THE BIOLOGY AND ECOLOGY OF THE SWEDE MIDGE,

CONTARINIA NASTURTII (KIEFFER),

(DIPTERA; CECIDOMYIDAE)

By

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INTRODUCTION

The swede midge Contarinia nasturtii (Kieffer), a member of the family Cecidomyidae (Order, Diptera), is a small insect, the larvae of which induce gall formation on numerous cruciferous plants. Kieffer (1888) first described the midge which he reared from larvae occupying the closed flowers of Nasturtium palustre D.C., now known to be Rorippa islandica (Oeder) Borbas. In subsequent years numerous authors have reported the midge, under various synonyms, as a frequent pest of several important cruciferous crops.

Synonymy:

Contarinia (Diplosis) nasturtii (Kieffer), 1888.
Contarinia torquens ..... Meijere, 1906.
Contarinia perniciosa ..... Rübsaamen, 1914.
Contarinia geisenheyneri ..... Rübsaamen, 1917.

Also, according to recent studies by Stokes (1953a),

Contarinia ruderalis ..... (Kieffer), 1890.
Contarinia isatidis ..... Rübsaamen, 1910.

A comprehensive account of the synonymy of C.nasturtii is given by Barnes (1950).

The swede midge is widespread in Europe and its life-history has been investigated by numerous authors:- In England (Taylor, 1912; Dry, 1915; Thomas, 1946), in Holland (Meijere, 1906; Leefmans, 1937, 1938 and 1939), in Denmark (Rostrup, 1928), in France (Olombel, 1931;

Mesnil, 1938) and in Germany (Roesler, 1937; Noll et al, 1942; Frickhinger, 1943 and Hornig, 1953). With little variation the life-cycle of the midge is given thus:- There are from three to five generations a year; adults of the first generation emerge from the soil in June and after mating, the female lays her eggs on the younger parts of the host plant. The larvae feed gregariously on the host causing abnormal growth resulting in the production of a gall. When fully developed the larvae leave the plant and burrow into the upper layers of the soil. Here they spin a membranous cocoon inside which they pupate. The pupa leaves the cocoon and makes its way to the soil surface where adult emergence occurs. The duration of each generation varies with seasonal conditions but usually lasts from four to six weeks. Larval cocoons of the final summer generation, as well as a variable proportion of those from previous generations, enter a diapause state in which they overwinter.

This then is the basic plan on which previous researchers have attempted to explain the extreme fluctuations in abundance known to occur both in space and time.

Briefly this thesis re-examines the life-cycle of the swede midge in detail and investigates the effect of what seem to be the important environmental factors on successive stages of development. After consideration

of the observed population fluctuations of C.nasturtii at Nafferton, Northumberland, during 1957-1960, the thesis concludes with a discussion of population change in relation to various components of the environment.

SECTION I. THE ADULT

1.1 Emergence.

The act of emergence may be considered to begin with the vacation of the cocoon by the pupa, since eclosion soon follows. The pupal cocoon is formed near the soil surface (for details see Section 4). When fully developed, the pupa bursts out of the cocoon, the initial break in the membrane being made by the forward projecting dorsal thoracic horns. Contraction, expansion and gyration of the abdomen causes the pupa to leave the cocoon and move upwards through the soil. In this the pupa exhibits strong negative geotaxis; if reversed, it reorientates itself in order to burrow upwards. At the surface the pupa protrudes from the soil, only the tip of the abdomen remaining firmly anchored. The pupal case then splits longitudinally along its dorsal thoracic surface and the head and thorax of the adult are withdrawn. Legs, antennae, wings and abdomen soon follow but not without considerable effort. Once free of the pupal case the adult is immediately active. Eclosion takes from 1-5 minutes at 20°C.; adults taking longer than this to emerge are invariably deformed and often fail to free themselves.

1.11 Effect of temperature on emergence.

Emergence will occur at all temperatures between about 10°C. and 30°C. Below 10°C. the pupa appears to be

inactivated and temperatures above 30°C. tend to induce high mortality just prior to emergence. It will be shown later that pupae develop at temperatures as high as 32°C. but successful emergence of the adult at this temperature is inhibited. The lower lethal temperature for pupae has not been precisely ascertained but limited observations show that temperatures as low as 5°C. can be withstood for at least 1-2 days. Prolonged exposure to this temperature results in death.

Barnes (1930) states that adults of several other midge species have a characteristic daily emergence curve which may differ for different species; that males emerge earlier in the day than females and that pupae of different species, and different sexes, are differentially affected by temperature. There is no specific reference to a diurnal emergence rhythm for C.nasturtii but Barnes, among others, thinks that such a rhythm may exist. He infers this from his observation that ovipositing females are more numerous during the late afternoon and early evening.

Figures 1 and 2 show the results of two experiments carried out in July 1959, with the aid of rearing tubes (see Tables 1 and 2 for complete details). The rearing tubes consisted of 2-inch lengths of 7/8 inch diameter glass tubing, the ends of which were capped with discs of phosphor-bronze gauze (100 mesh per inch) welded to

FIGURE 1.

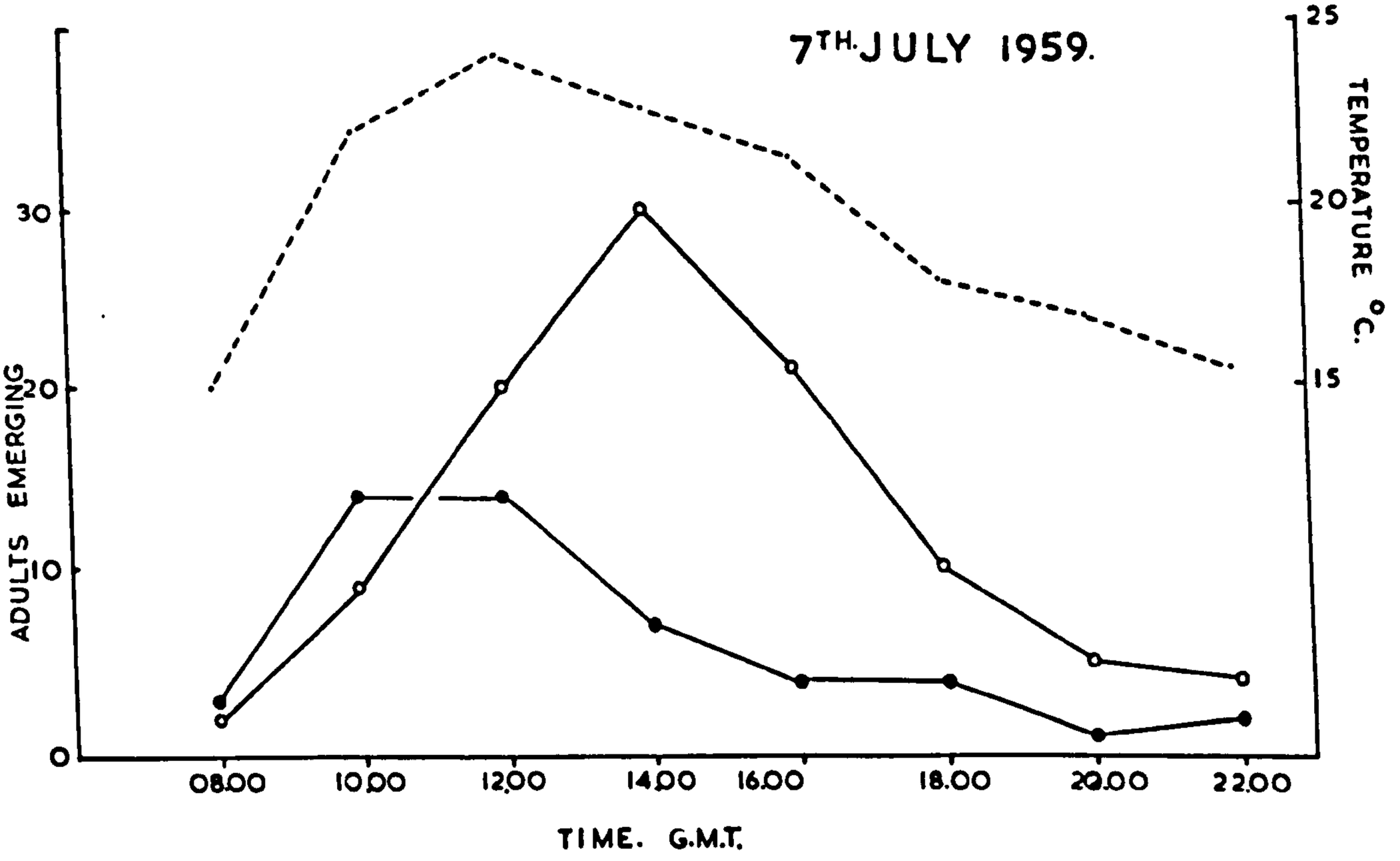
Emergence of adults of C.nasturtii in the field experiment of 7th July, 1959: (2-hourly totals from five replicates, each containing 200 larvae).

Open circles: females
Closed circles: males
Broken line: soil temperature °C. (2.5 cms. depth)

FIGURE 2.

Emergence of adults of C.nasturtii in the field experiment of 30th July, 1959: (2-hourly totals from five replicates, each containing 100 larvae).

Open circles: females
Closed circles: males
Broken line: soil temperature °C. (2.5 cms. depth)


7TH. JULY 1959.
ADULTS EMERGING
0
10
20
30
TEMPERATURE °C.
15
20
25
08.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
TIME. G.M.T.

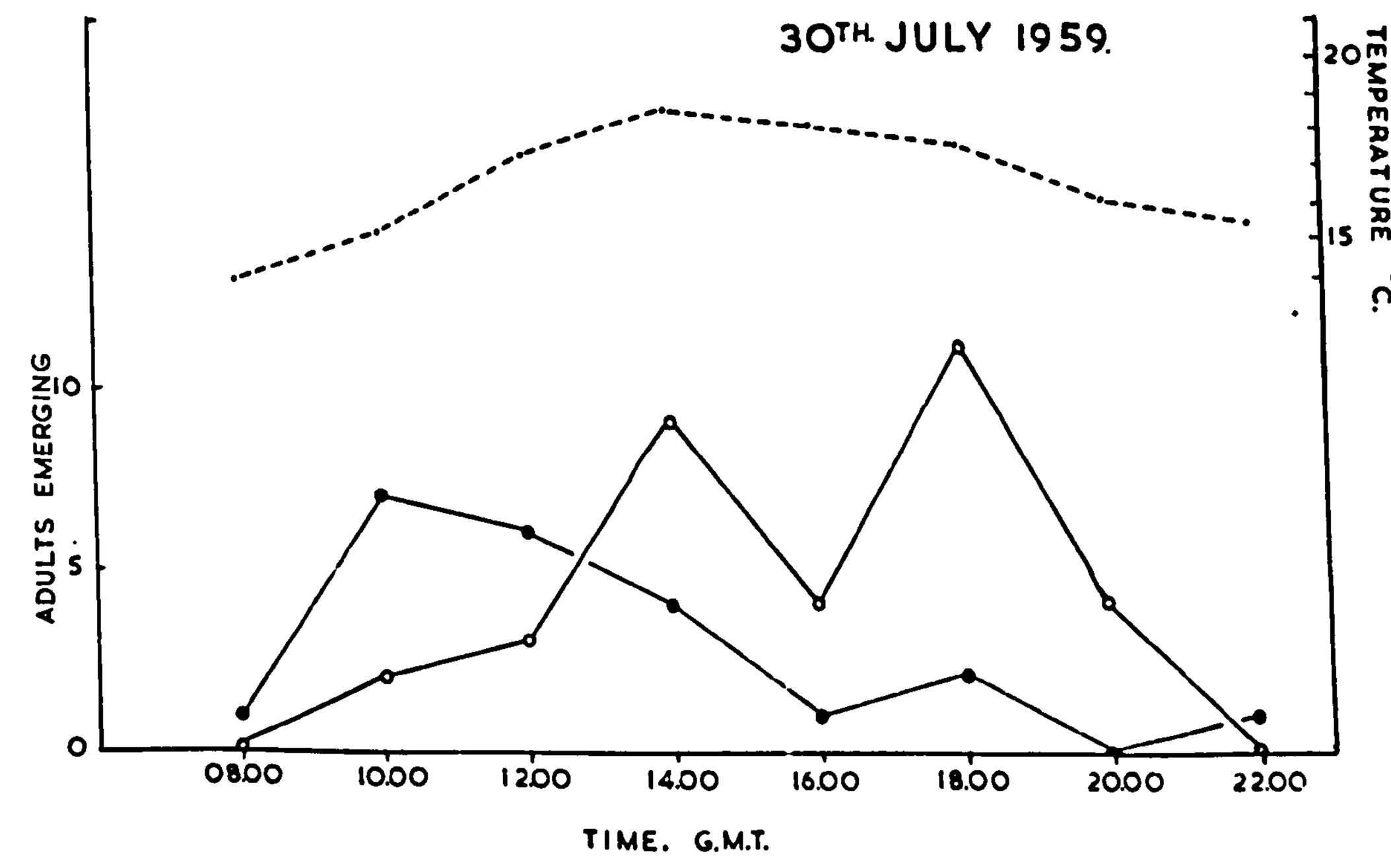

30TH. JULY 1959.
ADULTS EMERGING
0
5
10
TEMPERATURE °C.
15
20
08.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
TIME. G.M.T.

Table 1. Daily fluctuation of adult emergence in the field C.nasturtii, 7th July 1959

	Time (G.M.T.)																	
	0800		1000		1200		1400		1600		1800		2000		2200		Total	
Temp. °C.	15.0		22.1		24.2		22.9		21.5		18.0		17.1		15.5			
A*	**	-	3	1	1	4	-	7	1	2	-	4	-	1	-	-	5	19
B	1	2	1	3	7	7	4	10	-	8	1	1	-	-	-	-	14	31
C	1	-	5	2	3	5	1	6	1	1	3	2	1	2	1	1	16	19
D	-	-	1	3	-	3	-	5	1	3	-	-	-	1	-	1	2	16
E	1	-	4	-	3	1	2	2	1	7	-	3	-	1	1	2	12	16
Total	3	2	14	9	14	20	7	30	4	21	4	10	1	5	2	4	49	101
	5		23		34		37		25		14		6		6		150	

*Each replicate with 200 larvae

**Males on left, females on right

Table 2. Daily fluctuation in adult emergence in the field C.nasturtii, 30th July 1959

	Time (G.M.T.)								
	0800	1000	1200	1400	1600	1800	2000	2200	Total
Temp. °C.	14.0	15.1	17.2	18.5	18.0	17.5	16.0	15.4	
A*	** -	1 -	4	2 1 -	- - -	- 1 -	2 1 -		7 5
B	- -	3 -	1	1 - 5	1 - -	2 -	1 - -		5 9
C	- -	- 1	1 -	1 - -	4 -	3 - -	- - -		2 8
D	- -	1 -	- -	2 4 -	- 1	3 -	1 - -		4 8
E	1 -	2 -	- -	- - -	- - -	1	2 - -	- -	4 3
Total	1 -	7	2	6	3	4	9	1	4 2 11 - 4 1 - 22 33
	1	9	9	13	5	13	4	1	55

*Each replicate with 100 larvae.

**Males on left, females on right.

polythene collars fitting tightly over the glass tubing (Laughlin, 1958). In the first experiment (Fig. 1) five rearing tubes, each containing 200 larvae in moist soil, were sunk in the study field to the appropriate level. The larvae had been collected earlier from galled plants in this field and assigned at random to the tubes. On the day after emergence began the tubes were examined every two hours commencing at 0800 hours until 2200 hours (all times refer to G.M.T.) and the number and sex of the adults recorded. The experiment was repeated several days later with five replicates, each containing 100 larvae (Fig. 2). Both Figures show that males emerge a little earlier than females and that emergence is continuous throughout the day (0800-2200 hours). They also suggest that the amount of emergence increases as soil temperature rises and decreases as it falls throughout the day. This is more noticeable in Fig. 1 where the diurnal range in temperature was considerably greater than in Fig. 2.

The closeness of the relation between soil-temperature and emergence curves was examined in the laboratory. The experiment was carried out in the dark (except for actual counts which took only a few seconds every two hours). In each of 10 rearing tubes, 40 larvae were incubated at 20°C. Starting at 0800 hours on the day after emergence began, five of these tubes were subjected to the following

FIGURE 3.

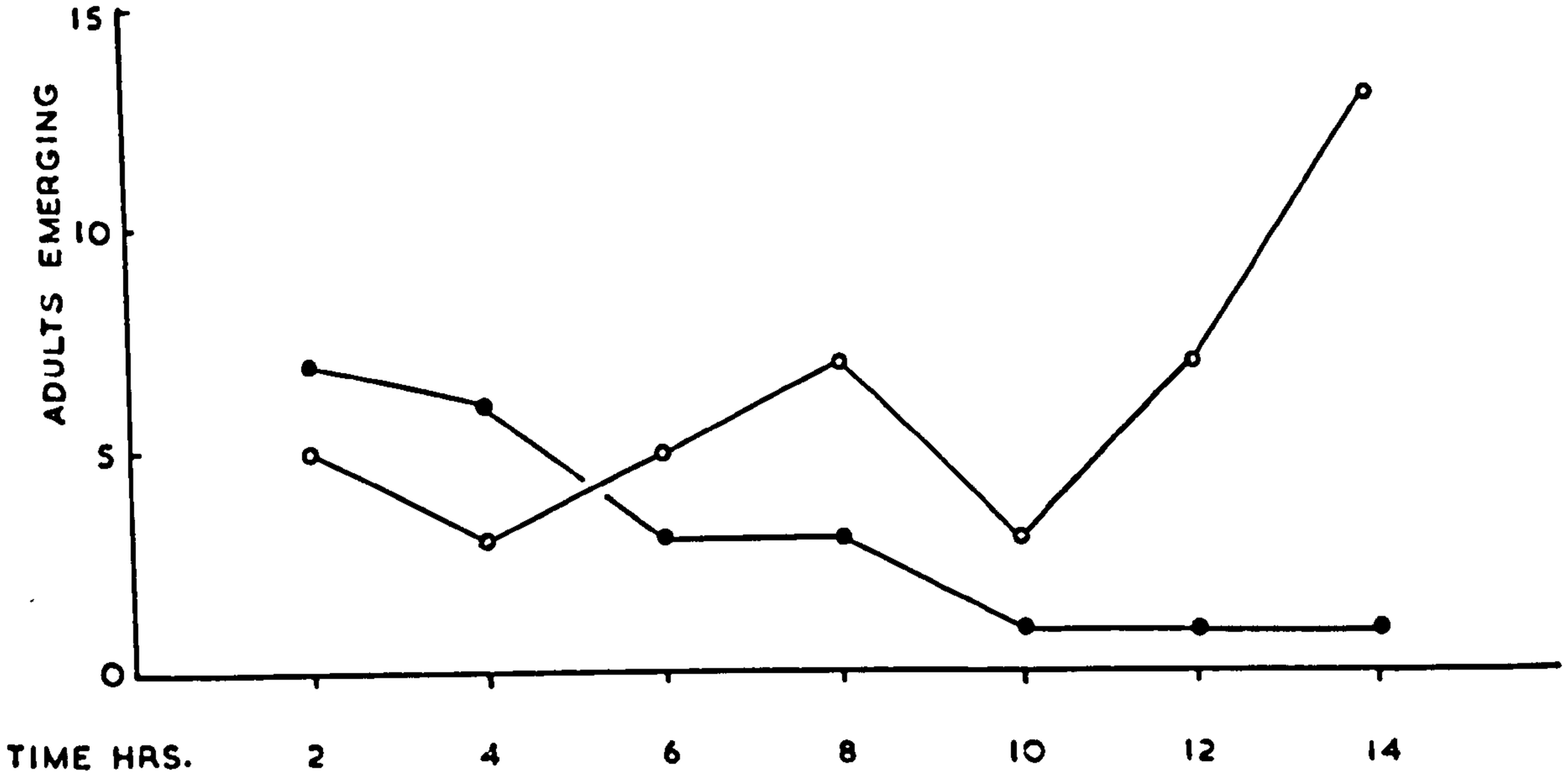
A. The emergence of adults of C.nasturtii
at constant temperature (20°C.)

B. The emergence of adults of C.nasturtii
in a rising and falling temperature
regime

Points represent the 2-hourly totals from
five replicates each containing
40 larvae

Open circles: females
Closed circles: males

CONSTANT TEMPERATURE 20°C.



FLUCTUATING TEMPERATURE

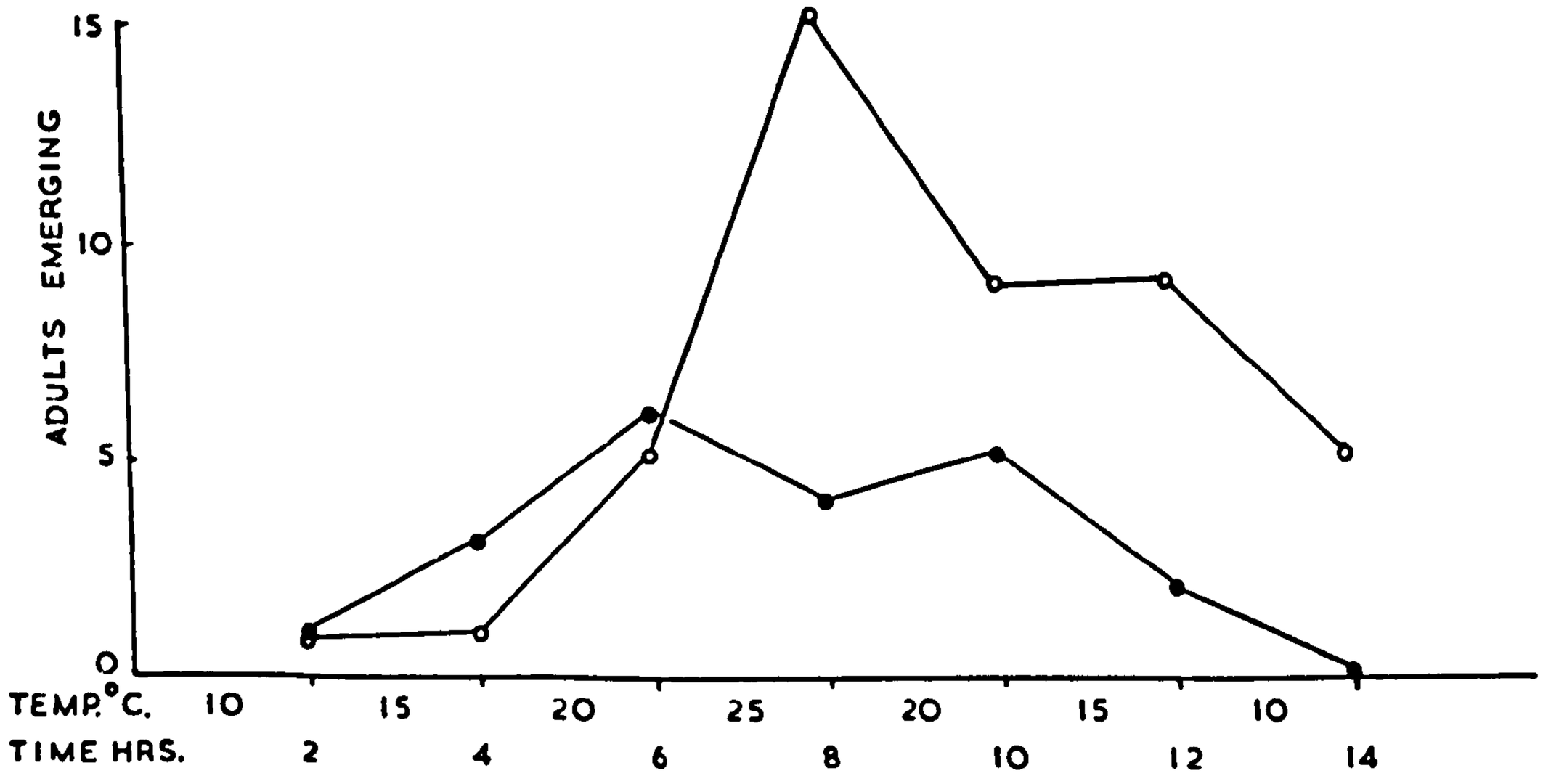


Table 3A. Adult emergence under a rising and falling temperature regime (C.nasturtii)

Time (hrs)	Temp. °C.	Tubes A*		B		C		D		E		Totals Male	Female
0-2	10	1**	-	-	-	-	-	1	-	-		1	1
2-4	15	1	-	1	-	1	-	-	-	-	1	3	1
4-6	20	1	4	2	-	-	-	1	-	2	1	6	5
6-8	25	-	3	-	4	2	-	-	1	2	7	4	15
8-10	20	-	1	-	5	4	-	1	2	-	1	5	9
10-12	15	-	2	1	-	1	2	-	4	-	1	2	9
12-14	10	-	-	-	-	-	1	-	2	-	2	-	5
	Totals	3	10	4	9	8	3	2	10	4	13	21	45

Table 3B. Adult emergence at constant temperature 20°C. (C.nasturtii)

Time (hrs)	Temp. °C.	Tubes A*		B		C		D		E		Totals Male	Female
0-2	20	**	-	1	3	4	2	-	-	2	-	7	5
2-4		2	1	-	-	4	1	-	-	-	1	6	3
4-6		-	1	-	-	1	-	2	1	-	3	3	5
6-8		3	2	-	1	-	1	-	-	-	3	3	7
8-10		-	7	1	5	-	1	-	-	-	-	1	13
10-12		-	2	-	1	-	-	1	4	-	-	1	7
12-14		-	1	1	2	-	3	-	1	-	6	1	13
	Totals	5	14	3	12	9	8	3	6	2	13	22	53

*Each tube started with 40 larvae.

**Males on left, females on right.

sequence of temperatures °C.: 10, 15, 20, 25, 20, 15, 10, two hours being spent at each temperature. At the end of each two-hour period, numbers and sex of emerged adults were recorded. The other five tubes remained at 20°C., but emergences were recorded at the same time intervals. The curves are compared in Fig. 3 (see Table 3 for full details). When temperature rises and falls, the emergence curve tends to rise and fall likewise (Fig. 3B). On the other hand, when temperature is constant there is no sign of any "peak" (Fig. 3A). These experiments therefore confirm that the peaked form of diurnal emergence in the field is due largely to soil-temperature.

1.12 Effect of soil-moisture on emergence.

Adults may emerge from a wide range of soil-moistures. In theory, two extremes (A & B) prevent emergence.

A. During periods of high temperature and low rainfall the moisture content of the upper layers of the soil (0-2.5 cm.) may be reduced to a point where the atmosphere of the pore spaces is unsaturated with water vapour. In such conditions pupae lose water and may die before they are ready to emerge.

B. Pupae may vacate their cocoons and surface when the soil is saturated with water. Under these conditions eclosion is inhibited. Table 4 shows the results of an

experiment which demonstrates the effect of soil saturation on emergence and the ability of pupae to survive short periods of immersion. 20 larvae were placed in moist soil in each of 10 rearing tubes and incubated at 20°C. On the day before emergence was due to begin, five of these tubes were stood in water. The level of the water was maintained about 0.5 cm. below the soil surface. During the next five days (Period I) the number of adults emerging from each tube was recorded. At the end of this time, the "wet" tubes were drained and observations continued for a further five days (Period 2). The numbers of adults emerging during each period from the control and the "wet" tubes are shown in Table 4. In the control tubes 82% of individuals emerged but only 32% in the "wet" tubes. Moreover most of the successful individuals in the control tubes (78 in 82) emerged in Period I while most in the wet tubes (29 in 32) emerged in Period 2. Clearly soil-saturation can cause the death of some individuals and retard the emergence of others.

1.13 Depth of pupal cocoon in the soil and its effect on adult emergence.

The results of two experiments which follow show conclusively that for successful emergence pupae must develop near the surface of the soil.

In the first experiment larvae were allowed to pupate

Table 4. Emergence of adults of C.nasturtii from moist (control) and saturated soil surfaces

Replicates (20 larvae per tube)	Number of adults emerging			
	Period 1 (0-5 days)		Period 2 (5-10 days)	
	Control	Wet	Control	Wet*
A	17	1**	1	5
B	19	0	0	7
C	15	1**	1	4
D	11	1**	2	3
E	16	0	0	10
Totals	78	3	4	29

* = drained

**adults emerged from pupae which climbed away from the wet soil surface.

in rearing tubes at their "natural level". Ten tubes were filled with soil to a depth of 2.5 cm. and 10 larvae added to each. After incubation for four days at 20°C. (when pupation would occur) five pairs of tubes were selected at random and the soil depth in four of them increased by 1.0, 2.5, 5.0 and 7.5 cm. through adding soil of uniform particle size (less than 2.0 mm.). Where necessary the tubes were extended in length to accommodate the extra depth of soil. Each tube was tapped for a standard number of times to effect uniform compaction of the soil. All tubes were then soaked, drained and incubated at 20°C. The number of adults and their day of emergence for each tube are shown in Table 5. Ten days after emergence started the soil in each tube was washed through a fine sieve (100 mesh per inch) and empty pupal cocoons and dead pupae counted (Table 5A).

No emergence occurred in the tubes with more than 1.0 cm. of additional soil. In these tubes nearly all the larvae pupated but the pupae died after vacating their cocoons (see numbers of empty cocoons and dead pupae, Table 5A). Three adults emerged after an extended period of time in the tubes with a 1.0 cm. layer of soil. Again, many dead pupae were recovered. In the controls emergence was complete, all but one larva which was parasitized, eventually producing adults.

Table 5. Adult emergence of C.nasturtii through different depths of soil.

Days after first emergence	Depth of soil (cms.) 0*		1.0		2.5		5.0		7.5	
0	2	1	-	-	-	-	-	-	-	-
1	2	2	-	-	-	-	-	-	-	-
2	5	7	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	1	1	-	-	-	-	-	-
10	-	-	1	-	-	-	-	-	-	-
Total	9	10	2	1	0	0	0	0	0	0

*Each treatment consisting of two replicates, each with ten larvae

Table 5A. Residual cocoons, pupae etc. remaining in soil

	Depth of soil (cms.) 0		1.0		2.5		5.0		7.5	
Empty pupal cocoons	9	10	10	10	9	10	10	10	10	10
Dead pupal cocoons .	0	0	0	0	1	0	0	0	0	0
Dead pupae	0	0	8	9	9	10	8	9	10	10
Parasitized larvae .	1	0	0	0	0	0	1	0	0	0
Adults emerged	9	10	2	1	0	0	0	0	0	0

It is clear that successful emergence can only occur when pupae develop near to the soil surface, at their "natural level".

It is conceivable that the ability of pupae to move up through soil may depend on the size of the soil pore spaces. The following experiment was designed to test this. Using sieves of the requisite mesh three grades of a soil were prepared with the following particle size:-

P_1 ..... less than 2.0 mm.
P_2 ..... 2.0-3.0 mm.
P_3 ..... 3.0-5.0 mm.

The procedure was the same as in the preceding experiment in that larvae were allowed to pupate at their "natural level" and then that level was changed by addition of soil. The three particle sizes were used with three depths (0.0, 1.0 and 2.5 cm.) of additional soil, and there were three tubes for each depth/particle-size treatment, making 27 tubes in all, each starting with 50 larvae allotted at random from a field collection. This experiment differed in that there were considerable amounts of both diapause and parasitism among the larvae. The parasitism provided an opportunity for comparing emergence of the adult parasite, Synopeas sp. B (see Section 5.1) with that of its host.

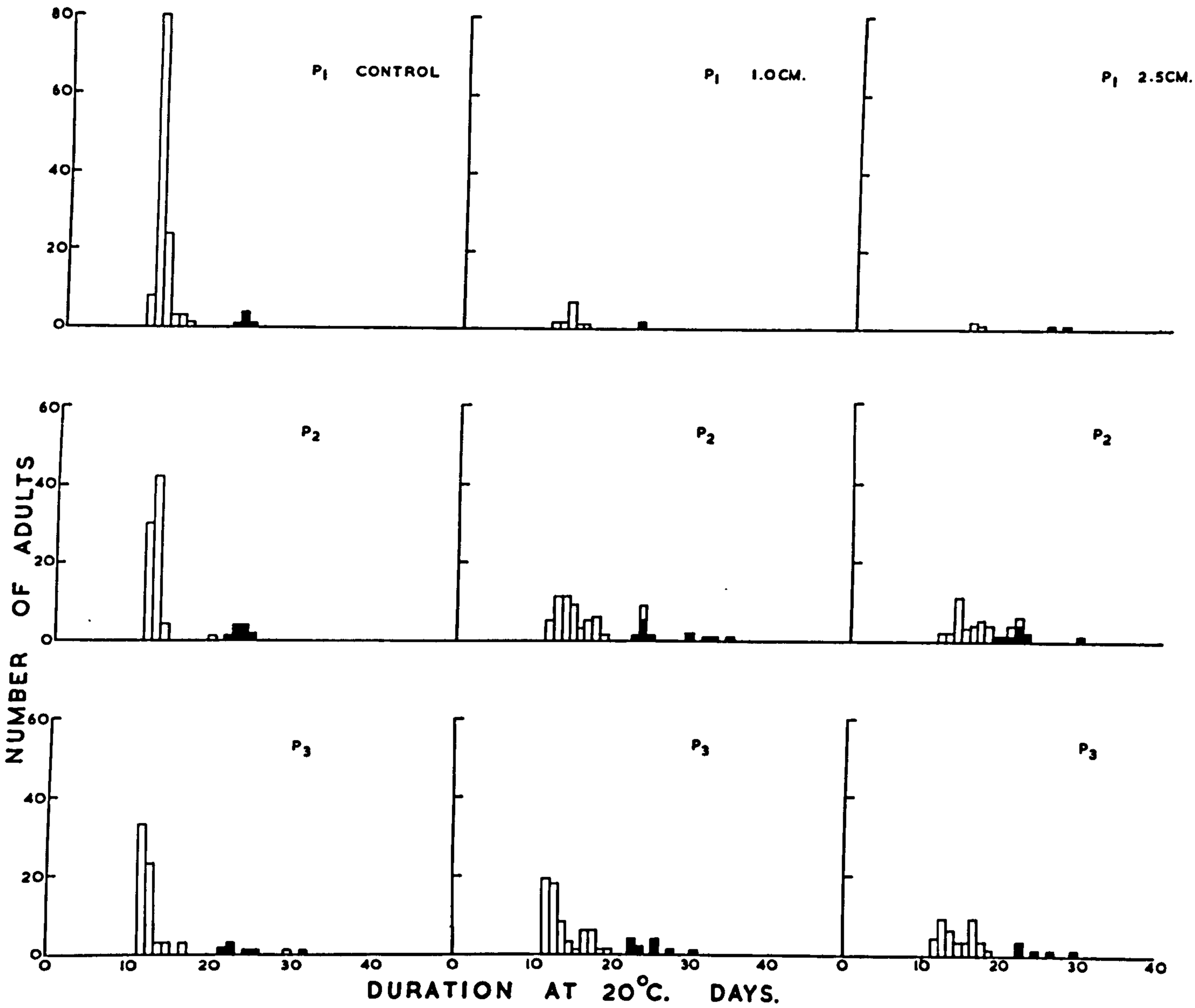
The total day by day emergence of adults from each group of tubes is shown in Fig. 4. It is apparent that

FIGURE 4.

The emergence of adults of C.nasturtii and a parasite, from three depths of soil of differing particle size (daily emergence totals from three replicates per treatment)

P_1, particles less than 2.0 mm.
P_2, particles 2.0-3.0 mm.
P_3, particles 3.0-5.0 mm.

White columns: adults of C.nasturtii
Black columns: adults of Synopeas sp. B


P1 CONTROL
P1 1.0CM.
P1 2.5CM.
P2
P3
NUMBER OF ADULTS
DURATION AT 20°C. DAYS.

the number of adult midges emerging diminished with increasing depth of soil cover, but that the reduction was less marked as the size of soil particle increased. In addition the time taken to emerge tended to increase as the soil depth increased. This is apparent in Fig. 4 and is made clearer by tabulating mean emergence times* from day 0 thus:-

	0.0	1.0	2.5
P_1	13.1 (112)	14.3 (13)	15.7 (3)
P_2	13.0 (78)	14.9 (56)	15.2 (37)
P_3	13.5 (66)	14.9 (63)	15.1 (38)

*mean emergence times in days; number of adults in brackets.

Obviously the reason is simply that pupae take more time to reach the surface through deeper layers of soil.

Table 6 shows full details of numbers of adults and of empty pupal cocoons recovered at the end of the experiment. The number of empty pupal cocoons invariably exceeded the number of emerged adults. This simply means that some death occurred after cocoon vacation. As would be expected, the proportion of deaths was greatest in the tubes with the deepest layer of soil of the smallest particle-size (P_1, 2.5 cm.). The higher proportion of deaths in P_2, 0.0 cm. and P_3, 0.0 cm. than in P_1, 0.0 cm., if significant, could be a matter of desiccating conditions

Table 6. Effect of soil depth and particle size on adult emergence of C.nasturtii
Each replicate, R, started with 50 larvae.
Number of adults shown with number of empty pupal cocoons

Soil particle size		Depth of soil cover (cm.) 0		1.0		2.5		
P_1, < 2.0 mm.	R1	43	(46)	7	(43)	3	(43)	
	R2	34	(43)	5	(44)	0	(41)	
	R3	35	(38)	1	(41)	0	(40)	
Total		112	(127)	13	(128)	3	(124)	128 (379)
P_2, 2.0-3.0	R1	24	(38)	16	(46)	12	(45)	
	R2	31	(42)	27	(44)	12	(46)	
	R3	23	(44)	13	(39)	13	(44)	
Total		78	(124)	56	(129)	37	(135)	171 (388)
P_3, 3.0-5.0	R1	9	(24)	22	(39)	19	(42)	
	R2	29	(40)	24	(37)	11	(41)	
	R3	28	(43)	17	(37)	8	(37)	
Total		66	(107)	63	(113)	38	(120)	167 (340)
Depth totals		256	(358)	132	(370)	78	(379)	466 (1107)

Table 6A. Analysis of Variance

Variation due to:	df	Sum of Squares	Mean Square	F	p
Depth of soil	2	1850.97	925.49	29.46	<.001
Particle size	2	125.41	62.71	2.00	<.1
Interaction (D x P)	4	1007.48	251.87	8.02	<.001
Error	18	565.33	31.41	-	
Total	26	3549.19	-	-	

arising in small amounts of soil (3-5 gm.) of large particle-size.

An analysis of variance (Table 6A) shows that a significant reduction in the number of adults emerging occurred with increasing depth of soil (V.R. = 29.46, with 2 and 18 df., $p < .001$). The effect of soil particle size alone on emergence was not significant (V.R. = 2.00, with 2 and 18 df., $p > 0.1$) but the highly significant interaction variance (V.R. = 8.02, with 4 and 18 df., $p < .001$) demonstrates that the success of pupae in emerging from unnatural depths really does partly depend on soil particle size, larger size permitting easier movement because of larger pore space.

Numbers of parasites (Synopeas sp. B) from each tube are given in Table 7. Analysis of variance (Table 7A) shows that neither depth of soil cover nor size of soil particle influenced their emergence. The adult parasite seems well adapted to movement through the soil by reason of the small size and general conformation of its body (see Plate B, Section 5.1).

Finally, the larvae remaining in diapause in each tube were recovered from the soil and counted (Table 8). Inspection of the results shows that the treatments had no effect on the incidence of diapause.

The foregoing experiments were undertaken partly to

Table 7. Effect of soil depth and particle size on emergence of Synopeas sp. B. (Hymenoptera, Proctotrupoidea), a parasite of C.nasturtii

Soil Particle size		Depth of soil (cm.) 0	1.0	2.5	
P_1, < 2.0 mm.	R1	0	2	3	
	R2	6	3	1	
	R3	1	2	0	
Total		7	7	4	18
P_2, 2.0-3.0 mm.	R1	3	3	4	
	R2	4	3	1	
	R3	4	3	4	
Total		11	9	9	29
P_3, 3.0-5.0 mm.	R1	1	5	3	
	R2	4	3	2	
	R3	3	4	2	
Total		8	12	7	27
Depth totals		26	28	20	74

Table 7A. Analysis of Variance

Variation	df	Sum of Squares	Mean Square	F	p
Depth of soil ..	2	3.86	1.93	0.87	>.1
Particle size ..	2	7.63	3.82	1.72	>.1
Interaction	4	3.70	0.93	0.42	>.1
Error	18	40.00	2.22	-	
Total	26	55.19	-	-	

Table 8. The numbers of diapause larvae in the soil-depth/particle-size experiment (see Fig. 4 & Tables 6, 7)

Soil particle size		Number of diapause larvae Depth of soil (cm.) 0	1.0	2.5	
	R1	2	6	6	
P_1, 2.0 mm.	R2	7	3	3	
	R3	10	6	7	
Total		19	15	16	50
	R1	10	4	4	
P_2, 2.0-3.0 mm.	R2	1	4	4	
	R3	2	6	6	
Total		13	14	14	41
	R1	2	4	5	
P_3, 3.0-5.0 mm.	R2	8	9	4	
	R3	5	8	7	
Total		15	21	16	52
Depth totals		47	50	46	143

explore the unexplained suggestion of Hörnig (1953) that some measure of control might result from intensive inter-row cultivation of swedes at the time when a large proportion of the midge population is in the pupal stage. Hörnig may have been thinking merely of the possibility of mechanical damage to pupae. But since moving the soil changes the depth at which cocoons lie, the present experiments show another way in which such cultivations could be effective: it could bury many cocoons too deep for successful emergence. It is also worth noting that inter-row cultivation could be expected to have a severer effect on the midge than on its parasite population.

1.2 Vertical distribution.

A restricted vertical distribution of adult activity is suggested from the field trapping results of 1959. Adults were trapped on transparent glass slides ($3\frac{1}{2}$ x $3\frac{1}{2}$ inch) coated on both surfaces with a uniform thin layer of a commercial tree-banding grease (so-called impaction traps). Each trap had one pair of plates at each of four heights above the ground - 12, 24, 36 and 48 inches. The plates were collected at weekly intervals, scanned beneath a binocular microscope and the number and sex of C.nasturtii counted. The totals of females caught at each height for each of 10 traps during 1959 are shown in Table 9A. Most females (98 in 106) were caught at or below 24 inches.

Table 9. Vertical distribution of adults in the field, 1959 (Wheldon, Nafferton) C.nasturtii

A - Females

Distance above the Soil surface	Trap number 1	2	3	4	5	6	7	8	9	10	Total	Mean	$s_{\bar{x}}$*
12"	16	3	4	7	6	7	7	6	7	14	77	7.7	1.300
24"	0	3	2	3	6	1	2	2	0	2	21	2.1	0.548
36"	0	2	1	0	1	1	0	0	1	0	6	0.6	0.221
48"	0	0	1	0	1	0	0	0	0	0	2	0.2	0.134
Total per trap	16	8	8	10	14	9	9	8	8	16	106		

* = Standard error of mean

Analysis of Variance

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between heights ...	3	360.1	120.03	23.35	< .001
Error	36	185.0	5.14		
Total	39	545.1			

Table 9B. Vertical distribution of adults in the field, 1959 (Wheldon, Nafferton)
C.nasturtii

B - Males

Distance above the soil surface	Trap number										Total	Mean	$s_{\bar{x}}$*
	1	2	3	4	5	6	7	8	9	10			
12"	3	0	3	0	3	1	0	3	0	4	17	1.7	0.518
24"	0	1	0	2	1	1	0	0	1	1	7	0.7	0.213
36"	0	0	2	0	0	1	0	0	0	0	3	0.3	0.213
48"	0	0	0	0	0	0	1	0	0	1	2	0.2	0.133
Total per trap	3	1	5	2	4	3	1	3	1	6	29		

* = Standard error of mean

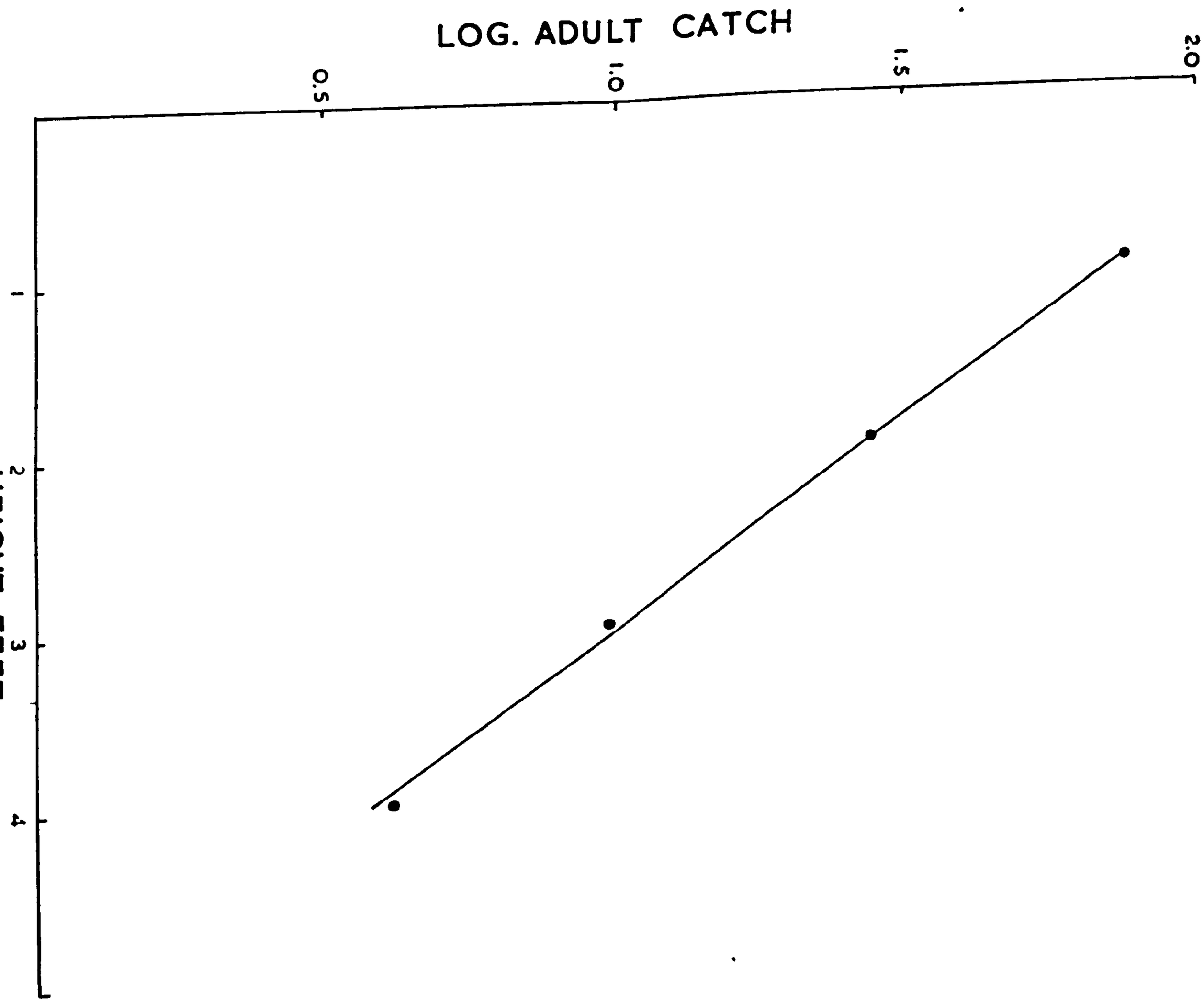
Analysis of Variance

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between heights	3	14.075	4.692	4.981	< .01
Error	36	33.900	0.942		
Total	39	47.975			

FIGURE 5.

The relationship between log. catch and height above ground

(*C.nasturtii* totals from weekly trapping data, W.Wheldon, 1959)


LOG. ADULT CATCH
0.5
1.0
1.5
2.0
1
2
3
4
HEIGHT. FEET.

The variation of catch with height was highly significant. (V.R. = 23.35, with 3 and 16 df., $p < .001$). Results for males (Table 9B) are similar, 24 in 29 being caught at or below 24 inches. This variation was also significant (V.R. = 4.98, with 3 and 36 df., $p < .01$).

The logarithm of total catch (males and females) is plotted against height in Fig. 5. The apparent linear relationship indicates that the decrease in catch with increasing height is exponential.

It seems that for the most part activity is confined within the canopy of the swede crop.

1.3 Mating.

Most species of midge mate soon after emergence (Barnes, 1956). For example, females of the raspberry cane midge, Thomasiniana theobaldi Barnes, occasionally mate before wing expansion is complete (within 2-5 minutes of eclosion) (Pitcher, 1952).

The following observations concern the post-emergence behaviour of C.nasturtii. Adults emerged in rearing tubes at constant temperature, usually 20°C. On eclosion both sexes run over the soil surface and up the tube sides to rest on the under surface of the upper gauze cap. Here their wings expand, the process being complete 2-5 minutes after eclosion. Females then remain at the top of the tube but males make erratic oscillatory flights with brief

rests on the tube sides. Both sexes readily drink from moist surfaces.

Immediately wing expansion was complete midges were transferred to 2 x 1 inch glass observation tubes containing ½ inch of moist sand. Unless otherwise stated, observations were made in day-light at room temperature, (17-20°C).

Copulation is brief (10-45 seconds) and is always preceded by a characteristic pattern of behaviour on the part of the male. He alights within 2 cm. of his intended mate but continues to vibrate his wings as if still in flight. After a few moments hesitation he approaches the female, still fluttering his wings, and mounts her, clasping her thorax with his pro- and mesothoracic legs and the sides of her abdomen with those of his metathorax. His abdomen is curved downwards and forwards to meet the extended terminal segment of the ovipositor.

Males are not attracted to every female. Virgin females less than about 6-8 hours old, and previously mated females, are completely ignored. The male usually but not invariably perceives and mates with virgin females more than 6-8 hours old. Males released into tubes which seconds earlier contained such females occasionally reacted as if the females were still present. This suggests that females produce a chemical attractant some time after emergence which is perceived and which

stimulates a strong mating urge in the male. Males may copulate with several females in quick succession. In a period of 10 minutes, one male mated with four virgin females (12-24 hours old), the duration of the successive matings being 15, 19 13 and 11 seconds. The fertility of such matings has not been tested.

Five observations of mating were made in the field; these were seen by chance in the course of other work. On three of the five occasions pairs were found <u>in copula</u>, two on the soil surface and one on the under surface of a swede leaf. In each case copulatiom continued for 5-10 seconds. After mating, the male took flight and was lost but the female remained in situ for a further 10 minutes when observation was discontinued. On the remaining two occasions the male perceived the female while he was flying, alighted near her and reacted just as he was seen to do in the observation tubes. Copulation lasted 21 and 39 seconds in each case. The males flew off immediately after copulation but the females remained at rest. After 15 minutes, in each case, I disturbed the female by tapping the leaf. One took flight and was lost at once. The other made a short flight to the under surface of a nearby leaf (about 18 inches distant). Here she remained, quite inactive apart from an occasional protrusion and retraction of the ovipositor, until observation was discontinued (a

further 15 minutes).

1.4 Oviposition.

Oviposition on the swede plant was observed in field and laboratory. The female remains inactive for 1-2 hours after copulation and during this time the ovipositor is alternately extended and retracted, this being the only visible sign of movement. The onset of egg-laying is sudden. The female makes short, dancing flights in among the foliage of the swedes. On encountering a suitable site for eggs, such as the young "heart" leaves of the plant growing point, or the unopened flower bud, she becomes most excited and walks up and down testing the cuticle surface with the extended valvular tip of the ovipositor. The eggs are always laid on the surface and nearly always within the folds of the younger leaves or well down into the unopened flower bud. To this end, the long sinuous ovipositor is inserted deep into the crevices and the eggs are extruded, one at a time, down the narrow vagina. The abdomen pulsates, rhythmically during oviposition and it is likely that this alternate lateral expansion and contraction of the abdomen assists the passage of eggs through the ovipositor. The eggs are deposited in batches held together and firmly attached to the plant cuticle by means of a thin "cement" layer. This layer prevents the eggs from being washed away by rain

droplets; even a powerful water jet failed to remove all the eggs from the growing point of a young swede plant.

A female has several bouts of egg-laying and each bout lasts 2-30 minutes. In one 10-minute bout observed in the field, over 50 eggs were laid. The female seems to restrict her oviposition to a few neighbouring plants, depositing several batches on one plant before flying to the next. The result of this behaviour is seen in the distribution of galled swedes in a field (see Section 3.6).

As mentioned above the female of C.nasturtii mates 6-8 hours after emergence and begins to lay eggs sometime after mating. The oviposition pattern was investigated as follows: 54 females and 34 males (emerged at 20°C. in the dark) were released at 0900 hours, (i.e., within 12 hours of emergence) into a cage containing ten swede plants. The cage walls were of glass (upper, front and rear) and terylene mesh (two sides) and the rear wall was placed next to an east-facing window. Throughout the experiment the cage interior received day-light and varied in temperature between 18 and 21°C. After 24 hours (i.e., at 0900 hours the next day) the ten plants were removed from the cage to a greenhouse and replaced with ten others of equal size and vigourl Despite precautions one or two of the more active males may have escaped from the cage during the transfer. The second group of plants were

removed from the cage at 0900 hours the following day and placed in the greenhouse. By this time most males and some females had died.

This experiment gave the females opportunity to mate and lay eggs over two consecutive 24-hour periods (Day 1 and Day 2), and the totals of eggs laid in each period would be reflected in the totals of larvae developing on the two groups of swedes. After several days, three of the first group of ten plants (Day 1) and five of the second (Day 2) developed leaf galls. The larvae from each infested plant were washed from their galls on to fine nylon gauze and counted. Results are shown in Table 10. More eggs had been laid on Day 2 (369 larvae resulting) than on Day 1 (157 larvae). Clearly C.nasturtii differs from C.triticii and S.mosellana (see Barnes, 1932). In the latter two species, the female not only mates and begins egg-laying within a few minutes of emergence but also completes oviposition in Day 1.

1.5 Productivity, fecundity and fertility.

For convenience, the terms productivity, fecundity and fertility are defined as follows:-

Productivity = number of mature eggs manufactured.
Fecundity .. = number of eggs laid.
Fertility .. = number of fertile eggs laid.

Table 10. Comparison of numbers of eggs laid on the first and second day after adult emergence (C.nasturtii)
Egg numbers reflected in larval numbers.

	No. of plants	No. of plants attacked	Larvae recovered
Day 1	10	3	157
Day 2	10	5	369

Table 11. Number of eggs produced by C.nasturtii

Type of female	Number dissected	Mean number eggs per female	Range	Standard error of the mean
Non-diapause .	50	93.4	38-134	3.11
Diapause	50	96.7	36-141	2.98
Total	100	95.1		

1.51 Productivity.

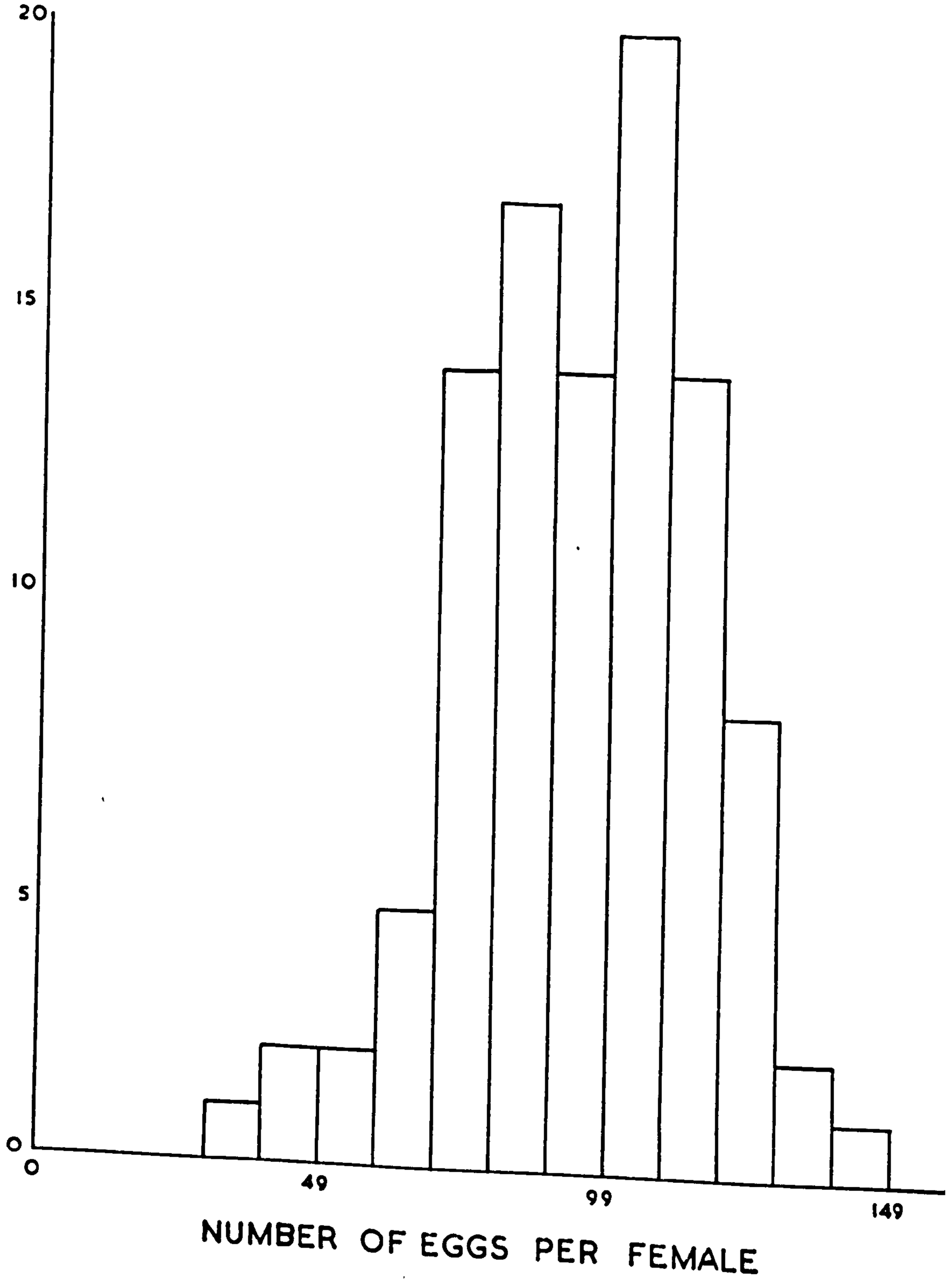
The teneral female (less than two hours old) has two compact ovaries containing many small immature eggs and she has a considerable amount of fat-body in the abdomen. Twelve hours later many eggs are mature and the fat-body is much diminished.

To estimate productivity, females were maintained in observation tubes at 20°C. for 24 hours after emergence. They were then dissected in water under a microscope (magnification X10). At this time the fat-body is practically exhausted and each ovary is neatly packed with mature eggs. The latter were separated for counting partly by squeezing the ovary gently (which caused some eggs to slip through the exit leading to the uterus), and partly by teasing the ovarian membrane (which released the remaining eggs). Results of 100 dissections (Table 11) show that an average female manufactures about 95 mature eggs. There was no significant difference between the mean productivities of females developing from diapause and non-diapause larvae. The combined data for 100 females are shown as a frequency histogram in Fig. 6. The distribution appears to be slightly negatively skewed. This departure from normality, if significant, could arise from discounting immature eggs. The females with fewer eggs may not have matured their full potential by the end of 24 hours.

FIGURE 6.

Frequency histogram showing the number of eggs produced by C.nasturtii

(100 dissections of females more than 24 hrs. old)


20
15
10
5
0
FREQUENCY
0
49
99
149
NUMBER OF EGGS PER FEMALE

1.52 Fecundity.

Unfortunately no data on fecundity are available. Although females may be induced to oviposit on swedes or other hosts, estimation of fecundity is hampered by the very small size, fragility and adhesiveness of the egg. The latter property causes the egg to stick to the plant cuticle and to other adjacent eggs in the batch. It is usually impossible to count the eggs *in situ*, and any attempt at separating them mechanically from one another invariably results in most of them disintergrating and becoming unrecognizable. Fecundity will be mentioned again in Section 1.54 (below).

1.53 Fertility.

The assessment of fertility given here is a by-product of the study of the incubation period at five constant temperatures (see Section 2.2). It is based on small batches or parts of batches of eggs which could be counted because they happened to be arranged symmetrically. These particular batches formed an unknown proportion of the total oviposition of an unknown number of experimental females. They were selected within two hours of being laid. The data are shown in Table 16. From a total of 410 eggs, 344 hatched. Assuming all eggs not hatching to be infertile, this represents a fertility of 83.9%. This assumption is obviously dubious. Some of the eggs that

did not hatch might well have been fertile, and this is certainly suggested by the differences in proportions hatching at different temperatures: 59%, 79%, 87%, 94% and 73% at 10°, 15°, 20°, 25° and 30°C. respectively (χ^2 = 22.75, df = 4, p <.001). It seems likely that our data under-estimate fertility. At all events, they suggest that fertility is quite high in C.nasturtii.

1.54 Progeny per female.

The number of progeny per female is the result of (a) productivity, (b) fecundity, (c) fertility and (d) mortality between fertilization and enumeration of the progeny.

An attempt was made to estimate larval progeny (first and second-instars) of 35 females under certain experimental conditions. Each female was released with one or more males into a cylindrical muslin cage (length 9 in., diam. 6 in.) covering the foliage of a swede plant growing in a 4-inch diameter plant pot. The cages were kept in day-light at room temperature (17-20°C.) and inspected daily. Detailed observation of each female was not possible but the following points were noted: many females (16 in 35) rested continually at the top of the cage; two females were observed in copula and three were seen laying eggs. When the female could no longer be detected (even after disturbing the foliage), the cage was removed and the

plant and soil surface searched. If found she was either dead or nearly so, and she was dissected for egg content. The plant was then removed to a greenhouse. Eventually (about three days after removal) 7 out of the 35 plants developed leaf galls and the larvae (first and second-instars) from each galled plant were washed on to fine nylon gauze and counted.

Results are shown in Table 12. Seven females had oviposited. The mean number of larval progeny per female was 78.7 (range 19-111). Only five of these females were recovered and they had a mean residual egg-content of 15.2 (range 1-46) after death. The data suggest that the number of progeny per female increases with length of adult life. For example, the female on Plant 2 produced 19 progeny, retained 46 eggs and lived for only two days, while the female on Plant 3 laid nearly all of its eggs (111 out of 112) and lived for five days.

Stokes (1953a) carried out similar experiments showing that C.nasturtii reproduces by means of unisexual families, i.e., the progeny of one female are all of one sex. This reproductive phenomenon was generally confirmed in the present work (see Section 3.6) except for one instance when a larva collected from a galled plant in September, 1959 developed into a gynandromorph. The adult head was that of a male, with typical bi-nodal antennal segments, but the

Table 12. Number of progeny (1st and 2nd instar larvae) per female C.nasturtii

Plant number	Larvae recovered	Mature eggs	Day female dissected
1	91	4	4
2	19	46	2
3	111	1	5
4	88	13	2
5	64	12	3
6	79	?	-
7	99	?	-
Total	551	76	

Mean number of progeny per female (n = 7) = 78.7 ± 11.4*
Mean number of eggs remaining ... (n = 5) = 15.2 ± 8.0

*Standard error of the mean.

Table 13. Number of progeny (adults) per female of C.nasturtii (From Stokes, 1953a)

Parent midges		Number and Sex of Progeny	
Male	Female	Male	Female
1	1	0	76
1	1	90	0
1	1	0	23
1	1	54	0
1	1	0	35
1	1	53	0
1	1	0	25
2	1	0	67
1	2	0	20
1	2	31	0
2	2	0	49
1	2	0	65
2	4	0	102
2	2	0	66
1	2	0	45
Totals		228	573
		801	

Mean number of progeny per experiment (n = 15) = 53.4 ± 6.35
Mean number of progeny per female (n = 8) .... = 53.0 ± 8.63

*Standard error of the mean.

remaining body, including the reproductive organs, was female. Other larvae from the same plant produced both normal male and female adults.

Stokes's results are shown in Table 13, and I have used them to calculate the mean number of <u>adult</u> progeny per female. In 8 of the 15 experiments a single female was released over a host plant and the resulting progeny reared to the adult stage in order to determine their sex. In the remaining 7 experiments, more than one female was involved (2-4) though the progeny resulting from each experiment were all of one sex, suggesting that only one female oviposited. However, disregarding these, the mean number of <u>adult</u> progeny per female (in 8 experiments) was 53 ± 8.63, as compared with 79 ± 11.4 for <u>larval</u> progeny (in our 7 experiments).

The variances in all the experiments in this section are comparatively large. Much larger samples would be required to show what one expects ecologically, namely, that productivity $>$ fecundity $>$ fertility $>$ larval progeny $>$ adult progeny.

<u>1.6 Length of adult life</u>.

The effect of temperature on length of life was investigated. Adults were isolated within 12 hours of eclosion in observation tubes containing $\frac{1}{2}$ inch of moist sand, and kept at one of the following temperatures ^{o}C.

10, 15, 20, 25 and 30, in the dark. They were examined every 24 hours and the number of deaths noted; the sand was re-moistened at each inspection. Results show that females live longer than males at all temperatures and that the life-span of both sexes decreases with increasing temperature (Table 14). These results agree with those of Hörnig (1953). When adequate moisture is available temperature speeds up the rate of metabolism with a consequent increase in the rate of depletion of food reserves; adults apparently do not feed though they readily drink from moist surfaces. The experiment was repeated with adults isolated in observation tubes without moist sand, i.e., water was withheld, at three temperatures, 10°, 15° and 20°C. The life-span of both sexes was shorter at all temperatures than in the preceding experiment but its decrease with increasing temperature was still apparent (Table 15).

In these experiments mating, oviposition and to some extent flight, were precluded. In the field, it may be assumed that the life-span is shortened by these activities (as it was in the laboratory, see Section 1.54). Catastrophic factors including predators, mechanical damage by rain drops or adhesion to wet surfaces must further reduce the mean length of life. However, observations of adult behaviour indicate that both sexes restrict their activities

Table 14. Duration of adult life at different constant temperatures (with water) C.nasturtii

Age (days)	Schedule of deaths* Temperature °C.									
	10		15		20		25		30	
0-2	4	1	6	1	4	3	16	13	19	11
2-4	9	5	10	4	8	7	10	19	4	9
4-6	7	3	5	14	7	15	1	6	-	2
6-8	3	7	1	17	1	10	1	3	-	1
8-10	1	10	3	23	3	8	-	5	-	-
10-12	-	11	-	11	1	5	-	-	-	-
12-14	-	9	-	-	-	5	-	-	-	-
14-16	-	1	-	1	-	-	-	-	-	-
Totals	24	47	25	71	24	53	28	46	23	23

*Males on left, females on right.

Table 15. Duration of adult life at different constant temperatures (without water) C.nasturtii

Age (days)	Schedule of deaths* Temperature °C.					
	10		15		20	
0-1	3	4	11	0	12	7
1-2	10	9	12	14	6	14
2-3	2	8	3	10	-	1
3-4	1	1	-	1	-	-
4-5	-	-	-	-	-	-
Total	16	22	26	33	18	22

*Males on left, females on right.

to within the leaf canopy of the swedes (see Sections 1.2, 1.3 and 1.4). There, humidity is higher and temperature usually lower than outside the canopy, and the leaves also provide shelter from rain and possibily to some extent from predation.

In general then, the leaf canopy provides a favourable environment for adult life. An exception to this occurred in July, 1960. After a brief heavy shower of rain at 1500 hours on 29th July, numerous females (normales were seen) were observed struggling on the surfaces of small pools of water which had collected on the leaves and in the leaf axils of the swedes. Occasionally the trapped female escaped the water-surface and continued ovipositing. It was impossible to estimate the proportion of trapped to active females; all that can be said is that the adult population (peak of adult Gen. II) was abundant, every other plant concealing one or more females. After a brief search (15 minutes) eight live females were collected from various pools and dissected. In order of magnitude their residual egg-contents were 4, 14, 19, 29, 34, 42, 57 and 91 (mean = 36.3). In the laboratory, males are frequently trapped on the moist sides of their container, old females (over 2-3 days) less frequently and young females (0-2 days) least of all. In the field, it appears that females of all age groups are equally susceptible to drowning in showery weather. Admittedly, the sample taken was small, but

"spent", "half-spent" and a female with most eggs still to lay are represented.

SECTION 2. THE EGG

2.1 General.

The mature unlaid egg is oval and contained in a delicate smooth chorion. In reflected light the egg is white and opaque but in transmitted light it is partly translucent. At the posterior pole (the pole pointing towards the ovarian exit to the uterus) it tapers abruptly to form a fine and almost straight appendage, the pedicel. Dimensions of 614 mature unlaid eggs (mean and standard error) were:-

Length ..... = 0.37 ± 0.06 mm. (range 0.25-0.41)
Width ..... = 0.09 ± 0.01 mm. (range 0.06-0.11)
Length of pedicel = 0.10 ± 0.02 mm. (range 0.06-0.15).

These values are slightly larger than those given by Olombel (1931) which are:- 0.27 x 0.08 mm. with pedidel 0.06 mm.; but Olombel does not indicate whether his measurements are means or whether they refer to laid or unlaid eggs.

The egg batches on the plant cuticle are just visible to the naked eye as whitish protruberances of the cuticle-surface. Eggs in a batch adhere together and to the cuticle by means of a thin glutinous "cement" layer which is secreted during oviposition. The "cement" is insoluble in water. Several authors state that there are 5-20 eggs per batch but in the present work batches with from 2 to more than 50 eggs were recovered from field and laboratory plants. Eggs in small batches (2-20) are easily counted

as they are neatly arranged, side by side, with their longitudinal axes parallel and pointing in the same direction. Larger batches have no such uniformity; the eggs appear to fuse together forming a dense disorganized mass, and only surface eggs are distinct. The "fusion" appears to be due to large amounts of the secretory "cement" substance.

The same factors which hampered estimation of fecundity (Section 1.54) hampered field sampling for eggs. However, feeding larvae remain near the site where they hatch and so the distribution of eggs is reflected in the distribution of feeding larvae (see Section 3.6).

2.2 Incubation period.

On several occasions an unknown proportion of eggs from an unknown number of experimental females were washed from infested swedes within two hours of being laid, collected on fine gauze and transferred to water. Single eggs and symmetrical batches were selected for ease of counting. These were placed under water in watch glasses and incubated in the dark at the following constant temperatures °C.: 10, 15, 20, 25 and 30. When hatching began watch glasses were inspected every 8 hours (20°, 25° and 30°C.) or 12 hours (10° and 15°C.), and first-instar larvae counted and removed. Eggs and larvae were handled with a fine glass pipette.

Results are shown in Table 16. Clearly the incubation period varies inversely with temperature, being shortest at 30°C. (26 hours) and longest at 10°C. (260 hours). The mean incubation period (hours) and its reciprocal multiplied by 100 (= "average percent. development per hour") are plotted against temperature in Fig. 7 and free-hand curves drawn through the points. The curves in Fig. 7 are typical of insects generally. Davidson (1942, 1944) proposed that the relation between rate of development and temperature might be expressed as the logistic equation

$$\frac{1}{Y} = \frac{K}{1 + e^{a - bx}}$$

where Y represents time required to complete development at temperature x (usually in degrees centigrade); a, b and K are constants; K defines the upper asymptote towards which the curve is trending; b defines the slope of the curve; and a relative to b fixes its position along the x axis. But on statistical analyses of his own and Davidson's best data, Browning (1952a) found that, although the observed points appeared to conform closely to the calculated curve, in fact they deviated significantly from the curve. However, Browning, among others, still maintains that from an ecological point of view the logistic curve still remains the most faithful representation of the trend in rate of development of insects (all stages) under

Table 16. Development of the egg at constant temperature (C.nasturtii)

Temp. °C.	Number of eggs incubated	Number of eggs hatched	Mean incubation period (hrs.)	Range	S*
10 ± 2.0	29	17	260.1	240-300	12.9
15 ± 2.0	94	78	144.4	120-180	12.6
20 ± 0.5	96	83	56.8	48-72	4.2
25 ± 0.5	100	94	31.8	24-48	4.4
30 ± 0.5	91	72	26.1	18-42	3.0
	410	344			

Mean Viability = 83.9%

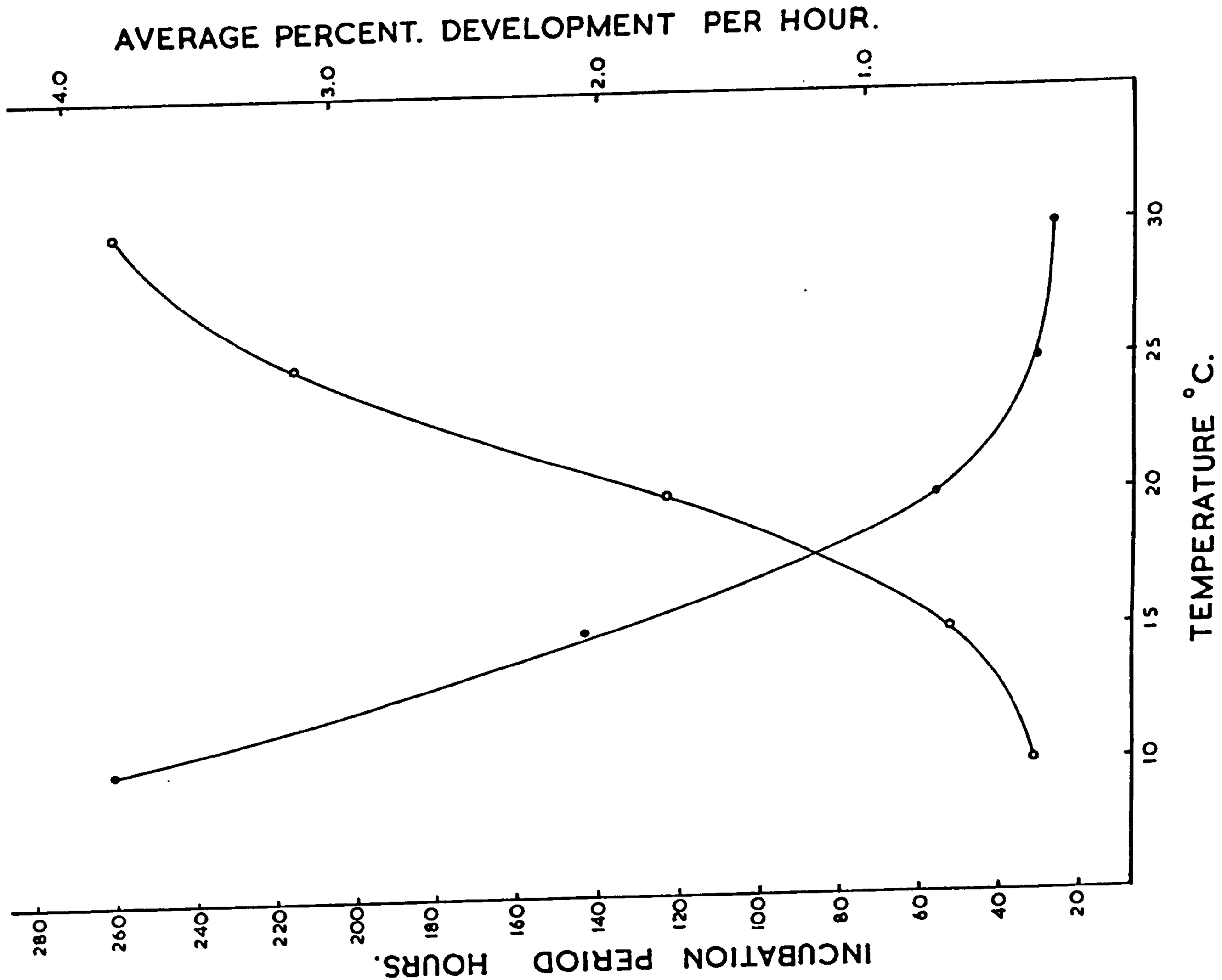
S* = standard deviation

FIGURE 7.

Development of the egg of C.nasturtii
at different constant temperatures

Open circles: average percentage development per hour.

Closed circles: mean incubation period (hours).


AVERAGE PERCENT. DEVELOPMENT PER HOUR.
4.0
3.0
2.0
1.0
30
25
20
15
10
TEMPERATURE °C.
INCUBATION PERIOD HOURS.
280
260
240
220
200
180
160
140
120
100
80
60
40
20

changing temperature conditions. Browning's paper shows that even a relatively simple process such as development of the egg can not be truly represented by a mathematical formula.

Hornig (1953) states that the eggs of C.nasturtii hatch after 5-6 days at 18-19°C. and 12-14 days at 10°C. Fig. 7 gives the mean incubation period at 18-19°C. as 70-80 hours (2.9-3.3 days) and at 10°C. as 260 hours (10.8 days). Hornig's estimates therefore exceed ours by about 2 days. A reason for this discrepancy might have emerged if Hornig had given details of his experimental method.

2.3 Effect of moisture on development.

The preceding experiments (Section 2.2) established that eggs can develop and hatch efficiently while totally immersed in water. Thus the percentages hatching under water at 10°, 15°, 20°, 25° and 30°C. were 59, 79, 87, 94 and 73% respectively, and the over-all mean was 84% (Table 16). It remains to investigate the effect of different relative humidities on eggs.

Four strips of epidermal tissue, bearing egg batches laid over a period of about 4-6 hours, were removed from experimentally infested swedes. Each strip was suspended in one of the following relative humidities: 75%, 85%, 95% and 100%, at 20 ± 0.5°C. in the dark. These humidities were maintained in desiccators (4-inch diameter)

containing appropriate solutions of sulphuric acid (for 75, 85 and 95%), or distilled water (for 100%). Eggs (i.e., the strips) were suspended about ½-1 inch above the liquid surface and inspected daily under a microscope (strips being removed from desiccators for about 2 minutes for this purpose). Briefly the results were as follows.

Eggs at 75% and 85% R.H. failed to develop; those on the outer surface of a batch collapsed after 24 hours and those adjacent to the epidermis lost turgor after 48 hours.

The strip in 95% R.H. bore two batches, one with 5 and the other with 17 eggs. All eggs retained their shape and lustre until the second day (48 hours) when 2 in the first batch and 6 in the second lost turgor; the latter eggs were furthest from the cuticle. After 72 hours the embryo was visible in some eggs and on the following day (96 hours) all eggs remaining turgid in the smaller batch and all but 3 in the larger batch had hatched. The 3 unhatched eggs were white and opaque and showed no sign of development. Thus at 95% R.H., 11 out of 22 (50%) of eggs hatched.

The strip over distilled water bore a single large batch containing about 50 eggs. After 72 hours about 45 eggs hatched leaving a cluster of translucent empty "shells" surrounding one or two opaque infertile eggs. After hatching was finished 43 live first-instar were

recovered from this desiccator.

Although the humidity conditions experienced by eggs in batches on strips of epidermis may be somewhat different from those of the surrounding air, these experiments show that eggs require saturated or near-saturated conditions for development. In humidities which deviate from saturation eggs on the batch surface are more susceptible to desiccation than those within the batch.

In the field, eggs are exposed to conditions of humidity which vary according to their situation on the plant. Most eggs are laid between the folds of the young "heart" leaves and here conditions may be expected to be continually saturated by reason of (a) plant transpiration during the day and (b) the ambient 100% R.H. during the night. Moreover, diurnal temperature fluctuations cause repeated condensation of moisture in the form of small droplets on the cuticle-surface, most evident in the early morning but persisting throughout the day, even in warm dry weather. Some eggs (a minority) are, however, laid on the surfaces of older leaves, and here their survival might be expected to depend on (a) batch situation in relation to ambient conditions (wind, humidity, temperature) and (b) position within the batch.

SECTION 3. THE FEEDING LARVA.

3.1 Hatching, feeding and development of gall.

The larva hatches from the anterior pole of the egg into an environment characterized by (a) abundant food, (b) high humidity and (c) a variable degree of protection from rainfall. As will be seen later, most larvae hatch on the surfaces of the compact "heart" leaves of the swede where humidities are near-saturated (see Section 2.3) and where the probability of being washed away by rainfall is very small; few hatch on older, less crinkled and thus more exposed leaf surfaces. Thus, after hatching C.nasturtii generally runs much less risk of desiccation or of being swept away than, say, the hessian fly, Mayetiola destructor Sayer, a gall-midge of wheat (Triticum). The latter has to journey a considerable distance on the exposed upper surface of the wheat leaf before it reaches its feeding site, the wheat stem (M^{c}Colloch and Yuasa, 1917).

Feeding begins as soon as the larva crawls from the egg. It has two well-developed salivary glands, one down each side of its body. The secretions of these glands dissolve the wax cuticle and "liquefy" underlying cells of the surrounding plant surface. From hatching to the final stadium the larva is immersed in a watery fluid consisting of cell contents, salivary secretions and

excretory products. This fluid is ingested into the alimentary tract for assimilation.

Larvae feed gregariously (eggs being laid in batches) causing destruction to considerable surface areas of host tissue. Bacteria and fungi were never observed invading occupied larval galls (although empty galls are invariably invaded) and it seems probable that larvae produce bactericidal and fungicidal agencies during the feeding phase.

The range of hosts infested by C.nasturtii is extensive (Stokes, 1953a) but the reaction of each host to infestation is similar. Leaves grow by cell extension, the ultimate leaf form being determined by the growth rates of its constituent cells. Feeding larvae destroy cells on one surface of the leaf (upper or lower) but surrounding cells, including those on the opposite surface continue to expand. Consequently larvae are gradually enclosed in the bases of U-shaped folds of the leaf surface. The degree of distortion produced depends partly on the number and distribution of larvae and partly on the growth rates of undamaged cells. Numbers of larvae and cell growth rate tend to be maximal in young leaves and minimal in old leaves. Thus larvae feeding on the young "heart" leaves of the host induce severe leaf distortion ("Central shoot Gall") whereas the

few larvae on old leaves induce only slight leaf distortion ("Outer Leaf Gall"). Outer leaf galls are usually less frequent and always less obvious than central shoot galls. Flower and leaf-bud galls also occur. Eggs are laid within the young buds and larvae feed on the surrounding meristematic tissues. Galled buds remain closed and abortive.

3.2 Growth rate.

A single swede plant bearing eggs laid over the previous 24 hours was kept in daylight at room temperature (18-21°C.) and inspected daily. On the day hatching began (Day 1) and on each subsequent day (until Day 10), 5 randomly selected larvae were removed and immersed in water (at 20°C.). This immersion gradually caused their activity to cease (after about 2 days) and then the fully extended length of each was measured. Results are shown graphically in Fig. 8. Initially (Day 1-3) increase in length is slow suggesting that larvae take some time to establish their feeding-milieu on the host. Growth is most rapid between the third and sixth day (from 0.5-1.7 mm.) and this corresponds approximately with the second stadium. Growth stops at about 2.0 mm. (Day 8-10). The first mature larva vacated the plant on Day 10.

3.3 Larval stadia.

Möhn (1955) has studied the morphology and taxonomy

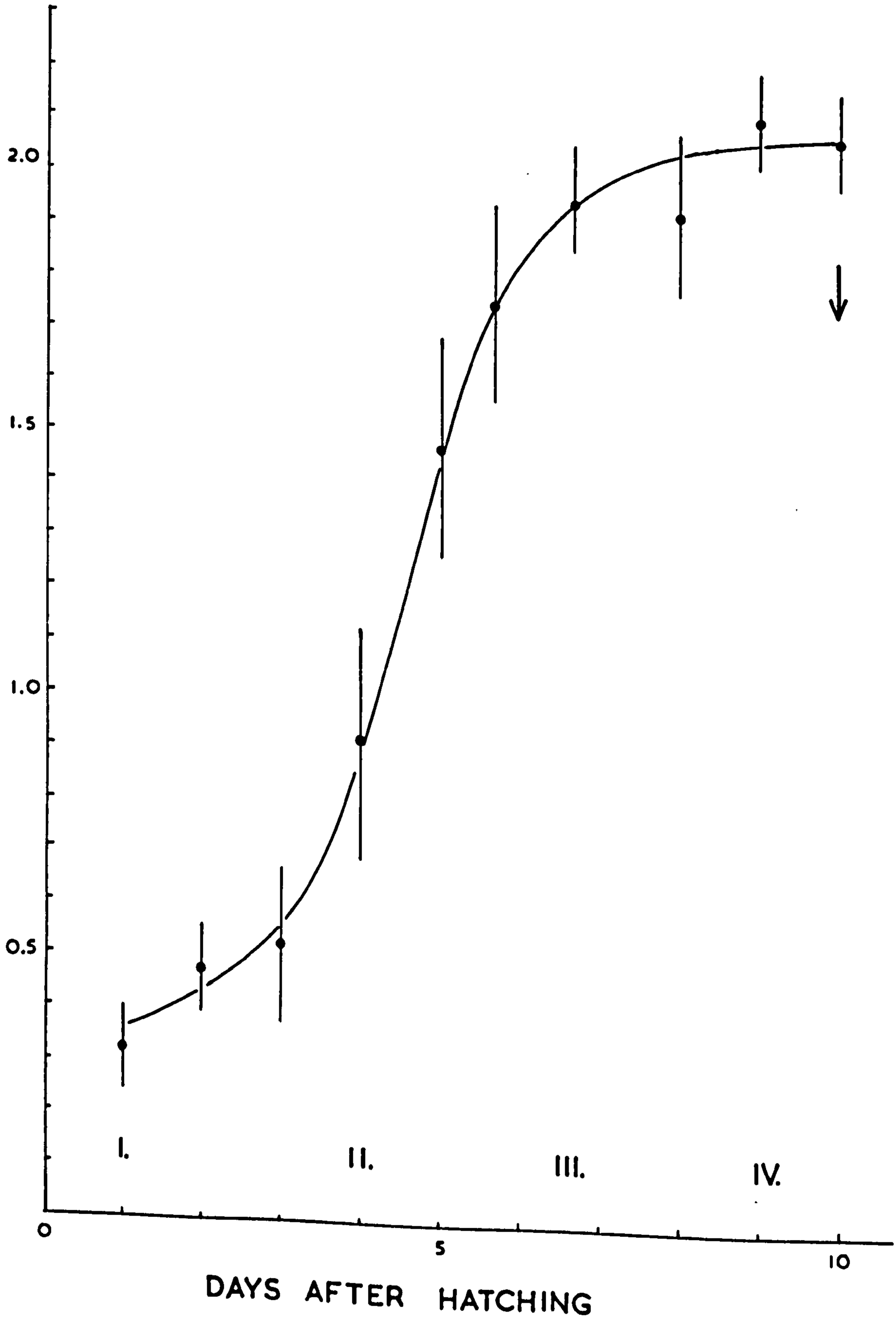
FIGURE 8.

Growth rate of feeding larva at room temperature (18-21°C.)

Mean denoted by dot, and twice its standard error by vertical line through dot (n = 5)

I-IV denote larval stadia

Arrow indicates commencement of larval migration from plant to soil


2.0
1.5
1.0
0.5
LENGTH MMS.
I.
II.
III.
IV.
0
5
10
DAYS AFTER HATCHING

of mature (final-instar) larvae of cecidomyids but preceding stadia have received little attention. Metcalfe (1933) briefly describes four larval stadia of Dasyneura leguminicola Lint. Pitcher (1952) states that T.theobaldi has only three stadia but does not describe them. So far, no author has ventured an opinion as to the number of larval stadia in C.nasturtii.

Field collections of feeding larvae of C.nasturtii occasionally contained individuals, varying considerably in size, actually in the process of moulting (the larval exuvium being still attached to the posterior segments of the body). This suggested the existence of several stadia in larval development. In the preceding growth experiment (Section 3.2), larvae in each age-group (i.e., Day 1, Day 2 ..... Day 10) were therefore examined under high magnification. This established that four stadia apparently occur, each of about 2 days duration at 18-21°C.

The newly hatched larva is extremely small and translucent. Its cuticle is smooth and laterally placed spiracles occur on segments 4-11 inclusive. Subsequent stadia (II-IV) have an additional pair of spiracles on the first "prothoracic" segment, as well as numerous transverse rows of minute cuticular denticles on the ventral anterior margin of each segment. The third stadium differs from the second only in that the faint outline of the sternal spatula (an obvious feature of Instar IV) can just be

discerned. The internal organs of the second and third stadia are clearly visible between the laterally accumulating fat-body. The fourth-instar contains a solid mass of yellow fat-body which completely masks its internal organs. On the ventral surface of segments I and II it also has the pigmented sternal spatula or "breastbone", a structure common to most mature gall-midge larvae. In C.nasturtii (as in other Contarinia spp.) the anterior section of the sternal spatula is bi-lobed.

3.4 Speed of development at constant temperature.

Three swede plants, each in a 4-inch diameter pot, were infested naturally with eggs over an 8-hour period and then kept in the dark (in an incubator) at constant temperatures, respectively 15°, 20° and 25°C. The pots were inspected daily. In each case, when larvae were almost mature, the plant was removed from the pot and its roots washed free of soil. It was then placed in a large beaker containing about 1-inch of water and returned to the incubator. Although the treatment caused abnormal plant growth (leaves became yellow and coarse textured), larvae matured, vacated the plant and were trapped in the water below. Numbers of larvae (in water) were recorded daily. The duration of the larval feeding phase at each temperature was estimated by subtracting the mean egg incubation period (see Section 2.2, Table 16) from the mean

Table 17. Speed of development of feeding larva at constant temperature, C.nasturtii

Temp. °C.	Method*	Mean duration of larval stage (days)	Range (days)	Number of larvae recovered
15	A	28	24-32	63
	B	27	24-29	29
20	A	11	10-14	94
	B	11	10-12	14
25	A	7	6-9	50
	B	7	6-8	7

*Method A - Reared from eggs; incubation period of egg accounted for.

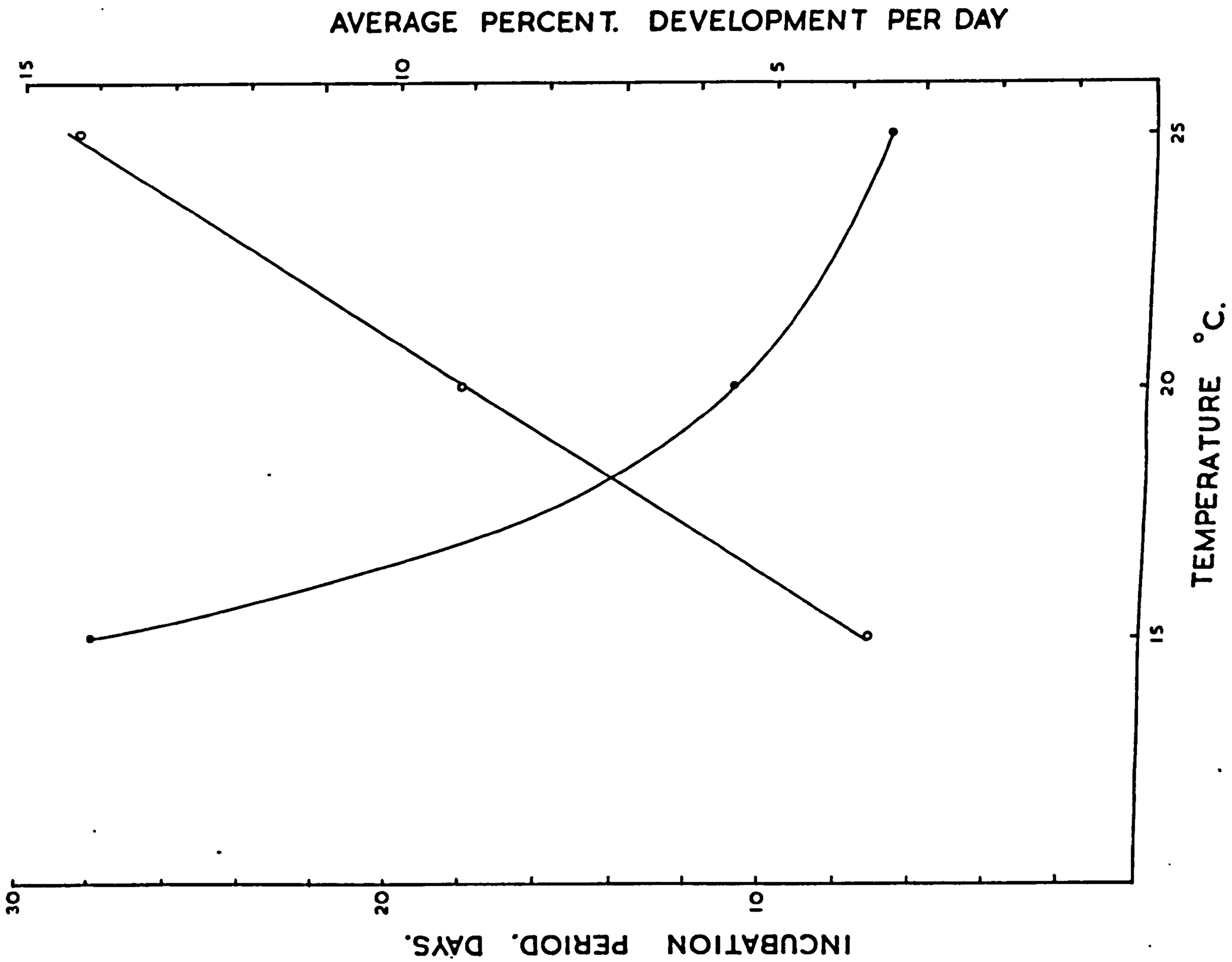
Method B - Reared from first-instar larvae.

FIGURE 9.

Speed of development of feeding larva
at constant temperature
(C.nasturtii)

o: average percentage development per day.

●: mean duration of feeding larval stage (days).


AVERAGE PERCENT. DEVELOPMENT PER DAY
15
10
5
0
25
20
15
TEMPERATURE °C.
30
20
10
INCUBATION PERIOD. DAYS.

time elapsing between oviposition and plant vacation. Results are shown in Table 17 (Method A). As one expects, the duration of the feeding period is longest and most variable at 15°C., the mean being 28 days (range 24-32), and shortest and least variable at 25°C., mean 7 days (6-9).

In a second experiment (Table 17, Method B), the procedure was identical in every respect except one, namely, the swede plants were artificially infested and not with eggs but with newly hatched larvae. These larvae were introduced in droplets of water. The results from Method B are practically the same as from Method A.

Results from Methods A and B combined are shown graphically in Fig. 9. Among others, Hörnig (1953), from field observations, suggested that rate of development varies inversely with temperature. Fig. 9 confirms this and shows that the relation is linear, at least between 15° and 25°C.

3.5 Speed of development in the field.

Previous estimates of the duration of the larval feeding period in the field vary between 7-9 days (Frickhinger, 1943) and 25-30 days (Hörnig, 1953), depending on local and seasonal weather conditions. For example, Hörnig (1953) states that the feeding period lasted 10-12 days in July (Generation I), 12-14 days in August

(Generation II) and 25-30 days in September (Generation III) of 1952 (observations in Schleswig-Holstein). It may be seen in Section 3.4, Fig. 9, that the range of feeding period durations given by Hörnig (10-30 days) would occur at constant temperatures between about 14° and 22°C.

On days between 14th June and 2nd July 1959, females of Generation I were observed apparently ovipositing on the central shoot leaves of several healthy swede plants in West Wheldon field (Fig. 10). Eleven of these plants were tagged and examined daily. Larvae hatched on nine of the eleven plants (see Table 18). Primary symptoms of attack (CI) were seen after 4-7 days as slight distortion and liquefaction of young leaf surfaces. These primary symptoms probably result from larvae that have hatched on the preceding day. Plants showed moderate crumple leaf, C2 (an arbitrary classifaction) after from 7-14 days, and finally, after 13-18 days, eight plants were classified as having severe central shoot galls (C3).

Migration of larvae from plant to soil began 3-4 days after C3 symptoms were recorded, and was followed by surrounding the base of each plant with a square sheet of black cardboard (48 x 48 inch) coated on its upper surface with a layer of tree-banding grease. Nearby plants were uprooted so as not to interfere with results. Each sheet

Table 18. Development of the feeding larva in the field, C.nasturtii

	swede plant								
Day	1	2	3	4	5	6	7	8	9
0	14/6*	21/6	23/6	23/6	23/6	23/6	24/6	30/6	2/7 1959
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	C1
5	C1**	-	-	C1	C1	-	-	C1	
6		-	C1			C1	C1		
7		C1							C2
8									
9						C2			
10	C2				C2		C2		
11			C2	C2					
12								C2	
13									C3
14	C3	C2							
15					C3	C3		C3	
16				C3			C3		
17									1
18	3***	C3							14
19	19								15
20	34				1			4	9
21	30		11	7	1	20		11	16
22	3		1	33	11	25	1	24	1
23	-	8	-	6	11	13	3	5	-
24	-	1	1	6	4	8	17	6	1
25	-	14	-	1	13	1	17	1	-
26	-	20	-	5	1	-	6	-	-
27	-	3	-	-	3	-	8	1	-
28	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	1	-	-
Total	89	46	13	58	45	67	53	52	57
Mean Temp. °C.	17.0	16.1	16.1	16.1	16.1	16.1	16.0	17.5	18.0

*Oviposition dates.

** Symptoms of larval attack .....
- C1, very slight crinkle of young leaves: eggs probably hatch day previously.
- C2, moderate crumple leaf.
- C3, severe distortion of leaves and petioles.

***Numbers of mature larvae leaving the plant.

had a 2-inch diameter hole at the centre to accomodate the plant stem (not yet a bulb) and had to be slit from one edge to the central hole in order to get it into position on the soil surface. In addition each sheet was marked out from the centre in a series of concentric circles (radial increments of 3 inches), the innermost circle being 8 inches diameter (including the central hole). Sheets were placed in position when C3 symptoms were noted, i.e. 3-4 days before larvae matured. Larvae vacating their galls adhered to the sheet surface where they fell and numbers in each concentric belt were recorded. Daily totals for each sheet (i.e. plant) are shown in Table 18. Totals at various distances (belts) from the plant stem are considered in Section 4.41.

Assuming that the feeding period commenced the day before primary symptoms (CI) were noted and terminated the day larvae vacated the plant, the mean duration of the feeding period varied between 16 and 19 days. The mean shade air temperature in which each plant's larval population developed (see Table 18) was calculated from thermograph charts, continuous records being made in a screen situated about 500 yards to the north of the study area. Larvae on plants 2-7 inclusive developed in almost the same mean ambient temperatures (16.0°C.), eggs being laid on these plants between 21st and 24th June. Larvae on

plants 1, 8 and 9 developed in ambient mean temperatures of 17.0°C., 17.5°C. and 18.0°C. respectively. Results in Table 19 suggest that the duration of the larval feeding phase at field temperature fluctuating with a mean x°C. is the same as at constant temperature x°C. This seems to be at variance with Uvarov (1931, p. 68) who says:

> "It is too early to attempt to draw any definite conclusions from the evidence ..... One point, however, is beyond dispute, namely that fluctuations in temperature are not without effect on the rate of development. This effect is often positive, particularly when a favourable temperature alternates with a lower one below the zero of development (but not low enough to be injurious), while an alternation with high temperature is usually harmful. In any case, the impossibility of using average temperatures in the exact estimation of the influence of actual weather conditions on the development of insects is abundantly clear".

But if fluctuation does not encompass temperatures outside the range where the rate/constant temperature relation is linear, there would be no difference between duration at fluctuating temperature with mean x°C. and at constant temperature x°C. (cp. Sanderson, 1908; Andrewartha & Birch, 1954). This may be the explanation of results in Table 19 because the above-mentioned range is at least 15-25°C. (see Fig. 9) while temperatures in the field never exceeded 25°C. and seldom descended very far below 15°C. (observed limit

Table 19. Duration of feeding larval stage at constant and field temperatures (*C.nasturtii*)

Mean field temperature °C.	Observed mean duration (and range) in field (days)	Calculated* mean duration at constant temperature** (days)	Difference
16.0	19 (16-24)	22	+3
17.0	17 (14-18)	18	+1
17.5	17 (15-23)	16	-1
18.0	16 (14-21)	15	-1

*Calculated from Fig. 9, Section 3.4

**Constant temperature equal to mean field temperature.

11.0°C.).

3.6 Spatial distribution.

In the past, distributional studies of C.nasturtii have been limited to estimation of percentage infestation of various cultivated host plants over fairly extensive areas (Damage Surveys). Results show generally what one expects ecologically, namely, that infestation is highest in areas containing the highest density of the most susceptible host plant. The following paragraphs show results from an intensive though restricted investigation at Nafferton during 1957-1960, dealing with distribution and susceptibility of wild and cultivated hosts, and distribution of feeding larvae on one host, the swede.

3.61 Distribution among wild host plants.

Stokes (1953a,b) established partly by experiment and partly by collection, that C.nasturtii occurs on about 100 species of Cruciferae. Several wild host species included in Stokes's list were common weeds at Nafferton, growing in arable fields, pasture and hedgerows. In particular, charlock (Brassica sinapistrum Boiss.), hedge-mustard (Sisymbrium officinale (L.) Scop.) and wild radish (Raphanus raphanistrum L.) were almost ubiquitous. These species were frequently examined for signs of midge damage (leaf or flower-bud galls) during the 1958, 1959 and 1960 seasons, with wholly negative results. In addition the

following quantitative estimate of distribution and susceptibility of charlock was made in July 1959. Three sites A, B and C were sampled (shown in Fig. 10).

A. Middleton field of spring wheat (1958 swedes)
B. West Wheldon field of swedes
C. West Wheldon, northern field-side of hedgerow.

At each of these sites five random area-samples were pegged out and numbers of charlock counted. Results are shown in Table 20. None of these plants was galled. At this time 10% of swedes in West Wheldon field (Sites B and C) contained feeding larvae*. Soil samples showed (Section 6.3) that adult midges (Generation I) actually emerged in Middleton where larvae from the 1958 population had overwintered (Site A; 0.318 charlock per sq.yd.), migrated to West Wheldon (Sites B and C, 0.156 and 1.34 charlock per sq.yd. respectively) to infest 10% of the swede crop there. The complete absence of galled charlock, particularly in Site A, where midges emerged and where there were no alternative host swedes, strongly suggests that (contrary to Stokes) charlock is not always infested in fields where C.nasturtii is present. In this connection it is interesting to note that Taylor (1912) and Dry (1915) hold that the alleged

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*Full details of swede infestation and density in Sections 3.632 and 6.2.

FIGURE 10.

Plan of part of Nafferton farm showing swede areas of 1958, 1959 and 1960.

Areas A, B and C sampled for charlock.

S_{1-6}, stratification of Middleton swede crop, 1958.

CSS, centric systematic area-sample units in W.Wheldon swede crop, 1959.

AREAS A. B. C. SAMPLED FOR CHARLOCK.

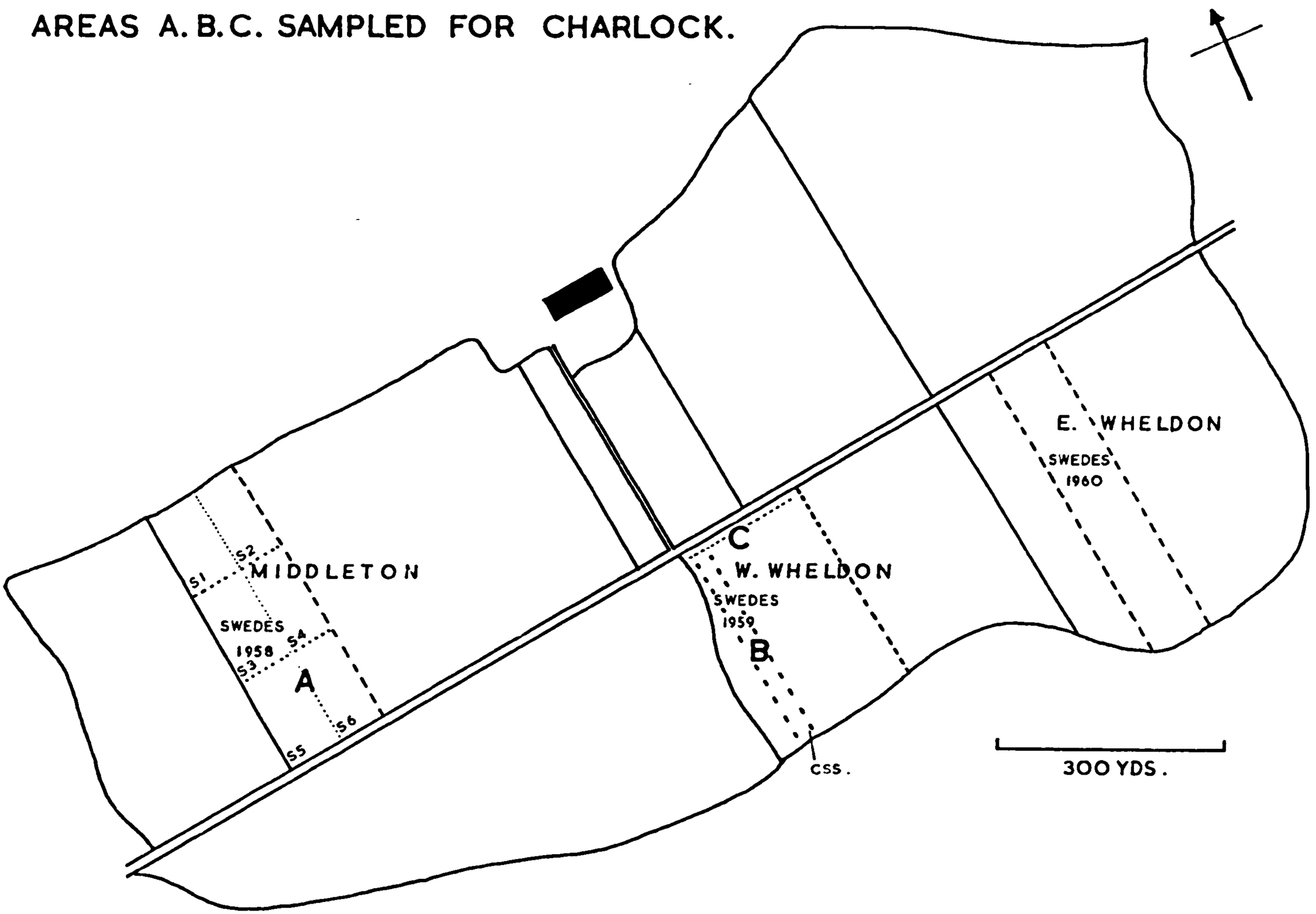


Table 20. Distribution of charlock (B.sinapistrum, Boiss.) at Nafferton, 1959.

Number of sample	Number of plants per sample*		
	Area A	Area B	Area C
1	23	11	7
2	48	19	15
3	17	29	14
4	34	6	21
5	37	13	10
Total	159	78	67
Mean	31.8	15.6	13.4
Density per sq./yd.	0.318	0.156	1.34

*Sample Unit - for A and B, 10 x 10 yards.
for C approx. 10 x 1 yards.

wild hosts of C.nasturtii are infrequently galled, even when adjacent swedes are heavily attacked. They both conclude that wild host plants are of little importance in the economy of C.nasturtii.

3.62 Distribution among cultivated host plants.

According to Stokes (1953a,b) most cultivated Cruciferae are hosts of C.nasturtii and reports of infestation of swedes, cauliflowers, savoys and various rapes are frequent in the literature. Cultivated hosts belong to three species of the genus Brassica

(a) B.Napus ..... swede and swede-like rapes.
(b) B.oleracea ..... cabbage, cauliflower, savoy and marrowstem kale.
(c) B.Rapa ..... turnip and turnip-like rapes.

Some of these plants were grown in a variety demonstration plot at Cockle Park, Northumberland, in 1958. Each plant in this small garden plot (12 x 6 yards) was inspected in August of that year and numbers and approximate positions of galled plants noted (see Fig. 11). Swede (all varieties), thousand-head kale, hungry-gap kale and rape (swede-like) were attacked, about 20% of plants containing feeding larvae (Generation II). Turnip (all varieties), marrowstem kale and kohl rabi were not attacked. Clearly, C.nasturtii had practised definite host selection during oviposition.

FIGURE 11.

Plan of variety demonstration plot of cultivated crucifers at Cockle Park Northumberland, showing approximate positions of plants galled (X) by C.nasturtii

PLANT.	VARIETY.	APPROXIMATE PLAN OF PLOT.	
TURNIP.	GREEN GLOBE, WHITE FLESH.	. . . . .	0
"	RED " , " "	. . . . .	0
"	GREEN " , YELLOW "	. . . . .	0
"	PURPLE TOP. " "	. . . . .	0
"	YELLOW TANKARD. " "	. . . . .	0
"	" " " "	. . . . .	0
SWEDE	ALBION. PURPLE TOP. GLOBE.	. . . X	1
"	" " " "	. X X X . . . X	4
"	MONARCH. PURPLE TOP. TANKARD.	. . . . . X . . . . X . . . X X	4
"	" " " "	X X . . . . . X X X	5
"	EXPRESS. BRONZE GREEN. GLOBE.	X . . . X . . . . . X X	4
"	" " " "	. . X . X . . . . X	3
"	KANGAROO. BRONZE GREEN. TANKARD.	. . . X X X . X	4
"	" " " "	. . . . . X . . . . . X .	2
KALE	MARROW STEM.	. . . . .	0
"	" "	. . . . .	0
"	THOUSAND HEAD.	X . . X X . . . . X X	5
"	" "	. X . X . . . . . X .	4
"	HUNGRY GAP.	. . X . X X . . X X . X . . . X . X X	9
"	" "	. X . . X . . . . . X . . . . . X . .	4
RAPE		. . . . X X . X . . . . . X	4
"		X . . X X . . . . . X . . . . . X X X	7
KOHL-		. . . . .	0
RABI		. . . . .	0

X ATTACKED

VARIETY DEMONSTRATION PLOT. COCKLE PARK. AUG. 1958.

General observations at Nafferton indicated that similar host plant preferences occur, turnip being rarely galled and marrowstem kale never galled, even though adjacent swedes were (or had been) attacked. Plant counts made in July (Generation I) and August (Generation II) of 1959, when midge numbers were relatively high, confirm that striking differences in host susceptibility occur. Rowed crops of swede, turnip and marrowstem kale and a broadcast mixture containing a swede-like rape, were grown in West Wheldon as shown in Fig. 12. On each of the two sampling occasions (i.e. at Gen. I and at Gen. II) ten sample-units of 50 plants from each crop were inspected and numbers galled noted. For row crops, samples were taken at a random point in a randomly selected row; for rape, samples included the nearest 50 plants to each of ten randomly chosen points. Results are shown in Table 21. In the first generation, 10.6% of swedes, 7.4% of rape and 1.6% of turnips were galled. In the second generation infestation levels were much higher being 40% of swedes, 35.6% of rape and 3.0% of turnips. Marrowstem kale was not attacked in either of the two generations. Density of swedes, turnips and marrowstem kale was fairly uniform (4-11 plants per sq. yd.) but that of rape (broadcast) was much higher (10-30 plants per sq. yd.). Unfortunately, as numbers of larvae per infested plant were determined for swedes only (see

FIGURE 12.

Plan of West Wheldon showing arrangement of cruciferous crops (1959).

CSS: systematic area-sample units (24 in all)

WEST WHELDON 1959.

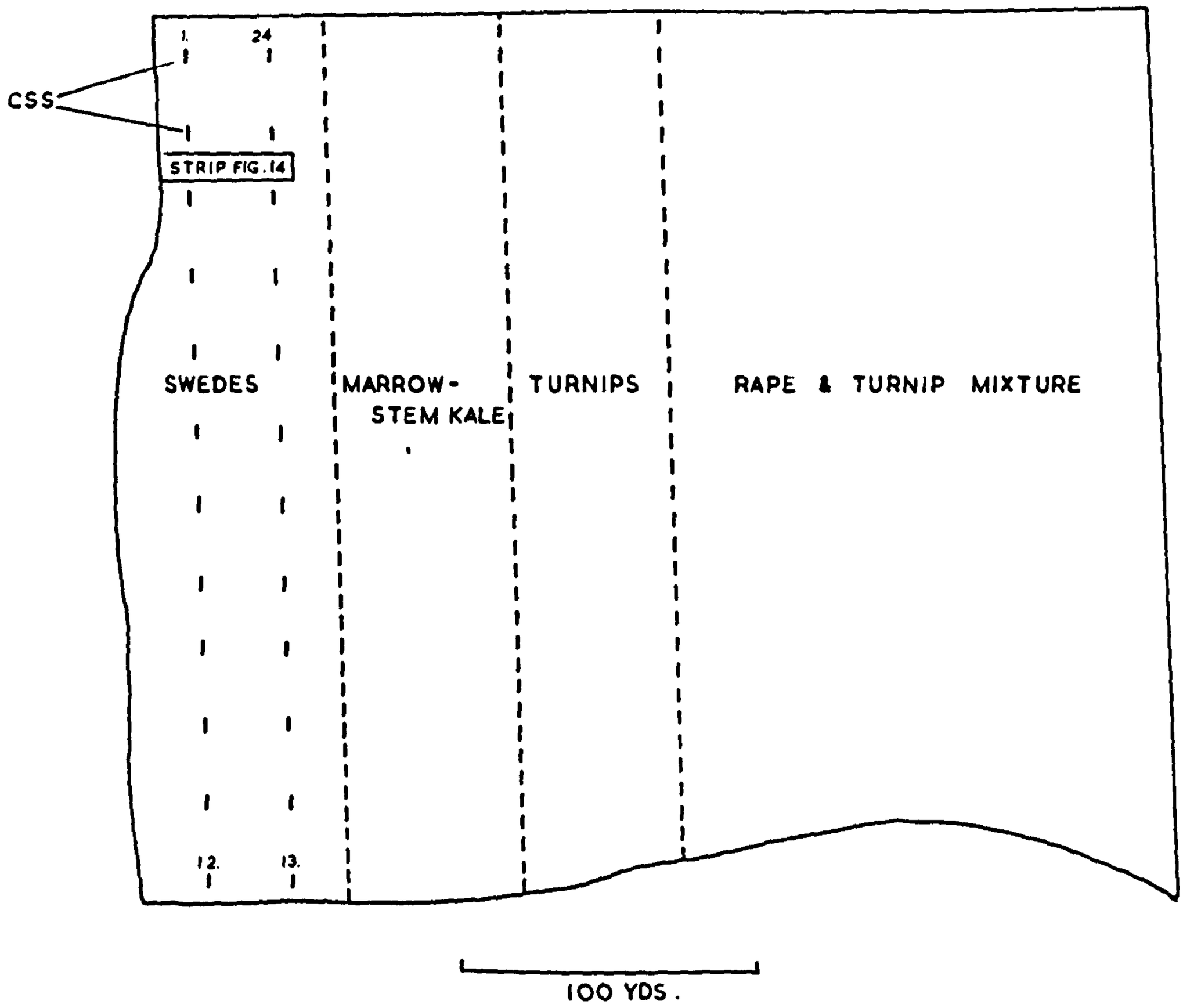


Table 21. Frequency of infestation of four cultivated crucifers by C.nasturtii

Host and Generation		Number of plants attacked per sampling unit										Total	% attack
		1	2	3	4	5	6	7	8	9	10		
Swedes	GI	0	0	11	7	0	1	14	5	9	6	53	10.6
	GII	16	21	19	13	31	6	24	24	34	14	202	40.4
Turnips	GI	0	0	0	0	1	3	0	2	1	1	8	1.6
	GII	4	3	0	0	0	7	1	0	0	0	15	3.0
Rape	GI	0	0	0	8	4	1	4	7	13	0	37	7.4
	GII	11	21	23	29	19	14	6	15	29	11	178	35.6
Marrow stem kale	GI	0	0	0	0	0	0	0	0	0	0	0	0
	GII	0	0	0	0	0	0	0	0	0	0	0	0

*Sample consists of 10 units of 50 plants each in Generation I (GI) and also in Generation II (GII) of C.nasturtii.

Section 3.632), it was not possible to estimate larval numbers per unit area for each crop. However, general observation showed that infested plants of rape and turnip contained considerably fewer larvae than those of the swede.

Finally, in June (Generation I) and August (Generation II) of 1960, percentages of galled plants in East Wheldon (Fig. 10) were, swede 24% and 74%, and turnip 2% and 5%; marrowstem kale remained completely free from attack.

3.63 Distribution of feeding larvae in the swede crop.

3.631 Distribution of galled swedes.

The distribution of potential host swedes is fixed at the beginning of the midge season (June) by mechanical and/or hand-singling operations. As midge numbers increase (from Generation I-III), the numbers of galled plants naturally also increase.

In 1958 the Nafferton swede crop was in Middleton field and there were no alternative hosts growing on the land immediately surrounding it. The crop was divided into six equal strata (see Fig. 10 Middleton, S1-6). At intervals during the summer, five random sample-units, each of 20 adjacent plants, were examined in each stratum and numbers of galled swedes counted. Total galled plants per stratum (100 plants) for each sample (600 plants) are shown in Table 22. Generally, infestation levels were low, being

Table 22. Total number of galled swedes in each of six strata of the 1958 crop.

Date		Strata						Date Totals
		1	2	3	4	5	6	
July	1	0	1	0	0	0	0	1
July	18	0	5	1	2	0	1	9
Aug.	26	4	8	2	12	6	6	38
Sept.	23	2	4	2	6	4	4	22
Oct.	28	1	2	0	1	0	1	5
Totals		7	20	5	21	10	12	75

Analysis of variance

Source of variation	df	Sum of Squares	Mean Square	F	p
Sampling occasion ..	4	30.33	7.58	19.4	<.001
Strata	5	8.86	1.77	4.5	<.001
Interaction .	20	9.51	0.48	1.22	<.01
Error	120	46.80	0.39		
Total	149	95.5			

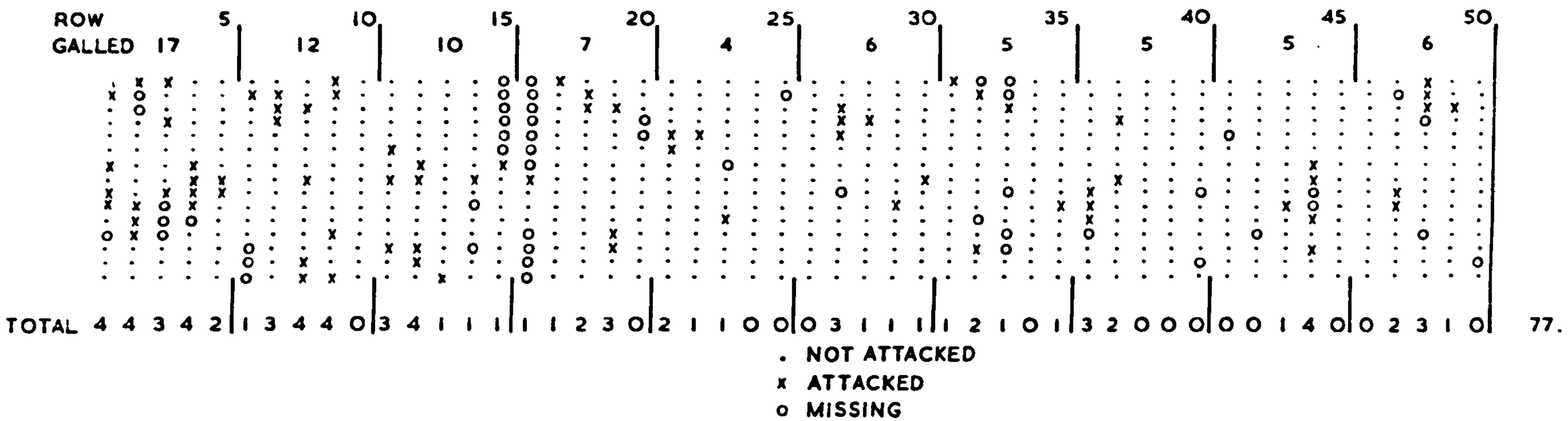
about 0.2% in Generation I (July) and 6.3% in Generation III (August-September). Despite this, however, it was possible to demonstrate significant differences of infestation levels in samples (time) and in strata (space). Analysis of variance (Table 22) shows that the 'Between Strata' variation was highly significant (V.R. = 4.5, with 5 and 120 df., $p < .001$). Positions of the strata (S1-6) are shown in Fig. 10. Only one plant of the 1st July sample was galled - this being from S2 (in north-east corner) but by the end of August infestation had spread over the whole field. That infestation became heaviest in S2 and S4 and lightest in S1 and S3 is probably explained by the fact that the prevailing S.W. winds tended to inhibit adult dispersal in the westerly direction. At any rate the results suggested that galled swedes do not occur over the swede crop at random and this suggestion obviously had to be investigated more fully in the 1959 swede crop which was grown in W.Wheldon. The investigation was made in two ways, (a) and (b).

(a) Enumerated strip.

A section of the 1959 crop was carefully singled by hand in early June to leave (as nearly as possible) one plant per foot of row. Later, when the first larval generation had reached its peak (July 1-7) a narrow strip

FIGURE 13.

Spatial distribution of galled swede plants
(Generation I, 1959) in strip
section of W.Wheldon field
(see Fig. 12)

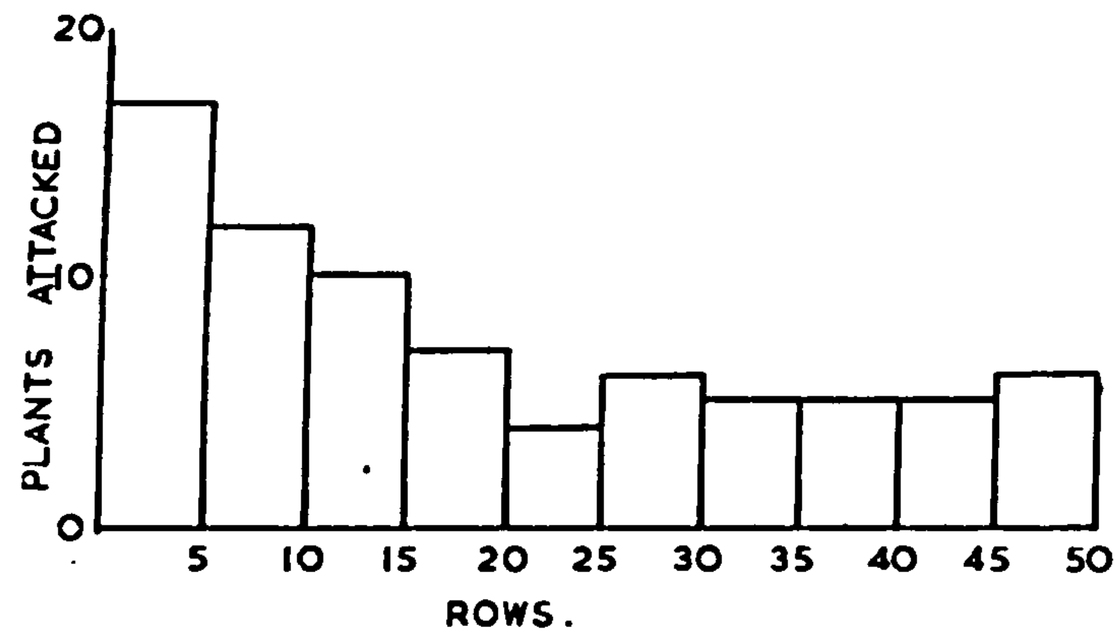


NEAREST NEIGHBOUR ANALYSIS.

N = 77
ϱ = 0.0513
$\sqrt{\varrho}$ = 0.2266
$\bar{R}_A$ = 1.7338
$\bar{R}_E$ = 2.2068

R = 0.7854

C = 3.60 , p<.001



SPATIAL DISTRIBUTION OF ATTACKED PLANTS. JULY 1959.

of this section was pegged out across the drills (Fig. 12). The strip was about 5 yards in width (i.e., 15 evenly spaced plants) and extended over 50 rows. Fig. 13 shows the positions of healthy, galled and missing plants to scale. The strip contained 703 plants of which 77 were galled. Inspection of the Figure suggests (i) that galled plants occur in clusters (i.e. tend to be aggregated) and (ii) that numbers of galled plants increase near the western margin of the field (see histogram). 'Nearest neighbour' analysis (Clark and Evans, 1954) confirms suggestion (i) because $\bar{R}_A/\bar{R}_E = 0.7854$ ($p < 0.001$), $\bar{R}_A$ being the actual mean distance between galled plants and $\bar{R}_E$ the theoretical mean distance expected from random distribution. Concerning (ii) above, taking each row as a replicate with 5 rows per 'treatment' (there being ten 'treatments', respectively Rows 1-5, 6-10, ... 46-50), an analysis of variance shows that there is a significant increase in numbers galled with decreasing distance from the western field margin (Row 1 is W, Row 50 is E), V.R. = 2.51, with 9 and ~~150~~ 40 df., $p < .05$.

Thus, in Generation I of 1959, galled plants were aggregated in clusters which were most frequent near to the western field margin.

(b) Routine sampling.

Distribution of galled swedes in the 1959 crop was studied further, using a centric systematic area-sample

which for statistical purposes may be treated as random, see (Milne, 1959). After singling was completed but before Generation I adults emerged, the crop was divided into 24 equal squares (25 x 25 yards) and a group of 30 plants at the centre of each square individually tagged for subsequent routine inspection. The arrangement of the sample is shown in Fig. 12. Numbers of galled plants in each unit at peaks of larval generations I, II and III are shown in Table 23. The sample is regarded as being in two strata; West (next to hedge) and East (next to marrowstem kale) with 12 units per stratum (i.e. 360 plants per stratum; 720 plants per sample). Analysis of variance shows a highly significant increase in numbers of galled plants with time (generations) which is to be expected, but shows no difference between infestation of the two strata (V.R. = 2.78, with 1 and 66 df., p = 0.1). However, when generations are considered separately, "t"-tests show that significantly higher numbers of galled swedes occur in the western stratum in Generation I ($\bar{x}_W - \bar{x}_E$ = 1.9 galled plants; t = 4.0, df = 22, $p < .001$) though not in Generations II and III. Thus, the second suggestion (ii) of method (a) above is independently confirmed, namely that galled plants are more frequent to the western side of the crop in Generation I. Obviously this difference is gradually ironed out as the season progresses (cp. strata totals for generations I, II and III).

Increased percentage attack of swedes and other hosts

Table 23. Number of galled swedes per sample unit* during 1959 (W.Wheldon)

(*30 plants per sample unit)

Generation	I		II		III	
Strata	West	East	West	East	West	East
	2	3	14	19	29	27
	2	5	19	14	29	27
	2	2	11	12	25	28
	4	1	15	12	26	26
	5	0	12	12	23	28
	4	0	17	12	26	22
	4	1	11	10	27	28
	3	1	16	7	21	23
	6	1	11	8	25	23
	4	0	11	10	26	28
	5	0	11	11	27	29
	3	5	10	14	28	28
Strata Totals	44	22	158	141	312	317

Analysis of variance

Source of variation	df	Sum of Squares	Mean Square	F	p
Generations .	2	6568.9	3284.5	571.2	< .001
Strata	1	16.0	16.0	2.78	= 0.1
Interaction .	2	117.2	58.6	10.2	< .001
Error	66	379.2	5.75		
Total	71	7081.3			

near field margins has already been noticed (Thomas, 1946; Hörnig, 1953), but no reference to aggregation can be found. Both phenomena probably result from adult dispersal and oviposition behaviour. As swedes (as well as other host crops) are rarely grown in the same field two years in succession (these crops being included in a four or more year rotation) midges of Generation I emerge in a non-host crop, usually a cereal, and have to travel varying distances to reach their hosts. On "discovering" these, the females alight and proceed to oviposit on plants in their vicinity (see Section 1.4). The 1959 crop (W.Wheldon) was situated about 500 yards to the east of Middleton (see Fig. 10) where a midge population had accumulated in 1958. More migrant females from Middleton would "discover" and alight on swedes in the western area of W.Wheldon (i.e. nearest to Middleton) than on swedes further to the east and this may be the explanation for the observed change in infestation levels across the drills (highest in West lowest in East).

3.632 Distribution of larvae on galled swedes.

Mean numbers of larvae per galled swede were estimated at intervals during 1958, 1959 and 1960. In 1958, galled swedes observed in the stratified samples and others were retained separately in polythene bags and returned to the laboratory. Larvae were washed from the plants and counted. Only ten galled plants were retained

for inspection when samples yielded more than this number. As plants in the 1959 systematic sample-units were not to be removed (these plants were tagged for routine observation) numbers of larvae per galled plant in that year were estimated from a random selection of galled plants collected over the whole crop (units and surrounding plants excluded). Numbers of plants examined varied between 6 and 20 according to population levels and time available for counting. Larvae were either washed from their galls (as in 1958) or allowed to mature and leave the plant naturally (for method see Section 3.4) for counting. A similar procedure was used in 1960 (Generations I and II only).

Numbers of larvae per galled swede (mean and standard error) and percentage crop attack (mean percentage of swedes galled and 95% fiducial limits) are shown in Table 24. Results show that numbers of larvae per galled plant were considerably higher in 1959 (144.8 larvae) and 1960 (98.6 larvae, Gen. I and II only) than in 1958 (15.5 larvae). But more interesting than this is the positive correlation between percentage crop attack and number of larvae per galled plant. This is shown graphically in Fig. 14. In 1958 plant infestation increased from 0.2% (Gen. I) to 6.3% (Gen. III) and numbers of larvae per galled plant remained fairly constant (about 10-20). In 1959, however, infestation levels and associated numbers of larvae per galled

Table 24. Number of larvae per galled swede (*C. nasturtii*) and percentage of swedes galled

Date 1958	Number of plants	Mean larvae per plant $\bar{x}$	$s_{\bar{x}}$*	Percentage attack Mean	Percentage attack 95% fiducial limits
July 1	1	14.0	-		
18	9	18.8	2.34	0.2	0-0.5
Aug. 15	4	14.3	6.37	1.5	0.7-2.3
19	4	48.5	17.71		
26	10	14.9	2.47	6.3	4.6-8.0
Sept. 2	8	22.2	4.51		
5	10	17.6	4.46		
12	10	20.4	4.70		
16	10	16.4	3.69		
19	10	11.4	2.19		
23	10	9.9	2.31	3.7	2.1-5.3
26	10	11.8	2.70		
30	10	12.0	3.94		
Oct. 3	3	3.7	0.67		
10	7	5.0	1.05		
14	5	11.6	5.55		
17	5	12.4	3.46		
21	2	22.0	3.00		
23	3	18.7	7.27		
28	3	11.7	5.04	0.8	0.1-1.5
31	1	33.0	-		
Mean for 1958 (n = 135) = 15.5 larvae per plant					
1959					
July 7	13	17.1	3.92		
7	11	24.3	3.23	9.2	7.0-11.4
28	8	93.4	16.35	41.5	37.2-45.8
Aug. 25	10	216.0	39.95		
27	20	283.4	29.53	87.4	84.1-90.7
Sept. 1	10	275.5	45.11		
17	10	86.6	15.57		
Oct. 1	6	9.8	2.39		
Mean for 1959 (n = 88) = 144.8 larvae per plant					
1960					
June 28	10	51.1	8.46	24.6	17.3-31.9
Aug. 6	5	193.6	54.23	74.0	
Mean for 1960 (n = 15) = 98.6 larvae per plant					

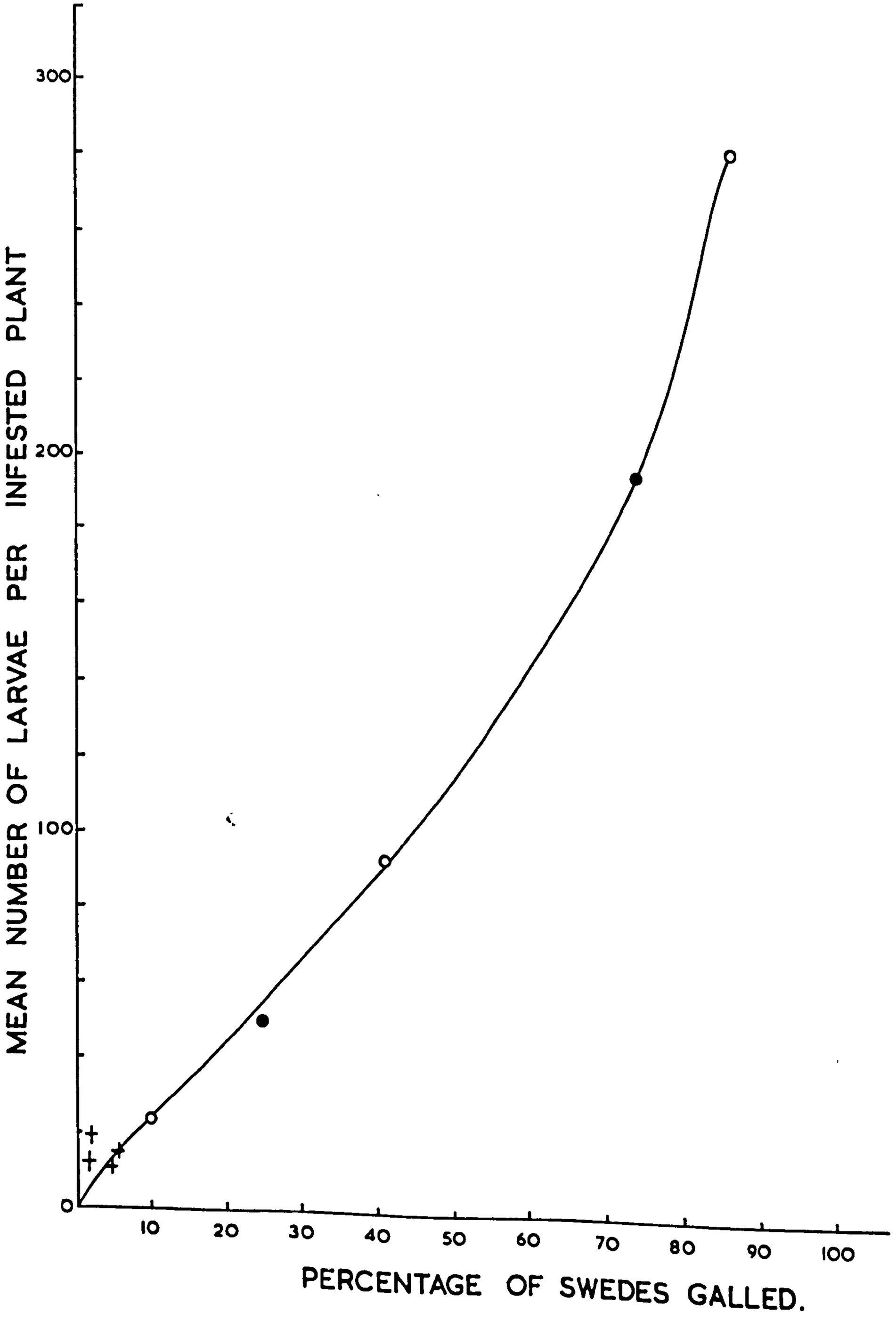
*$s_{\bar{x}}$, standard error of mean.

FIGURE 14.

Relation between percentage swede attack
and mean number of feeding larvae
per infested plant
(C.nasturtii)

Points represent mean values:

+ 1958, Middleton.
⊙ 1959, W.Wheldon.
• 1960, E.Wheldon.


MEAN NUMBER OF LARVAE PER INFESTED PLANT
300
200
100
0
10
20
30
40
50
60
70
80
90
100
PERCENTAGE OF SWEDES GALLED.

plant for Generations I, II and III were respectively 9.2% (24.3 larvae), 41.5% (93.4 larvae) and 87.4% (283.4 larvae). The intermediate values observed in 1960, i.e. 24.6% (51.1 larvae) and 74.0% (193.6 larvae) for Generations I and II respectively confirm the relation.

Results show what one more or less expects, namely, that when midge numbers increase in an area of constant food supply (i.e. number of swedes), numbers of larvae per galled plant also increase. When 100% of swedes are galled, numbers of larvae per plant continue to increase until the death of the plant and its larval population supervenes. The maximum holding capacity of the swede (i.e. the point towards which Fig. 14 trends) will vary with its size and vigour, being least in June (when midge numbers are low) and most in late August (when midge numbers are high). Intraspecific competition among feeding larvae (on the same swede) for food may be invoked at extremely high population levels but its effect is likely to be forestalled by the death of the swede, this causing the death of its larval population. Several galled plants of the 1959 and 1960 samples contained from 800-1200 larvae but although these plants were severely galled, none was anywhere near to death. Clearly, leaf tissues of one swede provide adequately for the successful growth and development of a very large number of larvae.

The effect of swede density on numbers of larvae per galled plant was investigated during Generation I 1959 (W.Wheldon). Several short drills at the western (hedge-side) margin of the field were purposely left unsingled (20-30 plants per sq. yd.) during the first generation attack. These rows and adjacent singled ones (5.0 plants per sq. yd.) were sampled at random at the peak of the first larval generation to estimate mean numbers of larvae per galled swede. Thirteen galled swedes from unsingled and 11 from singled rows were taken and results are included in Table 24 (7th July, 1959). There was no significant difference between the two sample means (24.3-17.1 = 7.2 larvae per plant; $t = 1.7$, $df = 22$, $p > 0.1$) but larger samples might have confirmed the suggestion that numbers of larvae per galled plant increase with decreasing plant density.

Until now no one has noticed the important fact that the average number of larvae per galled swede increases as the percentage of swedes galled increases. Previous authors have invariably estimated larval populations in the field on the basis of (a) percentage of swedes galled and (b) a fairly constant and low infestation rate per galled swede (10-50 larvae). Obviously many of their estimates must be unreliable. In fact, the rate of population increase (of feeding larvae) in any particular

year is likely to be far more rapid than has hitherto been realised. More will be said of this in Section 6.

As was previously mentioned, larvae from some galled swedes (those collected during Gen. II, 1959 and Gen. I, 1960) were allowed to vacate their hosts naturally before being counted. They were then placed in standard rearing tubes containing moist soil. The number and sex of adult midges developing from each plant's larval population, together with other details, are given in Table 25. Clearly, although the over-all sex ratio is nearly unity (see totals), the sex ratio for individual plants is extremely variable. The latter is to be expected since (a) C.nasturtii reproduces by uni-sexual families (see Section 1.5) and (b) the larval population of the individual swede can arise either from male-producing or from female-producing parents or from both. Again, since numbers of larvae per galled swede increase as the percentage of swedes galled increases, one would expect that a plant has less chance of being infested by only one female (i.e. with larvae all of one sex) at high field population (i.e. high percentage swede attack) than at low population. This is confirmed by the following tabulation of data taken from Tables 24 and 25:

Table 25. Sex ratio on individual galled swedes (C.nasturtii)

	Plant	Larvae per plant	Adults reared ♂♂	Adults reared ♀♀	Parasites	Diapause Larvae
	a	19	-	14	-	-
	b	88	16	16	-	2
	c	88	28	20	-	1
	d	49	22	20	-	1
Gen. I	e	75	-	44	-	-
1960	f	17	-	8	-	-
	g	56	-	10	-	-
	h	54	19	4	-	1
	i	22	12	4	2	-
	j	43	29	-	1	-
Total		511	126	140	3	5
	k	17	14	1	-	-
	l	47	45	-	-	-
	m	107	25	34	29	4
Gen. II	n	139	89	22	8	2
1959	o	171	28	117	15	-
	p	94	36	45	2	3
	q	52	16	23	6	-
	r	118	53	57	-	2
Total		745	306	299	60	11

Time	Percentage swede attack	swedes with one sex of larvae : swedes with both sexes
Gen. I, 1960	25%	5 : 5
Gen. II, 1959	42%	1 : 7

Note: Here galled swedes were taken at random over the field.

It was shown in Section 3.631 that swedes galled by the first generation tend to be aggregated in clusters and it was suggested that each cluster of galled swedes might well result from oviposition by a single female. If this were true (unfortunately it was not tested) larvae from all the galled swedes of a single cluster would be of one sex only. That is, the sexes of C.nasturtii would tend to be segregated in small isolated patches of the swede crop in Generation I. But segregation would tend to disappear as population gradually spread over the whole field in Generations II and III, and this in itself might well accelerate increase because the sexes would meet more readily.

3.7 Effect of infestation on the swede.

Larvae of C.nasturtii reduce the photosynthetic efficiency of the swede directly by destroying cells and indirectly by causing abnormal growth. In addition, it is alleged that 'secondary' bacterial and fungal diseases often follow galling, particularly in wet years (Leefmans, 1938). Walton (1927) and Davies (1931), however, could demonstrate no correlation between midge attack and

occurrence of 'secondary' diseases.

'Many neck' is a condition of the swede often attributed to galling, though any agency which destroys the central shoot may cause this. 'Many neck' plants usually, but not invariably, develop a small mis-shapen bulb with a hollowed crown which is particularly susceptible to bacterial or fungal infection. The centric systematic sample of 1959 (W.Wheldon) provided an opportunity to study the effect on swedes of galling in Generations I, II and III. Swedes in the sampling units were inspected at intervals during the summer and individual bulb weights were recorded (to the nearest 0.25 lb.) in October. Of the 720 swedes examined (30 in each of 24 sampling units), 89 (12.4%) were 'many necked' in October. Of these, 22 came from a total of 66 which had been galled by Gen. I larvae and the remaining 67 had either been galled by Generations II and III or not galled at all. Comparing 'many neck' frequency among (A) swedes galled by Gen. I and (B) swedes not galled by Gen. I, it is found that frequency is significantly higher in (A), $\chi^2 = 29.4$, $p < .001$. This result confirms the generally accepted view that swedes are more likely to become 'many necked' if galled at an early stage of growth (i.e. in July by Gen. I).

Thomas (1946) states that galled swedes with high larval numbers are more likely to become 'many necked'

than those with few larvae - his data are reproduced below:

Larvae per swede	swedes examined	% of swedes subsequently with 'many neck'
4-7	42	2
8-12	61	14
13-16	35	42
16	53	58

Thomas does not mention the size of swedes or the generation of larvae involved in his counts. Numbers of larvae per galled swede during all generations of 1959 were much higher than this (over-all mean = 144.8, see Table 24) when only about 15% of plants galled were 'many necked'. This suggests that Thomas either (a) underestimated numbers of larvae per galled swede or (b) neglected to consider some other factor causing 'many neck' which may have acted with increasing intensity as numbers of larvae per galled swede increased. At any rate it seems probably that a complex relation between galling and subsequent production of 'many neck' exists, in which size and growth rate of the swede and number and position of feeding larvae are involved.

Mean bulb weights of healthy and/or lightly infested swedes and of swedes with 'Central Shoot Galls' (i.e. severely infested) in one or any combination of Generations I, II and III, 1959 are arranged from highest to lowest in Table 26 (groups A-H). Bulb weights of plants with 'Outer Leaf Galls' are included in the 'unattacked' group (A) as

Table 26. Bulb weights of swedes from the centric systematic sample (W.Wheldon, 1959)

Group	"Plant history"	Number of swedes	Mean bulb weight, $\bar{x}$ (lbs)	$s_{\bar{x}}$
D	Galled* by G.III only ...	410	3.56	0.10
A	'Unattacked'**	130	3.35	0.20
F	Galled by G.I and G.III	22	3.26	0.44
B	Galled by G.I only	8	3.00	0.95
	$n_1 = 570$			
G	Galled by G.II and G.III	99	2.87	0.19
C	Galled by G.II only	45	2.44	0.25
H	Galled by G.I, II and III	5	1.95	0.44
E	Galled by G.I and G.II ..	1	0.25	-
	$n_2 = 150$			

*Galled - refers to 'Central Shoot Gall'.

**'Unattacked' - includes swedes with 'Outer Leaf Galls'.

Analysis of Variance

(excluding group E)

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between groups	6	89.3	14.88	3.03	$<.01$
Within groups (error)	712	3507.1	4.92		
Total	718	3596.4			

this type of damage would have a negligible effect on yield. Analysis of variance (excluding group E which has only one plant) shows that the variation between group means is highly significant (V.R. = 3.03, with 6 and 712 df., $p < .01$). Inspection of Table 26 suggests that the group means tend to fall into two classes, i.e. those from swedes galled by Gen. II (alone or in combination) - groups G, C, H and E ($n_2 = 150$), and those from swedes not galled by Gen. II (but galled by Gen. I and III or unattacked) - D, A, F and B ($n_1 = 570$). A 't-test' shows that the difference between the two class means (3.49-2.69) is highly significant ($t = 3.94$, $df = 718$, $p < .001$) and this represents a reduction of between 0.45 and 1.15 lbs. (5% fiducial limits) in the mean bulb weight of swedes galled by Gen. II.

A 'multiple range test' may be used to evaluate the significance of differences of means which are unequally replicated (Duncan, 1955; 1957). The difference between two ranked means is significant if it exceeds a 'shortest significant range', Rp, which is calculated from the error variance (from analysis of variance) and a value p, taken from Duncan's tables of the 'significant studentized range'. The test groups means which are not significantly different from one another into subsets and any two means not in the same subset are significantly different. However, the complete test (Duncan, 1957) makes allowance for testing

differences within a subset, the maximum difference of which does not exceed the adjusted value. For example, the difference between the highest (group D, n = 410) and the lowest (group E, n = 1) mean bulb weight is not significant so that D A F B G C H E is a subset. Using the complete test it is permissible to test inter-subset differences and this was done with the following result:

(D A F B H E) (G C)

Any two means <u>not</u> <u>appearing</u> <u>together</u> within the same brackets are <u>significantly</u> <u>different</u>. Any two means <u>appearing</u> <u>together</u> within the same brackets are <u>not</u> <u>significantly</u> <u>different</u>. Thus, apart from the inclusion of groups H and E (with only 5 and 1 replicates respectively) into the first subset (i.e. with group means of swedes not attacked by Gen. II), results agree with those of the 't-test', namely, that the mean bulb weights of swedes severely galled in Generation II were significantly less than those of swedes not so galled. Severe galling in Gen. I, as well as sometimes causing abnormal growth (see correlation between Gen. I attack with 'many neck') probably also reduces the final bulb weight, but too few weights were available to show this. Severe galling by Gen. III larvae alone had no effect on final bulb yield (group D) and this was expected as galling occurred towards the end of the growing season (September) when plants had already nearly

reached their maximum size.

Apart from general observations on associations between midge attack, many neck and 'secondary' diseases, previous authors give little information on the effect of infestation on swede growth and yield. Results above naturally only apply to the 1959 season, at Nafferton, a very dry year, when the incidence of secondary bulb rot was low. No attempt was made to correlate bulb rot with infestation by C.nasturtii.

SECTION 4. THE MATURE LARVA AND PUPA

4.1 General.

Previous authors, most of whom apparently accept Taylor's (1912) results, state that the mature larva vacates its gall, burrows into the upper 0-4 inch layer of soil and there constructs a spherical cocoon. Inside the cocoon the larva either (a) pupates almost at once (non-diapause larva) or (b) delays pupation until the following spring (diapause larva). Without direct proof, Bovien and Knudsen (1950) suspected what has recently been shown by Young *et al* (1954) for *S.mosellana*, namely that the overwintering larva may vacate its first cocoon in spring and pupate in a second cocoon near the soil surface. The proportion of diapause larvae increases towards the end of summer and is highest (100%) in Generation III (Bovien & Knudsen, 1950; Hörnig, 1953) but nothing is known of the factors inducing or terminating diapause. Hörnig (1953), among others, suggests that midge 'outbreaks' are likely to occur if the weather is warm and the soil moist when most of the population is in the soil. This suggestion is based partly on the results of his studies of midge abundance in relation to local weather and soil type, and partly on his observation that both larvae and pupae died in 'dry' sand. Clearly, Hörnig's suggestion deserves further investigation.

4.2 Non-diapause larva.

4.21 Speed of development at constant temperature.

Mature larvae, which were laboratory reared or came from Gens. I and II in the field and which had vacated their galls naturally, were pipetted into glass rearing tubes containing 1-inch of moist soil. Tubes were kept at one of the following constant temperatures: 12°, 15°, 20°, 25°, 30° and 32.5°C. All larvae burrowed into the soil at once and many constructed oval elongate cocoons just below the surface of the soil. Some of these cocoons were visible through the tube walls. Inside the cocoons the larvae moulted and pupated. When fully-developed, pupae burst out of their cocoons on to the soil surface where adults emerged (see also Section 1.1). Numbers and sex of adults were recorded each day until emergence ceased. The mean time spent in the soil varied inversely with temperature (Table 27 and Fig. 15), being about 34 days at 12°C. and 8 days at 30°C. Pupae developing at 32.5°C. died just prior to or during eclosion. At all temperatures below 30°C. the mean duration for the male was significantly shorter than that for the female; i.e. on the average, males emerged 12-24 hours before females (Table 27). The rate curve (i.e. 'average percentage development per day', regardless of sex, Fig. 15) appears to be almost linear between 12° and 30°C. It should be noted that this curve

Table 27. Speed of development of the mature larva (non-diapause) to adult stage at constant temperature, (C.nasturtii).

Temp. °C.	Sex	Number adults	Mean duration, $\bar{x}$ (days)	$s_{\bar{x}}$*	"t"	p**
12 ± 2	Male	43	33.7	0.165	3.49	<.001
"	Female	78	34.5	0.143		
15 ± 2	Male	60	25.6	0.110	2.98	<.01
"	Female	87	26.0	0.082		
20 ± 0.5	Male	85	13.2	0.062	5.67	<.001
"	Female	91	13.7	0.070		
25 ± 0.5	Male	61	10.1	0.072	5.07	<.001
"	Female	87	10.6	0.083		
30 ± 0.5	Male	19	7.9	0.329	1.91	>.05, <.1
"	Female	46	8.5	0.234		
32.5 ± 0.2	Pupae die,		7.0	-	-	-

*$s_{\bar{x}}$ = standard error of the mean.

**p, significance of the difference between the mean durations of development for the sexes.

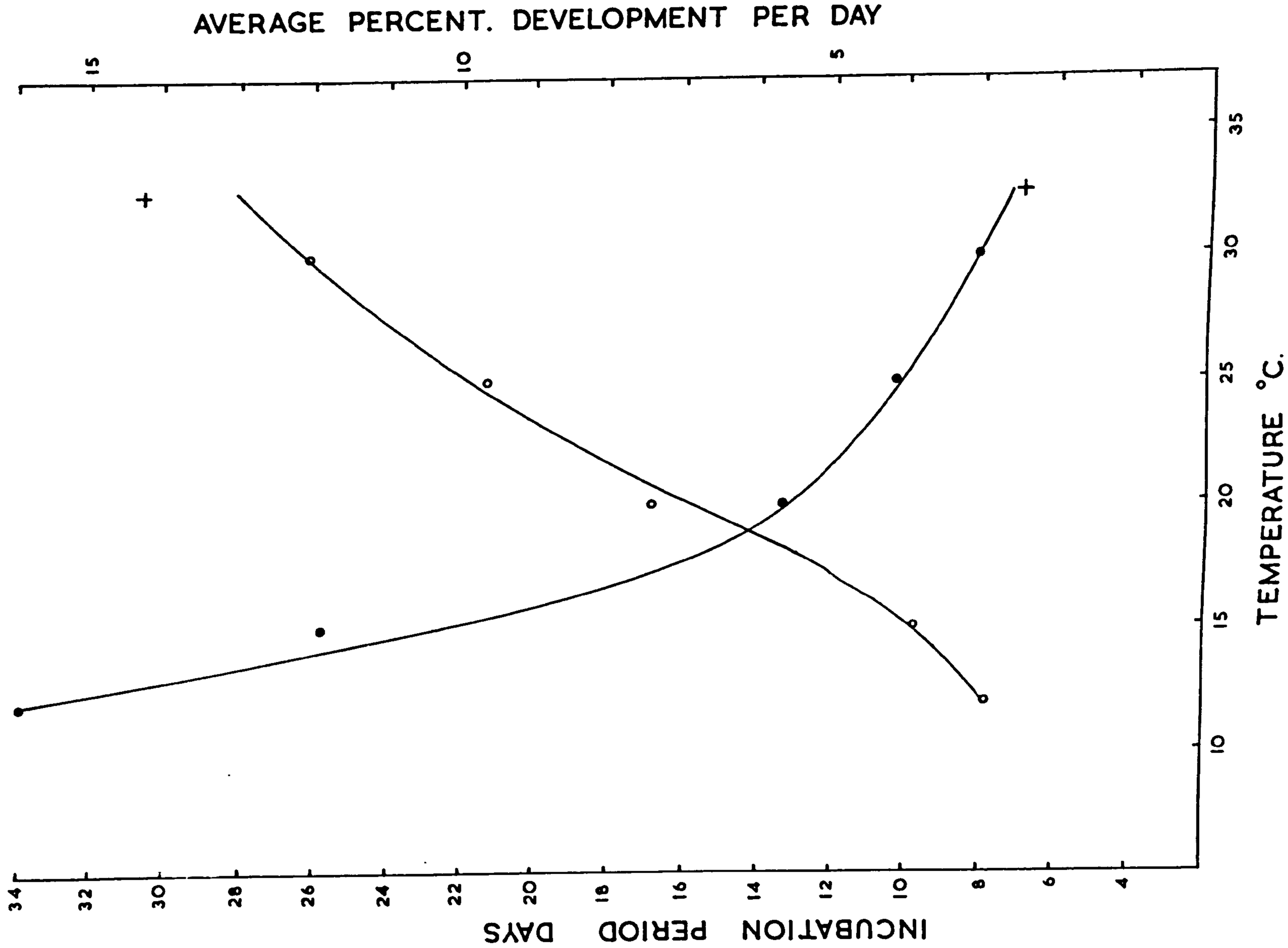
FIGURE 15.

Speed of development of the mature larva (non-diapause) to adult stage at constant temperature, (C.nasturtii)

O : Average percentage devebpment per day.

● : Mean duration (days).

+ : Pupae died on soil surface.


AVERAGE PERCENT. DEVELOPMENT PER DAY
15
10
5
35
30
25
20
15
10
TEMPERATURE °C.
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
INCUBATION PERIOD DAYS

involved not one (as in Figs. 7 and 9) but three phases of development, namely, (a) cocoon construction, (b) pupation and (c) emergence, each of which may have a different rate/temperature coefficient. Normally phase (b) lasts about 26 days, phase (a) about 36 hours and phase (c) only a few minutes.

4.22 Speed of development in the field.

On six occasions during 1959 batches of mature larvae were recovered from galled swedes which were collected at random in W.Wheldon. Several days after each collection date, larvae were assigned at random to rearing tubes (20 larvae per tube; 5 tubes per collection) which were soaked, drained and immediately sunk in W.Wheldon so that the level of the soil surface inside each tube was the same as that outside (i.e. larvae would pupate at their natural depth). Thereafter tubes were examined 2-4 times per week and numbers of emerging adults noted. Usually it was possible to ascertain the day on which most adults emerged ('peak emergence') from their body colour and activity. For each larval batch, time spent in the soil up to peak emergence and mean field temperature during this time are shown in Table 28.

The weekly mean temperature experienced by larvae during their sojourn in the soil was estimated with a temperature integrator (MacFadyan, 1956) by placing the

Table 28. Duration of development from mature larval to adult stage at constant and field temperatures
(*C.nasturtii*)

Date of peak adult emergence	Duration in field (days)	Mean field temperature °C.	Calculated* duration at constant temp.** (days)
26th July	17	18.5	16
18th Aug.	18	17.5	18
19th Sept.	22	15.0	25
14th Oct.	27	13.5	28
3rd Nov.	35	12.0	36

*Calculated from Fig. 15, Section 4.21.

**Constant temperature equal to mean field temperature.

'bead' of one thermistor (the present author's instrument included three thermistor circuits) in the upper ¼-inch layer of the soil of a blank rearing tube. The integrated mean temperature recorded was accurate to ± 0.1°C. and gave a very close estimate of the true mean temperature (see MacFadyan, 1956).

Results in Table 28 suggest that duration of development at field temperatures fluctuating with mean x°C. is almost the same as duration at constant temperature x°C. (cp. results for duration of feeding larval stage, Sections 3.4 and 3.5).

4.23 Effect of soil-moisture on development.

4.231 Laboratory experiments.

Series A. Extremes of soil-moisture

Larvae immersed in water do not develop though they may survive for several months providing the water is frequently aerated. This property was often used to accumulate large numbers of larvae for rearing experiments. Further, no development occurs in saturated soil or in petri-dishes containing saturated filter-paper. However, as soon as larvae are transferred from excessively wet conditions into moist soil, they construct oval cocoons and pupate almost at once.

It seems probable that 'wet' conditions prevent cocoon construction which apparently is necessary for successful

pupation.

Larvae do not pupate in 'dry' soil, though they may attempt and sometimes complete cocoons. These cocoons differ in size, shape and texture from those in which pupation occurs. To demonstrate this, two series of rearing tubes, M and D, were prepared with 1-inch of soil from a bulk of sieved light sandy loam taken from W.Wheldon field (particle-size < 2.0 mm.). Initially series M contained moist soil (23.0%)* and series D dry soil (8.0%). There were 6 tubes in each series. Twenty larvae were assigned at random to each tube; these larvae came from galled swedes in W.Wheldon on 27th August, 1959. Tubes were kept in the dark at 20°C. After 7 days, the soil in two tubes of the dry series (D2a,b) was moistened (i.e. soaked and then drained), and the soil in two tubes of the moist series (M2a,b) was dried (by passing dry air through for 24 hours). After 12 days, the same treatments were given respectively to tubes D3a,b and M3a,b. This left two tubes from each series as controls, D1a,b and M1a,b. All tubes were observed for a further 30 days when the soil in each was examined. Results are shown in Table 29 and

---

*Moisture expressed as percentage of dry weight.

Fig. 16.

Clearly, no emergence occurred in the dry controls (D1) whereas moistening of the dry soil after 7 (D2) and 12 (D3) days caused some larvae to pupate, midge emergence commencing respectively 7 and 12 days later than in the moist controls (M1). It is interesting to note that parasite emergence (Synopeas sp. B) was also delayed in D2 and D3 as compared with their emergence in M1. Emergence (midge and parasite) in tubes M2 and M3 was not delayed. This was expected because the soil in these tubes was initially moist and thus would not induce larval quiescence. However, emergence in these tubes was reduced because drying after 7 and 12 days caused some pupal death (Fig. 16).

Larvae remaining in the soil were either in spherical cocoons or 'naked'. Those from tubes D2, D3, M1, M2 and M3 (R = 13, 12, 11, 16 and 12 respectively) were all in diapause but those in D1 (R = 39) were either quiescent or in diapause. This was clearly demonstrated when larval cocoons were immersed in water; about 30 larvae from D1 vacated their cocoons immediately whereas all cocoons from the other treatments remained intact.

Another striking observation was the occurrence of almost equal numbers of two types of empty cocoons, spherical and elongate, in the soil of tubes D2 and D3. Obviously in dry soil larvae had first formed spherical

Table 29. Development of mature larvae in "dry" and "moist" soil (C.nasturtii)

Treatment*		Adults emerged	Parasites emerged	Larvae recovered	Total recovered (R)
D1	a	-	-	19	39
	b	-	-	20	
D2	a	7	1	7	13
	b	8	2	6	
D3	a	9	1	6	12
	b	5	2	6	
M1	a	8	4	7	11
	b	9	5	4	
M2	a	4	2	6	16
	b	-	-	10	
M3	a	6	-	7	12
	b	2	1	5	

*See text and Fig. 16 for details of treatments.

FIGURE 16.

Development of mature larvae in "Dry" (D_{1-3}) and "Moist" (M_{1-3}) soil (C.nasturtii)

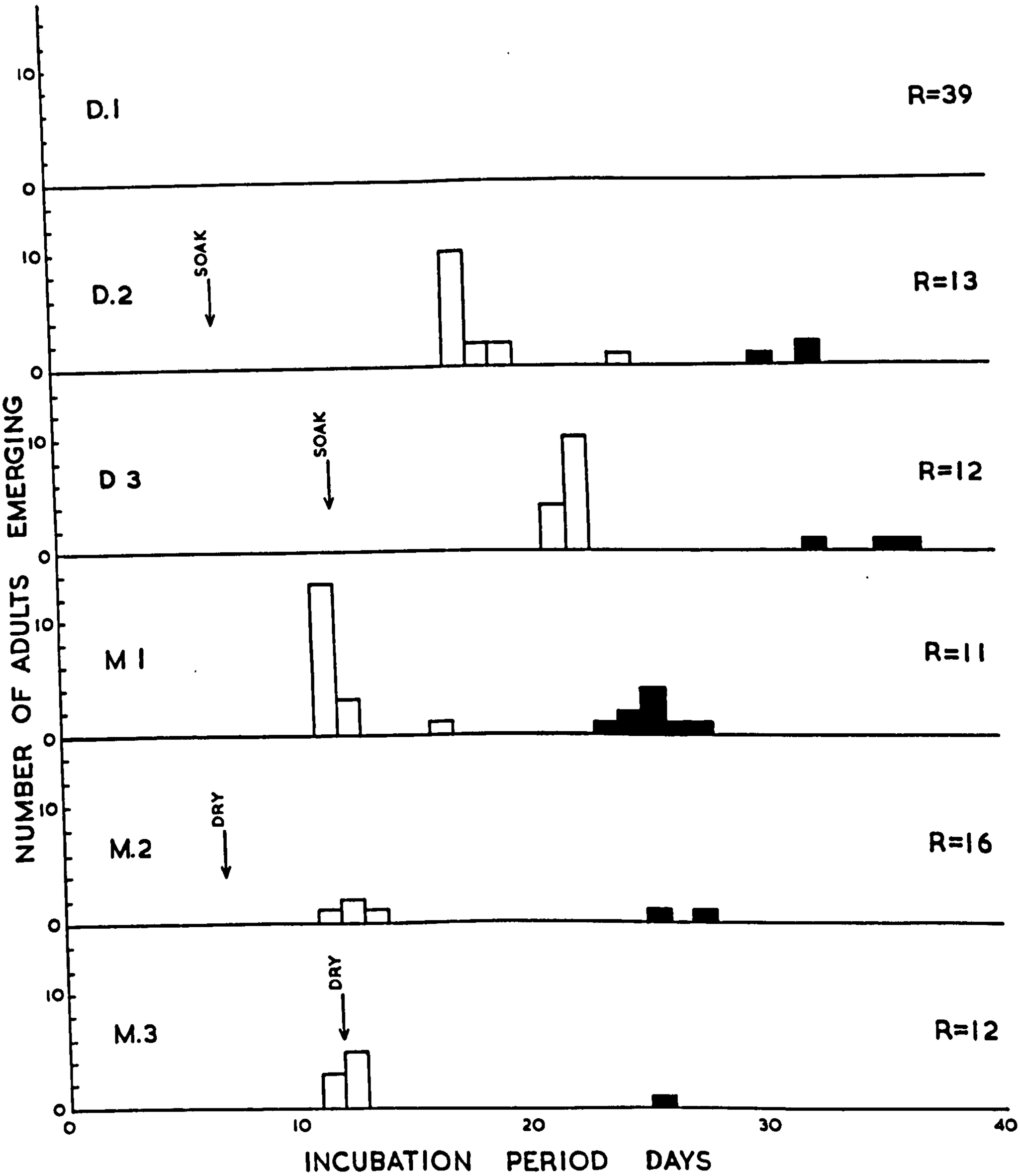
One pair of tubes to each treatment, 20 larvae per tube.

Day of "moistening" or "drying" indicated by andarrow.

White columns represent total number of adults of C.nasturtii emerging daily.

Black columns represent total number of parasites (Synopeas sp. B) emerging daily.

R denotes number of larvae recovered from the soil at the end of the experiment.


D.1
R=39
10
0
D.2
SOAK
R=13
10
0
D 3
SOAK
R=12
10
0
M 1
R=11
10
0
M.2
DRY
R=16
10
0
M.3
DRY
R=12
0
10
20
30
40
NUMBER OF ADULTS EMERGING
INCUBATION PERIOD DAYS

cocoons which they vacated when moisture became available (i.e. after 7 or 12 days) and then constructed elongate cocoons in which to pupate. Needless to say, only elongate (i.e. pupal) cocoons were found in tubes in which larval development was not delayed by dry conditions (M1, M2 and M3).

Naturally survival of larvae in 'dry' soil depends on their ability to withstand desiccation. In one experiment, 33 specimen tubes (3 x 1 inch) were prepared with 1-inch of dry soil (particle-size $<$ 2.0 mm., moisture content 8.7% i.e. 'dry') and 20 mature larvae (reared on laboratory swedes) were placed in each of 25 of these tubes. The remaining 8 tubes contained no larvae ('blanks') and these served to check moisture loss during the experiment. The soil in 5 tubes with larvae was moistened (Controls) and all tubes were tightly corked and kept in the dark at 20°C. The number of adults emerging and the number of larvae remaining in the controls after about 30 days gave an estimate of the proportion of non-diapause to diapause larvae in the original batch (Table 30, Control).

The tubes with dry soil were examined in groups of five after 50, 100, 180 and 240 days when, as shown by two 'blanks' in each case, moisture had fallen from 8.7% to 5.8, 5.6, 5.5 and 5.4% respectively. No adults emerged in the 'dry' tubes, but numbers of viable larvae present

Table 30. Survival of mature larvae in "dry" soil (C.nasturtii)

Days from beginning of expt.	Number of viable larvae recovered from each tube*					Total
	A	B	C	D	E	
50 days	19	16	19	19	18	91
100 days	19	12	14	14	14	73
180 days	11	9	13	15	9	57
240 days	4	10	11	8	9	42
Control (moist)	A	B	C	D	E	Total
Adults emerged	3	5	7	2	7	24
Diapause larvae	13	13	9	12	5	52
Survival (i.e. totals)	16	18	16	14	12	76

*Tubes - A-E each started with 20 larvae

Analysis of Variance (excluding Control)

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between durations	3	266.1	88.7	15.7	< .001
Error	16	90.2	5.6		
Total	19	356.3			

are shown in Table 30. The latter results suggest that larvae are able to survive 'dry' conditions of soil for considerable periods: 91%, 73%, 57% and 42% survived after 50, 100, 180 and 240 days respectively. Survival decreased significantly with increased duration in dry soil (V.R. = 15.7, with 3 and 16 df. $p < .001$).

As before, larvae from dry soil were either cocooned or 'naked'. Initially it was impossible to distinguish between dead and viable larvae (whether cocooned or not) as all larvae were more or less dehydrated and inactive. But after three days immersion in water, healthy larvae regained turgor and mobility whereas dead larvae decomposed. Thus, it appears that larvae of C.nasturtii can withstand partial dehydration without injury (cp. larvae of the chironomid, Polypedilum vanderplanki Hint. which survive almost complete dehydration (Hinton, 1960)).

Series B. Range of soil-moisture

Experiment 1.

The soil was a peaty clay from Prestwick Whins which on mechanical analysis had the following composition:

Coarse sand	17.7%
Fine sand	12.8%
Silt	13.0%
Clay	32.0%
Organic matter	25.1%

It was sieved (to give a particle-size < 2.0 mm.), moistened in bulk to a point above the highest moisture content desired and spread out on trays at room temperature to dry slowly. Soils with three different moisture contents were obtained by removing portions from the bulked soil at the appropriate stages of drying and storing them in air-tight jars. To ensure uniform distribution of moisture, the stored portions were thoroughly shaken once per day for a week before determining the actual moisture content.

Twelve specimen tubes (3 x 1 inch) were prepared with 1-inch of soil (4 tubes for each soil-moisture) and 20 mature larvae assigned randomly to each. These larvae were from a batch which had been collected in the field a few days earlier (W.Wheldon, 29th July, 1959). Tubes were corked, sealed with paraffin wax (to prevent moisture loss) and kept in the dark at 20°C. Numbers of adult midges and parasites (Synopeas sp. B) emerging in each tube were counted and after 40 days the soil in each tube was washed through a 100-mesh sieve in order to determine numbers of viable larvae ('free' and cocooned) and empty cocoons (elongate and spherical).

Results in Tables 31 and 32 show clearly that numbers of adults emerging increased with increasing soil-moisture; totals for 42.6%, 70.9% and 95.1% moistures were respectively

Table 31. Development of mature larvae in relation to soil-moisture (C.nasturtii)

Soil:- Peaty clay, Prestwick Whins.

Soil moisture (% d.w.)	Adults emerging* and larvae remaining** per tube				Total	Survival
42.6	4 (14)	4 (15)	4 (15)	7 (13)	19 (57)	76
70.9	15 (3)	1 11 (4)	13 (7)	1 11 (2)	2 50 (16)	68
95.1	12 (6)	19 (1)	16 (1)	16 (2)	63 (10)	73

*Adult midges on left; adult parasites directly above adult midges.

**Quiescent larvae in brackets.

Table 32. Analyses of Variance of data in Table 31.

A. Variation in adult emergence.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	2	262.17	131.09	31.44	<.001
Error	9	37.50	4.17		
Total	11	299.67			

B. Variation in numbers of quiescent larvae.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	2	327.17	163.59	43.62	<.001
Error	9	33.75	3.75		
Total	11	360.92			

C. Variation in survival (midges + parasites + larvae).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	2	10.50	5.25	2.20	>0.1
Error	9	21.50	2.39		
Total	11	32.00			

19, 52 and 63 (V.R. = 31.4, with 2 and 9 df. $p < .001$), and conversely, that numbers of quiescent larvae decreased with increasing soil-moisture; totals for 42.6%, 70.9% and 95.1% were 57, 16 and 10 respectively (V.R. = 43.6, $p < .001$). Soil-moisture had no significant effect on survival (i.e. midges + parasites + larvae) (V.R. = 2.2, $p > 0.1$).

The number of <u>empty elongate</u> (pupal) cocoons found in the soil-residue of each tube was almost the same as the number of adults which emerged. Occasionally however, such cocoons contained dead pupae.

Of the 57 larvae recovered from the lowest moisture (42.6%), 46 were 'free' and 11 were in spherical cocoons. Numbers of 'free' larvae to cocoons in the 70.9% and 95.1% moistures were 3 : 13 and 0 : 10 respectively. But soil-residues which contained 'free' larvae also had an equal number of <u>empty spherical</u> cocoons and this suggests that some larvae (most in the driest soil) had constructed spherical cocoons which they vacated as soon as the soil was washed at the end of the experiment.

Finally, the 'free' larvae and the larvae in spherical cocoons at the end of the experiment were reared separately in moist soil at 20°C. Adults emerged after 12-18 days from 'free' larvae but no emergence occurred among the others. Clearly, 'free' larvae had been quiescent through

lack of moisture and the larvae in spherical cocoons were quiescent through diapause.

Experiment 2.

The soil in this experiment was a light sandy loam from Nafferton (W.Wheldon) with the following composition:

Coarse sand	23.6%
Fine sand	37.1%
Silt	11.0%
Clay	22.0%
Organic matter	6.1%

Four specimen tubes were prepared for each of four soil-moistures (7.9%, 14.9%, 21.6% and 31.4%) and 20 mature larvae added at random to each. These were collected from W.Wheldon on 29th August, 1959. Tubes were corked, sealed and kept in the dark at 20°C.

Results agree nicely with those of the preceding experiment in that most adults emerged and least larvae remained quiescent at high soil-moistures (Tables 33 and 34). Excluding results from the lowest soil-moisture (7.9%), in which no emergence occurred, the effect of soil-moisture on emergence was just significant (V.R. = 4.44, with 2 and 9 df., $p < .05$), but its effect on larval quiescence was highly significant (V.R. = 12.8, $p < .01$). There was again no significant difference in survival (V.R. = 3.06, with 3 and 9 df., $p > .05$).

Table 33. Development of mature larvae in relation to soil-moisture (C.nasturtii)

Soil:- Light sandy loam, Nafferton

Soil Moisture (% d.w.)	Adults emerging* and larvae remaining** per tube				Total	Survival
7.9	- (19)	- (19)	- (19)	- (18)	- (75)	75
14.9	11 (6)	16 (1)	14 (6)	10 (7)	51 (20)	71
21.6	15 (5)	15 (4)	1 13 (4)	1 15 (4)	2 58 (17)	77
31.4	1 15 (1)	16 (1)	17 (1)	16 (1)	1 64 (4)	69

*Adult midges on left; adult parasites directly above adult midges.

**Quiescent larvae in brackets.

Table 34. Analyses of Variance of data in Table 33.

A. Variation in adult emergence (excluding lowest soil-moisture).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	2	25.17	12.59	4.44	<.05
Error	9	25.50	2.83		
Total	11	50.67			

B. Variation in numbers of quiescent larvae (excluding lowest soil-moisture).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	2	36.17	18.09	12.78	<.01
Error	9	12.75	1.42		
Total	11	48.92			

C. Variation in survival (midges + parasites + larvae) (including lowest soil-moisture).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between soil-moistures	3	10.00	3.33	3.06	>.05
Error	12	11.00	1.09		
Total	15	21.00			

On rearing the 'free' larvae and the larvae in spherical cocoons, the same results were obtained as in Experiment 1.

Experiment 3.

Eight specimen tubes were prepared for each of six soil-moistures, three from the light sandy loam and three from the peaty clay. Twenty larvae, which came from a large batch collected in W.Wheldon (28th August, 1959) and which had been in aerated water for about two weeks, were assigned at random to each tube, and all tubes were corked and sealed as before.

Tubes for each soil-moisture were divided at random into two groups of four, one group being kept at 15°C. and the other at 25°C., both in the dark. The soil in the tubes was washed 30 days after 'peak emergence'. Naturally the experiment lasted longer (by 16 days) at 15°C. than at 25°C.

Results in Tables 35 and 36 show what one now expects from the two preceding experiments, namely that most adults emerged and least larvae remained quiescent at high soil-moisture, viz. for adults V.R. = 88.9, with 5 and 36 df., $p < .001$; and for quiescent larvae V.R. = 54.8, $p < .001$. Similarly the effect of soil-moisture on survival was not significant (V.R. = 1.01, $p > 0.2$).

Temperature also caused significant amounts of

Table 35. Development of mature larvae in relation to soil-moisture and temperature. (C.nasturtii)

Soil Moisture (% d.w.)	Adults emerging* and larvae remaining** per tube									
Sandy-loam	15°				Total	25°				Total	Moisture Totals
6.8	1 (18)	1 - (16)	1 (16)	1 (19)	1 3 (69)	1 - (14)	- (17)	- (19)	- (16)	1 - (66)	2 3 (135)
12.4	2 11 (4)	8 (11)	2 6 (4)	9 (8)	4 34 (27)	13 (6)	1 12 (5)	1 12 (3)	2 12 (3)	4 49 (17)	8 83 (44)
13.5	1 9 (4)	15 (2)	10 (7)	1 14 (1)	2 48 (14)	2 13 (2)	15 (3)	15 (1)	15 (4)	2 58 (10)	4 106 (24)
Peaty clay											
31.2	1 2 (16)	- (18)	- (18)	- (17)	1 2 (69)	1 (16)	1 (15)	- (16)	2 (13)	- 4 (60)	1 6 (129)
43.9	5 (12)	2 (17)	4 (6)	3 (10)	14 (45)	1 7 (9)	1 9 (8)	7 (10)	1 2 (11)	3 25 (38)	3 39 (93)
56.5	1 8 (6)	1 9 (6)	11 (7)	1 12 (3)	3 40 (22)	1 8 (6)	10 (7)	13 (3)	3 10 (3)	4 41 (19)	7 81 (41)
Temperature Totals:					11 141 (246)					14 177 (210)	

*Adult midges on left; adult parasites directly above adult midges.

**Qhiescent larvae in brackets.

Table 36. Analyses of Variance of data in Table 35.

A. Variation in adult emergence.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Soil-moistures	5	1294.36	258.87	88.96	< .001
Temperatures	1	33.69	33.69	11.58	< .01
Interaction	5	33.18	6.64	2.28	> .05 n.s.
Error	36	104.75	2.91		
Total	47	1465.98			

B. Variation in numbers of quiescent larvae.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Soil-moistures	5	1411.5	282.3	54.82	< .001
Temperatures	1	27.0	27.0	5.24	< .05
Interaction	5	6.0	1.2		n.s.
Error	36	185.5	5.15		
Total	47	1630.0			

C. Variation in survival (midges + parasites + larvae).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Soil-moistures	5	17.85	3.57	1.01	> .2
Temperatures	1	0.18	0.18	0.05	> .2
Interaction	5	26.20	5.24	1.49	> .2
Error	36	126.75	3.52		
Total	47	170.98			

variation in both adult emergence (V.R. = 11.6, with 1 and 36 df., $p < .01$) and larval quiescence (V.R. = 5.24, $p < .05$), but again had no effect on survival (V.R. = 0.05, $p > 0.2$). This result anticipates what will be shown later (Section 4.31), namely that high temperatures (e.g. 25°C.) induce pupation of some larvae (particularly those collected late in the season) which at lower temperatures (e.g. 15°C.) would enter diapause (i.e. remain 'quiescent'). Unfortunately, in this experiment, no distinction was made between larvae which were quiescent through moisture-lack and those 'quiescent' through diapause.

An important point in experiment 3 (also evident when experiments 1 and 2 are compared) is that a range of 6.8-13.5% moisture in sandy loam gave much the same results as a range of 31.2-56.5% in peaty clay. This shows that 'quiescence' is not simply a matter of absolute moisture content.

4.232 Relation between pF and development.

It is clear from the preceding experiments that amounts of larval quiescence at equal soil-moistures must vary with soil type. As a matter of fact it has long been realised that absolute soil-moisture is not a measure of water available to soil-dwelling organisms.

In the past, attempts have been made to measure 'available water' in soil by relating moisture content to

various soil-moisture constants, e.g. saturation value, hygroscopic coefficients, permanent wilting point, moisture-equivalent and field capacity (Baver, 1948), but they have been unsuccessful because each so-called equilibrium point expresses only one relationship which may exist between soil and water. Schofield (1935), however, has got over this difficulty.

Schofield's pF scale has been widely used by plant physiologists, and Evans (1944) and Maelzer (1957) have shown it to be a convenient continuous measure of water available to insects in soil. Despite this, however, measurement of soil water on a 'free-energy' basis, i.e. on the pF scale, has been virtually neglected by animal ecologists.

pF is a measure of the energy with which soil holds water. It is defined as the logarithm of the height (cm.) of a water column that is necessary to produce the desired suction to remove water from the soil. It is directly related to

(a) Percentage relative humidity (R.H.) of the soil atmosphere,

$$pF = 6.5 + \log_{10} (2 - \log R.H.)$$

and (b) Osmotic pressure (O.P.) of any solution,

$$pF = 3 + \log_{10} O.P.$$

In several soil-dwelling organisms development appears to be related to pF. Wireworms (Agriotes) neither gain nor lose water at pF 3.9 (Evans, 1944). Eggs of Locusta pardalina (Walk.) are in equilibrium in a 0.65 M solution of sucrose (Mathee, 1951) and hatching of eggs of the beet eelworm, Heterodera schachtii Schmidt, ceases in solutions of 0.65 M sucrose and 0.42 M urea (Wallace, 1956). The osmotic pressure exerted by these solutions is approximately 16 atmospheres and corresponds to the free energy value of soil at wilting point, i.e. approximately pF 4.2. Maelzer (1957) could demonstrate no significant variation in percentage hatch of eggs of Aphodius howitti Hope at pF values 2.5, 3.0, 3.25, 3.50, and 3.75, although eggs at pF 3.75 absorbed least water; eggs lost water and failed to hatch at pF 4.0.

The pF curves for the two soils used in experimentsl, 2 and 3 (Section 4.231) were determined by the author with apparatus kindly supplied by the Department of Agricultural Chemistry (soils), King's College. Curves for each soil are shown in Fig. 17. Two methods were used, one for pF values between 3.0 and 4.5 and the other for values above 4.5.

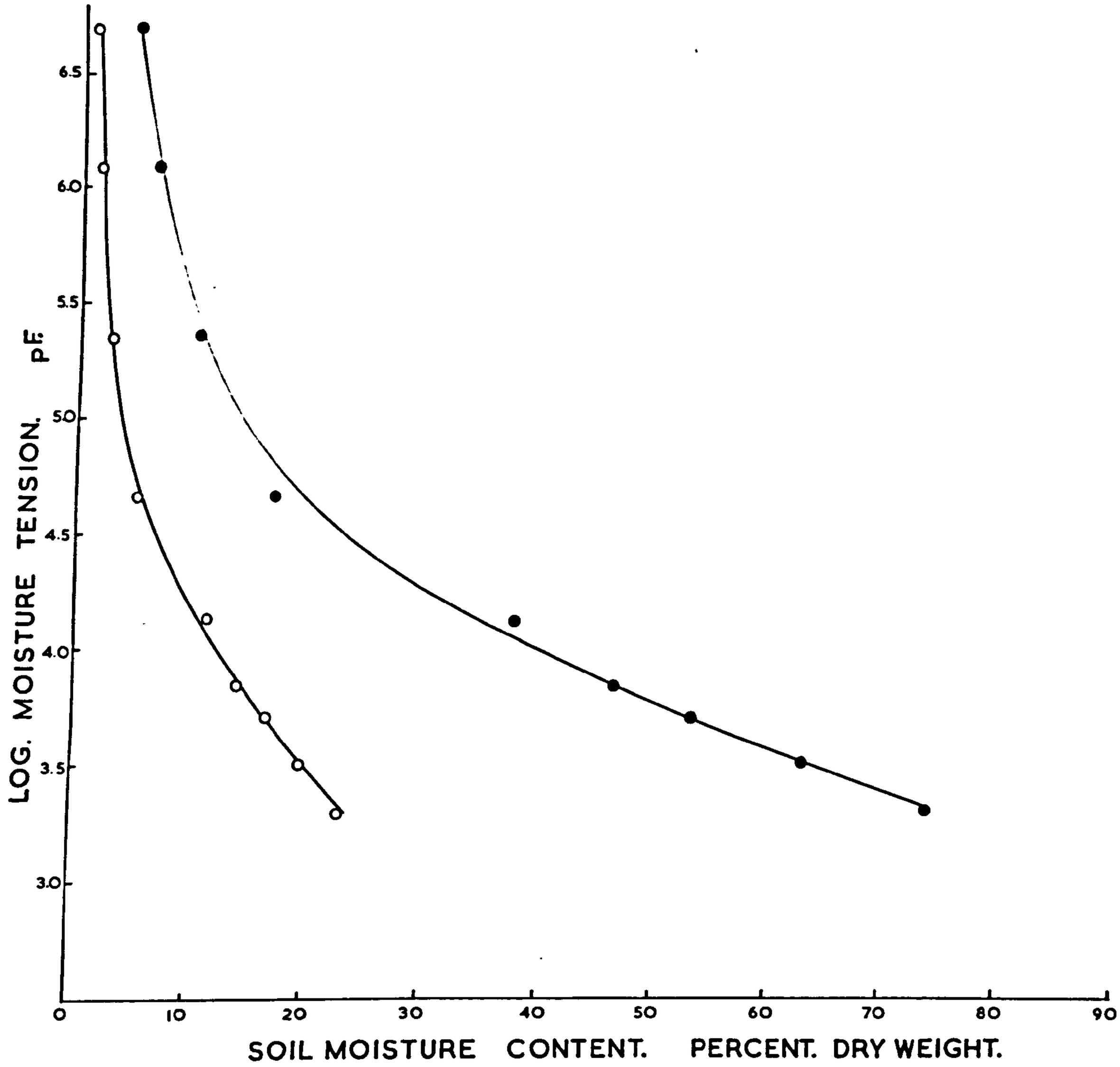
Briefly, the first method involves placing a thin (3/8 inch) layer of saturated soil over a membrane which is permeable to water but almost completely impermeable to

FIGURE 17.

Relation between soil-moisture and pF.

● : Peaty clay (Prestwick Whins)

o : Sandy loam (Nafferton).


LOG. MOISTURE TENSION. pF.
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
10
20
30
40
50
60
70
80
90
SOIL MOISTURE CONTENT. PERCENT. DRY WEIGHT.

air. A series of increasing constant pressures is applied to the soil by means of compressed air. Each pressure is maintained until no more water is expressed from the soil and the volume of expressed water measured. Full details of construction and use of the pressure-membrane apparatus are given by Richards (1947).

The maximum pressure in our experiments was 225 lbs. per sq. in. (≡ 15 Atmospheres ≡ pF 4.2), this being equivalent to the tension with which soil holds water at the 'permanent wilting point' (Richards and Weaver, 1944). The moisture content of the soil at this tension was then determined and, knowing the volumes of water expressed by each pressure increment, moisture-contents at lower tensions were calculated.

For pF values above 4.5, small air-dry samples of each soil were placed in four known humidities and moisture content determined at equilibrium. pF values equivalent to each humidity were calculated by substition in formula (a).

Unfortunately, curves in Fig. 17 were not completed in time to enable comparison of equal pF in different soils in relation to larval development. However, using Fig. 17, soil-moistures in experiments 1, 2 and 3 (Section 4.231) have been converted to pF and plotted against mean numbers of quiescent larvae, expressed as percentage of survival

(Fig. 18). It should be noted that in the case of experiment 3, the mean numbers are averages from the two temperature treatments.

Clearly, the free-hand curve in Fig. 18 shows that larval development is closely related to pF, irrespective of soil type. The steepness of the curve at pF values around 4.0 suggests that this value may be critical. At higher tensions, most larvae become quiescent and at lower tensions, most larvae pupate. The inclusion of a small proportion of diapause larvae in the means for quiescent larvae causes the curve to flatten at low pF values; naturally no amount of available water would induce these larvae to pupate. In fact, had there been no diapause in these experiments, the curve would probably have continued its steep descent to meet the abscissa at pF 3.7-3.9.

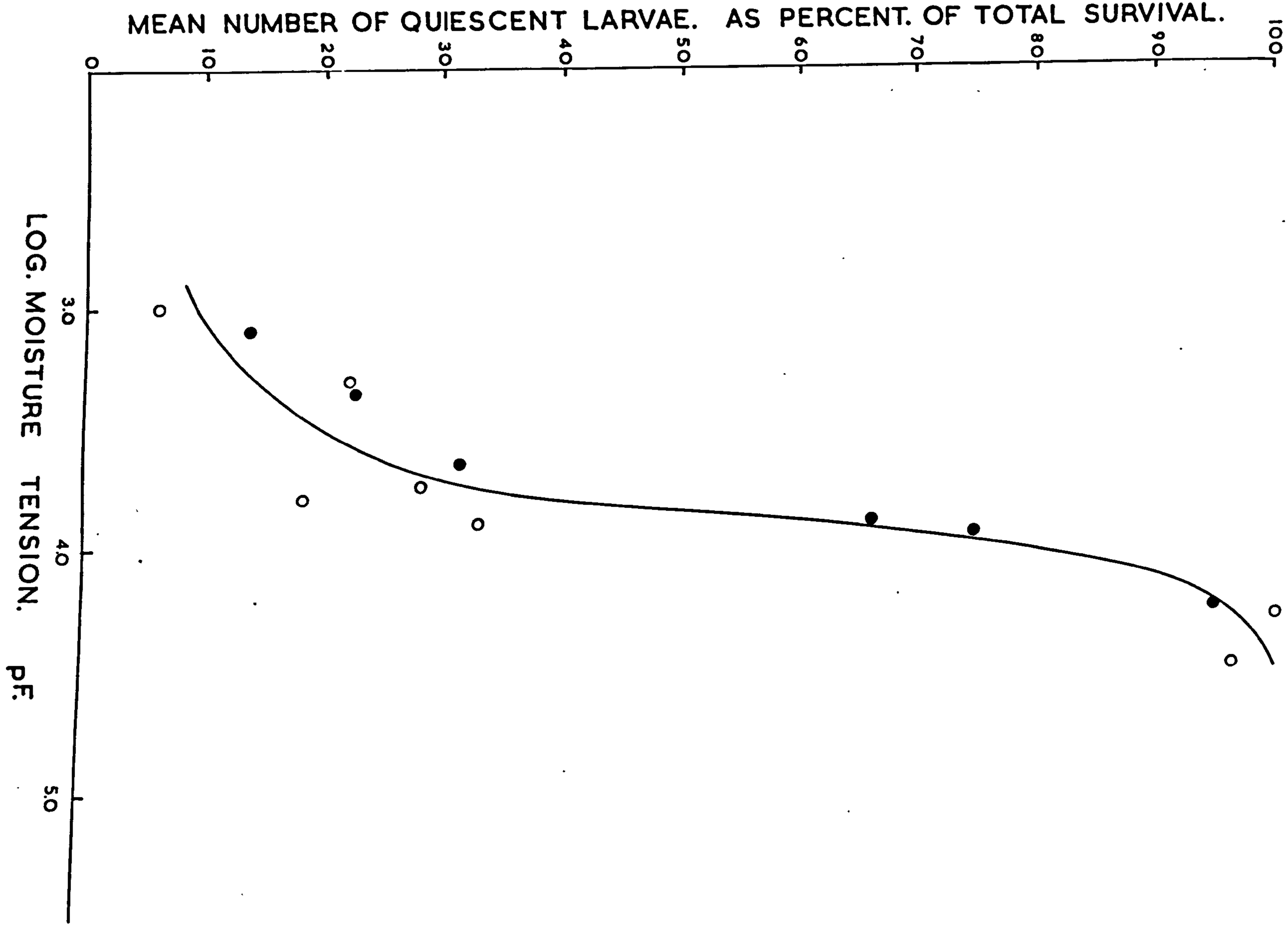
Our results suggest that pF influences larval development of C.nasturtii, not as one might expect through its effect on pupation, but apparently by influencing cocoon building behaviour. At pF below 3.7 most larvae build oval (i.e. pupal) cocoons but as pF rises above 3.7 there is an associated rapid increase in the proportion of larvae building spherical cocoons. Since quiescent larvae never pupate inside spherical cocoons, even when the soil is moistened, it seems improbable that high moisture tension directly inhibits metamorphosis.

FIGURE 18.

Development of non-diapause larvae in relation to pF.

o : larvae in sandy loam.

● : larvae in peaty clay.


MEAN NUMBER OF QUIESCENT LARVAE. AS PERCENT. OF TOTAL SURVIVAL.
0
10
20
30
40
50
60
70
80
90
100
LOG. MOISTURE TENSION. P.F.
3.0
4.0
5.0

It is interesting to note that the spherically shaped cocoon is associated with 'quiescence' (i.e. non-pupation), whether this is due to moisture-lack or diapause.

4.233 Field observations.

1959 was a warm dry year. Gen. I adults emerged from overwintering larvae in Middleton (wheat) and later the midge established itself in W.Wheldon (swedes). Soil-moisture was therefore measured first in Middleton (until Gen. I emergence ceased) and then in W.Wheldon. Since the soil in both fields was the same as the sandy loam used in earlier experiments (Section 4.231 and 4.232), soil-moistures were converted to pF from Fig. 17.

Ten tins of soil were taken over the whole field at approximately weekly intervals and each tin contained 5 soil cores (2 inch deep, 1 inch diam.) which were taken at equal intervals across two drills.

Mean moistures (% d.w.) and equivalent pFs are given in Table 37; daily maximum temperatures (^{o}F.) and rainfall (inches), and mean soil-moistures are also illustrated in Fig. 30 (Appendix I).

Significant amounts of rain (i.e. > 0.02 inches) fell during the following periods:- A (9 May), B (4-9 June), C (21-22 July), D (16-18 July), E (26-28 July), F (14 August) and G (20-24 September).

Table 37. Soil-moisture and pF, Nafferton, 1959

Date	Field	Crop	Mean Moisture-content, $\bar{x}$, (% d.w.)	$s_{\bar{x}}$*	Equivalent pF
2/6	Middleton	Wheat	11.4	0.96	4.10
9/6	"	"	21.1	0.49	3.35
16/6	"	"	10.7	0.50	4.15
23/6	W.Wheldon	Swedes	21.2	0.54	3.35
30/6	"	"	25.8	0.72	3.15
8/7	"	"	15.8	0.45	3.70
15/7	"	"	13.9	0.74	3.80
21/7	"	"	13.7	0.57	3.80
30/7	"	"	22.8	0.67	3.30
4/8	"	"	14.9	0.45	3.75
13/8	"	"	11.5	0.44	4.10
20/8	"	"	9.4	0.28	4.30
25/8	"	"	9.5	0.37	4.30
1/9	"	"	8.5	0.31	4.35
8/9	"	"	8.1	0.21	4.40
15/9	"	"	7.2	0.31	4.50
22/9	"	"	21.7	0.62	3.35
29/9	"	"	15.2	0.45	3.75
6/10	"	"	12.0	0.40	4.00
13/10	"	"	12.7	0.50	3.95

$s_{\bar{x}}$* = standard error of the mean
(n = 10)

Larvae of Gen. I and Gen. II dropped from their host swedes to the soil during periods C and E-F respectively, when, for most of the time, pF was below 3.8. As one would expect from Section 4.232 there was no delay in their development (see Fig. 28, Section 6). But Gen. III larvae vacated their hosts during a long dry spell, between periods F and G, when pF gradually increased to 4.5. About 20-30% of these larvae were non-diapause (see Section 4.3) and their development (i.e. pupation) would be delayed until rain fell on 20th September. By then, however, field temperatures had fallen so that pupation and emergence would extend well into November and this would lead to 100% pupal and/or adult mortality either because pupae can not over-winter or because Gen. IV adults would occur out of season.

A short period of rain in early September would probably have allowed an 'effective' fourth generation to occur in early-mid-October. Support for this view comes from field rearing experiments (Section 4.22): Gen. III larvae in <u>moist</u> soil (rearing tubes soaked, drained and then sunk in W.Wheldon) gave rise to adults which emerged from mid-September onwards (Table 28).

4.3 Diapause larva.

4.31 Onset of diapause.

Fig. 19 shows adult emergence from three batches of mature larvae collected at intervals during 1958 (from galled swedes in Middleton). Larvae were kept in rearing tubes containing moist soil at 20°C. Obviously, as the season progressed, emergence became increasingly irregular. The upper curve (19th August) is typical for larvae collected during generations I and II.

It was also noticed that an increasing proportion of larvae entered diapause as the season progressed. These larvae were found in spherical cocoons 50-70 days after emergence had started. Moreover, the incidence of diapause among larvae collected on the same day appeared to increase, the lower the subsequent rearing temperature (10°-30°C.).

Clearly, factors influencing diapause deserved further investigation.

In 1959 four large batches of mature larvae were recovered at intervals during the summer. On each occasion galled swedes were collected at random from W.Wheldon and returned to the laboratory where larvae were allowed to leave them naturally. Larvae from each batch were assigned at random to 25 rearing tubes (20 larvae per tube, i.e. 500 larvae per collection). The tubes were

FIGURE 19.

Adult emergence from three batches of larvae collected at intervals during 1958 and kept at 20°C., (*C.nasturtii*)

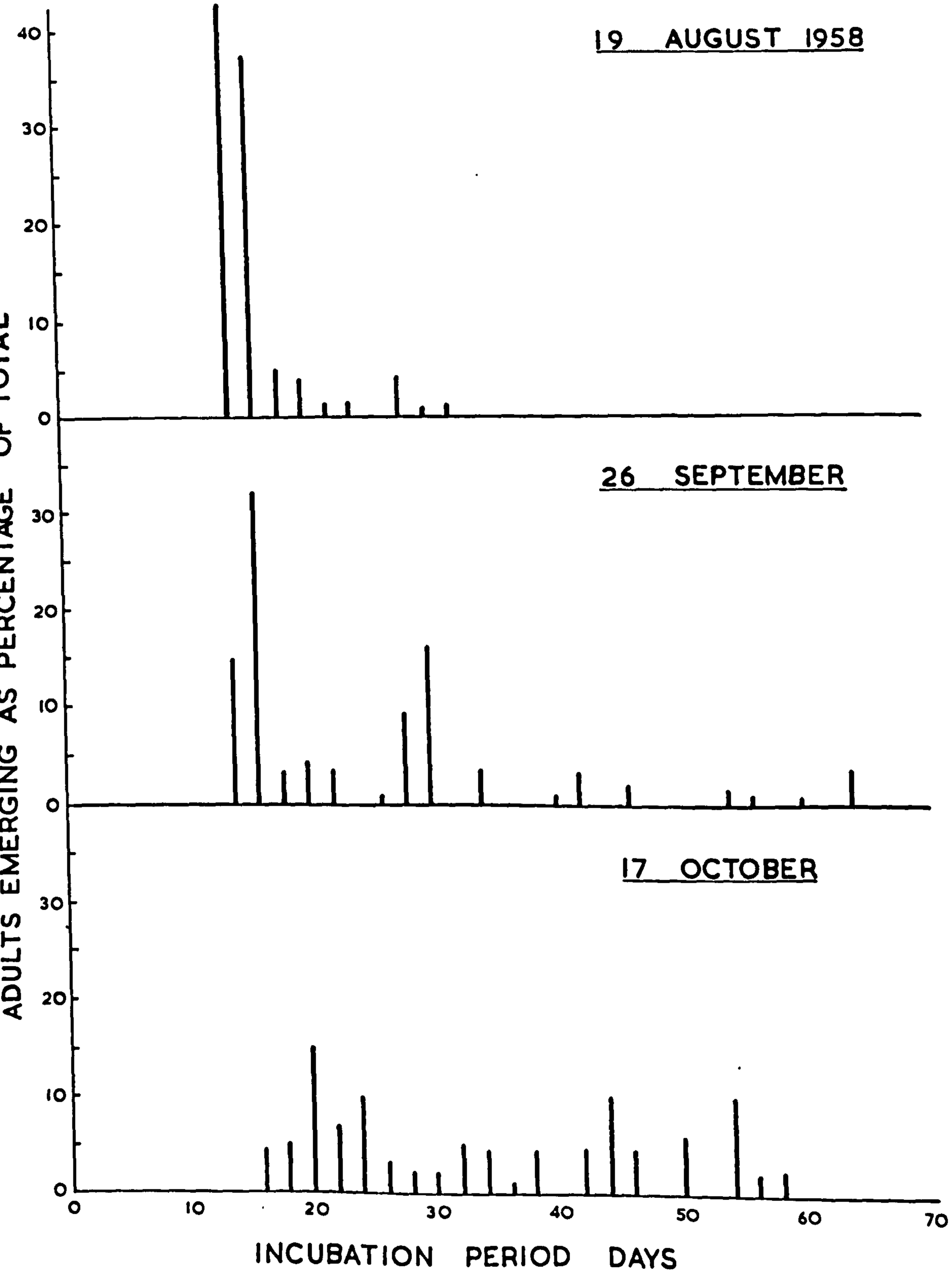

19 AUGUST 1958
26 SEPTEMBER
17 OCTOBER
PERCENTAGE OF TOTAL ADULTS EMERGING AS PER
40
30
20
10
0
INCUBATION PERIOD DAYS
0
10
20
30
40
50
60
70

Table 38. Effect of constant and field temperatures on the induction of diapause in mature larvae collected at intervals during 1959. (*C. nasturtii*)

Date of Collection		10°C.					T*	15°C.					T*	20°C.					T*	25°C.					T*	Field temperatures					T*
7/7/59	Midges	9	14	16	14	14	67	16	15	9	16	16	72	10	14	12	10	19	65	10	14	13	14	19	70	14	7	9	8	14	52
	Parasites	0	0	0	0	2	2	4	3	3	0	2	12	6	0	0	1	1	8	6	4	4	5	1	20	2	3	1	0	1	7
	Diapause	10	2	3	5	4	24	0	1	2	1	1	5	0	2	1	1	0	4	0	0	1	0	0	1	0	0	1	0	1	2
	Survival	19	16	19	19	20	93	20	19	14	17	19	89	16	16	13	12	20	77	16	18	18	19	20	91	16	10	11	8	16	61
28/7/59	Midges	10	11	10	8	13	52	14	12	16	17	11	70	14	16	17	13	14	74	15	11	12	10	16	64	18	15	16	12	15	76
	Parasites	0	0	0	0	0	0	0	0	0	0	2	2	1	3	2	0	2	8	2	3	5	2	4	16	0	0	2	4	0	6
	Diapause	8	8	3	5	2	26	3	5	2	2	2	14	5	1	0	4	2	12	0	0	1	1	0	2	0	2	1	2	1	6
	Survival	18	19	13	13	15	78	17	17	18	19	15	86	20	20	19	17	18	94	17	14	18	13	20	82	18	17	19	18	16	88
27/8/59	Midges	10	8	8	12	10	48	10	9	11	16	14	60	15	12	14	15	14	70	12	12	11	14	11	60	8	8	7	8	8	39
	Parasites	1	0	1	1	0	3	3	1	0	0	3	7	0	2	1	0	1	4	1	0	1	0	1	3	0	0	2	1	1	4
	Diapause	8	9	7	4	7	35	6	6	7	3	2	24	3	5	2	3	5	18	3	5	3	2	4	17	5	5	5	7	2	24
	Survival	19	17	16	17	17	86	9	16	18	19	19	91	18	19	17	18	20	92	16	17	15	16	16	80	13	13	14	16	11	67
8/9/59	Midges	1	4	4	3	1	13	2	6	3	7	2	20	7	7	11	8	9	42	14	12	8	9	6	49	5	8	1	6	5	25
	Parasites	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Diapause	14	14	14	15	14	71	10	9	10	10	18	57	8	8	5	7	5	33	0	4	8	2	7	21	9	8	13	8	10	48
	Survival	15	18	18	18	15	84	12	15	14	17	20	78	15	15	16	15	14	75	14	16	16	11	13	70	14	16	14	14	15	73

*T = Totals

5 tubes at each temperature; each tube started with 20 larvae.

soaked, drained and then kept in groups of five, four in incubators at constant temperatures 10°, 15°, 20° and 25°C., and one in the field (the 5 tubes here being sunk in the soil of WaWheldon). Thereafter, tubes at constant temperature were soaked and drained about once per week but the moisture content of 'field' tubes, although initially high, later fluctuated according to weather. The initial high moisture content ensured that larvae would not become quiescent through moisture lack.

Tubes were inspected daily (constant temperature) or 2-4 times per week ('Field') and emergences (midge and parasites) noted. Numbers of diapause larvae remaining in each tube were counted about 30 days after emergence began. Occasionally viable pupae were recovered ('late developers') and these were included in the midge totals, i.e. considered as being non-diapause. Complete results are shown in Table 38. 'Field' results are considered first since these are hardly comparable with those of constant temperatures.

Mean numbers of diapause larvae and mean survival (midges + parasites + diapause larvae) for 'field' tubes are plotted against collection dates in Fig. 20. The gradual rise in the incidence of diapause with time is quite evident (Table 39, V.R. = 41.3, with 3 and 16 df., $p < .001$). Naturally, the converse is also true in that

FIGURE 20.

Onset of diapause.
Larvae collected and reared in the field
(W.Wheldon, 1959)
(*C.nasturtii*)

o diapause larvae.

X survival = midges + parasites + diapause larvae.

MEAN SURVIVAL FOR EACH COLLECTION DATE.

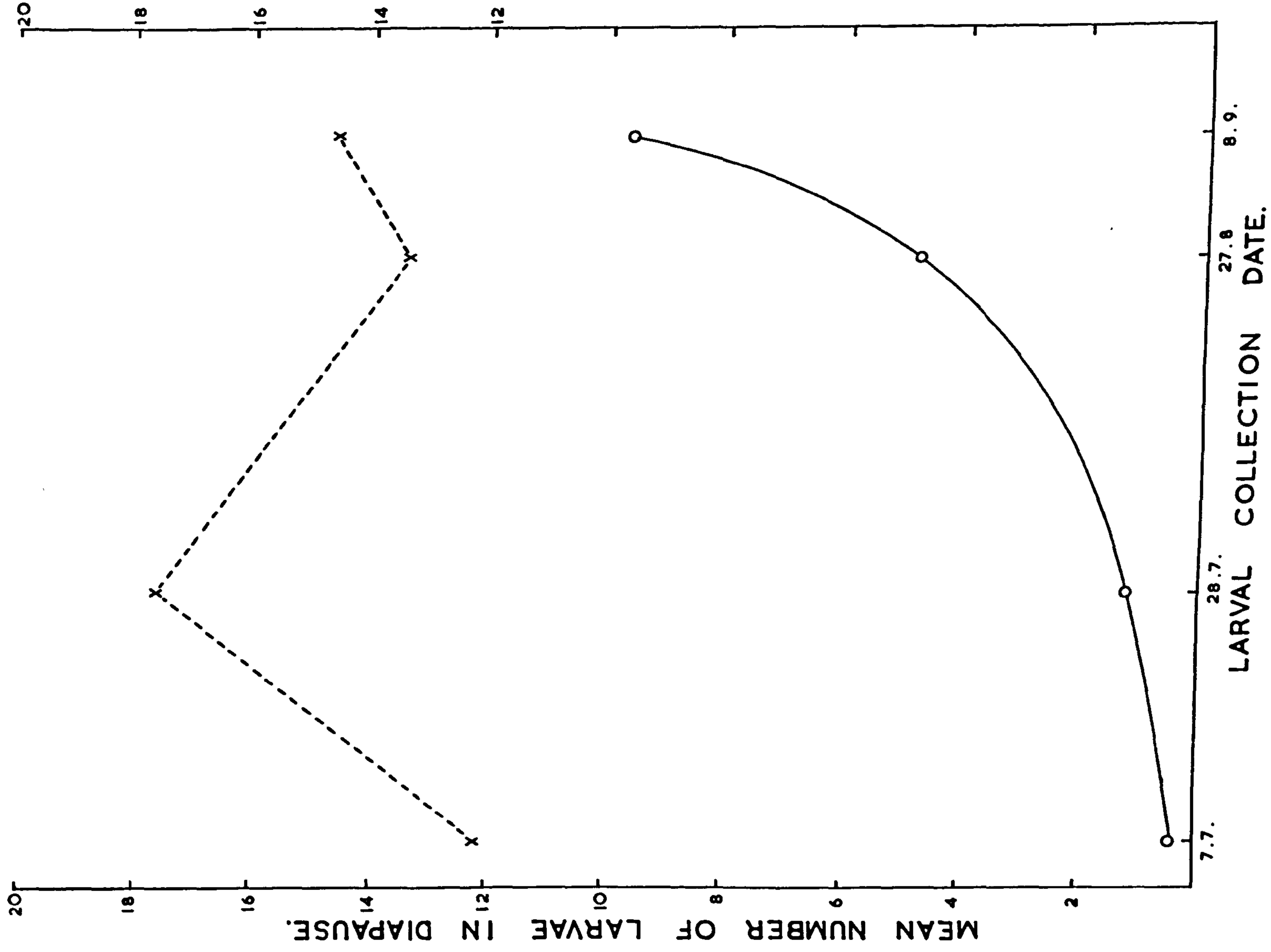


Table 39. Analyses of variance of 'Field data' in Table 38.

A. Emergence of adult midges and parasite (non-diapause).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Collection dates	3	351.75	117.25	22.00	<.001
Error	16	85.20	5.33		
Total	19	436.95			

B. Diapause larvae.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Collection dates	3	264.0	88.00	41.31	<.001
Error	16	34.0	2.13		
Total	19	298.0			

C. Total Survival (midges + parasites + diapause larvae).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Collection dates	3	80.95	26.98	5.83	<.01
Error	16	74.00	4.63		
Total	19	154.95			

the incidence of non-diapause larvae (those giving rise to midges or parasites) decreases with time (V.R. = 22.0, $p < .001$).

However, the survival of different batches varied significantly (V.R. = 5.83, $p < .01$) and this was probably due to fluctuation in soil-moisture; tubes of 7th July and 27th August were out during particularly dry weather. Examination of the soil-residues of these tubes revealed that at least 84.6% and 78.8% respectively of the total mortality (20-survival) was in fact due to pupal death (i.e. non-diapause), which meant that larval deaths (diapause) were fairly infrequent. This might have been expected from

(a) the smoothness of the diapause curve in Fig. 20 as compared with the irregular survival curve.

(b) the reduced variance ratio for non-diapause individuals (22.0) as compared with that for diapause (41.3) - see Table 39; naturally pupal death would only affect the former,

and (c) the fact that larvae survived for considerable periods in 'dry' soil (Section 4.231, Table 30).

The experiment also confirms the last suggestion of the 1958 results, namely that the temperature at which mature larvae are kept <u>after</u> they leave the plant further influences the onset of diapause. Reverting to Table 38, totals for diapause larvae at 10°, 15°, 20° and 25°C. for the first larval batch (7th July, 1959) were respectively

24, 5, 4 and 1, while totals for the last batch (8th September, 1959) at the same temperatures were respectively 71, 57, 33 and 21. The inverse relation between temperature and diapause is illustrated in Fig. 21 in which the mean number of diapause larvae per temperature is shown for each batch. Mean survival at each constant temperature, irrespective of collection date, is also shown and clearly this did not vary.

Analyses of Variance for the whole experiment (excluding 'Field' results) are set out in Table 40. The 'between collection' variances for both diapause and non-diapause individuals were highly significant and this is what one expects from 'field' results shown earlier. But clearly, the 'between temperature' variances were also highly significant, viz. for diapause larvae V.R. = 30.8, with 3 and 64 df. $p < .001$; and for emergence V.R. = 16.5, $p < .001$)

Obviously high temperature had induced some larvae to pupate which at lower temperature would have entered diapause.

It is interesting to note that the incidence of diapause increased abruptly between collection dates of 27th August and 8th September, particularly at the low temperatures (10° and 15°C.). This is evident when the mean number of diapause larvae for each temperature is

FIGURE 21.

Effect of collection date and subsequent constant temperature on induction of larval diapause,
(C.nasturtii)

○ ○ ● mean number of diapause larvae.

x mean survival for each constant temperature.

MEAN SURVIVAL FOR EACH TEMPERATURE.

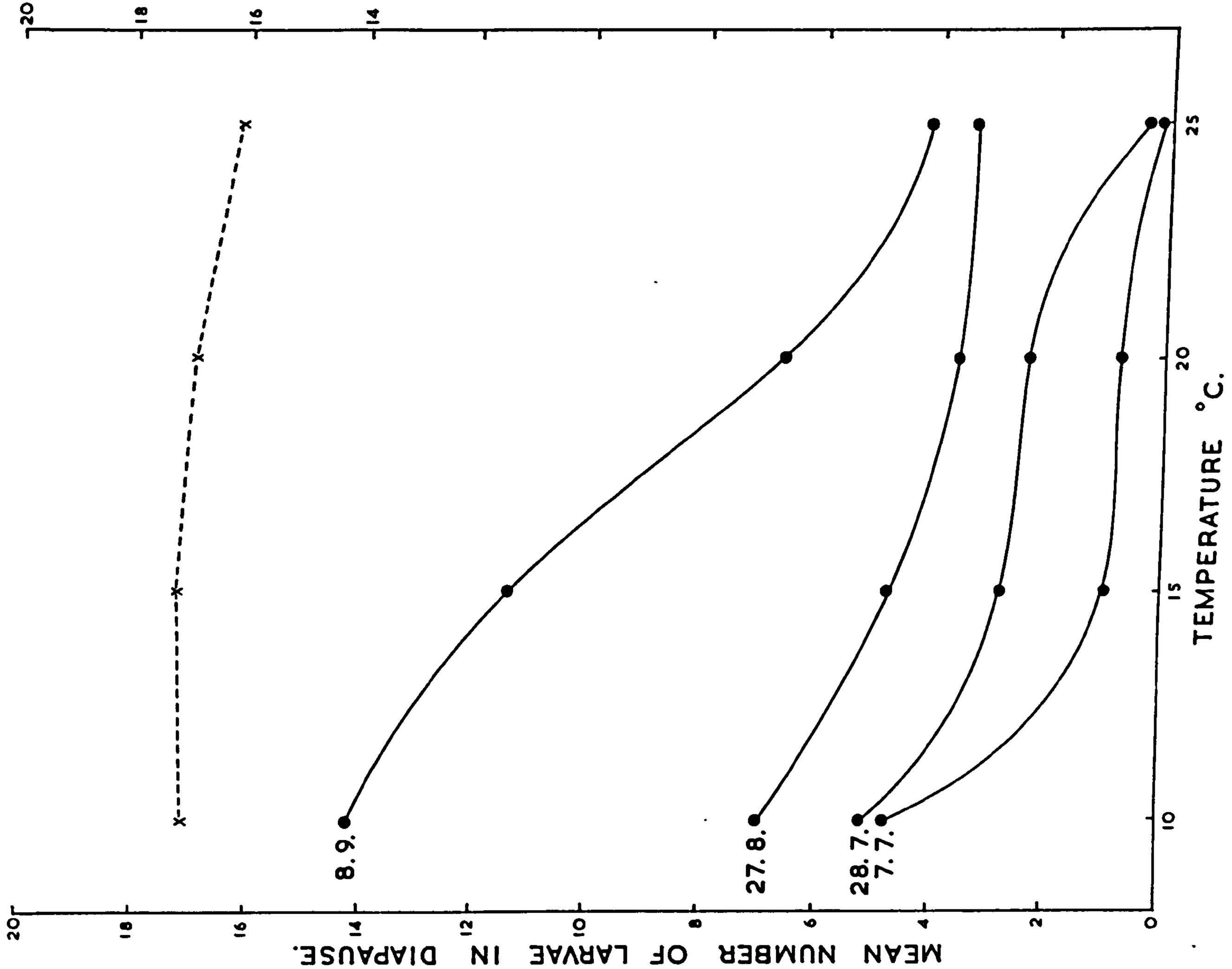


Table 40. Effect of different constant temperature on induction of larval diapause (C.nasturtii)

Analyses of Variance (from data in Table 38)

A. Emergence of adult midges and parasites (non-diapause).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperature	3	282.25	94.08	16.53	<.001
Collection dates	3	1061.05	353.68	62.16	<.001
Interaction	9	116.25	12.92	2.27	<.05
Error	64	364.40	5.69		
Total	79	1823.95			

B. Diapause larvae.

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperature	3	369.1	123.03	30.83	<.001
Collection dates	3	645.4	215.13	53.92	<.001
Interaction	9	103.7	11.52	2.89	<.01
Error	64	255.6	3.99		
Total	79	1073.8			

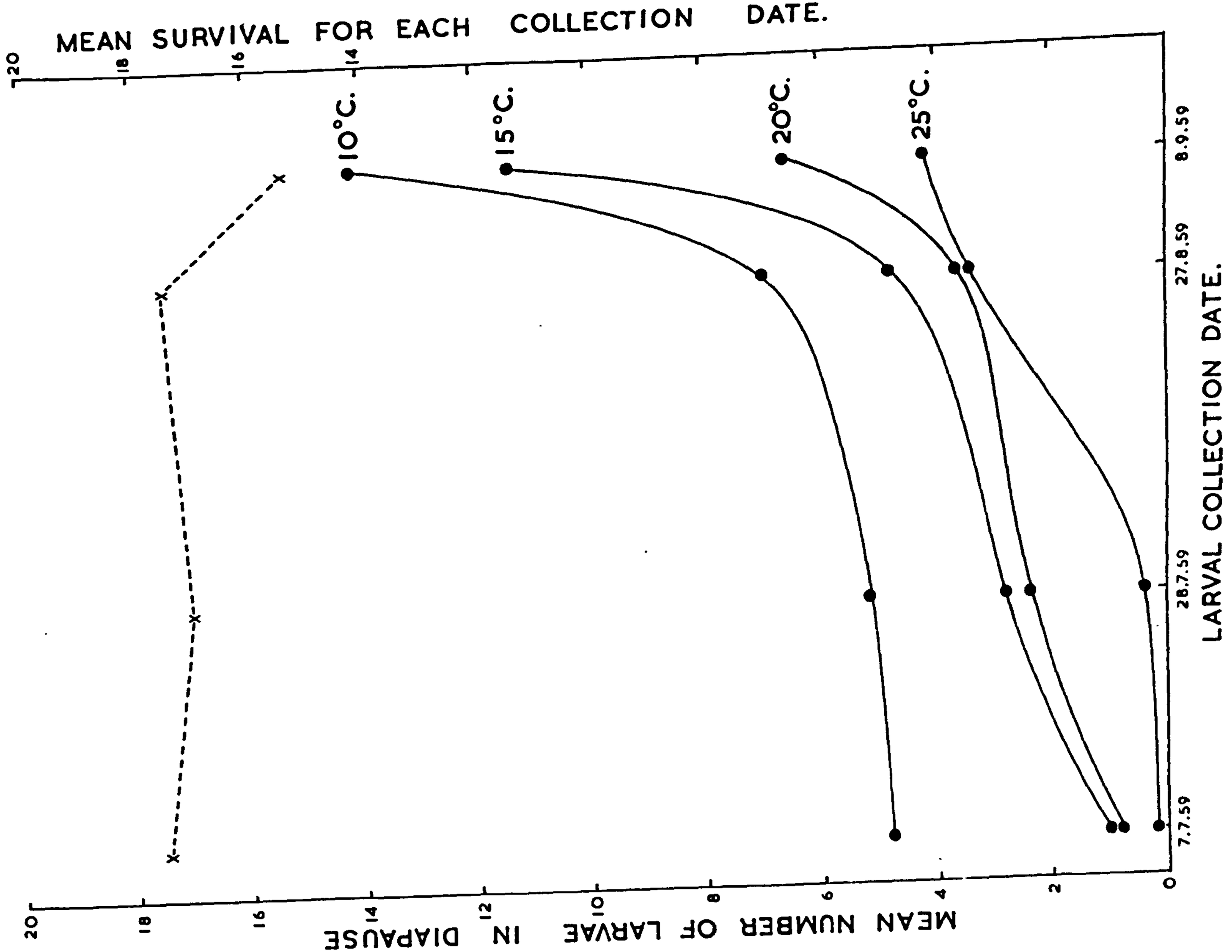
C. Total survival (midges + parasites + diapause larvae).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperature	3	13.05	4.35	1.13	>.2
Collection dates	3	61.05	20.35	5.27	<.01
Interaction	9	84.65	9.41	2.44	<.05
Error	64	246.80	3.86		
Total	79	405.55			

FIGURE 22.

Effect of constant temperature and collection date on induction of larval diapause, (C.nasturtii)

- ● mean number of diapause larvae.
- x mean survival for each collection date.


MEAN SURVIVAL FOR EACH COLLECTION DATE.
20
18
16
14
10°C.
15°C.
20°C.
25°C.
8.9.59
27.8.59
28.7.59
7.7.59
LARVAL COLLECTION DATE.
MEAN NUMBER OF LARVAE IN DIAPAUSE
20
18
16
14
12
10
8
6
4
2
0

plotted against time (i.e. collection dates) as in Fig. 22. Note also the reduced mean survival of the batch collected on 8th September as compared with survival among earlier collected batches. In fact, the variation in survival between collection dates was significant (V.R. = 5.27, $p < .01$). It may be that 'forced' pupation of late collected larvae at high temperature (20° and 25°C.) is associated with high mortality; at all events, metamorphosis is a particularly susceptible phase in development.

Larvae in the above experiments, being collected at four different dates, obviously had spent their feeding phase in four different sets of field conditions. It was therefore decided to investigate the effect of temperature on diapause induction among a large number of larvae which had all undergone the same conditions during the feeding phase, i.e. laboratory conditions.

Five hundred mature larvae from a large batch, which were reared on swedes kept at room temperature (17.2°-19.8°C.) and in normal day light (20th October-3rd November) in the laboratory were assigned to 25 rearing tubes (20 larvae per tube) at random. Groups of 5 tubes were then kept at four constant temperatures 10°, 15°, 20° and 25°C. and in the field (W.Wheldon), starting on 3rd November, 1959. Diapause larvae were counted 50 days after the start of emergence or, where there was no emergence, counts were made 100 days after the experiment began.

Results (Table 41) show that all larvae entered diapause in the 'field' and 10°C. tubes; totals recovered 100 days after the start of the experiment were 100 and 96 respectively. From the 15°, 20° and 25°C. tubes, emergence totals and diapause totals (in brackets) were respectively 2 (96), 19 (69) and 59 (29). Analyses of variance in Table 42 (excluding 'field' results) show that temperature had significant effects on emergence and diapause, but not on survival. Clearly, these results agree with those from field collected larvae in that fewer larvae entered diapause at high temperature.

However, another interesting point was that amounts of diapause in this experiment were just what one would expect had the larvae been collected from the field during September-October (cp. results for field collected larvae: Table 38 and Figs. 22 and 21). Obviously a relatively high rearing temperature during the feeding phase (room temperature 17.2°-19.8°C.) did not prevent a comparatively massive incidence of diapause, particularly among the 'field' and 10°C. treatments. This suggests that the main factor controlling diapause is not temperature, but something that changes to a much greater degree than temperature as the season progresses, irrespective of whether larvae are reared in the field or laboratory. Clearly, day length during the larval feeding period seems to possess the

Table 41. Effect of constant and field temperatures on induction of larval diapause: Larval feeding phase on laboratory swedes (17.2-19.8°C.) in normal day light, October-November, 1959.

Temp. °C.	Adults* emerging and larvae** remaining per tube					Total	S+
Field	- (20)	- (20)	- (20)	- (20)	- (20)	- (100)	100
10	- (18)	- (19)	- (20)	- (19)	- (20)	- (96)	96
15	- (20)	1 (19)	- (19)	- (20)	1 (18)	2 (96)	98
20	3 (15)	6 (13)	7 (10)	1 (16)	2 (15)	19 (69)	88
25	13 (7)	7 (10)	12 (4)	14 (5)	13 (3)	59 (29)	88

*Adults on left (non-diapause).

**Larvae in brackets (diapause).

+S = S represents survival.

Table 42. Analyses of variance of data in Table 41.

A. Emergence of adults (excluding field treatment).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperatures	3	449.2	149.73	40.69	< .001
Error	16	58.8	3.68		
Total	19	508.0			

B. Diapause larvae (excluding field treatment).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperatures	3	601.8	200.6	54.2	< .001
Error	16	59.2	3.7		
Total	19	661.0			

C. Survival (excluding field treatment).

Source of Variation	df	Sum of Squares	Mean Square	F	p
Temperatures	3	14.55	4.85	2.85	> .05
Error	16	20.40	1.70		
Total	19	34.95			

Table 43. Relation between day length (for the feeding larva), temperature (for the mature larva) and the incidence of diapause in 1959 (*C.nasturtii*)

Larval collections (or rearing*) date	Day length during feeding period (hours)	Percentage diapause at 10°C.	15°C.	20°C.	25°C.	'Field'
7th July	16.5	24	5	4	1	2
28th July	16.0	26	14	12	2	6
27th August	14.0	35	24	18	17	24
8th September	13.0	71	57	33	21	48
3rd November*	10.0	100	96	69	29	100

*larvae reared on laboratory swedes in day light (17.2°-19.8°C.)

properties that would make it mainly responsible for diapause control. Day length gradually decreases as the incidence of diapause increases (i.e. from the end of June onwards), and, moreover, it would have the same effect on laboratory reared larvae as on those reared in the field, since swedes were grown in normal day light in both cases. Tabulation of available data supports this view (Table 43). Obviously, although the percentage of diapause larvae varied with temperature at which the mature larvae were kept, it also increased regularly from batch to batch, as day length during each batch's feeding period decreased. The data therefore suggest that amounts of diapause will increase markedly if day length during the feeding phase falls below 14 hours per day. This suggestion would need to be tested by further experiments on the effect of photoperiod and temperature on the development of the feeding larva.

4.32 Termination of diapause.

Diapause larvae kept in moist soil at high temperature do not develop and since there is usually a close connection between climate and the physiological requirements for diapause (Lees, 1955) it might be expected that low temperature (as in winter) would favour diapause development.

During 1958 diapause larvae in spherical cocoons, which came from galled swedes in Middleton, were kept in moist soil at 20°C. until required. A batch of 400 cocoons were assigned at random to 20 rearing tubes (20 cocoons per tube) which were soaked, drained and then kept at 5°C. After 20, 40, 80 or 160 days at this temperature, five tubes were soaked, drained and transferred to 20°C. for a further 60 days. During this time adult emergence was recorded daily and then diapause larvae and empty cocoons remaining in soil-residues counted.

Fig. 23 shows that the proportion of larvae remaining in diapause decreased with increased duration at 5°C.; almost 100% completed diapause after 160 days at 5°C. Vertical lines on either side of each mean are standard deviations: obviously means for 160 and 80 days are significantly different from each other and also from means for 20 and 40 days.

Mean survival (adults and remaining larvae) is also shown in Fig. 23 and this likewise decreased with increasing duration at 5°C. The variation in survival was significant (V.R. = 9.8, with 3 and 16 df., $p < .001$) and was probably due to higher mortality among batches pupating because metamorphosis is a particularly susceptible stage in development.

To determine the temperature most favourable for

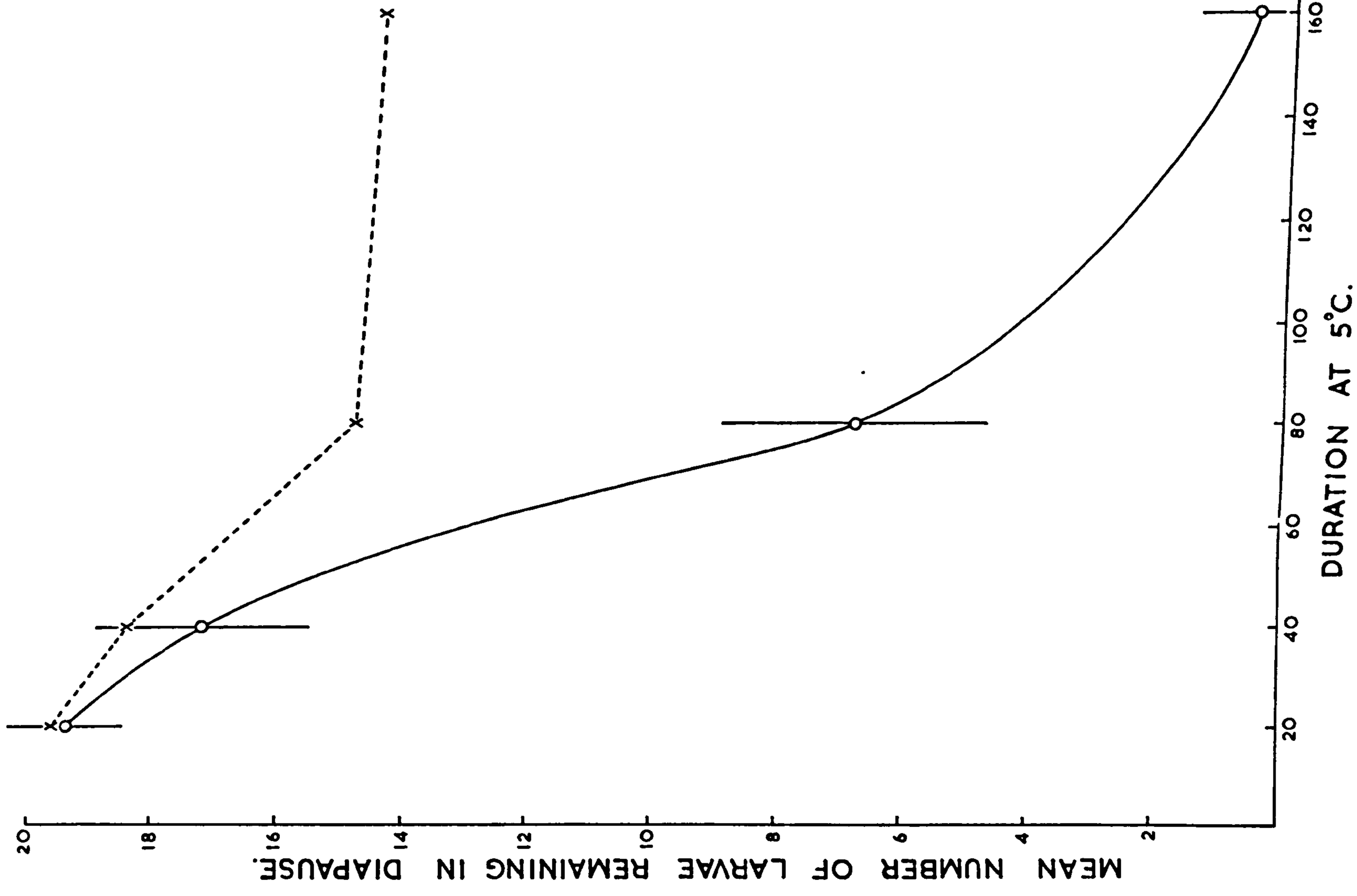
FIGURE 23.

Diapause development at 5°C.
(C.nasturtii)

o mean number of larvae remaining in diapause after final incubation at 20°C. for 60 days. Each mean is derived from 5 tubes of 20 larvae, and is delimited on either side by the standard deviation.

x means survival.

MEAN SURVIVAL FOR EACH DURATION.



diapause development, diapause cocoons were kept at 2°, 5° and 15°C. for 100 days and then at 20°C. for a further 60 days. There were three rearing tubes, each containing 10 cocoons, per temperature. Rearing tubes were soaked and drained before and after low temperature incubation. Fig. 24 shows the mean number of adults emerging and the mean number of larvae remaining in diapause. Obviously, 100 days at 2° and 5°C. caused 100% of the larvae to pupate, since none were recovered at the end of the experiment. On the other hand, little diapause development occurred at 15°C. since nearly all the larvae remained in diapause. This suggests that diapause development proceeds most rapidly at about 0°-5°C., the rate falling as temperature rises; it appears that no diapause development is possible at temperatures above 15°C. Clearly the temperature range favourable for diapause development is much lower and separate from that for morphogenesis (cp. Andrewartha, 1952).

In the two preceding experiments, soil-residues of tubes in which adults emerged invariably contained almost equal numbers of empty oval and empty spherical cocoons. Microphotographs of these are shown in Plate A. Obviously, larvae had left their spherical cocoons and pupated in oval cocoons. This is just what happened when 'dry' soil, containing non-diapause quiescent larvae (also in spherical

FIGURE 24.

Influence of temperature on diapause development

(*C.nasturtii*)

o mean number of larvae remaining in diapause after final incubation at 20°C. for 60 days.

● mean number of adults emerging.

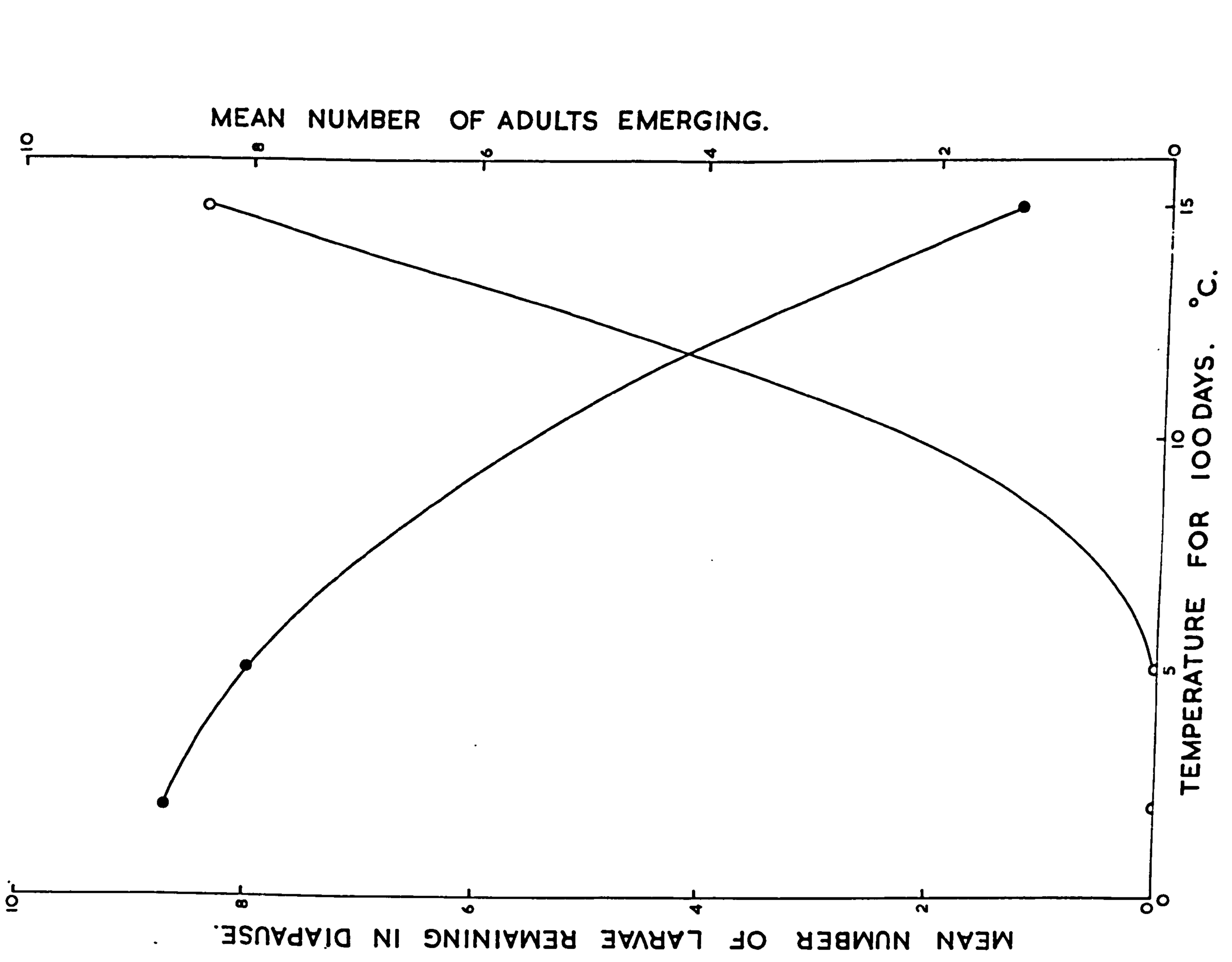
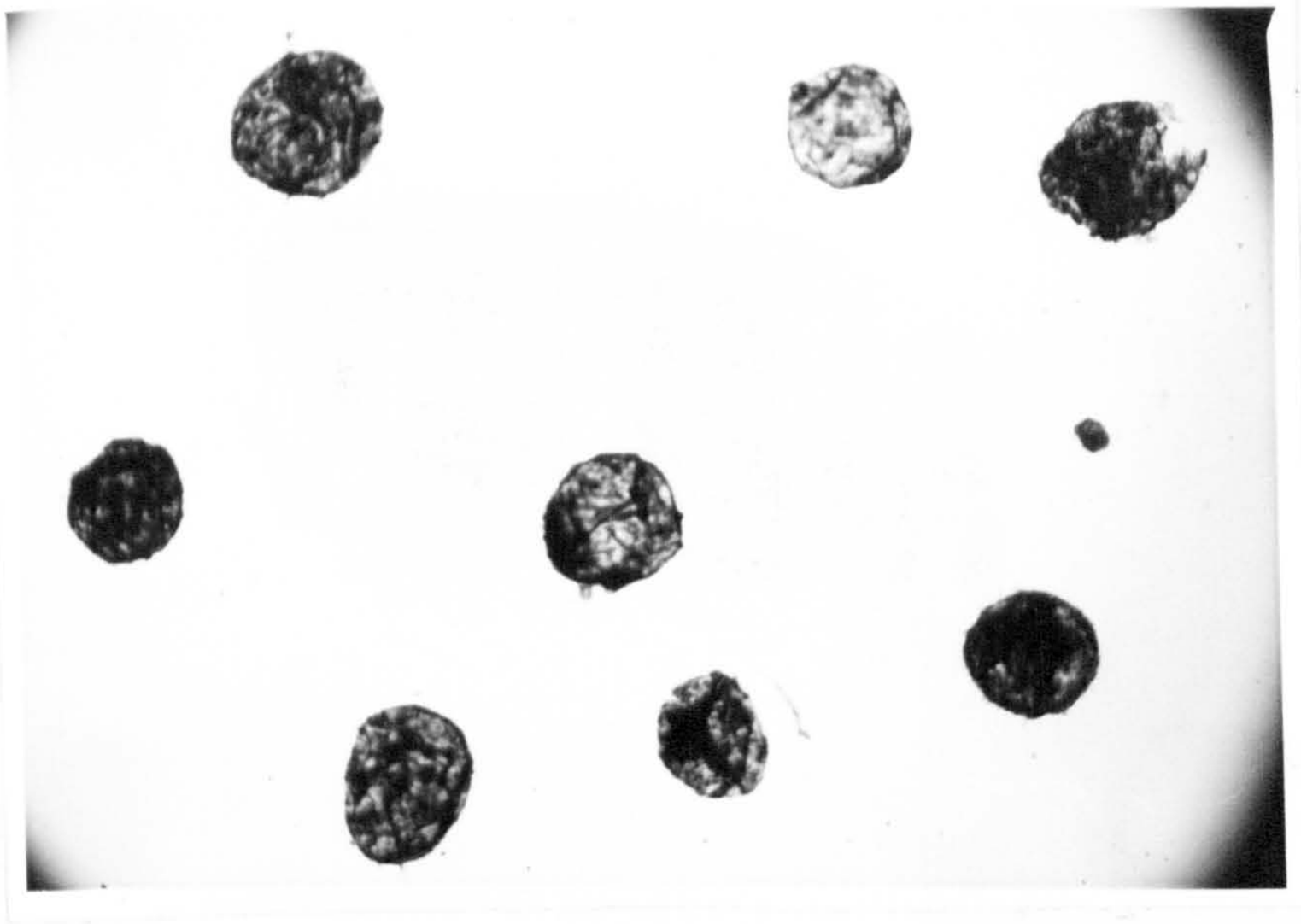
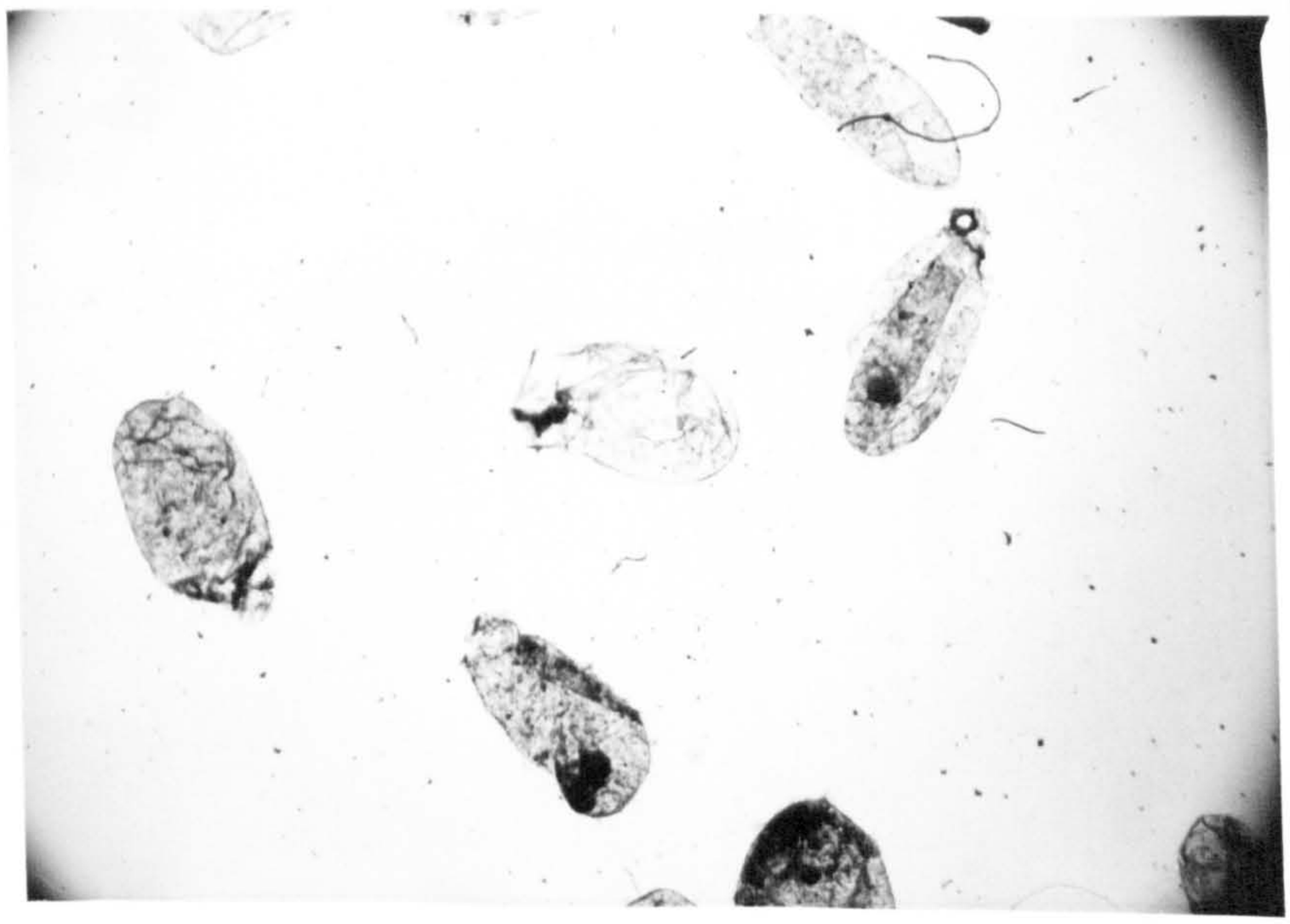

MEAN NUMBER OF ADULTS EMERGING.
10
8
6
4
2
0
TEMPERATURE FOR 100 DAYS. °C.
15
10
5
0
MEAN NUMBER OF LARVAE REMAINING IN DIAPAUSE.
10
8
6
4
2
0

Plate A. Empty cocoons of C.nasturtii



Larval



Pupal

5.0 MM.

cocoons), was thoroughly wetted (Section 4.23). But in the experiments described above, tubes were frequently soaked so that quiescence through moisture lack could not occur. Later experiments show how soil-moisture may influence post-diapause development.

For convenience let development at the subsequent high temperature (20°C.) be called post-diapause development in the case of individuals which ultimately pupated. Then the mean time for completion of post-diapause development (i.e. the mean time elapsing between the start of high temperature and the emergence of the adult) was 47.7 days (N = 91, range = 37-60). The emergence curve reached a peak after about 42 days, then fell abruptly and emergences continued sporadically for the duration of the experiment. In another experiment, post-diapause larvae were kept at 25°C. and the mean time to emergence was 42.7 days (N = 27, range = 32-57). At both temperatures males emerged a little earlier than females. The mean time to emergence may be extended indefinitely by keeping post-diapause larvae at low temperature or by keeping them in 'dry' soil at high temperature. However, it was not possible to curtail this period, either by incubation at higher temperatures (post-diapause larvae) or by prolonged exposure to low temperature during diapause development. And since the duration of the pupal period at 20°C. and 25°C. is less

than 14 and 10 days respectively (Section 4.21), then, at high temperature, the post-diapause larva must spend about 30 days in a quiescent state unable to leave its spherical cocoon: for example, at 20°C., time spent in cocoon equals 47.7 - 14 = 33.7 days and at 25°C., 42.7 - 10 = 32.7 days. During this 30-day period the post-diapause larva may be eliminating a substance produced during diapause development (at 0°-5°C.) which inhibits further development, i.e. cocoon vacation and pupation. At all events, observations suggest that a period at high temperature is a necessary phase of development preceding pupation.

The following experiment concerned both onset and termination of diapause and gave results which were rather unexpected. Perhaps this was because larvae had spent their feeding phase at high temperature (17.2°-19.8°C.) in the laboratory (Section 4.31, Table 41). When mature, they were kept in moist soil at one of four constant temperatures, 10°, 15°, 20° and 25°C. (100 larvae per temperature) for 100 days. Some adults emerged and diapause larvae were recovered at the end of the experiment (R, left half of Fig. 25). These diapause larvae were given further treatments (as shown in the right half of Fig. 25): all the larvae from 25°C. were left at 25°C. for a further 80 days, but larvae from the other three temperatures were in each case split into two groups at random, one of which

FIGURE 25.

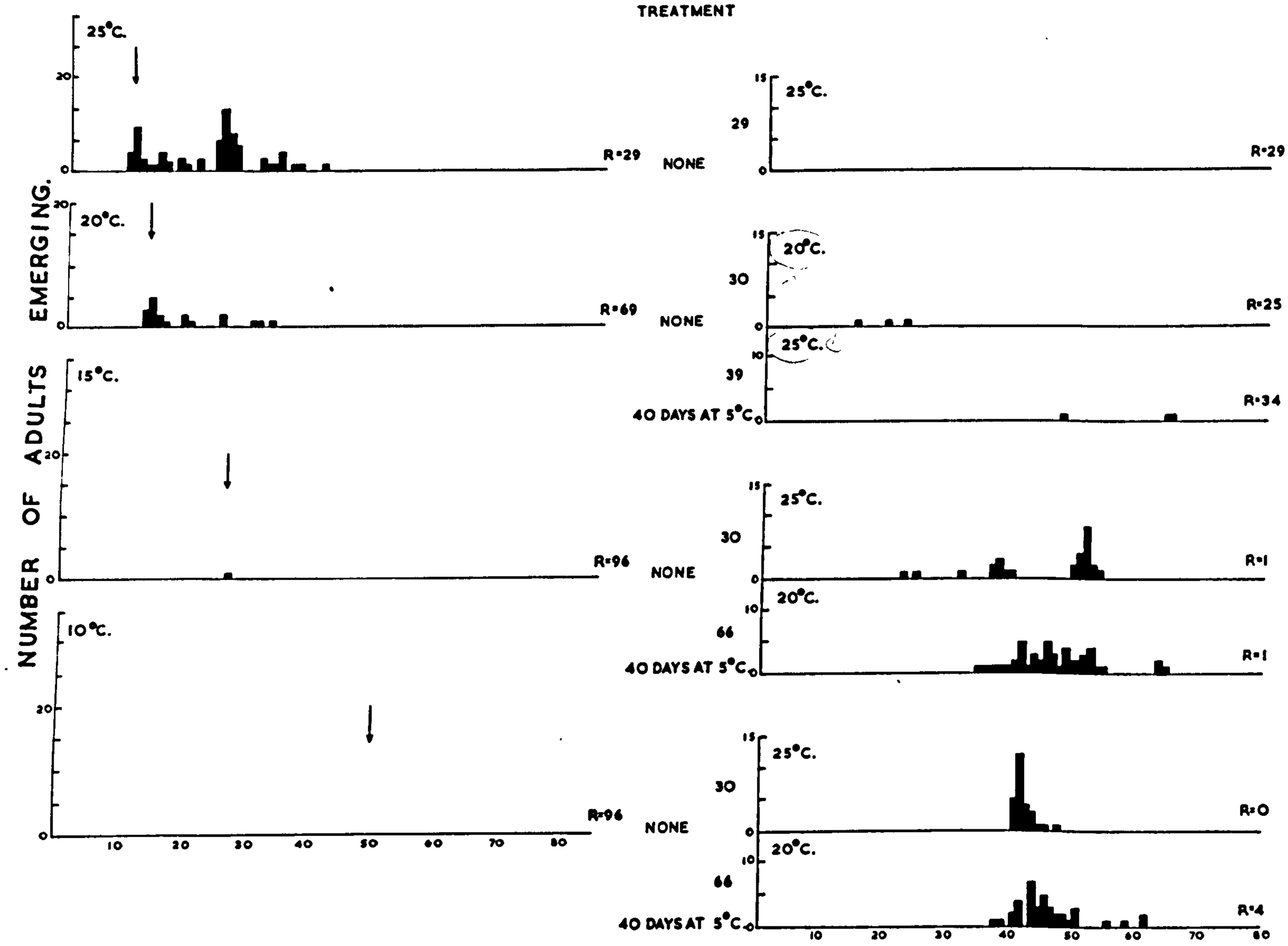
Onset and termination of diapause
(*C.nasturtii*)
Larvae reared on laboratory swedes
(17.2°-19.8°C.) in normal day light

Initial treatment (left half of Figure): larvae at 10°, 15°, 20° and 25°C. in moist soil. Arrows indicate normal peak day of emergence (non-diapause).

Further treatment (right half of Figure): larvae kept at 25°C., or first at 5°C. for 40 days and then at 20°C.

R diapause larvae recovered at the end of each treatment.

Black columns: daily adult emergence.


TREATMENT
NUMBER OF ADULTS EMERGING.
25°C.
R=29
20°C.
R=69
15°C.
R=96
10°C.
R=96
NONE
25°C.
R=29
NONE
20°C.
R=25
25°C.
R=34
40 DAYS AT 5°C
NONE
25°C.
R=1
20°C.
R=1
40 DAYS AT 5°C
NONE
25°C.
R=0
20°C.
R=4
40 DAYS AT 5°C
INCUBATION PERIOD DAYS.

was kept at 25°C. for 80 days, and the other kept first at 5°C. for 40 days and then at 20°C. for 80 days. All larvae were in rearing tubes of soil which were soaked and drained once per week. In the right half of Fig. 25, larvae starting in each treatment are shown next to the respective ordinates, and R shows numbers recovered at the end of the experiment. These were still in diapause.

Results suggest that larvae which entered diapause at 10° and 15°C. were able to complete diapause development at these temperatures since nearly all of them produced adults 40-60 days after entry into high temperature (20° and 25°C.). The extra 40-day term at 5°C. had little effect on emergence. On the other hand, larvae entering diapause at 20°C. were not able to complete diapause development, even after 40-days at 5°C. Similarly, no diapause development occured at 25°C.

Since larvae in this experiment completed diapause development in less than 100 days at 15°C. whereas those in earlier experiments did not (Fig. 24), it may be that the firmness of diapause varies, possibly with conditions during the onset of diapause. It is interesting to note that (a) the incidence of diapause among eggs of Gryllulus commodus Walker varies inversely, and that (b) the firmness of diapause varies directly, with the incubation temperature of the egg (Browning, 1952 b). Results in Section 4.31 show that (a) is

equally true for larvae of C.nasturtii and results of the last experiment suggest that (b) may also apply. At all events, it appears that the firmness of the larval diapause does vary, probably with the incubation temperature.

4.33 Effect of soil-moisture on post-diapause development.

In preceding experiments, post-diapause larvae were kept in rearing tubes of soil which were soaked at least once per week. In these conditions the mean time to emergence was 47.7 days, 30 days of this period being spent in the larval stage. The following experiment shows how soil-moisture may effect this phase of development.

Larvae in spherical cocoons which had completed diapause development (100 days at 5°C.) were assigned at random to three series of tubes, A, B and C. Series A and Series B each had five specimen tubes (3 x 1 inch) with 1-inch of 'dry' (8.0% water) and 1-inch of 'moist' (20.1%) soil respectively (20 cocoons per tube). Tubes were corked, sealed with wax and kept at 20°C. Series C tubes (9 in all) were rearing tubes (2 x 1 inch) with 1-inch of 'dry' (8.0%) soil (10 cocoons per tube). These were also kept at 20°C. The soil in all tubes was the light sandy loam used earlier (Section 4.23).

Groups of Series C tubes (3 tubes per group) were soaked and drained after intervals of 20, 30 and 40 days at 20°C. Series A and B tubes were unsealed and their

soil soaked and drained after 50 days at 20°C. No adults emerged prior to soaking in any of the tubes.

Soon after soaking (1-24 hours) numerous 'free' larvae were observed in the soil of all tubes, except those of the first group of Series C (20-day soak), and it was clear that soaking had induced post-diapause larvae which had spent at least 30 days at 20°C. to leave their spherical cocoons. From Fig. 26, showing adult emergence in each Series, it is clear that vacation of spherical cocoons was immediately followed by pupation in oval cocoons, since in every case (again except for the first group of Series C), emergence began about 14 days after soaking and duration of the pupal stage at 20°C. is known to be about 14 days (Section 4.21). Obviously, post-diapause larvae in 'dry' or even in 'moist' soil could not vacate their cocoons until the surrounding soil was thoroughly wetted. However, the Figure also shows that post-diapause larvae, irrespective of soil-moisture, do not leave their cocoons until about 30 days at 20°C. have elapsed; for example, *soaking after 20 days at 20°C. (group 1 Series C) did not stimulate larvae to vacate their cocoons at once, and consequently did not reduce the 'normal' mean time to adult* emergence. Larvae in these tubes left their cocoons several days after soaking, since emergence began on day 40, i.e. more than 14 days from soaking on day 20.

FIGURE 26.

Effect of soil-moisture on post-diapause development (*C.nasturtii*)

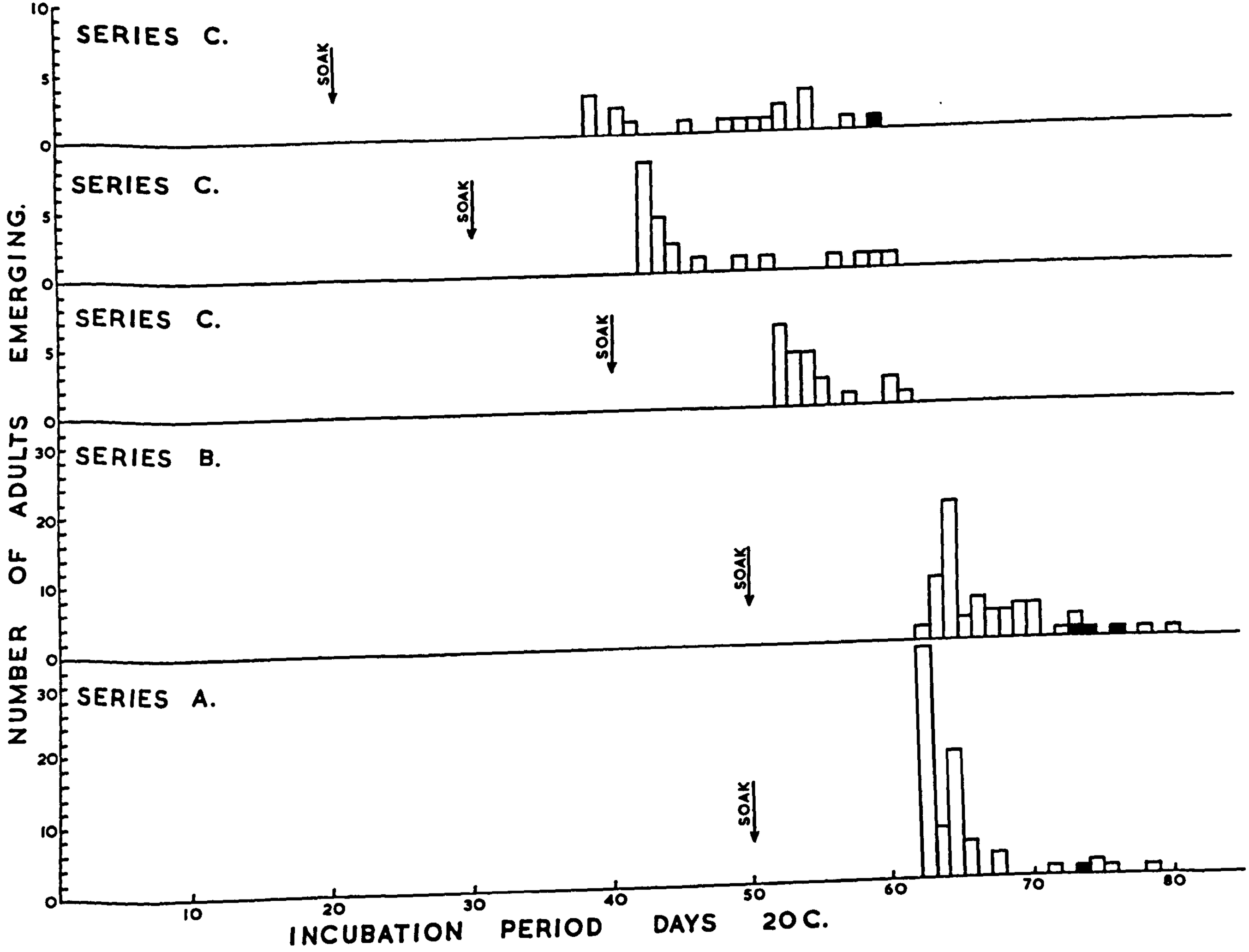
Series A 5 tubes with 1-inch of 'dry' soil at 20°C., 20 cocoons per tube. Soaked and drained after 50 days.

Series B 5 tubes with 1-inch of 'moist' soil at 20°C., 20 cocoons per tube. Soaked and drained after 50 days.

Series C 9 tubes with 1-inch of 'dry' soil at 20°C. Groups of 3 tubes soaked and drained after 20, 30 or 40 days.

White columns: midge emergence.

Black columns: parasite emergence (*Synopeas*)


SERIES C.
SOAK
SERIES C.
SOAK
SERIES C.
SOAK
SERIES B.
SOAK
SERIES A.
SOAK
NUMBER OF ADULTS EMERGING.
0
5
10
0
5
10
20
30
0
10
20
30
10
20
30
40
50
60
70
80
INCUBATION PERIOD DAYS 20 C.

Results therefore show that post-diapause larvae spend about 30 days at 20°C. before they are ready to vacate their cocoons. This period occupies most of the time spent in the soil when moisture is not limiting. However, after this period, further development depends mainly on soil-moisture. Spherical-cocoon vacation and therefore pupation, is delayed unless the soil becomes thoroughly wet. Even in 'moist' soil, equivalent to pF 3.4, spherical-cocoon vacation was delayed until the day of soaking (day 50). It is worth noting that the wetting of cocoons which have spent more than 30 days at 20°C. in 'dry' soil leads to mass spherical-cocoon vacation and therefore mass emergence. This is evident from the 'peaked' emergence curves in Series A, B and the last two groups of Series C. The "peaking" is obviously due to moisture lack being a developmental barrier which allows later developers to catch up.

4.4 Spatial distribution of mature larvae and pupae.

4.41 Horizontal.

The mature larva crawls away from the slimy surface of its gall on to an exposed leaf. Sometimes it simply falls from there to the soil. In most cases, however, it 'leaps' downwards by curling up and then suddenly straightening out so that it flips into the air, the process being repeated as often as is necessary. Once on the soil it immediately burrows out of sight. Observations indicate that larvae do not move far in soil, except perhaps when leaving their spherical overwintering cocoons in early summer.

Galled swedes of Gen. I, at least, tend to be aggregated in clusters (Section 3.631), therefore mature larvae in soil tend to be similarly distributed. The following experiment indicates how distribution varies in soil round the bases of galled swedes.

Larval migration from 9 galled swedes was observed in June-July, 1959, in the field (Section 3.5). These swedes were isolated, there being no other swede plants within 2 ft. radius and no other galled swede plants within 4 ft. radius. All but one of the experimental swedes had severe central shoot galls; in fact numbers of larvae per galled swede were somewhat higher than average for that particular generation. Larvae vacating their hosts

were trapped where they fell on sheets of sticky cardboard which lay over the soil surface round the base of each swede and which were marked out in 3-inch wide concentric belts. Numbers caught in belts 0-3, 3-6, 6-9, ... 21-24 inches from the stem of each swede are shown in Table 44. Most larvae fell within 9 inches of the swede stems and numbers decreased as distance away from the stem increased. This is more evident when results are expressed as larval densities: thus, beneath the average isolated galled swede (N = 9), mean numbers of larvae per square inch landing on successive belts were respectively 0.25, 0.15, 0.07, 0.02, 0.02, 0.007, 0.002 and 0.0008.

In the field, however, swedes are grown about 12 inches apart in drills 24 inches apart so that larval density on the drills tends to be much higher than that between the drills. This suggests that a suitable sampling method for larvae in soil is one whereby each sampling unit has two recording units (soil cores), one being on the drill and the other midway between the drills. Numbers recovered from such units would be less variable and therefore more representative of population (for a given number of units) than those from single units of the same area taken at random points.

Non-diapause larvae pupate near their point of entry *to the soil so that one might expect pupal distribution in*

Table 44. Horizontal distribution of mature larvae around the bases of nine galled swedes, C.nasturtii

Belts	Galled swede									Belt
(inches)	1	2	3	4	5	6	7	8	9	Totals
0-3	9	13	11	10	12	4	3	18	29	109
3-6	21	29	2	3	13	23	19	18	18	146
6-9	26	4	-	31	13	19	11	1	1	106
9-12	16	-	-	3	1	10	2	11	3	46
12-15	9	-	-	9	3	3	14	4	1	43
15-18	6	-	-	1	-	7	4	-	1	19
18-21	2	-	-	1	1	1	-	-	3	8
21-24	-	-	-	-	2	-	-	-	1	3
Larvae per swede	89	46	13	58	45	67	53	52	57	

a horizontal plane to be the same as that of the larvae.

4.42 Vertical.

The pupal cocoon is constructed just below the soil surface. Larval cocoons (spherical) in laboratory rearing tubes were invariably constructed 0-2 inch below the surface. Cocoon depth was unaltered in either negative or positive vertical moisture gradients, though these tended to have abrupt moisture divisions. Field samples from undisturbed soil beneath previously galled swedes showed that 'free' larvae and larval cocoons were restricted to 0-3 inch depth. Cultivations, however, bury cocoons at greater depths so that sampling depth is determined by depth of cultivation (e.g. ploughing depth) rather than the 'natural' vertical distribution of the population.

In early summer or after rain following a period of drought, larvae which have been quiescent through diapause or moisture-lack leave their spherical cocoons (which may have been buried), move to the soil surface and then re-enter to construct their pupal cocoons.

SECTION 5. PARASITES AND PREDATORS.

5.1 Parasites.

Three species of parasitic Hymenoptera (Proctotrupoidea) were reared from mature larvae during this study. These are now represented in the world collection of the Commonwealth Institute of Entomology as

Species A *Platygaster* sp.
Species B *Synopeas* sp.
Species C *Synopeas* sp.

(List no. 1716 (Europe), Collection no. 16922).
Adults of species A and B are shown in Plate B.

The "unidentified proctotrupids" mentioned by Dry (1915) and the "platygasterines" mentioned by Hörnig (1953) might well be members of these genera. However, the chalcid *Pirene exima* Halliday recorded by Bovien and Knudsen (1950) was not found here.

The life-cycles of these three proctotrupid species have received little attention but our observations suggest that they may be similar: Adults emerge from their host larvae in the soil and crawl to the soil surface. After mating, the female lays one egg in each of the feeding host larvae she discovers. The parasite egg hatches only when its host larva is about to pupate. Up to this time there are no obvious differences in behaviour or appearance between parasitized and healthy larvae. Both enter the soil and, according to their type (diapause or non-diapause) and moisture content of the soil, construct oval or

Plate B. Adult Parasites of C.nasturtii

2.0 MM.

Platygaster sp. A. (male on left)

Synopeas sp. B. (male on left)

spherical cocoons. Just before moulting, i.e. after the larva has made its oval pupal cocoon, the parasite larva hatches from the egg, devours the surrounding tissues, pupates and emerges as an adult. Factors which delay host pupation, such as diapause or moisture lack, obviously also delay parasite development by similar amounts (see Figs. 16 and 26).

5.11 Speed of development at constant temperature.

Section 4.21 shows how temperature influenced development of the host larva. These and other experiments often involved a proportion of parasitized larvae whose development was also studied. Table 45 compares the mean times to emergence for the host midge and its parasites (A, B and C) at various temperatures (time being measured from day that mature C.nasturtii larvae entered the soil). Observations for Synopeas B are more numerous (since this species was the most abundant of the three) and are graphed in Fig. 27. Obviously, the correspondence between the two free-hand curves suggests that the host's pupal period and the parasite's developmental period (egg to adult) have the same rate/temperature coefficient. This is what one expects if a parasite is to survive in successive generations of its host. It is interesting to note that at 20°C. the mean time to emergence (non-diapause) of Platygaster A (35 days) is much longer than that of Synopeas B (25 days).

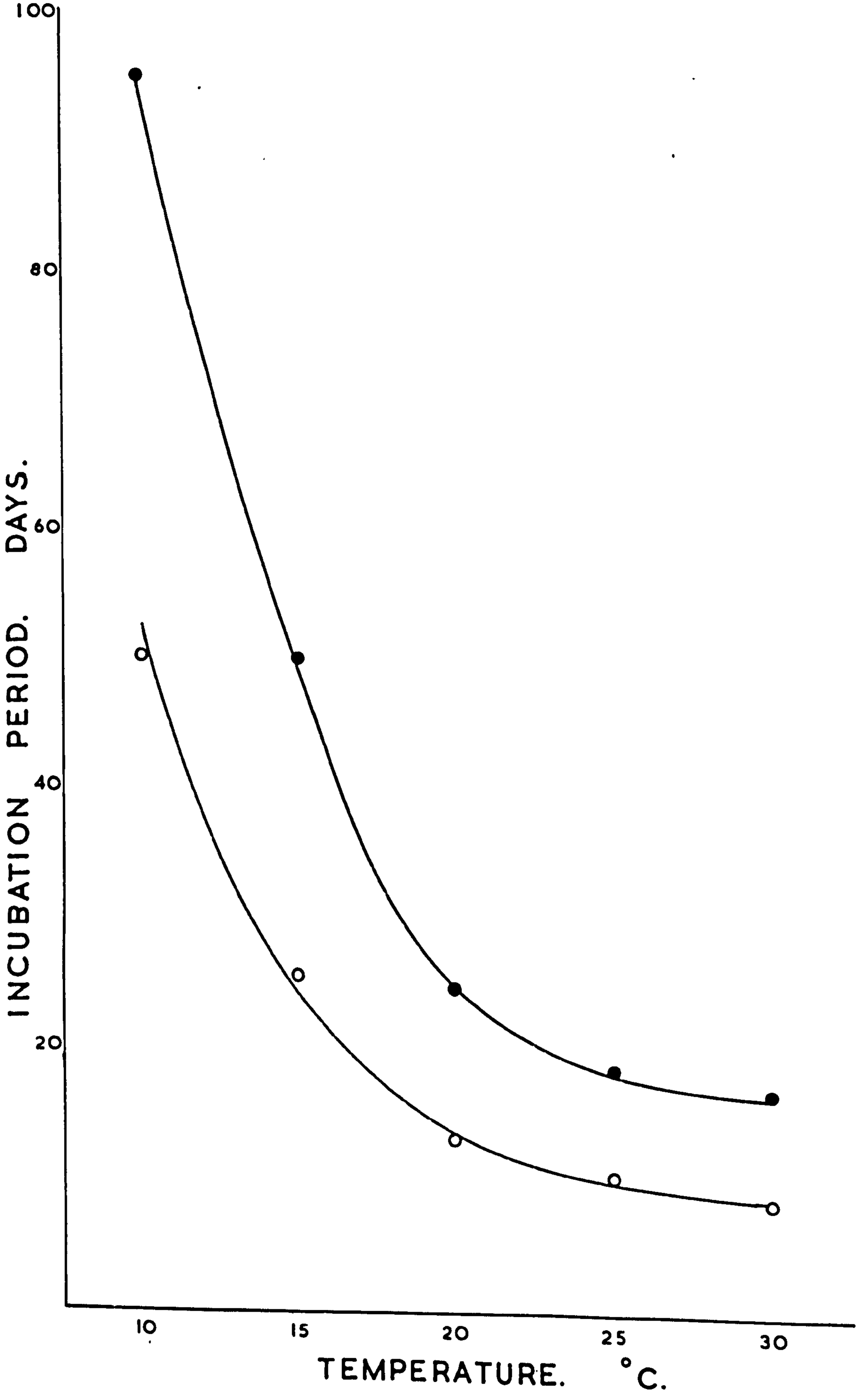
Table 45. Time needed to develop from larva to adult in both C.nasturtii and its parasites. Time measured from day that mature C.nasturtii larvae entered the soil.

Temp. °C.	Mean time to adult emergence (days) Number of observations in brackets					
	Non-Diapause				Post-Diapause	
	Host	Platygaster A.	Synopeas B.	Synopeas C.	Host	Synopeas B.
10	50	-	97 (3)	-	-	-
15	26 (147)	-	50 (7)	-	-	-
20	14 (176)	35 (29)	25 (123)	28 (1)	48 (91)	58 (16)
25	10 (148)	-	18 (27)	-	43 (27)	-
30	8 (65)	-	16 (11)	-	-	-

FIGURE 27.

Speed of development of the mature host larva
(C.nasturtii)
and of its parasite (Synopeas B)
at constant temperature

o Mean time to adult emergence (host)
● Mean time to adult emergence (parasite)

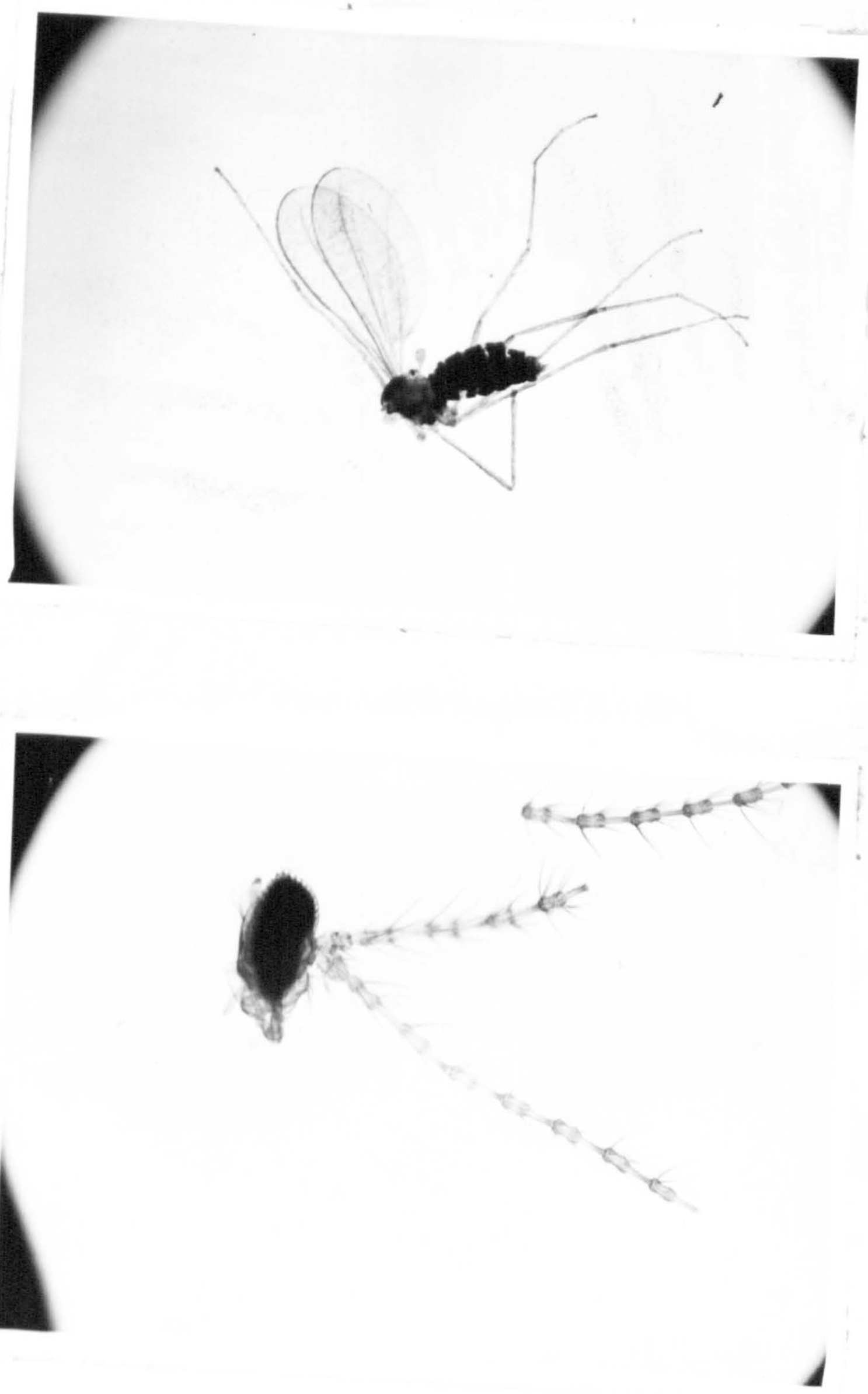

100
80
60
40
20
INCUBATION PERIOD. DAYS.
10
15
20
25
30
TEMPERATURE. °C.

This may partly account for the apparent success of Synopeas B compared with Platygaster A. Another point to note in Table 45 is that at 20°C. Synopeas B emerges about 10 days after its host, irrespective of delay in pupation through diapause development.

5.2 Predators.

Gall midge predators include other cecidomyids, insects, spiders, mites, birds and browsing animals (Barnes, 1956). During this study, Dry's (1915) observation that adult midges were occasionally caught in spider's webs was confirmed, and another cecidomyid, Lestodiplosis sp., was found occupying the same gall as C.nasturtii. However, there was no evidence to show what Barnes categorically states, namely that larvae of Lestodiplosis sp. are predacious. In any case, only two swedes containing both species in the same gall were found and these came from W.Wheldon in Gen. III 1959, when the larval population of C.nasturtii was at its peak. Numbers of Lestodiplosis and Contarinia on the two plants were 13:102 and 6:26 respectively. Adults of Lestodiplosis are shown in Plate C. Summing up: although C.nasturtii may have numerous non-specific predators and perhaps one specific predator, the effect of predation on population increase was thought to be negligible during this study.

Plate C. Adult of predacious Lestodiplosis sp.



Female.

Plate C. Adult of predacious Lestodiplosis sp.

Male.

SECTION 6. DISTRIBUTION IN TIME

6.1 The adult.

Seasonal fluctuation in abundance was followed with so-called impaction traps which have been described in Section 1.2. There were 5 traps in 1958 and 10 in 1959 and 1960. Traps were spaced uniformly over the swede crop at the end of May and remained *in situ* until October (June in 1960) when trapping was discontinued. Mean catch of *C.nasturtii* and mean catch of other insects per trap per week are shown in Fig. 28.

No Gen. I adults were trapped in 1958, simply because very few adults had reached the crop; the nearest field where Gen. I adults emerged, i.e. the nearest population of overwintering larvae, was about one mile away. The Figure suggests that emergence peaks of Gen. II and Gen. III were respectively about early August and mid-September, time between peaks (i.e. the generation interval) being 5-6 weeks.

Catches in 1959 showed three main peaks, these obviously corresponding to peaks of Gens. I, II and III emergence, occurring respectively in June, late July and late August. Clearly, considerable numbers of Gen. I adults arrived in W.Wheldon from Middleton (1958 swedes) situated only 400-500 yards to the west. The two small peaks comprising Gen. I emergence were probably due to soil-moisture fluctuation in May and June (see Fig. 30,

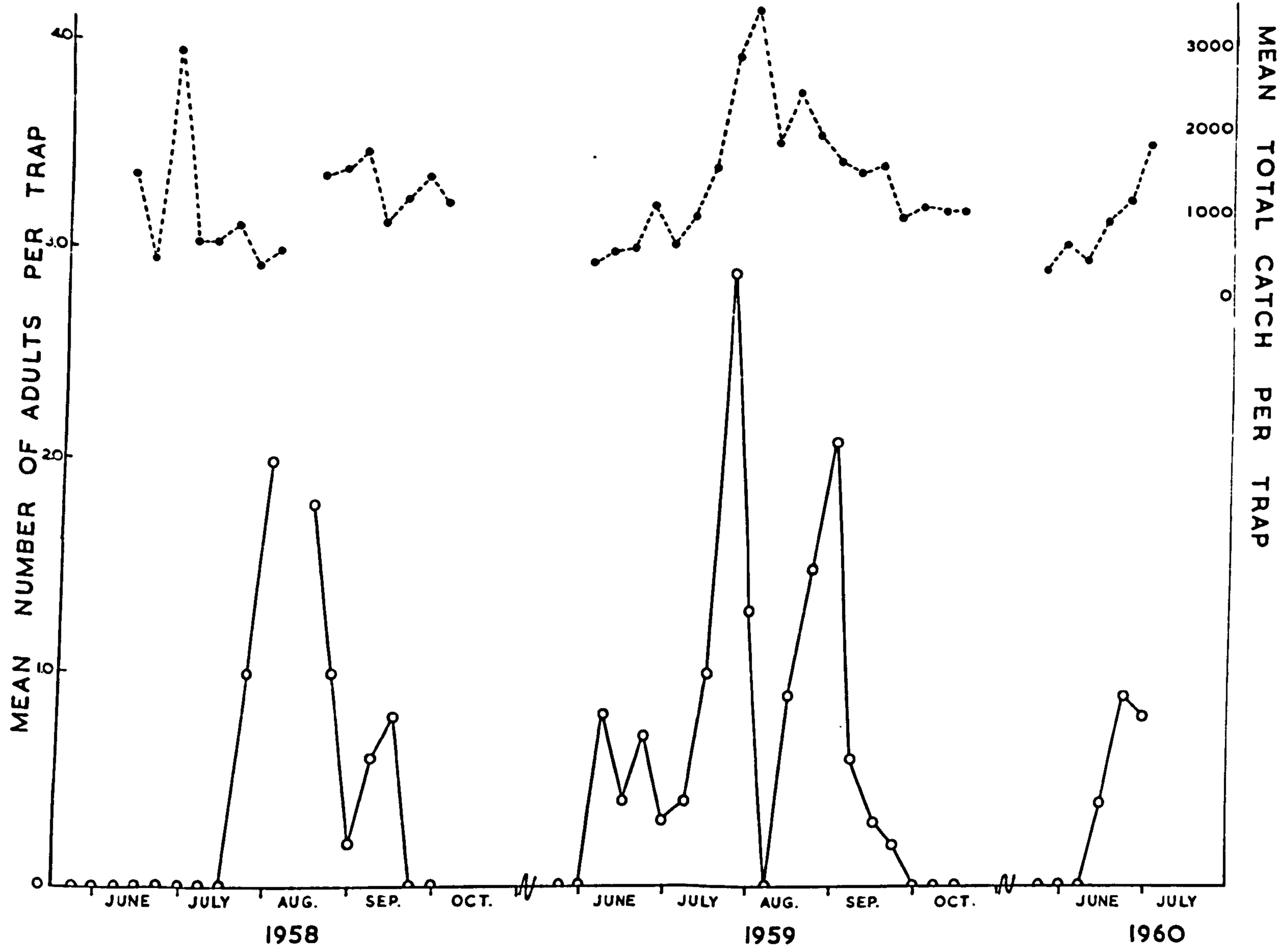
FIGURE 28.

Mean catch of C.nasturtii and of other insects per trap per week during 1958, 1959 and 1960

o : mean numbers per trap per week of C.nasturtii.

● : mean number per trap per week of other insects.

1958. Five traps in Middleton.
1959. Ten traps in West Wheldon.
1960. Ten traps in East Wheldon.


MEAN NUMBER OF ADULTS PER TRAP
30
20
10
0
MEAN TOTAL CATCH PER TRAP
3000
2000
1000
0
JUNE
JULY
AUG.
SEP.
OCT.
1958
JUNE
JULY
AUG.
SEP.
OCT.
1959
JUNE
JULY
1960

Appendix I) which may have caused some post-diapause larvae to pupate two weeks earlier than others (see Section 4.33). At all events, Gen. II adults were mainly the progeny of late emerging Gen. I adults only, because the swedes were too small to be galled when the first arrivals entered the field in early June. However, rain on 4-9th June stimulated rapid swede growth so that by the end of June, plants were well advanced and attractive to ovipositing midges. Intervals between Gens. I, II and III peaks were therefore 4 and 4-5 weeks respectively.

The 1960 swedes (E.Wheldon) were just as near the preceding year's swede field (W.Wheldon) and naturally, because of this and because the larval population in W.Wheldon was high, considerable numbers of Gen. I adults arrived and were trapped in E.Wheldon. Emergence started during the second week of June when swedes were well advanced, reached a peak the following week and then declined; traps were removed at the end of June. The main points arising from trapping are

1. Gen. I emergence, being in June, is usually but not invariably synchronous with swede growth.
2. Numbers of Gen. I adults arriving in the swede crop depend on the availability of overwintering larvae, i.e. partly on a nearby source of infestation and partly on numbers of overwintering larvae there.

3. Generation intervals vary, being 4-5 weeks in a warm year (1959) and 5-6 weeks in a cold year (1958).
4. Generation peaks are about two weeks earlier in a warm year (1959) than in a cooler year (1958).

The mean weekly catch of other insects fluctuates widely, probably in relation to local weather. There is no obvious correlation between total catch and midge catch. This may be partly the result of midges tending to restrict activities to beneath the swede canopy (Section 1.2), i.e. to an environment less affected by local weather.

6.2 The feeding larva.

Tables 22 and 23 (Section 3.631) summarize results of sampling for galled swedes at intervals during 1958 and 1959 respectively. Each sample has a mean (a) of galled swedes with a variance ($V_{(a)}$) calculated from 30 sample units of 20 plants in 1958, and 24 units of 30 plants in 1959. Comparable data for mean numbers of larvae per galled swede (b and $V_{(b)}$) can be extracted from Table 24 (Section 3.632). Obviously the product of means a and b, for any particular sampling occasion, equals the mean number of larvae per 20 (1958) or 30 (1959) swedes. Table 46 gives the complete statistical data justifying Table 47 and the following conclusions.

Larval density increased up to Gen. II in 1958 and up to Gen. III in 1959, and was very much greater in 1959 than

Table 46. Numbers of feeding larvae during 1958 and 1959 (C.nasturtii)

Date	Galled swedes per 20 plants			Larvae per galled swede			Number of larvae per 20 plants				5% fiducial limits of a.b.**
1958	n_1	a	$V_{(a)}$	n_2	b	$V_{(b)}$	a.b	a^2	b^2	$V_{(ab)}$*	
July 1	30	0.03	0.001	1	14.0	-	0.42	0.001	196	0.216	± 0.95
18	30	0.33	0.007	9	18.8	5.47	6.20	0.109	353	2.86	± 3.43
Aug. 26	30	1.26	0.028	10	14.9	6.10	18.8	1.605	222	16.0	± 8.12
Sept. 23	30	0.73	0.024	10	9.9	5.34	7.23	0.537	98	5.22	± 4.63
Octo. 28	30	0.17	0.005	3	11.7	25.4	1.99	0.028	137	1.40	± 2.40
1959	per 30 plants								per 30 plants		
July 7	24	2.75	0.103	11	23.4	10.4	67	7.56	590	140	± 24
28	24	12.5	0.383	8	93.4	267.3	1164	155.3	8724	44839	± 424
Aug. 27	24	26.2	0.283	20	283.4	872.0	7428	687.0	80316	617777	± 1572

*Calculated from the relation $V_{(ab)} = a^2V_{(b)} + b^2V_{(a)} + V_{(a)} \cdot V_{(b)}$

**Calculated as $\pm t \sqrt{V_{(ab)}}$ where t is read for $n_1 + n_2 - 2$ degrees of freedom at the 5% level of probability.

Table 47. Numbers of feeding larvae per square yard and percentage of swedes galled during 1958 and 1959 (C.nasturtii)

Generation	Date	Percentage of swedes galled	Larvae per square yard (i.e. per 5 swedes)
	1958		
I	July 1	0.14	0.1
II	Aug. 26	6.3	4.7
III	Sept. 23	3.7	1.8
	1959		
I	July 7	9.2	11
II	July 28	41.5	194
III	Aug. 27	87.4	1238

1958. Moreover the data for 1959 suggest that the numbers of swedes galled increases linearly while the numbers of larvae increase exponentially.

6.3 The mature larva and pupa.

6.31 Method.

Larvae and pupae were recovered from soil samples using a modified flotation-separation method, flotation being in a saturated solution of magnesium sulphate (sp.g.1.2) (Salt and Hollick, 1944). The 'float' was examined in a tray of water under a binocular microscope (mag. X10). Golightly (1952) and Bevan and Uncles (1958) used the same method for wheat midge and pea midge cocoons respectively, except that in both cases the 'float' was examined with the naked eye. Swede midge cocoons, particularly those of larvae, were not easily seen among the float debris, and, in any case, they had to be carefully scrutinized to check identification. This last point is emphasized by Aitkenhead *et al* (1955) since they found at least ten species of midge larvae in samples from the Broadbalk wheat field at Rothamsted. Golightly (*loc. cit.*), with no mention of species other than *S.mosellana* and *C.tritici*, claims that his method was more than 95% efficient.

The efficiency of the method was investigated in two ways (a) and (b):

(a) 54 larval cocoons (diapause) were picked out of soil from rearing tubes and sprayed with a powerful water jet until all obvious soil-particles were removed from their membranes. They were then placed in a saturated solution of magnesium sulphate (sp.g.1.2) when it was noted that some sank: actually cumulative totals sinking after 0, 10 and 15 minutes were 17, 23 and 25 respectively. The entire 54 cocoons were then re-examined. All contained a small air bubble but those (25) which sank had one or more silica crystals attached to their membranes. These crystals, being transparent, had escaped notice at the jetting. On removing the silica crystals, 23 out of the 25 now floated; the remaining two cocoons still did not float but they had very small air bubbles and also thick 'dirty' membranes. The membranes of all 54 cocoons were then dissolved in a 5% solution of sodium hypochlorite and the 'free' larvae returned to the magnesium sulphate solution; all of them floated.

Although no comparable test was made for pupal cocoons, observation showed that these usually float in water, their buoyancy being partly due to large bubbles of air.

(b) In September 1959 a sample of soil, 6 inches square and 2 inches deep, was taken within 9 inches of the stem of each of ten swedes which earlier had had severe central shoot galls. Samples were flooded, stored at -5^{o}C. for three

weeks and then run through the flotation-separation process three successive times, numbers of individuals in each 'float' (I, II and III) being counted. Numbers in 'floats' were as follows:

	I	II	III	Totals
Larval cocoons	29	14	3	46
Pupal cocoons	7	0	0	7
'Free' larvae	36	2	0	38
'Free' pupae	2	0	0	2
'Float' totals	74	16	3	93

These results confirm that the method is least efficient for larval cocoons.

The two experiments, (a) and (b), therefore suggest that the flotation-separation process is about 60% efficient for larval cocoons and almost 100% efficient for pupal cocoons, 'free' pupae and 'free' larvae.

6.32 Results.

Random, stratified random or systematic samples of 10-30 units (each comprised of two recording units) were taken in the 1957 (E.Hemmel), 1958 (Middleton) and 1959 (W.Wheldon) swede fields. Middleton and W.Wheldon were sampled before, during and after the midge season but E.Hemmel was sampled only after the crop had been harvested (i.e. after the midge season). The recording units were two 2½ inch diameter soil cores, one taken on the drill and the other midway between the drills as suggested in

Section 4.41. Where there were no drills, i.e. after ploughing, cores were taken 12 inches apart across the direction the drills had originally followed. Sampling depth was increased from 3 inches in summer to 4-6 inches after ploughing.

For each sample, mean number of C.nasturtii (larvae and pupae) recovered per unit and its 5% fiducial limits are shown in Table 48. Means are also expressed as (a) numbers recovered per square yard and (b) numbers per square yard assuming 60% extraction. This value was chosen because it seems to apply to larval cocoons and these were in the majority for most samples.

Table 48 confirms what is to be expected from Sections 4.3 and 6.2, namely that numbers in the soil increase from zero in June to a maximum in late autumn, remain fairly constant over winter and spring and then fall rapidly in May and June to zero again. It also shows that numbers in the 1959 swede field (W.Wheldon) were far higher than in the 1958 or 1957 fields (cp. results for feeding larvae Table 47).

Several other midge species were recovered from samples, larval cocoons of S.mosellana being particularly abundant. S.mosellana larvae may spend up to 14 winters in the soil before pupation (Barnes, 1952; Barnes and Arnold, 1960) and this explains their occurrence in fields which had not grown their host, wheat, for 3-4 years.

Table 48. Numbers of C.nasturtii in the soil, 1957-1960.

Date	Field	Sampling method	Units per sample	Individuals recovered per unit mean	± L*	Individuals recovered per square yard mean	Population per square yard (assumed 60% extraction) mean
Nov. 15, 1957	E.Hemmel	Random	20	0.56	± 0.45	73	122
Feb. 18, 1958	"	"	20	0.41	± 0.35	53	89
April 14	"	"	20	0.31	± 0.22	40	67
May 14	"	"	20	0.10	± 0.15	13	22
June 6	"	"	20	0	-	0	0
July 22, 1958	Middleton	Stratified Random	30	0	-	0	0
25	"	"	30	0.03	± 0.07	4	7
Sept. 22	"	"	30	0.24	± 0.22	31	52
April 3, 1959	"	"	10	0.36	± 1.14	47	78
June 1	"	"	10	0.08	± 0.32	10	17
July 3	"	"	10	0	-	0	0
May 30, 1959	W.Wheldon	Random	20	0	-	0	0
July 22	"	Systematic	24	0.25	± 0.25	33	55
Aug. 13	"	"	24	0.55	± 0.42	72	120
Oct. 13	"	"	24	3.07	± 0.98	399	666
Dec. 12	"	"	24	4.17	± 3.06	540	902
May 18, 1960	"	Random	10	4.08	± 3.64	529	883
25	"	"	10	2.45	± 0.99	317	529
June 1	"	"	10	1.74	± 1.09	225	376
9	"	"	10	0.73	± 0.62	94	157
23	"	"	10	0	-	0	0

*L = 5% fiducial limits.

6.4 Parasites.

Only Synopeas B occurred in larval samples taken during 1958. From 1,199 larvae of C.nasturtii collected in Middleton between July and October, 11 parasites and 572 midges emerged at 15°, 20° and 25°C., i.e. 11/(572 + 11) or about 2% parasitization; 616 larvae either died or entered diapause.

Synopeas B was by far the most numerous parasite encountered in the 1959 midge season (See Tables 38 and 49). From all the C.nasturtii larvae reared (> 20,000) only 29 adults of Platygaster A and 1 adult of Synopeas C emerged and these came from Gen. III larvae in early September, i.e. when numbers of host feeding larvae were maximum.

Table 38 (Section 4.31) shows numbers of parasites (all Synopeas B) and midges emerging from four batches of C.nasturtii larvae (500 per batch) which were selected at random from larger samples, themselves collected at random from galled swedes in W.Wheldon, at intervals during 1959. Groups of 100 larvae from each batch had been kept in rearing tubes at four constant temperatures, 10°, 15°, 20° and 25°C. and at field temperatures. From the number of parasites and midges emerging in each group (see the five totals (T) for each batch), the number of parasitized larvae in each group at the start is

$$\frac{\text{parasites}}{\text{parasites + midges}} \times 100$$

on the assumption that the proportion parasitized is the same in diapause larvae and in larvae disappearing without trace as it is in the non-diapause larvae. The only data for diapause larvae concern 40 of the 71 figuring in 10°C., 8/9/59, Table 38; these 40 (used in an experiment on diapause termination) gave rise to 27 midges and 2 parasites, a proportion which is not significantly different from 13 midges and 0 parasites for non-diapause larvae. Obviously, no data can be got for larvae that die and disappear without trace, but there seems to be no strong reason to suppose that the proportion should be different among them. Thus it seems fairly safe to calculate the number of parasitized larvae in each group at the start as indicated above. Results are shown in Table 49. The last column gives mean percentage parasitization, and analysis of variance reveals a significant different between these means (V.R. = 3.75, with 3 and 16 df., $p < .05$). Now the three larval midge batches (7/7, 28/7 and 27/8/59) were collected at peaks of Generations I, II and III, and the densities of midge larvae per square yard at these three peaks are given in Table 47 (Section 6.2). Hence it is possible to tabulate as follows:

	Gen.I	Gen.II	Gen.III
Midge larvae	11	194	1238
Percentage parasitization ..	12.4	8.0	7.2

Clearly, percentage parasitization decreased as midge numbers rose from generation to generation during 1959.

Table 49. Parasitism among mature larvae of C.nasturtii collected from galled swedes in W.Wheldon at intervals during 1959

Date of Collection (batches)	Parasitized larvae and adult parasites (in brackets) per group (each group starting with 100 larvae)					Batch means
	10°	15°	20°	25°	Field	
7/7/59	2.90 (2)	14.3 (12)	11.0 (8)	22.2 (20)	11.7 (7)	12.4 (9.8)
28/7/59	- (0)	2.78 (2)	9.76 (8)	20.0 (16)	7.32 (6)	8.0 (6.4)
27/8/59	5.88 (3)	10.5 (7)	5.41 (4)	4.76 (3)	9.30 (4)	7.2 (4.2)
8/9/59	- (0)	4.76 (1)	- (0)	- (0)	- (0)	0.95 (0.2)

Analysis of Variance

(parasitized larvae)

Source of Variation	df	Sum of Squares	Mean Square	F	p
Between batches ..	3	334.3	111.4	3.75	<.05
Error	16	475.5	29.7		
Total	19	809.8			

DISCUSSION

The short duration and limited scope of the present study obviously precludes dogmatic conclusions on the causes of population distribution, growth and control. However, the available data do suggest that wind, temperature and moisture seem to be the most important circumstances in the ecological economy of C.nasturtii.

It is interesting to discover that C.nasturtii can make two types of cocoon, one oval and the other spherical. The oval cocoon is constructed near the soil surface for pupation and the spherical cocoon at greater depth for 'quiescence'. The diapause larva always makes first a spherical cocoon and then an oval cocoon. The non-diapause larva makes a spherical cocoon only when the soil conditions are rather dry ($pF > 3.7$). 'Quiescence' in the spherical cocoon aids survival in drought conditions and tends to synchronize midge-development with swede growth.

The interplay of temperature and moisture is also very interesting. Thus, if not too dry, a 'warm' year gives rise to more than three generations because the duration of each stage varies inversely with temperature (Sections 1.6, 2.2, 3.4 and 4.21). Again, if not too dry, a 'warm' year increases the turnover of individuals from generation to generation because the proportion of non-diapause larvae in any particular generation varies directly with temperature (Section 4.31). Extra generations and enhanced

generation turnover must accelerate population growth but this tendency can be curbed to some extent by the dryness which sometimes accompanies warmth (Section 4.23).

On the farm scale, spatial distribution of C.nasturtii at Nafferton is markedly discontinuous. Practically all the C.nasturtii at Nafferton are in the swede crop, other alleged hosts being of little or no importance (Sections 3.61-2). The swede crop, however, is moved every year, the distance between swede fields of successive years varying considerably. This means that adults of Generation I have to travel varying distances to reach their chief host. Spatial distribution within the swede field is also markedly discontinuous - in the early part of the season. At this time galled swedes are understandably most frequent on the field side nearest to the infested site of the previous year; and these galled swedes tend to be aggregated in clusters because the female tends to lay all her eggs on a few adjacent plants (Section 3.631). Moreover, since the progeny of a female are either all male or all female, the sexes themselves are segregated in different swede clusters. As generation succeeds generation, the number of larvae per galled swede increases and the galled swede clusters tend to merge together. This tendency for spatial discontinuity and sexual aggregation to disappear may be assisted or hindered by the prevailing

wind (Sections 3.631-2). Spatial distribution of C.nasturtii is therefore mainly a matter of first colonization of and then spread through the swede field of the particular year.

With regard to time distribution, the results for 1958 and 1959 suggest that the population level attained in any particular year depends partly on the availability of overwintering larvae and partly (perhaps mainly) on weather. Thus the low population of 1958 was associated with greater distance from the source of infestation (1 mile from nearest 1957 swede field) and a cooler season, while the very much higher population of 1959 was associated with a shorter distance ($\frac{1}{4}$ mile down the prevailing wind) and a warmer season which was wet at the appropriate times.

During 1957-60, parasites seemed to be relatively unimportant as controlling agencies. Percentage parasitization by the commonest parasite, Synopeas B, actually decreased as midge numbers increased from generation to generation in 1959. The reason for this appears to have been weather affecting the midge and its parasite unequally. Although 1959 was droughty, brief periods of rain did happen to coincide with the times when most midge larvae of Generations I and II dropped to the soil. The midge larvae pupated at once, emerged before the soil became too dry and regained the moist environment provided by the

swede plant. On the other hand, the parasite probably suffered lethal desiccation in many cases because its sojourn in the soil (10 days longer than its host) extended well into the intervening periods of drought.

In conclusion the author is of the opinion that the kind of year most favourable for increase of C.nasturtii is one in which

(a) a dry spell in April-May is followed by a brief warm period of rain (this causing Generation I adults to emerge en masse 10-20 days later)

and (b) the summer is warm and relatively dry, except for brief periods of rain coinciding with the times when most larvae are dropping to the soil (this obviating larval quiescence).

SUMMARY

The Adult.

Pupation occurs near the soil surface. When depth below surface is artificially increased after pupation, successful emergence is reduced or inhibited altogether, this depending partly on depth and partly on pore-size of the soil. Normally, factors inhibiting emergence are low temperature (< 10°C.), dry soil during the pupal stage and saturated soil during emergence. Adults emerge throughout the day (0800-2200 hours) but the diurnal curve of numbers emerging appears to rise and fall in harmony with the soil temperature curve.

Both sexes appear to restrict activity to below the leaf canopy of the swede crop, a favourable environment in all but showery weather.

Females do not mate immediately after emergence but rest for several hours. Eventually (after 6-8 hours at 20°C.) they produce a chemical attractant which stimulates a strong mating urge in males.

The average female manufactures about 95 mature eggs. Longer-lived females lay nearly all of these eggs. The eggs are laid in several batches on the younger parts of the host plant. Most eggs laid seem to be fertile (at least 83% at 20°C.).

The midge breeds unisexual families and the mean number of larval progeny per female was 79 ± 11.4 in one

experiment at room temperature.

Length of adult life in the field is unknown. In the lab. individuals lived for 1-16 days, life-length decreasing with increasing temperature (10^{o}-30^{o}C.) and females living longer than males.

The Egg.

The unlaid egg is 0.37 x 0.09 mm. with a 0.10 mm. pedicel. As laid on the plant host, batches vary from 2 to more than 50 eggs.

Eggs removed from the plant and incubated in water developed and hatched at constant temperatures between 10^{o} and 30^{o}C. The incubation period varies inversely with temperature (26 hours at 30^{o}C., 57 hours at 20^{o}C. and 260 hours at 10^{o}C.). The relation between rate of development and temperature is typical of insects generally. Graphically this relation is an S-shaped curve which seems to be logistic in form.

Eggs can develop and hatch under water and in saturated or near-saturated humidities. These moisture conditions occur among the young "heart" leaves where most eggs are laid.

The Feeding Larva.

Since eggs are laid chiefly in the heart leaves of the swede, most larvae hatch into a favourable environment with respect to humidity and protection from being swept away by

rain.

Larvae feed gregariously, producing secretions which dissolve the wax cuticle and liquefy the underlying cells of the surrounding plant surface, so that they are eventually immersed in a nutrient watery fluid. Destruction of host cells results in distortion, severest in young leaves where both larval numbers and cell growth rate tend to be maximal. Leaf-bud and flower-bud galls also occur causing the buds to remain closed and abortive.

At 18°-21°C. larvae mature in about 10 days. Initially growth is slow (Day 1-3) but rapidly increases (Day 3-6) and then declines (Day 6-10) as larvae become mature.

Four instars occur, each lasting about 2 days at 18-21°C. The feeding phase occupies about 28 days at 15°C., 11 days at 20°C. and 7 days at 25°C. The relation between duration of development and constant temperature (15-25°C.) is inverse and linear. Larval development in the field (June and July) seems to proceed at a rate equivalent to that which occurs in the laboratory at constant temperature of the same value as the field mean temperature.

Numerous species of wild and cultivated Cruciferae are alleged to be host plants of C.nasturtii. At least three species of "wild host" were relatively abundant at Nafferton but none of these was ever infested. Several brassicas were grown together at Cockle Park (1958) and

Nafferton (1957-1960) and of these, swede and swede-like rapes and kales were frequently galled, turnip much less frequently and marrowstem kale not at all. Clearly, C.nasturtii favours swede and swede-like plants as hosts.

Galled swedes tend to be aggregated in clusters and, at least in Generation I, are more frequent on the field side of the crop which is nearest to a site infested the year before (usually also swedes). Galling spreads outwards from plants attacked in Gen. I.

Numbers of larvae per galled swede were highest in 1959 (Gen. III) and lowest in 1958, and increase as the percentage of swedes galled increases. This was unknown to previous workers and hence many of their population estimates must be suspect.

The overall sex ratio of larvae is close to unity but that of larvae from individual swedes varies very widely. Swedes galled with larvae of one sex only are most frequent at low larval population. The reasons for this are discussed.

Swedes galled by Gen. I larvae are more likely to become 'many-necked' through destruction of the central shoot than those unattacked or those galled by later generations. Bulbs of swedes with severe 'Central Shoot Galls' in Gen. II were significantly lighter than those of swedes not so galled.

The Mature Larva and Pupa.

In favourable conditions of soil-moisture the mean time spent in the soil by the non-diapause larva varies inversely with constant temperature (12^{o}-30^{o}C.). Males develop faster than females by about 12-24 hours at all temperatures below 30^{o}C. Pupae of both sexes die just prior to or during eclosion at 32.5^{o}C.

In 1959 the time spent in the soil by non-diapause larvae at field temperature fluctuating with mean x^{o}C. was almost the same as the time at constant temperature x^{o}C.

Development of the non-diapause larva is arrested by excessive wetness or dryness of the soil but it proceeds normally as soon as moisture conditions become favourable. In 'dry' soil, larvae may construct spherical cocoons in which they quiesce but never pupate; as soon as the soil becomes sufficiently moist, they vacate these spherical cocoons and construct oval elongate cocoons in which they pupate. Larvae survive for considerable periods in 'wet' and also in 'dry' soil, though the latter condition causes partial dehydration.

The proportion of quiescent larvae increases as soil-moisture ~~increases~~ decreases. However, results for two different soil types, a sandy loam and a peaty clay, indicate that larval quiescence is not simply a matter of absolute moisture content.

pF is the best measure of water available to insects in the soil. The same moisture content in different soils does not have the same pF. The proportions of larvae becoming quiescent are the same at the same pF in different soils. In the laboratory, at pF below 3.7, most non-diapause larvae build oval (i.e. pupal) cocoons; as pF rises above 3.7 there is an associated rapid increase in the proportion of larvae building spherical cocoons (i.e. quiescent). It is therefore suggested that the tension with which soil holds water influences cocoon building behaviour, not, as one might expect, subsequent metamorphosis.

Although 1959 was a very dry year, pF was less than 3.8 (due to brief periods of rain) when larvae of Generation I and II dropped to the soil and so their development was not delayed; with Generation III, however, pF was 4.1-4.5 and therefore no 'effective' fourth generation developed.

The adult emergence period becomes more protracted and the incidence of diapause increases from generation to generation within the year. The onset of diapause seems to be controlled partly by temperature but mainly by day length during the feeding phase. Diapause development proceeds fastest at 0^{o}-5^{o}C., the rate decreasing as temperature rises. The data suggest that the firmness of diapause varies according to temperature experienced during the onset of diapause. Diapause larvae,

previously kept at low temperature (0°-5°C.), remain in their spherical cocoons for about 30 days after entry to high temperature (20°C.); they then vacate the spherical cocoons and pupate in oval cocoons. Larvae which have completed post-diapause development at high temperature (30 days at 20°C.) vacate their spherical cocoons (in order to pupate) only when the soil becomes wet. This lack of soil-moisture can be a developmental barrier causing mass-emergence of adults.

Most larvae fall to the soil within 9 inches of the stem of their host swede. Numbers decrease as distance from the stem increases.

Oval pupal cocoons are made just below, and spherical larval cocoons 0-2 inches below, the surface of the soil. Cultivation, however, tends to bury cocoons to greater depths.

Parasites and Predators.

Three species of hymenopterous parasites were reared from larvae of C.nasturtii and their life-cycles appear to correspond closely with that of the host.

Duration of parasite development varies inversely with temperature and at 20°C. is 10-20 days longer, depending on the species, than the host's pupal stage. The rate/ temperature coefficient for development of Synopeas B appears to be almost the same as that for the host's pupal stage.

Although C.nasturtii may have numerous non-specific predators and perhaps one specific predator, the effect of predation on population increase was thought to be negligible during this study.

Distribution in Time.

Generation I emergence, being in June, occurs usually but not invariably when swedes have grown to the stage suitable for attack. Numbers of Gen. I adults arriving in the swede crop depend mainly on the availability of overwintering larvae, i.e. partly on proximity of the crop to a source of infestation and partly on numbers of overwintering larvae there. In warm years, peaks of emergence are earlier and less widely separated than in cooler years.

Density of feeding larvae increased up to Gen. II in 1958 and up to Gen. III in 1959, and was very much greater in 1959 than 1958.

A flotation-separation technique is briefly described and found to be about 60% efficient for extracting larval cocoons and almost 100% efficient for pupal cocoons, 'free' larvae and 'free' pupae.

Numbers of C.nasturtii in the soil increase during the summer, remain fairly constant in winter and spring and fall to zero in May and June. Numbers were far higher in 1959 than in 1958 or 1957.

Synopeas B was the only significant parasite encountered

Percentage parasitization decreased as midge numbers rose from generation to generation in 1959.

Discussion.

The short duration and limited scope of the present study obviously precludes dogmatic conclusions on the causes of population distribution, growth and control. However, the data do suggest that wind, temperature and moisture seem to be the most important circumstances in the ecological economy of C.nasturtii.

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APPENDIX I. METEOROLOGICAL DATA 1958-1960.

FIGURE 29.

Daily maximum shade temperature (°F.) and
rainfall (inches)
1958
(Whittle Dean Resevoir)

DAILY MAX. TEMP. °F.

80
70
60
50

DAILY RAINFALL. INS.

.80
.60
.40
.20

MAY JUNE JULY AUGUST SEPTEMBER

1958

FIGURE 30.

Daily maximum shade temperature (^{o}F.) and rainfall (inches) at Whittle Dean Resevoir, and mean percentage soil-moisture in West Wheldon 1959

DAILY MAX. TEMP. °F

80

70

60

50

SOIL MOISTURE. %.

20

0

RAINFALL. INS.

.40

.20

MAY

JUNE

JULY

AUGUST

SEPTEMBER

1959

FIGURE 31.

Daily maximum shade temperature (^{o}F.) and
rainfall (inches)
1960
(Whittle Dean Resevoir)

DAILY MAX. TEMP. °F.

80
70
60
50

DAILY RAINFALL. INS.

80
60
40
20

MAY JUNE JULY AUGUST SEPTEMBER

1960

APPENDIX II. REFERENCES

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