend ongeveer 5 dagen na het uitkomen uit de pop.

In een geografisch ras afkomstig uit Zuid-Frankrijk (43° 35' N.B.) lag de kritieke fotoperiode tussen 12 en 14 uur. In dit ras werd bij 10 en 12 uur fotoperiode in resp. slechts 15% en 17% der kevers activiteit verkregen, vergeleken met 100% en 85% bij kevers verzameld bij Wageningen (51° 57' N.B.).

De diapauze wordt verbroken door een korte dag behandeling en verlengd door een lange dag.

In de ovariën van 24 dagen oude kevers, gekweekt bij lange dag, werden geen oöcyten in het afdalend stadium gevonden. De kernen van de trophocyten namen toe in diameter van 5–6 μ bij een jonge imago tot 10 μ bij ♀♀ in diapauze en 15–20 μ bij leggende kevers.

Beide rassen zijn goed aangepast aan de jaarcyclus van hun waardplanten; het herfst-ras aan de natuurlijke vegetatieve groeiperiode van kool en koolzaad in de herfst en het voorjaarsras aan die van *Sinapis arvensis*. De cultuur van koolsoorten gedurende vrijwel het gehele groeiseizoen heeft de periode van het jaar waarin deze waardplanten in een geschikt stadium verkeren, sterk verlengd. Beide rassen hebben zich hieraan nog niet aangepast, al komt het voorjaarsras lokaal wel op kool voor.

Beide rassen konden worden gekruist; de nakomelingen bleken fertiel te zijn. Bij de hybriden duurde de diapauze (bij 13 uur fotoperiode en 21°C) slechts 40 à 50 dagen, vergeleken met 75–80 dagen bij het voorjaarsras onder dezelfde omstandigheden.

De twee rassen van *C. pleurostigma* vormen waarschijnlijk een voorbeeld van het voortbestaan van oorspronkelijk geografische rassen, die door migratie nu niet meer geografisch gescheiden zijn. Hun reproductieve isolatie wordt in stand gehouden door een verschil in de fenologie van de reproductieve periode als gevolg van een verschil in fotoperiodieke reactie.

4. *Jaarcyclus van Psylliodes chrysocephala*. Bij deze soort zijn twee ovipositieperioden bekend, één in de herfst en één in het voorjaar. In continentale gebieden is de laatste het belangrijkste voor de vermeerdering. De eieren worden in de grond gelegd, de larven boren in de hoofdnerf van het blad en in de stengel, verpoping vindt plaats in de grond. De imagines verschijnen in juni, zij aestiveren en worden weer actief tegen het einde van de zomer.

In laboratoriumproeven kon een invloed van de fotoperiode op de diapauze worden vastgesteld. Vlak na het uitkomen uit de pop is een periode van grote voedingsactiviteit waar te nemen. De duur hiervan neemt af bij stijgende temperatuur, de snelheid van voedselopname neemt toe, maar de totale hoeveelheid opgenomen voedsel in deze periode neemt af naarmate de temperatuur

stijgt. Hierna volgt steeds een periode van geringe activiteit, welke bij fotoperioden van 13 uur eerder wordt verbroken dan bij fotoperioden van 20 uur. Vervolgens begint ook een zichtbare groei van de oöcyten.

5. *Conclusie*. Verscheidene voorbeelden van soorten met een ovipositieperiode in de herfst zijn bekend onder de Coleoptera. Deze soorten behoren veelal tot genera welke voornamelijk univoltine soorten bevatten. Van sommige soorten zijn rassen bekend, die een ovipositieperiode in het voorjaar of in de herfst hebben. Deze rassen worden veelal in geografisch verschillende gebieden gevonden.

Bij *C. pleurostigma* komen – mogelijk door een verandering in het areaal van de voedselplant – deze rassen thans in hetzelfde gebied voor. Hun voortbestaan als ras hangt nu af van hun reproductieve isolatie door de tijd, veroorzaakt door hun verschil in reactie op de fotoperiode.

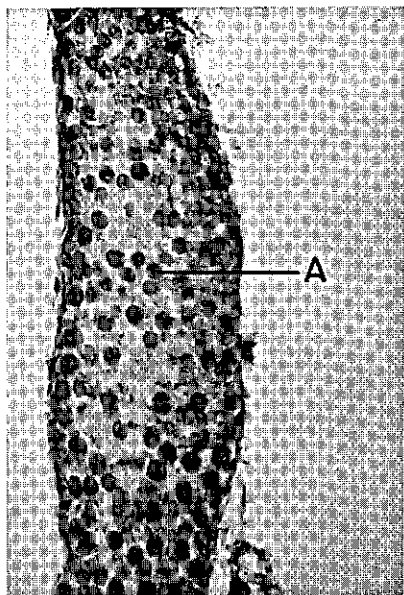
Het lijkt waarschijnlijk, dat korte dag insekten zoals *C. pleurostigma* zich niet zullen kunnen handhaven, wanneer zij van een hogere naar een belangrijk lagere breedtegraad worden overgebracht. Zij zullen dan namelijk niet tijdig in aestivatie-diapauze kunnen gaan door de te korte dag gedurende het voorjaar. Anderzijds zullen dieren van een lagere breedte naar een hogere breedte overgebracht hun activiteit slechts wat later in de nazomer beginnen dan de autochtone populatie. Het omgekeerde geldt voor lange dag dieren.

REFERENCES

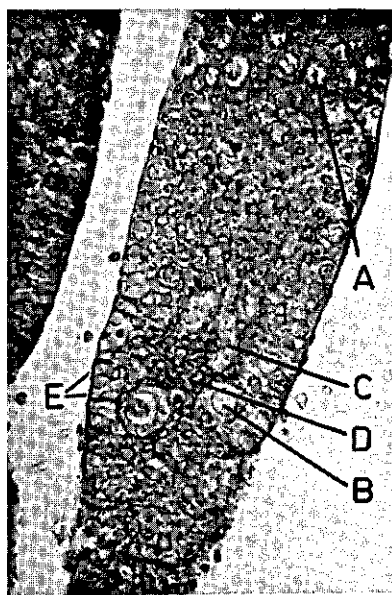
- ADKISSON, P. L. (1961). Effect of larval diet on the seasonal occurrence of diapause in the Pink Boll worm. *J. econ. Ent.*, **54**, 1107–1112.
- ANDREWARTHA, H. G. (1952). Diapause in relation to the ecology of insects. *Biol. Rev.*, **27**, 50–107.
- ANKERSMIT, G. W. (1952). Life history and control of the soya bean leaf beetle *Phaedonia inclusa* (Stål) (Coleoptera Chrysomelidae). *Contrib. gen. agric. Res. Sta. Bogor*, **129**, 1–34.
- ANKERSMIT, G. W. (1956). De levenswijze en de bestrijding van de koolzaad snuitkever (*Ceuthorrhynchus assimilis* Payk.) en de koolzaadgalmug (*Dasyneura brassicae* Winn.). *Versl. Landbouwk. Onderz.*, **62.9**, 1–59.
- ANONYMUS, (1958). In copertina. Lotta Antiparassitaria, **10**, 10, 11.
- BONDARENKO, N. V. (1958). Particularities in the origin of diapause in the mite *Tetranychus urticae*. *Zool. Zhur.*, **37**, 1012–1023.
- BONNEMAISON, L. et JOURDHEUIL, P. (1954). L'altise d'hiver du Colza (*Psylliodes chrysocephala* L.). *Ann. Epiphyt.*, Série C, **5**, 345–524.
- BONNEMAISON, L. (1957). Le charançon des siliques (*Ceuthorrhynchus assimilis* Payk.) biologie et méthodes de lutte. *Ann. Epiphyt.*, **4**, 387–543.
- BÖRNER, C., BLUNCK, H., SPEYER, W. und DAMPF, A. (1921). Beiträge zur Kenntnis vom Massenwechsel (Gradation) schädlicher Insekten. *Arb. biol. Reichsanst., f. Land u. Forstw.*, **10**, 405–466.
- CARLSON, E. C., LANGE, W. H., and SCIARONI, R. H. (1951). Distribution and Control of the Cabbage Seedpod Weevil in California. *J. econ. Ent.*, **44**, 958–966.
- DANILEVSKY, A. S. (1957). Photoperiodism as a factor of formation of geographical races in insects. *Entomol. Obozrenie*, **36**, 5–27.
- DANILEVSKY, A. S. (1960). Intraspecific Physiological Adaptations of Insects towards Zonal-

- Geographic Peculiarities of Climate (In Russian). Proc. Intern. Symp. Ontogeny Insects, Praha, 1959, 293–296.
- DANON, M. (1953). Prilog biologigi i suzby anju kupusne pipe siškarice. Plant Prot. Beograd, 19, 3–16.
- DEUBERT, K. H. (1955). Beiträge zu den Beziehungen zwischen *Ceuthorrhynchus napi* Gyll. (Col. Curc.) und Winterraps hinsichtlich der Gallenbildung mit Ovarienuntersuchungen an verschiedene *Ceuthorrhynchus*-Arten. Wiss. Z. Martin Luther Univ. Halle-Wittenberg Math-Nat., 4, 909–932.
- ERMOLAJEW, W. and WASSILJEV, W. (1935). Zur Kenntnis schädlicher Lepidopteren siberischer Nadelhölzer. Z. angew. Ent., 21, 560–565.
- FOLSOM, J. W. (1909). The insect pests of clover and alfalfa. Univ. of Illinois Agric. Exp. Sta. Bull., 134, 113–196.
- GEISPITZ, K. F. (1953). Reactions of monovoltine butterflies to prolongation of daylength (In Russian). Entomol. Obozrenie, 33, 17.
- GRISON, P. A. (1952). Relations entre l'état physiologique de la plante hôte, *Solanum tuberosum* et la fécondité du Doryphore, *Leptinotarsa decemlineata* Say. Trans. Ninth Int. Congr. Ent., 1, 331–337.
- GROSSHEIM, N. A. (1928). Data for the study of the genus *Sitona* Germ. (In Russian). Bull. Mleev. Hort. Expt. Sta, 17, 1–57, (ref: Rev. appl. Ent., 17, (1929), 434–436).
- HARPAZ, I. (1961). Some observations on the role of diapause in the phenology of insects in semi-arid zones. Proc. Symp. Cryptobiotic stages in biological systems. 5th Biol. Conf. 'Oholo' 1960, 154–158.
- HEGI, G. (1906). Illustrierte Flora von Mittel-Europa. Lehman, München, 4, 1 Teil, 229–268.
- HEYMONS, R. (1922). Mitteilungen über der Rapsrüssler *Ceuthorrhynchus assimilis* Payk. und seinen Parasiten *Trichomalus fasciatus* Thoms. Z. angew. Ent., 8, 93–111.
- HORSFELD, REINER (1963). Synökologischer Vergleich der Fauna von Winter- und Sommer-rapsfeldern. Z. angew. Ent., 52, 209–254.
- IACOPINI, P. (1957). Alcuni aspetti della lotta contro il *C. pleurostigma* Marsh. Rivista di Ortoflora frutticoltura italiana, 61, 625–631.
- ISAAC, P. V. (1923). The turnip gallweevil *Ceuthorrhynchus pleurostigma* Marsh. (Coleoptera Curculionidae). Part I. Life history and bionomics. Ann. appl. biol., 10, 151–170.
- JOHANSSON, A. S. (1958). Relation of nutrition to endocrine reproductive functions in the Milkweed-bug, *Oncopeltus fasciatus* (Dallas). Nytt. Mag. Zool., 7, 1–132.
- JUNK, W. und SCHENKLING, S. (1930). Coleopterorum Catalogus (von DALLA TORRE, K. W. et HUSTACHE, A. Curculionidae: Ceuthorrhynchinae, 43). Junk, Berlin, 30, 113.
- JUNK, W. und SCHENKLING, S. (1939). Coleopterorum Catalogus. (HEIKERTINGER, F. et CSIKI, E.: Chrysomelidae: Halticinae I, 539). Junk, 's-Gravenhage 25.
- KAUFMAN, O. (1923). Beobachtungen und Versuche zur Frage der Überwinterung und Parasitierung von Ölfruchtschädlingen aus den Gattungen *Meligethes*, *Phyllotreta*, *Psylliodes* und *Ceuthorrhynchus*. Arb. Biol. Reichsanst., 12, 109–169.
- KAUFMANN, O. (1940). Neue Gedanken und Erkenntnisse über den Rapserrdfloh (*Psylliodes chrysocephala* L.). Nachr. Bl. dtsh. Pfl. Sch. Dienst, Berl., 20, 1–3.
- KAUFMANN, O. (1941a). Zur Biologie des Rapserrdflohes (*Psylliodes chrysocephala* L.). Z. Pflkrankh., 51, 305–324.
- KAUFMANN, O. (1941b). Epidemiologie und Massenwechsel des Rapserrdflohes (*Psylliodes chrysocephala* L.). Z. Pflkrankh., 51, 342–369.
- LAMPA, Sven, (1894). Berättelse angående resor och förrättningar underår 1893 af kongl. landtbruksstyrelsens entomolog. Ent. Tidskrift, 15, 1–40.
- LEES, A. D. (1953). Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite *Metatetranychus ulmi* Koch (Acarina: Tetranychidae). Ann. Appl. Biol., 40, 449–486.
- LEES, A. D. (1955). The Physiology of Diapause in Arthropods. Cambridge Univ. Press. 1–151.
- LÉNKOVA, A. (1949). The structure of the female reproductive organs in some gonohorristic and parthenogenetic species of the genus *Polydrosus* Germ. (Coleoptera Curculionidae). Bull. int. Acad. pol. Sci. Série B, sci.naturelles 2.

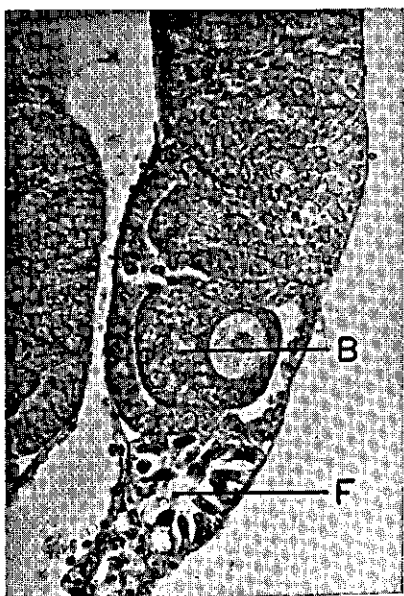
- MASAKI, S. (1961). Geographic variation of diapause in insects. Bull. Fac. Agric. Hirosaki Univ. 7, 66-98.
- MELIS, A. (1940). Contributo alla conoscenza del Bombice del Pino (*Dendrolimus pini* L.). Redia, 26, 73-175.
- MEUCHE, A. (1940). Untersuchungen am Rapserdfloh (*Psylliodes chrysocephala* L.) in Ostholstein. Z. angew. Ent., 27, 464-495.
- MEUCHE, A. (1944). Zur Überwinterung des Rapserdflohs (*Psylliodes chrysocephala* L.). Z. Pflkrankh., 54, 138-153.
- MÜLLER, H. J. (1957). Über die Diapause von *Stenocranus minutus*. Beitr. Ent., 7, 203-226.
- PIETRI-TONELLI, PIETRO DE (1950). Contributo alla conoscenza della biologia del *Ceuthorrhynchus pleurostigma* Marsh.. Redia, 35, 85-128.
- RISBEC, J. (1952). Contribution à l'étude de *Ceuthorrhynchus assimilis* Payk., Charançon des siliques du Colza. Rev. Path. Veg., 31, 137-174.
- SANT, L. E. VAN 'T (1955). Boorsnuitkevers (*Ceuthorrhynchus*-soorten) in kool en koolrapen. Jaarversl. Inst. Pltztkt. Onderz., 61-62.
- SANT, L. E. VAN 'T (1956). Boorsnuitkevers (*Ceuthorrhynchus*-soorten). Jaarversl. Inst. Pltztkt. Onderz., 42-43.
- SANT, L. E. VAN 'T (1957). Boorsnuitkevers (*Ceuthorrhynchus pleurostigma* Marsh.). Jaarversl. Inst. Pltztkt. Onderz., 47.
- SANT, L. E. VAN 'T (1958). Boorsnuitkevers (*Ceuthorrhynchus*-soorten). Galboorsnuitkever (*Ceuthorrhynchus pleurostigma* Marsh.). Jaarversl. Inst. Pltztkt. Onderz., 46.
- SANT, L. E. VAN 'T, VIJZELMAN, H. E. en BETHE, J. G. C. (1961). Levenswijze en bestrijding van de galboorsnuitkever. Meded. Dir. Tuinb., 24, 27-32.
- SANTANIELLO, M. (1938). Contributo alla conoscenza del Punteruolo del cavolo. L'Ortofrutticoltura Italiana, Firenze, 140-142.
- SCHEIDING, U. (1956). Untersuchungen zur Biologie des Kohlgallenrüsslers *Ceuthorrhynchus pleurostigma* Marsham. Z. angew. Ent., 39, 186-228.
- SCHLOTTMAN, LOREN L. and BONHAG, PHILIP F. (1956). Histology of the ovary of the adult mealworm *Tenebrio molitor* (Coleoptera Tenebrionidae). Univ. Calif. Publ. Ent., 11, 6, 351-394.
- SORAUER, P. (1953). Handbuch der Pflanzenkrankheiten, (Heddergott, H. Menhofer, H. Muller, F. P. und Speyer, W.: Superfamilie: Macrofrenatae.) 5e Aufl. Parey, Berlin und Hamburg, 4, 2 Lieferung, 415, 432.
- SORAUER, P. (1954). Handbuch der Pflanzenkrankheiten (Dosse G. Curculionidae). 5e Aufl. Parey, Berlin und Hamburg, 5, 2 Lieferung, 483-484.
- SPEYER, W. (1923). Kohlschotenrüssler (*Ceuthorrhynchus assimilis* Payk.) Kohlschotenmücke (*Dasyneura brassicae* Winn.) und ihre Parasiten. Arb. Biol. Reichsanst. 12, 79-108.
- STOKES, PEARL and VERKERK, K. (1951). Flower Formation in Brussels sprouts. Meded. Landb.-Hogeschl. Wageningen, 50, 141-160.
- STRÜBING, H. (1960). Eiablage und photoperiodisch bedingte Generationenfolge von *Chloriana smaragdula* Stål und *Enidella speciosa* Boh. (Homoptera Auchenorrhyncha). Zoöl. Beitr. (Neue folge), 5, 301-332.
- TASCHENBERG, E. L. (1879). Einführung in die Insektenkunde. M. Heinsius, Bremen, 165-166.
- VEENENBOS, J. A. J. (1953). Bestrijdingsproeven tegen enkele voor koolzaad schadelijke insecten. Tijdschr. PlZiekt., 59, 35-50.
- WEISZ, H. A. VON (1940). Beiträge zur Biologie und Bekämpfung wichtiger Oelfruchtschädlinge. Zur Biologie und Bekämpfung von *Ceuthorrhynchus assimilis* Payk. und *Meligethes aeneus* Fbr. Z. angew. Ent. 26, Beih. 14, 1-131.
- WILDE, J. DE, DUINTJER, C. S. and MOOK, L. (1959). Physiology of diapause in the adult Colorado beetle. I The photoperiod as a controlling factor. J. Ins. Physiol., 3, 75-85.
- WILDE, J. DE and J. A. DE BOER (1961). Physiology of diapause in the adult Colorado beetle. II Diapause as a case of pseudo-allatectomy. J. Ins. Physiol., 6, 152-161.
- WILDE, J. DE (1962). Photoperiodism in insects and mites. Ann. Rev. Ent., 7, 1-26.



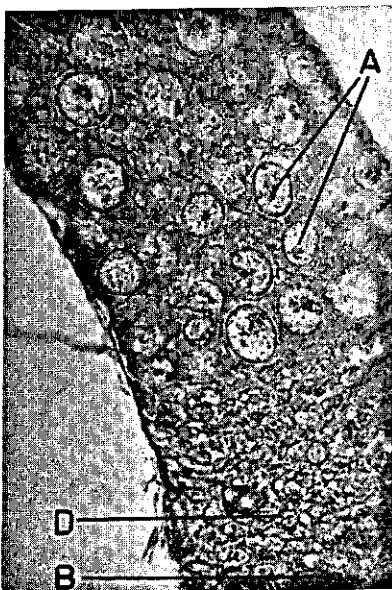
1



2



3



4

PLATE 1. Germarium of *C. assimilis* 27 days after emergence from the pupa. Reared at a 10-hour photoperiod and 17°C. Small trophocyte nuclei.

PLATE 2. Germarium of *C. assimilis*. Weevil kept at 10°C until November 30th. Afterwards reared under natural daylength at 20°C uptill December 18th. Trophocyte nuclei slightly grown. Nutritive cords are present. Oöcytes not yet in the descending stage.

PLATE 3. Other ovariole of the same weevil as in plate 2. Small oöcytes surrounded by follicular epithelium. Cells of this epithelium are still small.

PLATE 4. Germarium of weevil with strongly growing oöcytes. Note large trophocyte nuclei.

A Trophocyte nuclei

B Oöcyte

C Nutritive cords

D Prefollicular tissue

E Nuclei endothelial sheath

F Epithelial plug

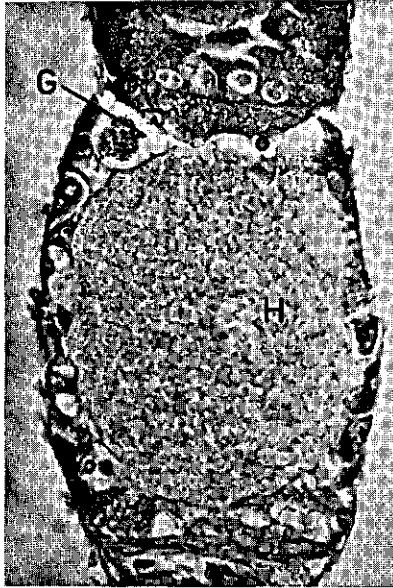


PLATE 5. Growing oöcyte. Same weevil as in plate 4. Cells of follicular epithelium greatly enlarged.

All photographs are of the same magnification

100 μ .

G Follicle epithelium

H Growing oöcyte