# THE BIOLOGY AND POPULATION DYNAMICS OF *SITONA REGENSTEINENSIS* HBST. (COL. CURCULIONIDAE)

by

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## ADDENDUM

The Mymarid egg parasite of *Sitona* has been identified by Dr. R.L.Doutt, Berkely, California as

*Patasson diana* (Girault)

= *Anaphes* (*Patasson*) *lameerei* Debauche.

## ERRATUM

Page 144 Fig. 41 : In the second illustration (23 oct 63), for"DLM" read "DVM".

Page 176 line 21 : For "Allea et al.," read"Allee et al.,"

Page 196 line 1 : For"sec" read "See".

## ABSTRACT

The population dynamics of the Curculionid beetle, *Sitona regensteinensis* Herbst was studied during the period from October 1962 to May 1965 in a small area of broom (*Sarathamnous scoparius* L. Wimm.), at Silwood Park, Berkshire.

*Sitona* is univoltine with an obligatory imaginal diapause in winter. The adult beetle feeds on the green parts of broom. Oviposition takes place from March to July; the eggs are dropped from above on the soil surface. There are four larval instars. The larvae are subterranean and feed on the bacteria-containing cells of the root nodules of broom. Pupation takes place in soil and the new adults emerge from September to November. In winter these adults move from the plant to hibernate among the litter underneath and re-emerge when temperature becomes favourable in spring.

Predation was the main cause of mortality of immature stages. Mites, Staphylinids and Carabids are the important predators. A considerable loss to the adult population occurs during the winter by death and in spring by emigration. Adults are also killed by a braconid parasite, *Centistes excrucians* Haliday. The "balance" of the population during the period under investigation appeared to have been maintained by changes in the fecundity and egg mortality. The latter was caused by sterility and predation.

The adult beetle is dimorphic with a non-migrant form and an

obligatory migrant; the former is brachypterous and the latter macropterous. The migratory form is almost devoid of flight muscles at the time of emergence. The flight muscles grow during the autumn feeding period and are maintained through the winter unchanged. The growth of the ovary and egg production of the migratory forms are retarded by some weeks, during which flight occurs. Migration takes place within a short period in spring. The studies on migration and reproduction support some current hypotheses on insect migration.

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# 1. INTRODUCTION

This is primarily a study of the changes of a self-contained population of Sitona regensteinensis Herbst, a Curculionid beetle that lives on the broom plant, Sarothamnous scoparius (L.) Wimm. An attempt is made here to measure the changes in the population, to assess and interpret natality and mortality quantitatively and finally to construct a life-table. The work extended between October 1962 and May 1965.

The population of Sitona was a small one, composed of approximately thirteen to seventeen thousand adults when they were at the maximum, living in a more or less closed habitat of 138 broom plants. The estimates of the adult numbers as well as those of eggs were made by several methods which acted as mutual checks to one another. All known causes of mortality were studied quantitatively wherever possible and the predators of the immature stages were determined serologically. Some attention was paid to the Braconid parasite, Centistes excrucians Hal. which was responsible for a proportion of the adult deaths.

During the course of these investigations it became evident that certain other aspects of the biology of Sitona needed detailed study, not only to solve some of the problems of this species, but also to clarify some of the available information on the biology of the genus Sitona, many of which are well known pests of leguminous crops. Thus a modest portion of this work is devoted to studies on reproduction, migration and the larval feeding habits.

Sitona regensteinensis is dimorphic, with a brachypterous non-migratory form and a macropterous migratory form. The studies on the reproductive biology of the two forms and the flight activity of the migratory form were carried out with special reference to the modern concepts of insect migration. The seasonal changes in the flight muscles and the growth of female reproductive organs have been studied quantitatively and the knowledge so obtained provides a better understanding of the phenology of the insect.

The definite feeding site of the larva was established during the work on the larval feeding habits. It was seen that the larvae feed selectively on the bacteria containing tissue of the root nodules. This may help to clarify the differing opinions on the larval feeding habits of various species of Sitona.

There are only two published accounts on Sitona regensteinensis; all the work was done by Scherf (1958a, b) in Germany and deals with a general account of the adult and larval behaviour, the anatomy of the reproductive organs, description of the immature stages, and a short note on a Braconid parasite, Leiphron muricatus Hal. Scherf's work is qualitative and therefore differs greatly from that described in this thesis.

The literature on the genus Sitona amounts to approximately 76 papers published over the past 51 years. The bulk of these papers deal with the general biology of Sitona lineatus (L.), S. hispidulus (F.), S. cylindricollis Fahr. and S. crinitus Hbst. and their parasites. Although these species are economically important as pests of peas, beans, clover

and alfalfa, none of the published work deals with their population dynamics. A probable reason for this is that eggs of all Sitona spp. are laid on soil, the eggs and small immature stages have to be extracted from it, and the methods of extraction in the recent past were not good enough for this. This problem was effectively solved at the commencement of this work by modifying the Salt and Hollick extraction technique. The above modification is expected to have wider applications to include other soil arthropoda.

## 2. THE HOST PLANT

Sitona regensteinensis is commonly found on a leguminous shrub, broom (Sarothamnus scoparius (L.) Wimm.) and to a much lesser extent on gorse (Ulex europaeus L.). It has also been reported on other leguminous plants: Lupinus (Jackson, 1922), Ulex nanus Forster, Laburnum anagyroides Medic., and Genista cinerea D.C. (Hoffmann, 1950). Though Sitona does not appear to be strictly monophagous the main host plant seems to be Sarothamnus.

Broom is a much branched perennial shrub with green, glabrous, 5-angled twigs. The leaves are small, appear in spring, and may start falling as early as the middle of September. The lower leaves are shortly stalked and consist of three small, obovate leaflets. The upper leaves are single and sessile. Flowering takes place sometime in May, but the time and intensity of flowering varies from year to year. The flowers are large, bright yellow and occur singly or in pairs. These are borne on slender pedicels, in the axils of the old leaves, forming handsome leafy racemes along the upper branches. The pod is flat, measuring about 1.5 to 2 inches in length. The adult Sitona feeds on the leaves, flowers and green parts of the twigs, with a preference for leaves and flowers when available.

There are two main growth periods in the year, one in spring just before flowering and another after pod formation in summer when most of the growth takes place. In older bushes heavy flowering may result in the

loss of the growing points of many branches. The green stems of these branches may persist in the following year alive but often without any shoot growth.

Generally, a shrub grows to the height of about 6 to 8 feet or more. The length of life of a broom bush is usually about 10 to 15 years (Fourt, D.F. in Richards and Waloff, 1961). Broom is strongly calcifuge, found on heaths, waste ground and in woods. It readily grows on disturbed ground, but it is usually replaced as the natural vegetation regenerates. Areas of broom are therefore often relatively temporary habitats.

Sarothamnus is widely distributed in the British Isles, except for Orkney and Shetland. Its European distribution extends from Scandinavia to Spain and to the Canary Islands, but does not extend eastwards of Poland and Hungary (Clapham, Tutin and Warburg, 1952).

## 3. THE HABITAT

The site chosen for this study comprised a small area of broom in the grounds of Imperial College Field Station, Silwood Park, Sunninghill, Berkshire. Fig. 1 is a sketch map of the Field Station. The study area is located in the Rookery slope where the broom had been planted in 1958 and 1959. Altogether there are 138 bushes scattered either singly or in groups of 2 to 8 forming 36 separate units. The scatter of the bushes is illustrated in Fig. 2. The amount of ground occupied by this 36 units of broom is approximately 6875 square feet. The total area beneath the individual bushes is 3659 square feet.

The surrounding area up to a radius of about 100 feet is mainly grassland which then continues on 3 sides, except towards the south, as parkland (or woodland). The south side is bordered by a foot path which separates it from a private farm.

Since the study area is situated on a piece of grassland, the undergrowth and the rest of the vegetation is predominantly graminaceous. The commonest grasses are Dactylis glomerata (L.), Poa pratensis (L.) and Deschampsia caespitosa (L.). During spring and summer, a considerable number of flowering plants appear among the grasses. Carduus pratensis Huds. (Meadow Thistle), Achillea millefolium L. (Milfoil or Yarrow), Milium effusum L. (Spreading Milium), Potentilla verna L. (Spring Potentilla) and Rubus ideaus (Raspberry) are the most prominent of these. The undergrowth usually dies around the bases of the broom stems and accumulates as litter.

This litter serves as a hibernation site for the adult beetles.

In Silwood Park there are two other areas of broom which are larger. One of these is a 2.5 acre plot planted in 1957. This is situated about 300 yards away from the study area, towards the north, in Gunnes's Hill. In between is parkland with tall trees (sweet chestnut, sycamore, elm, oak and horse chestnut). The other is a piece of natural broomland, about 2 acres in extent, and lies 500 yards away from the study area towards the east, in the Heath. This area of broom is commonly known as 'Old Broom', since many of the broom bushes there are older than any other at Silwood Park. The area between Rookery Slope (location of the habitat) and the Old Broom is occupied by a meadow, buildings and woodland.

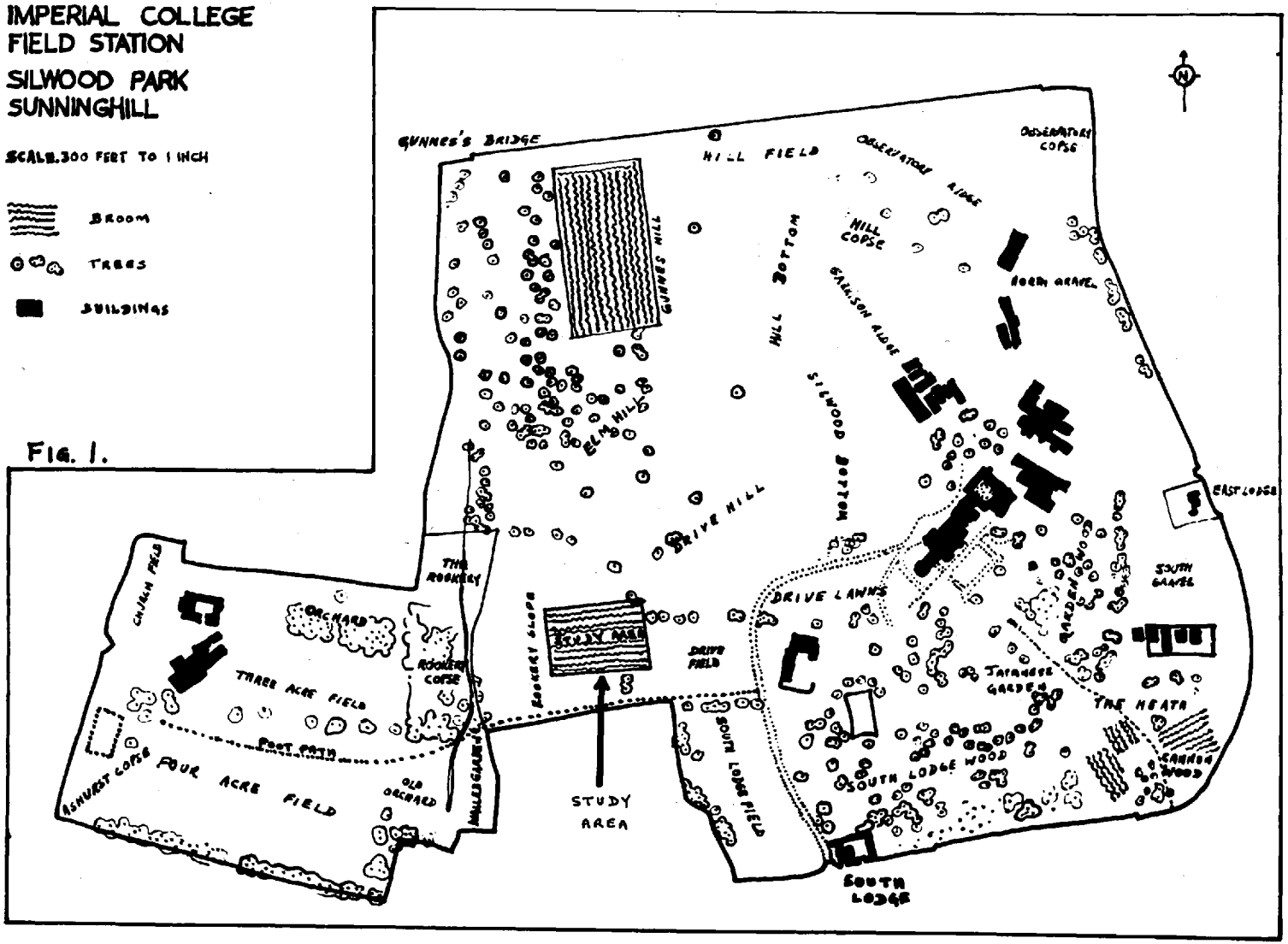
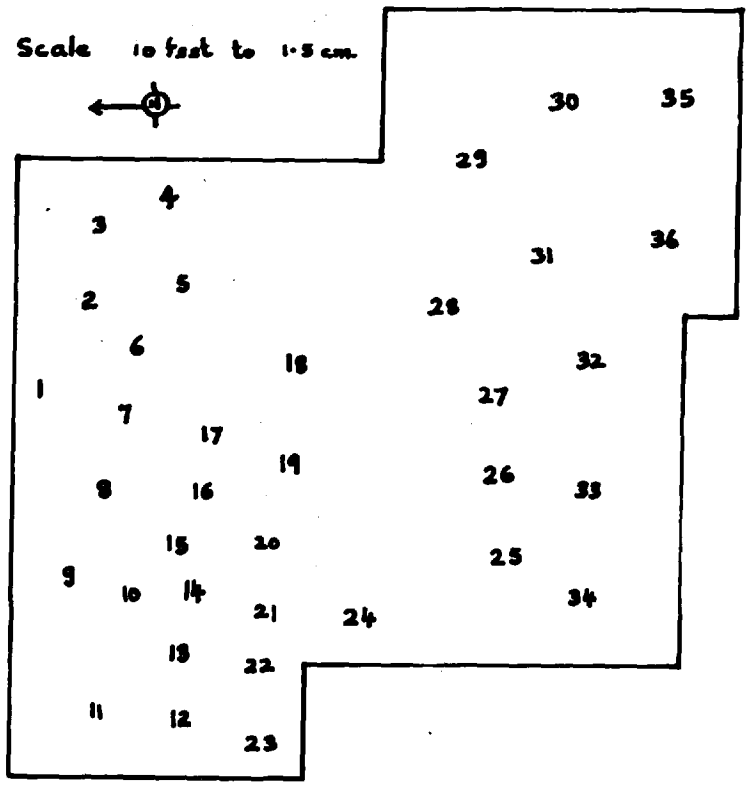
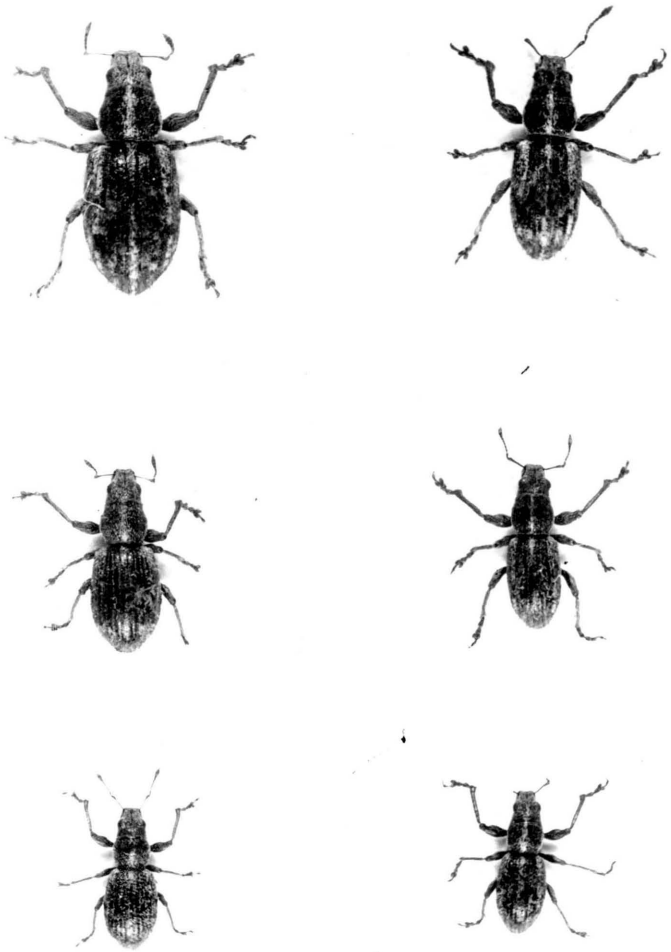

IMPERIAL COLLEGE
FIELD STATION
SILWOOD PARK
SUNNINGHILL
SCALE. 300 FEET TO 1 INCH
BROOM
TREES
BUILDINGS
GUNNES'S BRIDGE
HILL FIELD
OBSERVATORY COPSE
OBSERVATORY RIDGE
GUNNES HILL
HILL BOTTOM
HILL COPSE
GARLSON RIDGE
ROBIN GRAVEL
ELM HILL
SILWOOD BOTTOM
DRIVE HILL
EAST LODGE
CHURCH FIELD
THE ROOKERY
ORCHARD
ROOKERY COPSE
THREE ACRE FIELD
FOOT PATH
ASHURST COPSE
FOUR ACRE FIELD
OLD ORCHARD
ROOKERY SLOPE
STUDY AREA
DRIVE FIELD
SOUTH LODGE FIELD
DRIVE LAWNS
SOUTH GRAVEL
JAPANESE GARDEN
THE HEATH
SOUTH LODGE WOOD
SOUTH LODGE

Fig. 1.

FIG. 2. Showing the scatter of bushes in 36 groups in the habitat.




| Group No. | No. of bushes | Group No. | No. of bushes |
|---|---|---|---|
| 1 | 2 | 19 | 7 |
| 2 | 7 | 20 | 1 |
| 3 | 5 | 21 | 4 |
| 4 | 6 | 22 | 2 |
| 5 | 5 | 23 | 2 |
| 6 | 5 | 24 | 2 |
| 7 | 8 | 25 | 2 |
| 8 | 2 | 26 | 8 |
| 9 | 2 | 27 | 3 |
| 10 | 1 | 28 | 4 |
| 11 | 1 | 29 | 4 |
| 12 | 2 | 30 | 4 |
| 13 | 1 | 31 | 5 |
| 14 | 2 | 32 | 6 |
| 15 | 6 | 33 | 4 |
| 16 | 5 | 34 | 6 |
| 17 | 5 | 35 | 4 |
| 18 | 3 | 36 | 7 |




FEMALE MALE

SITONA REGENSTEINENSIS HERBST

## 4. LIFE HISTORY OF SITONA REGENSTEINENSIS HERBST.

Sitona regensteinensis Hbst. has a univoltine life cycle. There are 2 periods of emergence from soil a year and to distinguish these from one another they have been designated as the first generation and the second generation of adults.

The first generation adults begin to emerge from hibernation very early in spring during February and March when the soil temperature rises above 5°C., but return to soil again whenever the temperature drops. Thus there is a fluctuation in numbers of adults on broom until the temperature begins to rise steadily as the season advances. By the middle of April almost the whole population is found on broom, although there are many simultaneous movements up and down from broom to soil.

The beetles start feeding and copulating immediately after emergence. There are a few individuals capable of laying eggs at emergence, but oviposition generally commences about 2 weeks following it, towards the end of March. The rate of egg laying increases with temperature and attains a peak sometime in May or June. The oviposition period of the population of beetles lasts about 4 months and tails off in July. As a result there is much overlapping of stages. In winter a few individuals may be seen on broom on warm days if the soil temperature rises above 3 - 5°C. This happens especially when the warm period is preceded by a very cold one.

Development of all stages from egg to adult takes place in the

soil. The eggs are dropped on to the soil surface. The small first instar larvae hatch out and find their way into the bacterial nodules of the broom plant. There are four larval instars. These feed inside the nodules for some time and then as they grow bigger on the outside. The mature fourth instar larvae pupate in earthen cells and give rise to the adults of the second generation; these emerge from early September to the beginning of November. A very small number of late fourth instar larvae and pupae remain in soil throughout the winter, as they are unable to develop further at low temperatures. These emerge as first generation beetles in the spring of the following year.

From mid-June onwards many of the first generation adults begin to die of old age. Some of the survivors cease to feed and disappear for a period of about two months. These old beetles are seen again in autumn. They feed for a while and return to soil to overwinter for the second time. When these individuals emerge in the following spring they oviposit for a second time. It may be possible that some of these may survive for yet another season, but there is no evidence of this.

Beetles of the second generation, i.e. the newly emerging autumn generation, increase in numbers within a few weeks and attain a peak in early November. They feed, accumulate fat bodies, but remain sexually immature and in this condition gradually descend to the soil with the onset of winter. Diapause occurs throughout the winter. Most of the beetles are found in the upper two inches of soil, among litter and grass roots. There is evidence that feeding takes place during the period of

diapause.

There are slight differences in the life cycle of the macropterous forms. A build-up of flight muscles takes place during the autumn feeding period. These muscles are not used up during the winter. Migratory flights occur after a short feeding period in spring, in April and May. The egg production in the macropterous females is delayed by over a month and consequently oviposition is postponed until after the migratory flight.

## 5. DESCRIPTION OF STAGES.

### 5.1. The Adult.

Sitona regensteinensis was first described by Herbst in 1794. According to Hoffmann (1950), it has been subsequently described under the synonyms S. pleuriticus, S. femoralis and S. ulicis by Stephens (1831) and as S. globulicollis Gryll. by Schonher (1834) and Hustache (1925). Taxonomic descriptions and keys to the identification of Sitona species are given in Fowler (1891), Joy (1932), Hoffmann (1950) and Kevan (1959). S. regensteinensis is a well-defined species and no difficulty was experienced in its identification. No other species of Sitona were found on broom except for one or two specimens of S. lineatus collected in large scale sampling.

The adult S. regensteinensis is black, but variegated with coppery, metallic green or greenish grey scales. Differences in densities of these scales give rise to several colour varieties, but the majority of the adult beetles appear to be of metallic brown colour. The scales are denser on the head and thorax where they form median and two lateral bands. The thorax is convex and arched, forming a distinct angle with the elytra. In addition to scales, distinct, erect setae arise from the elytra; the setae are most prominent when viewed from the side.

The adult beetles vary considerably in size. Variation in size is a common feature among the genus Sitona and appears to be of genetical origin. As a rule, the male is smaller, with elytra more

parallel-sided. The female is usually larger with the elytra more convex, wider and less parallel.

The size is also related to the alary dimorphism found in this species. There is a macropterous form with well formed wings, which is capable of flight and a brachypterous form with vestigal wings. The non-flying brachypterous form accounted for about 89 per cent. of the individuals encountered in this study. Wing dimorphism is also found in several other species of Sitona (Jackson, 1928; Loan, 1963). In S. hispidula (F.), the brachypterous condition has been shown to behave as a simple Mendelian dominant (Jackson, 1928).

Figs. 3 - 6 illustrate the comparative distribution of size in males and females of the dimorphic forms. The lengths have been measured from the anterior margin of the eye to the tip of the abdomen. The mean lengths with their 95 per cent. fiducial limits are:

Brachypterous male: $4.17 \pm 0.90$ mm.

Brachypterous female: $4.54 \pm 1.10$ mm.

Macropterous male: $4.96 \pm 0.66$ mm.

Macropterous female: $5.49 \pm 0.92$ mm.

These lengths differ significantly from one another at $P < 0.001$ level.

Since the lengths of males range from 3.2 mm to 5.8 mm, and that of females from 3.4 mm to 6.8 mm, size cannot be taken as a criterion for separating the sexes. Fowler (1891) indicated that there is a certain difference in the formation of the terminal spines of tibiae of male and

Figs. 3-6. Distribution of size in the dimorphic forms.

Fig. 3.

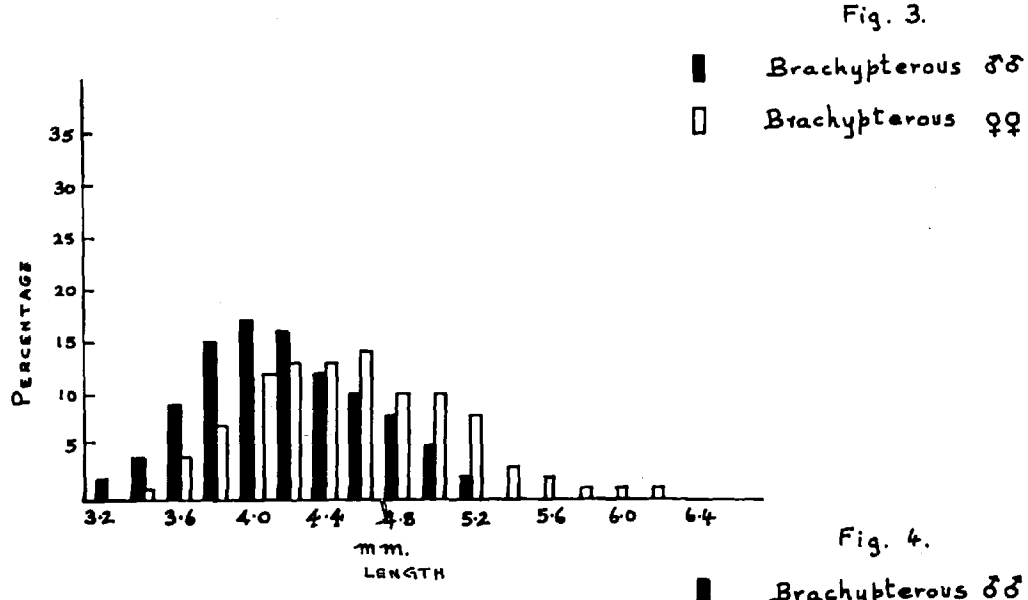
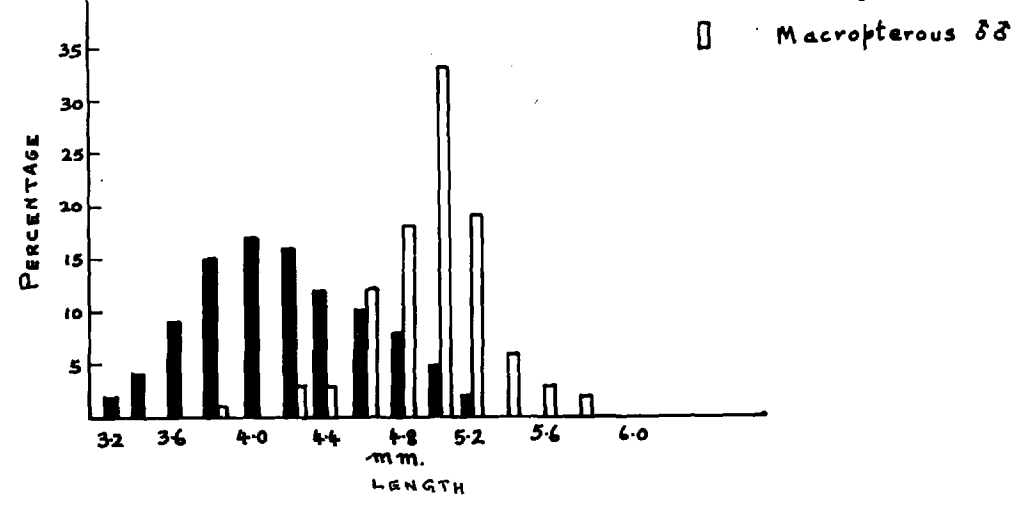



Fig. 4.



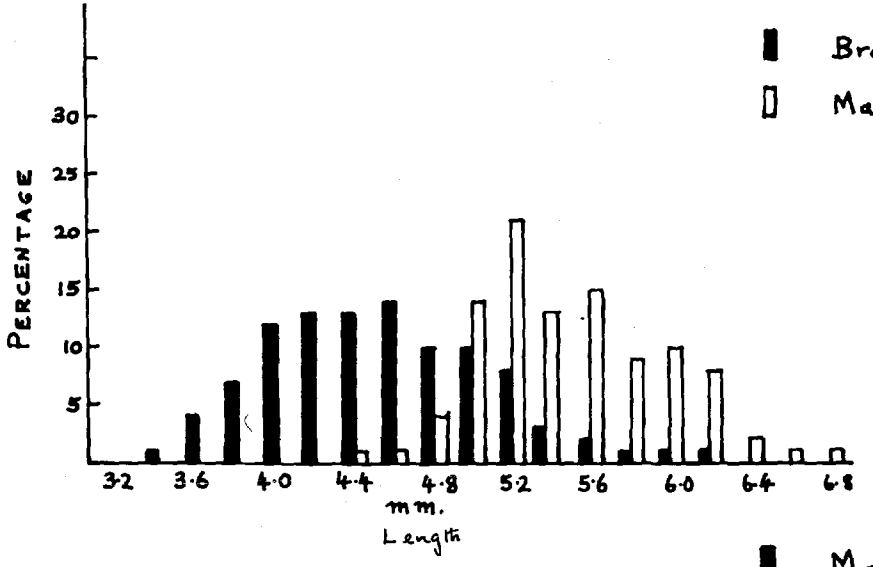

Brachypterous ♀♀
Macropterous ♀♀
PERCENTAGE
30
25
20
15
10
5
3·2
3·6
4·0
4·4
4·8
5·2
5·6
6·0
6·4
6·8
mm.
Length

Fig. 5.

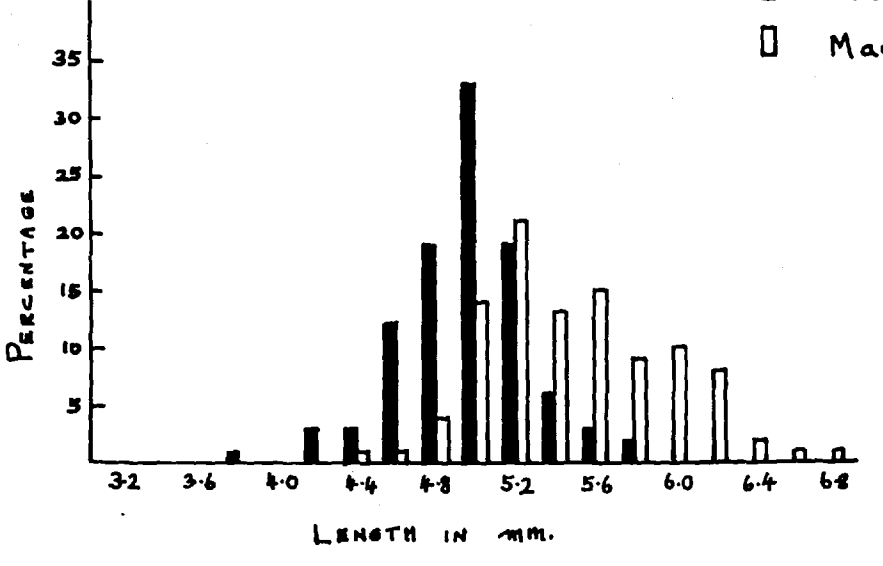

Macropterous ♂♂
Macropterous ♀♀
PERCENTAGE
35
30
25
20
15
10
5
3·2
3·6
4·0
4·4
4·8
5·2
5·6
6·0
6·4
6·8
LENGTH IN mm.

Fig. 6.

female S. lineatus. Jackson (1920) and Anderson (1931) pointed out the uncertainty of this characteristic for separating the sexes. They found good morphological differences in the terminal segments of the abdomen. The pygedium of the male is large and overlaps the hypopleurites of each side. In the female, the pygedium is small and does not overlap the hypopleurites. When viewed ventrally, in the male, the hind margin of the seventh sternite is seen to be truncated and hence the pygedium is visible. The seventh sternite of the female is rounded and consequently the pygedium is not visible. Jackson's method of identification of sexes has been shown to be applicable to S. regensteinensis by Scherf (1958). Live beetles could be sexed with great accuracy by the examination of the ventral segments of the abdomen (Fig. 7).

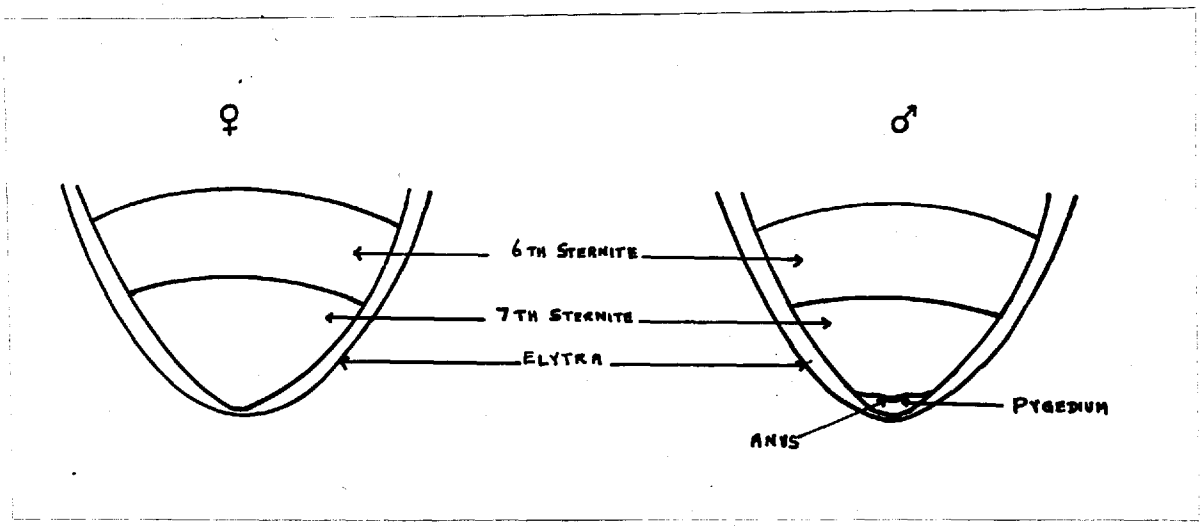



Fig. 7. Diagrams of the ventral view of posterior abdominal segments.

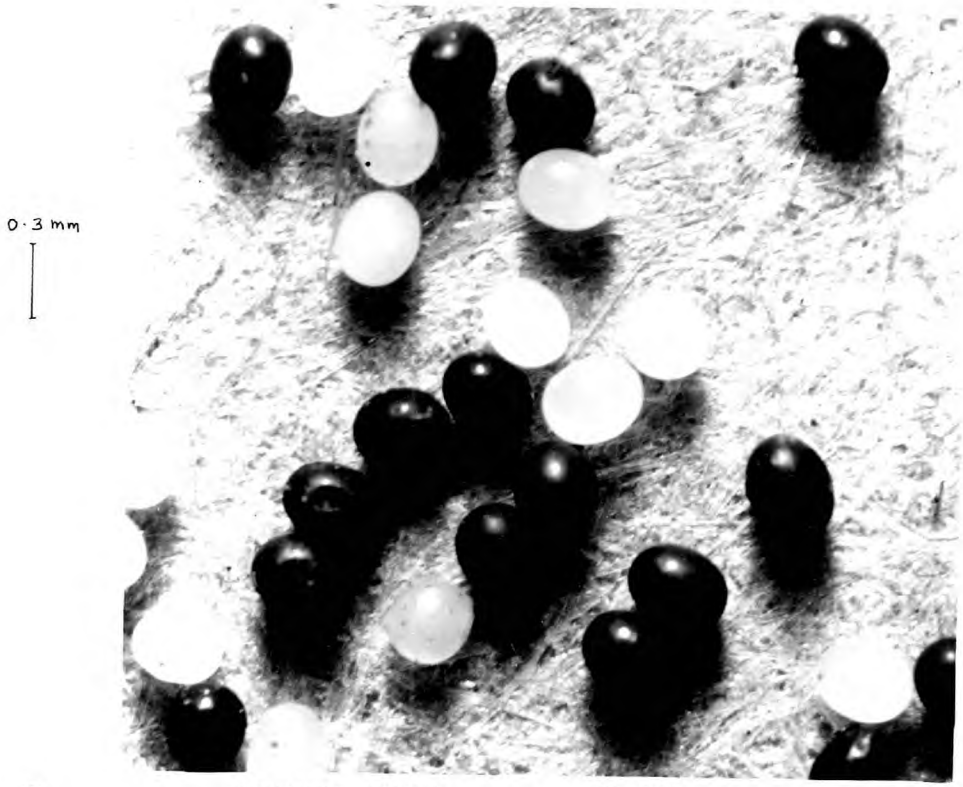



FIG. 8. SITONA EGGS. X 37


FIG. 12. SOIL SAMPLER

5.2. Eggs.

The newly laid eggs are white, glossy and oval, but few eggs laid at the start of oviposition period are slightly oblong and pointed at the ends. The average size of an egg is 0.35 mm long and 0.32 mm broad. The variation in length ranges from 0.33 mm to 0.39 mm and the diameter from 0.28 mm to 0.37 mm. The size and shape do not alter in the course of development, but there is a profound colour change. Newly laid white eggs change to grey and finally to black within some hours to a few days depending on the temperature. This change of colour is gradual; at 25°C. it is completed in about 20 hours whereas at 20°C. it takes about 28 hours. Since sterile eggs show no colour change, this phenomenon is an indication of the viability of eggs. Sterile eggs remain white, then turn yellow and shrivel after a few days. Many of the eggs laid during the last days of the oviposition period are sterile. At 100 per cent. relative humidity, the incubation period is 9 days at 25°C. and 14 days at 20°C. The emerging larva leaves the egg through a hole cut at one of the poles (see Fig. 8).

5.3. Larvae.

Some measurements of the head capsule widths in the successive larval instars and the total lengths of the larvae are summarised in Table 1. These measurements are in good agreement with Dyar's law.

A detailed description of the first instar larva of S. regenstein-ensis and a key to the identification of 8 species of Sitona larvae are

Table 1. Widths of head capsules and total lengths of larvae in mm with the 95% fiducial limits.

| Instar | Number measured | Head capsule width (mm) ± 95% fiducial limits | Body length (mm) ± 95% fiducial limits |
|---|---|---|---|
| I | 50 | 0.159 ± 0.038 | 1.048 ± 0.148 |
| II | 17 | 0.269 ± 0.087 | 1.495 ± 0.435 |
| III | 28 | 0.468 ± 0.183 | 2.142 ± 0.192 |
| IV | 42 | 0.849 ± 0.140 | 4.783 ± 1.683 |

given in a paper by van Emden (1952).

The first stage larva is white, with a transparent light amber head capsule which is slightly darker anteriorly and at the lateral margins. The body is slender, almost linear and slightly fusiform. The head and the body segments are provided with long colourless setae; the setae of the last segment are particularly long. Though legless and blind, they are extremely agile. Locomotion is by peristalsis, the single segments being extended or contracted alternatively. The last abdominal segment serves as "proleg", aiding in locomotion. It is likely that the long setae also help the first stage larvae to move in addition to the protection which they give during their passage among soil particles. The first instar larvae live for 6 - 9 days without feeding, provided there is adequate moisture.

The older larvae are creamy white, with comparatively short setae

distributed sparsely over a distinctly segmented body. The strongly chitinised head is small in relation to the body. A taxonomic description of the final instar larva is found in Scherf (1958).

The mature larvae are generally slow in their movements. These movements consist of bending the body, making searching movements with the head and prothorax and change of position; they may be said to exhibit klinotactic behaviour. When disturbed the larva loops the body ventrally in the manner of many caterpillars. The older larvae are usually found in soil in this attitude, which is also the characteristic posture assumed by the dead larvae. Larvae of all stages are thigmotactic and also exhibit negative phototaxis. Prior to pupation, the mature larva converts its last abode into a pupation chamber.

5.4. Pupa.

An account of the external features of the pupa is given in the paper by Scherf (1958). The pupa is soft skinned and creamy white. As development proceeds, the colour becomes dark. First it turns yellow and then gradually becomes brown. The length of the pupa varies from 3.5 to 6.0 mm. The head is curved ventrally and is not seen from above. Nearly half the length of the body is taken by the thorax. The wing buds traverse along the back for some distance and then curve ventrally between the femora of the second and third legs. The duration of the pupation period is about 20 days. The young adult spends a few days in the pupal cell before emergence.

## 6. METHODS OF ESTIMATING POPULATION SIZE.

### 6.1. Methods of Sampling Adults.

#### 6.11. Beating Method.

This method has been successfully used in a study of a natural population of Phytodecta olivacea (Forster), a chrysomelid beetle living on broom, by Richards and Waloff (1961).

The adult Sitona have a tendency to exhibit thanatosis when disturbed; in doing so they often fall on their backs and remain motionless for some time. In the beating method, this behaviour is made use of to sample the adult population. A quantity of broom measuring about one-eighth of a bush is shaken over a cloth tray. The beetles that fall on to the tray are counted, sexed and released. As mentioned in section 3 (page 6), there are 138 bushes scattered in 36 groups in the study area. Since beating involves considerable disturbance of the habitat, only one beat of each group of bushes can be done per occasion. During the early stages of this study (October to December, 1962), 30 of these groups were selected randomly and a single beat was done on each and the numbers of beetles recorded. From 1963 onwards each of the 36 groups of bushes was beaten so that the sampling covered the whole of the study area. Thus each sample comprised 36 units of one-eighth bushes. From the numbers of beetles collected from this known quantity of broom estimates of the adult Sitona population were obtained, since the total number of bushes was known. This method of sampling is systematic and covers the whole area and

is similar to the centric systematic area-sample described by Milne (1959).

6.12. The Method of Marking and Re-capture.

In this method, a number of beetles were marked with artists oil paint on the elytra and scattered over the study area. After 48 hours, a sample was taken by the beating method and the total number in the sample and the number of re-captured marked individuals noted. An estimate of the population was made by using the formula of Bailey (1952).

Since some of the beetles marked and released on one day were re-captured on subsequent weekly routine sampling days, it was also possible to analyse this data by using Jackson's 'positive' method (Jackson, 1939).

6.13. Emergence Trap Method.

In this method, the number of adults emerging from soil was estimated by the use of emergence traps based on the Varley pattern (c.f. Southwood and Jepson, 1962). Each trap consisted of a 2 feet square 'dural' alloy box with 4 inches-high sides. The corners of the open end were provided with 4 short spikes. Two (3 × 1)inch specimen tubes projected from holes in diagonally opposite corners (see Fig. 9). The grass under the selected broom bush was trimmed and the box inverted and pushed into the soil. Any gaps along the sides of the box were then sealed with clean sand.

Beetles emerging from the soil under a trap, are strongly positively phototactic, and make their way to the tubes which provide the

FIG. 9

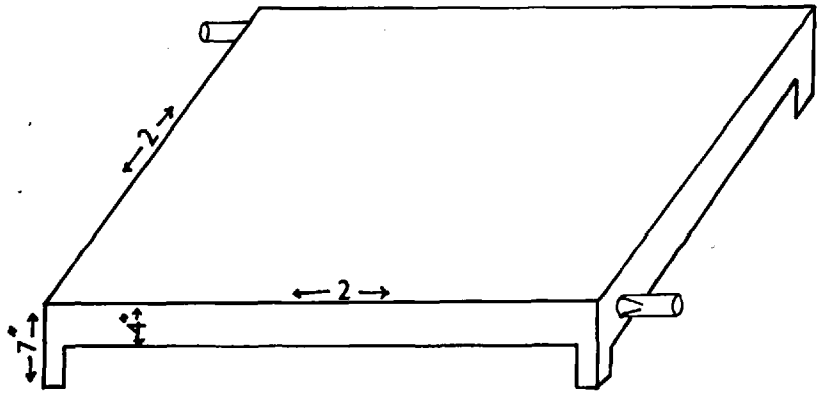
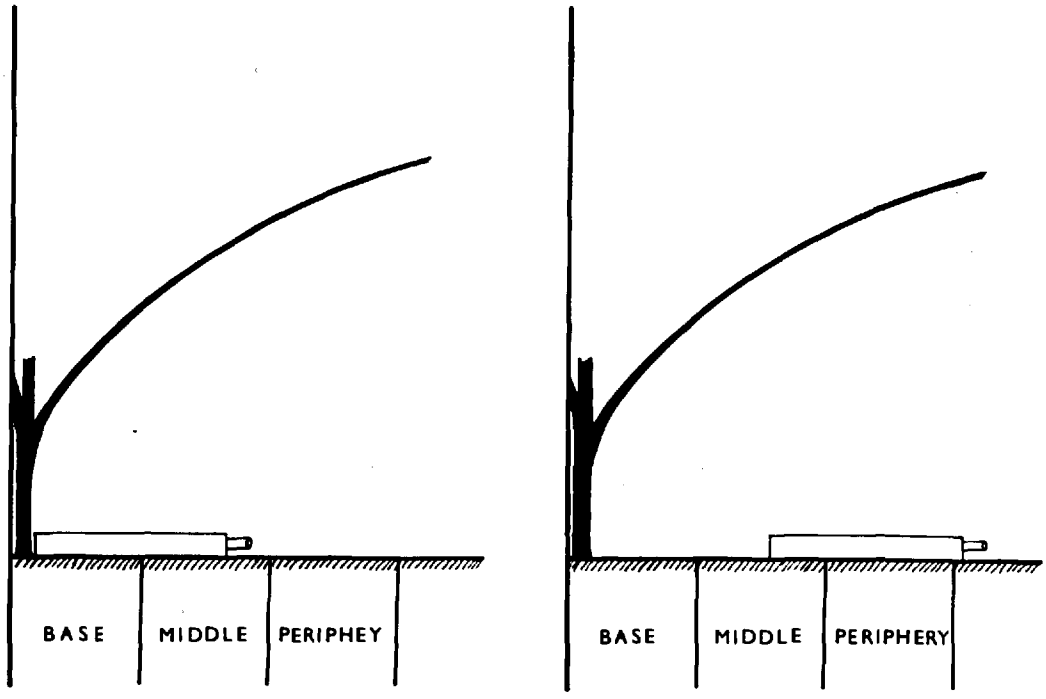



FIG. 10



only source of light.

The life history of Sitona provides two occasions a year when this method can be used to estimate the size of the population; one is when the first generation of adults emerges in spring after hibernation and the other occasion is when new adults or the second generation of adults emerge for the first time in autumn. The emergence traps were therefore placed in position once towards the end of winter and again soon after the egg laying period was over. Altogether 20 traps were placed under 20 randomly selected bushes. The trapped beetles were collected once in every 3 days.

A single trap does not cover the whole area under the branches of a broom bush. It will be shown later that the accumulation of eggs in soil is not uniform under a broom plant. There is a negative correlation between the numbers of eggs found in soil and the distance away from the stem. This relationship made it necessary to divide the area under a bush into 3 approximately equal regions for purposes of sampling. These three regions have been designated as the basal, middle and peripheral regions (see Fig. 10). Since the distance between the base of the stem to the periphery of a bush of average size is about 4 feet, each of these regions cover about 16 inches along the radius. This distance varies slightly as it depends on the size of the bush. The 20 emergence traps were placed in such a way that 10 of them covered the basal and middle regions and the other 10 covered the middle and peripheral regions of soil under the individual bushes. It was thought that this method of placing the traps would prevent or reduce any variability of emergence brought

about by the distribution of eggs in soil.

The area covered by 20 emergence traps is equal to 80 square feet and the total area under broom is 3659 square feet (section 3). If the number of Sitona collected over the whole emergence period is x, the population estimate will be $\frac{x}{80} \times 3659$.

6.14. Comparison of Results from Different Methods of Sampling Adults.

Population estimates by the three methods are presented in Table 2. The results from different methods seem to agree fairly closely. The series of estimates by independent methods can be considered to provide a mutual check independent of information given by fiducial limits.

Reliability of beating as a method of sampling is demonstrated in Table 3. The general rise and fall in adult numbers in each generation in the 2½ years as derived from beating is shown in Fig. 11. Where they overlap, they were assigned to the correct generation by dissecting a sample of 30 males and 30 females (see section 14, page 153). The number of Sitona found at any time on broom branches is greatly influenced by the prevailing air temperature and soil temperature; the beetles tend to stay in soil during cold periods. As seen in Fig. 11 (also see section 11), the marked fluctuations in the numbers soon after the spring emergence are brought about by the unsettled weather conditions during early spring. Population estimates derived by beating during cold periods can therefore be considered to be estimates of the numbers on the branches since this excludes those that stay in soil. However, when it is warm, almost the

FIG II SEASONAL CHANGES IN THE NO. OF SITONA ON BROOM

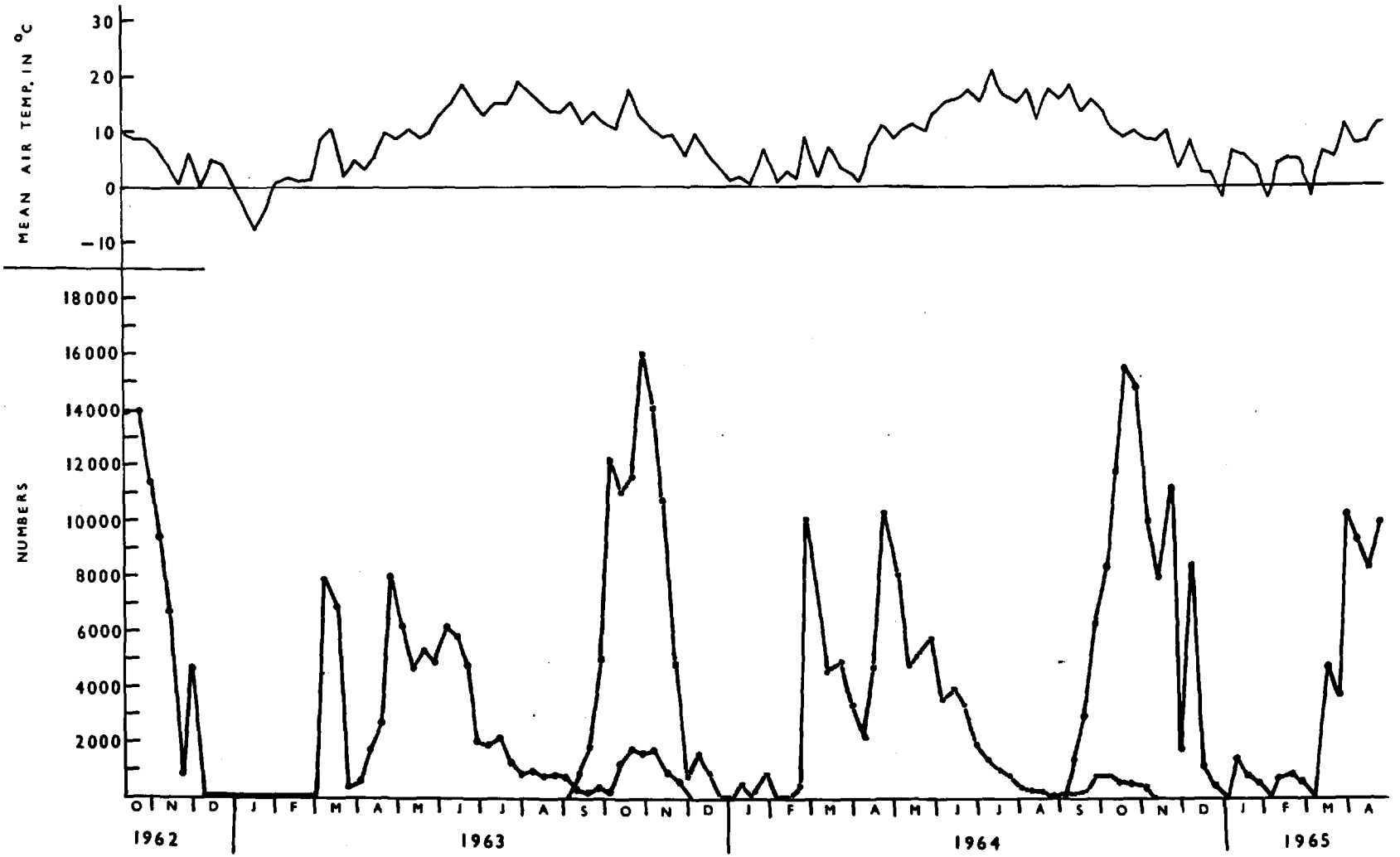


Table 2. The maximum estimates of the population of adult Sitona by the different methods, with their 95% fiducial limits.

| Date of maximum estimate by beating and marking | Estimate by* beating at maxima | Estimate by marking at maxima | | Estimate from* emergence traps Total emergence up to date |
|---|---|---|---|---|
| | | Bailey's Method** | Jackson's Method | |
| 15.10.62 | 13,726 ± 1,462 | 15,079 ± 10,304 | 14,706 | - |
| 22.10.62 | 13,836 ± 1,478 | 8,352 ± 4,123 | 7,567 | - |
| 19. 4.63 | 7,985 ± 1,112 | 6,524 ± 4,860 | - | 5,991 ± 1,091 |
| 25.10.63 | 15,572 ± 1,591 | 17,084 ± 2,026 | 24,125 | 14,777 ± 1,578 |
| 17. 4.64 | 10,212 ± 1,149 | 11,613 ± 4,572 | - | 8,812 ± 1,331 |
| 23.10.64 | 15,424 ± 1,420 | 20,120 ± 14,829 | 17,173 | 16,003 ± 1,813 |
| 31.10.64 | 14,720 ± 1,398 | 17,374 ± 10,243 | - | 16,735 1,832 |

* Fiducial limits based on negative binomial distribution (see section 6.15).

** Fiducial limits calculated as described by Bailey (1952).

Table 3. Showing the reliability of the beating method for sampling Sitona adults.

| Date | Number of beats | Total caught | Mean number of Sitona per beat ± 95% fiducial limits | Standard error | Standard error as % of mean |
|---|---|---|---|---|---|
| 15.10.62 | 30 | 373 | 12.43 ± 4.29 | 2.100 | 16.89 |
| 17.10.62 | 30 | 282 | 9.40 ± 3.04 | 1.486 | 15.81 |
| 22.10.62 | 30 | 376 | 12.53 ± 4.37 | 2.140 | 17.07 |
| 30.10.62 | 30 | 310 | 10.33 ± 3.521 | 1.722 | 16.66 |
| 13.11.62 | 30 | 182 | 6.07 ± 2.60 | 1.271 | 20.94 |
| 22.11.62 | 30 | 22 | 0.733 ± 0.43 | 0.212 | 28.92 |
| 6. 5.63 | 36 | 202 | 5.60 ± 1.926 | 0.953 | 17.01 |
| 27. 9.63 | 36 | 402 | 11.16 ± 3.79 | 1.869 | 16.75 |
| 11.10.63 | 36 | 433 | 12.03 ± 3.82 | 1.893 | 15.73 |
| 18.10.63 | 36 | 574 | 15.93 ± 4.03 | 1.997 | 12.53 |
| 17. 4.64 | 36 | 333 | 9.25 ± 2.82 | 1.390 | 15.03 |
| 26. 6.64 | 36 | 42 | 1.17 ± 0.44 | 0.216 | 18.46 |
| 23.10.64 | 36 | 503 | 13.97 ± 4.42 | 2.180 | 15.60 |
| 31.10.64 | 36 | 480 | 13.33 ± 3.12 | 1.535 | 11.51 |

whole population appears to stay above ground, and this happens throughout late spring, summer and early autumn (from mid-April to end of October; see Fig. 11). This leads to the assumption that sampling done by the beating method during this period gives satisfactory estimates of the real population. The comparative data in Table 2 seem to confirm this fact. Seasonal changes in the adult numbers as given by routine sampling by beating can therefore be considered to give a correct impression of the real fluctuations in the adult population, once the effects of the weather factors are realized.

When the marking and re-capture experiments were carried out it was seen that the necessary conditions were generally satisfied. The colony of Sitona under investigation consisted of a more or less closed population. The proportion of the migratory form was small and flight occurred only during a short period in spring towards the end of April. There is not much to choose between the results from the two techniques of analysing the data (Table 2). Though the confidence limits appear to be wide, the mean population estimates agree favourably with those from other methods.

In the use of emergence traps, fractions of the population were caught as they emerged, and the 20 traps covered a comparatively large part of the total area under the broom. Moreover, the traps prepared for autumn emergence did not sample the survivors from the previous generation. Population estimates of the new autumn generations as given by this method appear to show good agreement with those from the other methods. When all

factors are evaluated the estimates by this method for autumn generations seem to be the best to warrant use in the construction of life tables. Spring generation traps however, under-estimated the numbers that emerged to a small extent. A possible reason for this is that small numbers of beetles were seen to feed on and off during the winter and consequently left out when the traps were placed in position. The emergence trap data are summarised in Table 4.

Table 4. Summary of Data from Emergence Trap Sampling.

| Date | Total Sitona trapped in 20 traps | No. of Sitona per trap ± 95% F.L. | Standard error | Standard error as % of mean |
|---|---|---|---|---|
| 19. 4.63 | 130 | 6.50 ± 3.4 | 1.63 | 25.07 |
| 25.10.63 | 323 | 16.15 ± 9.59 | 4.59 | 28.42 |
| 17. 4.64 | 193 | 9.63 ± 4.12 | 1.97 | 20.46 |
| 23.10.64 | 350 | 17.49 ± 8.59 | 4.11 | 23.49 |
| 31.10.64 | 366 | 18.29 ± 7.48 | 3.58 | 19.57 |

6.15. Distribution of Adult Sitona in the Field.

In studying the occurrence of adults in the field, a sample of 100 beats was taken on 25.10.63 and the number of individuals in each beat recorded. The results are expressed as a frequency series, with the number of beats containing 0, 1, 2 ..... n individuals tabulated in order in Table 5. This frequency distribution does not fit a Poisson series and

Table 5. Fitting the negative binomial to Sitona counts (100 beats done on 25.10.63) and the goodness-of-fit tested by $\chi^2$.

| No. of Sitona per beat x | Frequency f | Negative Binomial Expectations ø | $\frac{(f-ø)^2}{ø}$ | No. of Sitona per beat x | Frequency f | Negative Binomial Expectations ø | $\frac{(f-ø)^2}{ø}$ |
|---|---|---|---|---|---|---|---|
| 0 | 2 | 1.94 | 0.0110 | 21 | 0 | 1.84 | |
| 1 | 3 | 3.30 | | 22 | 2 | 1.66 | |
| 2 | 6 | 4.24 | 0.6386 | 23 | 1 | 1.49 | 0.8576 |
| 3 | 4 | 4.85 | 0.1489 | 24 | 1 | 1.34 | |
| 4 | 6 | 5.21 | 0.1198 | 25 | 1 | 1.20 | |
| 5 | 5 | 5.38 | 0.0268 | 26 | 0 | 1.07 | |
| 6 | 5 | 5.40 | 0.0296 | 27 | 1 | 0.97 | 0.7305 |
| 7 | 6 | 5.32 | 0.0869 | 28 | 1 | 0.87 | |
| 8 | 6 | 5.16 | 0.1367 | 29 | 0 | 0.78 | |
| 9 | 4 | 4.94 | 0.1788 | 30 | 0 | 0.69 | |
| 10 | 2 | 4.68 | 1.5347 | 31 | 2 | 0.62 | 2.9882 |
| 11 | 5 | 4.40 | 0.0882 | 32 | 1 | 0.55 | |
| 12 | 6 | 4.11 | 0.8691 | 33 | 2 | 0.49 | |
| 13 | 3 | 3.82 | 0.3704 | 34 | 2 | 0.44 | |
| 14 | 6 | 3.53 | | 35 | 0 | 0.39 | |
| 15 | 2 | 3.25 | 0.0085 | 36 | 0 | 0.34 | |
| 16 | 4 | 2.98 | | 37 | 2 | 0.30 | 3.0237 |
| 17 | 1 | 2.72 | 0.0077 | 38 | 0 | 0.26 | |
| 18 | 4 | 2.48 | | 39 | 0 | 0.23 | |
| 19 | 1 | 2.25 | 0.3879 | 40 | 0 | 0.20 | |
| 20 | 2 | 2.04 | | 41 | 1 | 0.18 | |
| | 83 | 82.00 | 4.6436 | | 17 | 15.91 | 7.6000 |
| | | | | Brought over | 83 | 82.00 | 4.6436 |
| | | | | Total | 100 | 97.91 | 12.2436 $\chi^2$ |

Mean = 12.6
Variance = 93.21

k = 1.9695 Standard Error of k = 0.3953

$\chi^2$ = 12.2436

D.F. = 19

hence cannot be considered to show random distribution; the occurrence of low numbers and high ones are far too common. It is well known that animals are only rarely distributed at random in their habitats, their distribution being practically always aggregated.

A distribution which is generally found to be useful in assessing biological aggregation is the negative binomial which has proved applicable to a wide diversity of biological data, particularly for the analysis of insect counts (Anscombe, 1949; Bliss and Fisher, 1953).

The data from the 100 beats fitted the negative binomial very satisfactorily. The value k estimated by the method based on the relation between the variance and the mean, was found to be 1.9695. The best estimate of k is obtained by the method of maximum likelihood (Bliss and Fisher, 1953), but Anscombe (1949) has shown that if $k > 1$, the former method gives about 90% efficiency. The negative binomial expectations and the test of goodness-of-fit by $\chi^2$ for the Sitona data are presented in Table 5. When the Sitona population was low, the distribution approached randomness and fitted the Poisson series, but still a better fit was found with the negative binomial.

Walters (1959) has shown that the parameter k is a valid measure of aggregation. Its values can range from zero, where aggregation is extreme, to infinity, which defines a purely random distribution. In actual practice, any large value of k indicates an approach towards randomness. The value of k also indicates the relative degree of aggregation for the conditions involved. If the emergence trap data given below are

examined, the aggregation of Sitona on the plant, shown by the beating method appears to be actually a reflection of aggregation in soil. The numbers of individuals emerged in the 20 traps in autumn 1963 are as follows:

| Trap No. | Covering base and middle | Trap No. | Covering middle and periphery |
|---|---|---|---|
| 1 | 2 | 11 | 0 |
| 2 | 6 | 12 | 0 |
| 3 | 6 | 13 | 1 |
| 4 | 9 | 14 | 1 |
| 5 | 21 | 15 | 2 |
| 6 | 24 | 16 | 2 |
| 7 | 28 | 17 | 3 |
| 8 | 39 | 18 | 9 |
| 9 | 66 | 19 | 15 |
| 10 | 69 | 20 | 20 |
| Total | 270 | Total | 53 |

The above figures show that 83.6 per cent. of all individuals emerged in the traps covering the basal and middle regions. The variation within each column itself is wide. Traps covering the base and middle show a variance of 589.55 (mean = 27). The figures in the right hand column have a variance of 49.34 with 5.3 as the mean. In both cases, the variance is much greater than the mean which is a feature of departure from randomness. The emergence trap data strongly suggests aggregation since the estimate of the k value is 0.6355. This aggregation of adult Sitona in soil prior to emergence (or at emergence) is obviously a result of aggregation during immature stages. It will be shown later (in section 10) that this aggregation is brought about by the distribution of eggs

(oviposition) and root nodules (the food of larvae) in soil. Reproduction behaviour and responses to the host plant, or parts thereof, are included among the truly biological bases of aggregation by Walters in his paper on aggregation in insects (Walters, 1959).

It is relevant at this stage to discuss how aggregation may influence the analysis of sampling data from beating and emergence trap methods. Aggregation results in a condensation of the population in certain parts of the habitat and a corresponding depletion in others. This is clearly seen in the frequency distributions shown by the 2 methods (Table 5 and page 35). A random sample from such a population will contain an excess of empty or nearly empty sampling units while other units will have an excessive number of individuals. This results in a greater variance than would be expected from a random distribution. Debauche (1962) has shown that this variance consists of two components: (1) the normal variance which results from habitual fluctuation about the mean, and (2) an increment proportional to the degree of aggregation existing in the population sampled. Thus if the distribution is of the negative binomial form the total variance is

$$s^2 = \bar{x} + \frac{\bar{x}^2}{k} \qquad \text{where } \bar{x} = \text{mean}$$

the second term, $\bar{x}^2/k$, representing the increment caused by aggregation. Debauche has also shown that this increment appears as a constant characteristic of the species at the time of sampling; it is not aleatory and should not be included in the error. The significance of these principles

in analysing Sitona data becomes clear if figures from the 100 beats and from the 1963 autumn emergence traps are again considered.

Variance calculated for the 100 beats in the usual way (on the assumption that the distribution is approximately normal with variance independent of the mean) is 93.21 (mean = 12.6). The variance for the same data based on the negative binomial distribution is 12.6 since

$$s^2 = \bar{x} + \frac{\bar{x}^2}{k}$$

$$= 12.6 + 80.5$$

$$= 93.1.$$

Thus a greater part of the variance (80.5) is as a result of aggregation. In the case of emergence trap data, the variance calculated in the normal way is 426.6 (mean = 16.15). The variance based on the negative binomial distribution is 16.15 since

$$s^2 = \bar{x} + \frac{\bar{x}^2}{k}$$

$$= 16.15 + 410.4$$

$$= 426.55.$$

This shows that the amount of error involved in sampling by beating and emergence trap methods as indicated by the normal method of analysis is far greater than that actually occurring. Therefore, the fiducial limits for population comparisons (in Table 2) have been based on the negative binomial distribution.

6.2. Methods of Sampling Immature Stages.

Preliminary investigations were carried out first to find a suitable and a reliable method for the routine sampling of immature stages, particularly the eggs. In the first two methods described below, the eggs were sampled as they were dropped from the foliage. In the third method, the eggs that accumulated in soil were sampled by taking soil cores and processing them to separate the eggs and larvae from soil. This also served as a method of sampling the root nodules of the broom plants as well as some predatory mites.

6.21. Method 1.

Fifteen 14" × 12" white enamel trays were placed under 15 bushes to cover the three regions under the broom plant. The numbers of eggs that collected on these trays were noted.

6.22. Method 2.

In this method, instead of trays, 50 glass funnels, 4 inches in diameter each and fitted with 3" × 1" specimen tubes on to the stem of each were placed upright and pushed slightly into soil. The eggs that dropped from above collected on the inner walls of the funnels or were washed into the tubes by rain water. At regular intervals, those that were still on the funnel walls were washed in with a jet of water from a wash bottle, the tubes exchanged for fresh ones, and the eggs that collected were counted.

6.23. Method 3.

In this method, a sample of 60 soil cores was taken with a sampler that removes a core of soil 1-inch in diameter up to a depth of 18 inches. This sampler, originally designed by Dr. N.G.M. Hague for nematode sampling, consists of a steel tube sharpened at one end and provided with a handle of the same material at the other (see Fig. 12) p. 19). One half of the wall of the tube is removed along its length, leaving 1.5 inches at the cutting end. This allows removal of the soil core and reduces compression of soil while sampling. A one-inch scale is marked along the length of the sampler to enable the depth of soil to be measured.

For the preliminary sample, 20 bushes were randomly selected, and from the area covered by each bush 3 soil cores were taken, one from the basal region, one from the middle region and the third from the peripheral region. The sample thus consisted of 60 soil cores, each one inch in diameter and 12 inches long. These were collected in separate polythene bags, labelled and stored in a deep freeze or a refrigerator until processed.

The method that was used to separate the eggs and larvae from soil was a combination of two well known techniques of extraction; but as this method has not been used hitherto for the extraction of insect eggs from soil, it is intended here to review the available methods briefly and then describe the modifications that have been introduced.

Although many methods are available for the extraction of arthropoda from soil, their suitability as far as arthropod eggs are

concerned is questionable, particularly when small eggs, barely visible to the eye need to be separated from soil. Hand sorting and sifting are the traditional methods of separating large eggs. Centrifugal floatation which is widely used by parasitologists for extracting protozoan cysts and helminth eggs from faeces has been used for soil acarina, insects and their eggs by several workers (see Murphy, 1962; Muller, 1962). This process, though efficient, can be used only for small samples of about 5 g. or less. There is need for an efficient method of extracting arthropod eggs from soil samples of moderate or fairly large size.

The Salt and Hollick extraction technique (Salt and Hollick, 1944) is well known for its efficiency in extracting soil arthropoda from agricultural soil (see Raw, 1962). Raw (1955) published an account of a scaled-down version of the apparatus for the extraction of micro-arthropods from soil samples of about 100 $cm^3$. It is only the outline of the Salt and Hollick technique given here, as well-illustrated details of the method and its refinements are found in many of the recent books on soil zoology (c.f. Kevan, 1955, 1962; Kühnelt, 1961; Murphy, 1962).

Briefly, the Salt and Hollick method consists of wet sieving or washing the soil through one or two sieves for the removal of unwanted coarse particles and mixing the filtered small particles with a magnesium sulphate solution of specific gravity 1.2. On settling, the soil particles sink and all organic matter floats to the surface.

To separate the animal from the vegetable matter, the floating material is subjected to an oil separation stage during which the float is

removed, rinsed free of magnesium sulphate, shaken in a vessel containing benzene (or Xylene) and water and then allowed to settle. Benzene wets the cuticle of most arthropods which settle out at the benzene-water interphase, while the vegetable debris which is wetted by water settles at the bottom. It has been pointed out that the oil separation stage is not suitable for some arthropods, particularly dipterous larvae, Isopoda, Myriapoda and insect eggs as these are not wetted sufficiently to float the specimens (Macfadyen, 1955; Murphy, 1962) and this was found to be the same with Sitona eggs. The following procedure was adopted to overcome this disadvantage of the oil separation stage.

The material collected after floatation is washed free of magnesium sulphate and centrifuged with a saturated solution of sodium chloride (specific gravity 1.18) at 1500 r.p.m. for 5 - 10 minutes. The Sitona eggs, larvae and other arthropods float at the top of the centrifuge tube while the vegetable matter is deposited at the bottom. For best results, only up to one-third of the centrifuge tube must contain the material and the rest is filled with salt solution. After centrifuging, the supernatant liquid is poured into a shallow glass dish; all floating material can be transferred by rotating the centrifuge tube between the fingers while pouring. The eggs and larvae are then picked up under a low power microscope. The efficiency of the method may be hindered by small amounts of vegetable debris floating as a result of trapped air bubbles. This, however, can be easily overcome by impregnating the vegetable matter with water by freezing and thawing several times prior to centrifugal floatation.

If the eggs are required alive for various purposes, for instance to determine their viability or to obtain parasites, freezing is replaced by soaking in water overnight so as to render the material soggy. The method is efficient for both hatched and unhatched eggs. Several specimens of a mymarid parasite emerged from Sitona eggs obtained in this way. It was also observed that other insects, acarina and their eggs floated up during the process; these were mostly alive. The method therefore seems to be applicable to arthropods and eggs that cannot be obtained satisfactorily by the other method as it depends on the specific gravity rather than on differential wetting. Among the advantages of this modification are its simplicity, avoidance of harmful and unpleasant handling of benzene, and the ability to obtain specimens alive. These observations suggest that this appears to be a promising replacement for the oil separation stage in Salt and Hollick extraction technique.

6.24. Method 4.

This method was primarily designed to give information regarding the oviposition rate of the field population and also to serve as a check for the routine sampling method. Basically, the method consists of counting the eggs laid by a known number of caged beetles maintained in the field. The beetles are changed at regular intervals so that the information is from a cross-section of the population and not merely restricted to that from a single set of beetles.

A cage was constructed to the specifications given in Fig. 13.

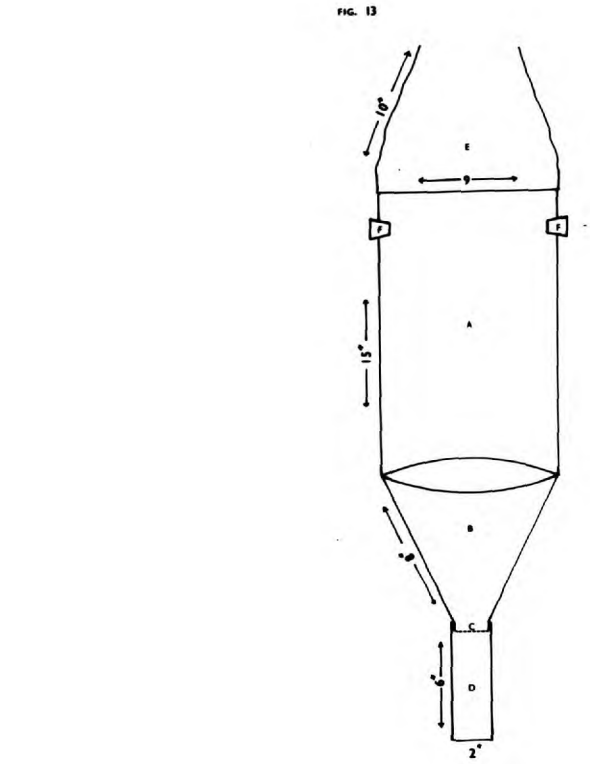



FIG. 13. The field egg laying cage.

This consists of a celluloid (cellulose acetate) cylinder A, to one end of which is fused a celluloid funnel B. The funnel is provided with a short stem C and the end of the stem is covered with nylon net with 0.5 mm × 0.7 mm apertures. This net acts as a sieve which permits eggs to pass through, but retaining the beetles. A detachable tube D is fitted to the stem of the funnel. The bottom of this tube is covered with organdie. The open end of the cylinder is extended with a sleeve E made of fine net. The upper end of the cylinder is also provided with 2 holes which are kept closed with 2 rubber stoppers F.

A small branch from a broom plant is introduced into the cylinder through the sleeve and is kept in position by tying the sleeve tightly round it. A known number of male and female Sitona is introduced through one of the holes and the rubber stopper replaced. The eggs laid within the cage fall through the net and collect in the tube D. The organdie bottom of this tube permits rain water to drain through. The eggs that adhere to the inner walls of the cage are brought down with a jet of water from a wash bottle just before they are collected. Once the eggs are collected in the tube, it is replaced with a similar fresh one. The tube with eggs is taken to the laboratory and the eggs counted.

Altogether 20 males and 20 females were used in this apparatus, but were changed by a different set of beetles from the field once a week. The eggs laid in the previous 24 hours were collected every morning between 9 a.m. and 10 a.m. These were counted and laid out on moist filter paper in petri-dishes. A count of the number of sterile and viable eggs was

made after 1 - 2 days, once the colour change was complete.

6.25. Comparison of results from different methods of sampling eggs.

The estimates of eggs by the first 3 methods of sampling are assessed as:

$$\text{Total number of eggs} = \frac{\text{Average number per core (or tray or funnel)}}{\text{Area of a single core (or tray or funnel)}} \times \text{Area under broom}$$

The total number of eggs is estimated by method 4 in the following way:

$$\text{Total egg number} = \frac{\text{Number of eggs collected}}{\text{Number of caged females}} \times \text{Total number of females in the field}$$

Results obtained in this way during the period 1.4.63 to 19.4.63 are given in the following table.

Table 6. Total number of eggs estimated by four methods.

| Method | Total area of the sample | Estimates of total egg number |
|---|---|---|
| 1. (Trays) | 2520 in.$^2$ | 31,676 |
| 2. (Funnels) | 628.4 in.$^2$ | 63,706 |
| 3. (Soil cores) | 47.14 in.$^2$ | 156,434 |
| 4. (Caged females) | - | 204,369 |

These preliminary results provided the following information: the estimates from the tray and funnel methods are very much lower than those from the other two methods; results from soil sampling gave a closer estimate to that from caged females. The estimate from method 4 can be considered to serve as a reliable check for the other methods, as

it is based on the fecundity of a known number of females. Soil sampling thus stands out as the best of the three methods for routine sampling of eggs in soil.

These results also indicate that oviposition may not be merely restricted to eggs dropped from above. Another factor that became apparent was that there is some relationship between the number of eggs in the soil, the root nodule content and the location of the sample. This aspect of the problem will be reconsidered in a later section.

The tray and funnel methods were discontinued from 19.4.63 onwards. Soil sampling was selected for routine sampling of immature stages. Method 4 in which caged females were used was continued so as to provide a check to soil sampling and also to obtain further information on oviposition.

If the statistical aspects of soil sampling are considered, then it is considered that the difficulties encountered have been more of an entomological nature than statistical. It must be mentioned here that the processing soil for eggs and larvae is rather laborious and time consuming; each soil core takes approximately one hour for the whole process. The soil sampling plan has been based mainly in relation to the biology and ecology of the beetle and to some extent on the economy of time. Finney (1946) in his paper "Field sampling for the estimation of wireworm populations" states that "The statistician concerned with economic entomology is frequently asked to recommend a sampling technique for estimating the density of an insect population. The principles to be adopted in devising a suitable technique are now fairly well understood; their object is to obtain, by a method economical of time and effort, an estimate known to be sufficiently

accurate for practical use, and, as a secondary consideration, some measure of that accuracy".

The reliability of soil sampling as a sampling method is shown in Table 7. The accuracy of estimates from soil sampling can also be checked with those based on the fecundity of caged females. The sampling plan in 1963 as well as in 1964 can be classed as a stratified random sampling method. It was indicated in section 2.13 that there is much aggregation of the immature stages in soil. Simple random sampling is mostly satisfactory when the population is homogeneous or not highly variable, as the selection of the sample is left entirely to chance. In stratified random sampling, by dividing a heterogeneous population into parts or strata each of which is fairly homogeneous, some precision is gained over random sampling (see Cochran in Snedecor, 1962). In this instance, the basal, the middle and the peripheral regions under the broom plant were considered to be the three strata from which soil cores were taken at random.

In 1964, the size and the number of soil cores were altered with the intention of obtaining some additional information. Samples were taken with a 2-inch diameter sampler of the same design. The soil cores were taken up to a depth of 12 inches and the top 4 inches (= 81.1 $cm^3$ of soil) were processed separately so as to minimise the volume of soil that had to be examined for eggs. The frequency of sampling was 10 days. Each sample consisted of a soil core from each of the 36 groups of bushes. The location of soil core in each group was rotated, for instance, on the first sampling occasion, a soil core was taken from the basal region from

Table 7. Showing the reliability of soil sampling.

| Date | Soil Core Size | No. of Cores per Sample | Total No. of Eggs | Mean No. of Eggs per Core ± 95% Fiducial Limits | Standard Error | Standard Error as Percentage of Mean |
|---|---|---|---|---|---|---|
| 27.4.63 | 1" | 30 | 12 | 0.4 ± 0.27 | 0.132 | 32.95 |
| 27.4.63 | 1" | 60 | 21 | 0.35 ± 0.153 | 0.076 | 21.86 |
| 5.6.63 | 1" | 60 | 66 | 1.1 ± 0.322 | 0.166 | 15.07 |
| 15.6.63 | 1" | 60 | 92 | 1.53 ± 0.434 | 0.217 | 14.18 |
| 25.6.63 | 1" | 60 | 85 | 1.42 ± 0.420 | 0.210 | 14.83 |
| 6.7.63 | 1" | 60 | 52 | 0.867 ± 0.351 | 0.175 | 20.22 |
| 27.4.64 | 2" | 36 | 83 | 2.305 ± 1.091 | 0.540 | 23.43 |
| 15.5.64 | 2" | 36 | 137 | 3.805 ± 1.299 | 0.643 | 16.90 |
| 16.7.64 | 2" | 36 | 138 | 3.833 ± 1.48 | 0.733 | 19.13 |
| 26.7.64 | 2" | 36 | 124 | 3.44 ± 1.699 | 0.841 | 24.45 |

group 1, from the middle region from group 2, from the peripheral region from group 3 and from the basal region from group 4 and so on; on the second sampling occasion, the soil core from group 1 was from the middle region and on the third occasion, was from the peripheral region, as shown in the following data:-

| Group No. | Occasion 1 | Occasion 2 | Occasion 3 | Occasion 4 |
|---|---|---|---|---|
| 1 | B | M | P | B |
| 2 | M | P | B | M |
| 3 | P | B | M | P |
| 4 | B | M | P | B |
| ↓ | | | | |
| 36 | P | B | M | P |

B = base; M = middle; P = periphery.

Thus the sample consisted of 36 units of 2-inch diameter and 12-inch long soil cores, 12 from each stratum. The 36 soil cores are equivalent to an area of 113.1 $in.^2$

## 7. ANALYSIS OF SAMPLING DATA

### 7.1. Survival and Mortality of Adults.

The trend in adult numbers illustrated in Fig. 11 shows that they reach the highest peak in autumn, but suffer appreciable mortality in winter during the hibernation period. The difference between the peak number of adults in autumn and the peak number in spring provides the number of winter deaths. The spring generation can be considered to be the more important one, since oviposition occurs during that period of life. The mortality of spring adults is low at the beginning, but increases rapidly with age. The trend of this differential mortality is more apparent when the logarithm of the total adult numbers (from Fig. 11) is plotted against time (i.e. the age of the beetles). This relationship is nonlinear, but, of the theoretical equations, the second degree polynomial,

$$Y = a + bX + cX^2,$$

was found to fit the data satisfactorily.

The survivorship curves or the lx curves obtained in this way for the 2 years are shown separately for the males and females in Figs. 14 and 15 respectively. To facilitate comparison, the age of beetles for both years have been calibrated by taking the 28th of February as the first day. The equations for these curves are as follows:

1963 ♀: $Y = 3.4342 + 0.003306\ X - 0.00005420\ X^2$

1964 ♀: $Y = 3.6312 + 0.003440\ X - 0.00008521\ X^2$

1963 ♂: $Y = 3.6050 + 0.003595\ X - 0.00006135\ X^2$

1964 ♂: $Y = 3.7343 + 0.004400\ X - 0.00009244\ X^2,$

FIG. 15. SURVIVORSHIP CURVES (FEMALE).

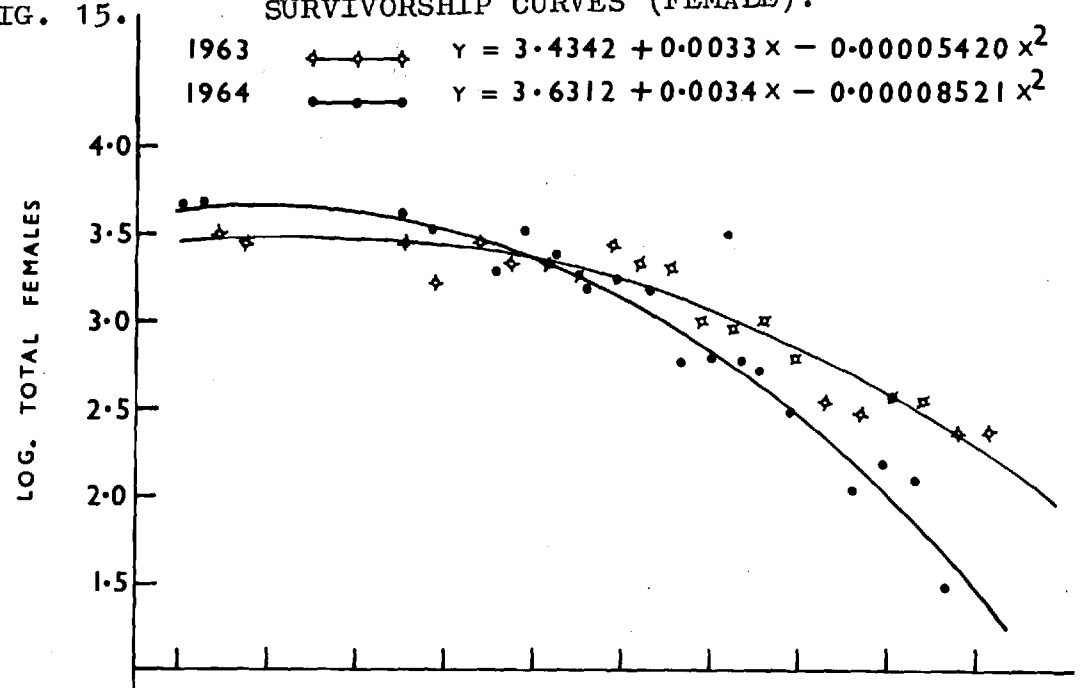
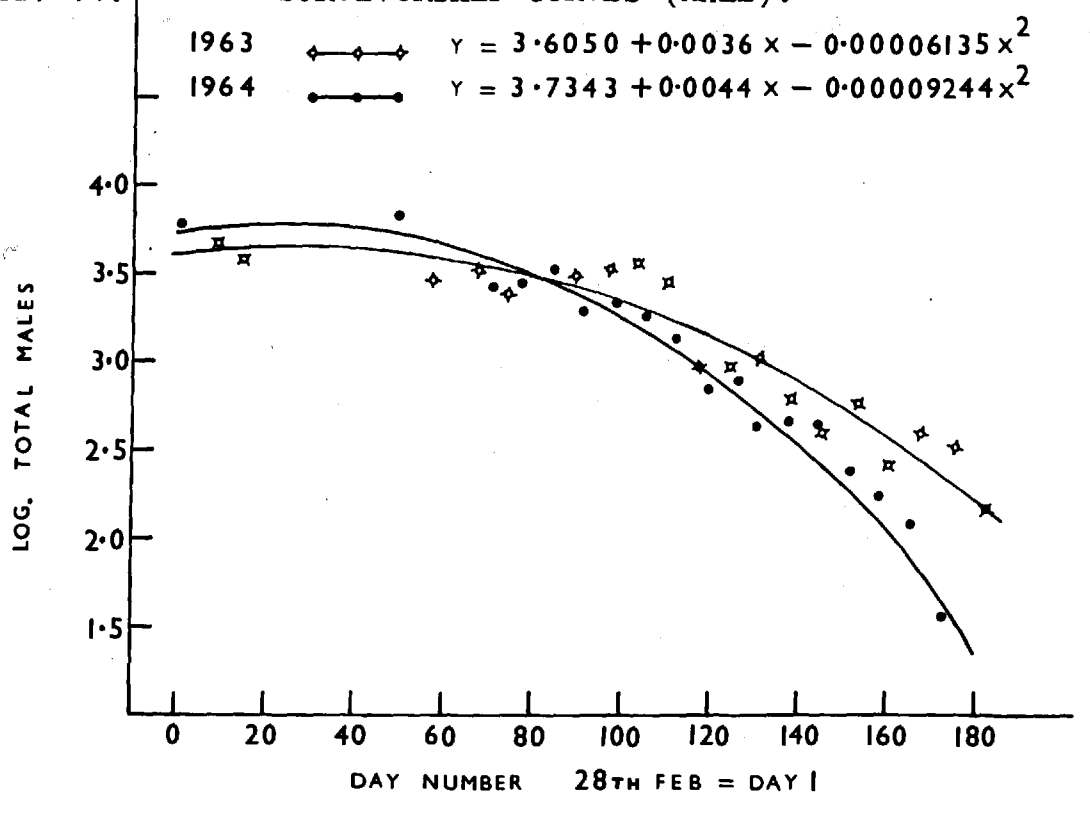



FIG. 14. SURVIVORSHIP CURVES (MALE).



where Y is the logarithm of the adult population on day X and X is the day number. Variance ratio calculations show that the curvilinearity of these regressions are significant at the 1% level. Figs. 14 and 15 as well as the four equations show that the survival of adults was greater in 1963 than in 1964. Since the values of the first coefficients (coefficients of X) in the four equations are very similar, it is of interest to note that it is the second coefficients (coefficients of $X^2$) which differ markedly in the two years. These negative coefficients may be then considered to give some idea of the death rate. The reason for treating the sexes separately and also for this endeavour to show different survival rates for the two years will be made clear in the discussion on fecundity, in the next section. It should be noted that in fitting equations to the data, the values between the day 15 and day 50 (see Figs. 14 and 15) have been justifiably omitted, since during this period, sampling by beating did not give real estimates of the adult numbers, as a result of weather conditions.

## 7.2. Estimation of Natality and Mortality of Immature Stage.

Owing to the long oviposition period of Sitona, the egg and many of the larval stages occur simultaneously. The analysis of data from a population of this type is difficult, since the numbers of any one stage are being reduced by moulting and death, and at the same time are being increased by oviposition, hatching or moulting. There are five known methods for the estimation of recruitment of individuals to the population and estimation of mortality in each stage, from such data. Three of these

have been described by Richards and Waloff (1954), Richards, Waloff and Spradbery (1960) and Dempster (1961). The latter publication also contains a short review of the former two methods. The other two methods (Dempster, unpublished; Southwood and Jepson, 1962) which are less accurate and have been considered to be crude are used only when the data is not suitable to be analysed more accurately. Of these, the method by Richards and Waloff was found to be by far the most suitable for the analysis of Sitona data. This method is based on the calculation of the slope of the fall in the population after the peak in numbers has been reached. By taking logarithms of the numbers, the fall-off is converted into an approximate straight line, and if this line is produced back to the beginning of the generation, the mortality and the initial number of individuals may be estimated. The method works well for populations in which there is a rapid build-up and a well-defined peak in numbers. This method was originally used in the study of grasshopper populations (Richards and Waloff, 1954) and has been tested by Dempster (1956) on a laboratory population of Locusta migratoria.

The theory of Richards and Waloff's method, which is also known as the regression method is as follows. If it is assumed that once egg laying, hatching or moulting is completed, there is an approximately steady mortality, the time trend of the population will fit the formula $Y = nk^x$, where Y is the population occurring on day x, n is the total number of eggs laid or larvae hatched (or moulted whatever the case may be), and k is the fraction surviving each day. The logarithm of Y (for the values after the peak) will follow a straight line since

$$\log Y = \log n + x \log k.$$

A linear regression equation can be determined for the logarithm of successive population estimates in conjunction with values of x, and the regression coefficient is the logarithm of the average fraction of the population surviving each day. From this regression coefficient, the size of the initial population (that occurring on day 0) can be estimated. These calculations are made for each stage and the mortality occurred in any one stage is estimated from the difference between the initial numbers of two successive stages. A calculation for the population less egg or first instar (i.e. for successive accumulated totals of the first or second instars depending on whether eggs or first instar larvae are treated as the first stage) gives an estimate of the total number of larvae which have entered the first or second instar. A corresponding total can be determined for each subsequent instar.

In the treatment of Sitona data, the value of x at the beginning of any stage was taken as that of the day preceding that on which the stage was first found. The estimates of the egg numbers as derived from the soil sampling data for the two years are shown in Figs. 16 and 17. The same data is presented in the logarithmic form in Figs. 18 and 19. These show that the fall-off after the peak can be considered to be approximately steady. The only obvious deviation is the day 87 in 1963 and the day 71 in 1964. The marked drop that occurred on these two days is more apparent in the untransformed data. The fact that this occurred on both years and also the relationship between the number of eggs in soil and the rate of oviposition (Figs. 20 and 21) show that the drop is real and not caused

FIGS. 16 & 17

FIG. 16: SOIL SAMPLING DATA FOR 1963

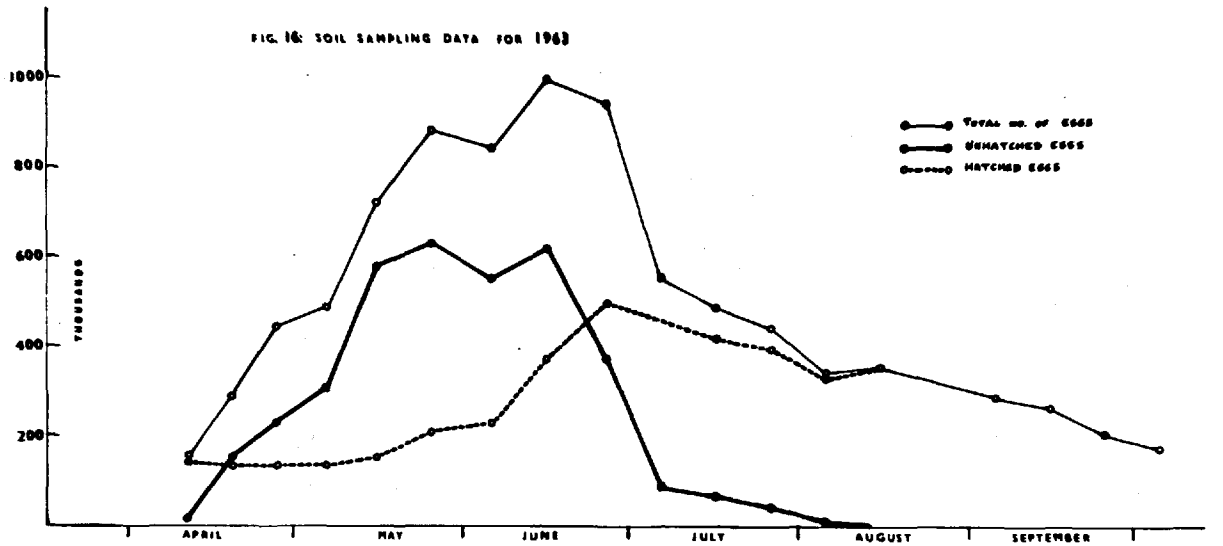



FIG.17: SOIL SAMPLING DATA FOR 1964

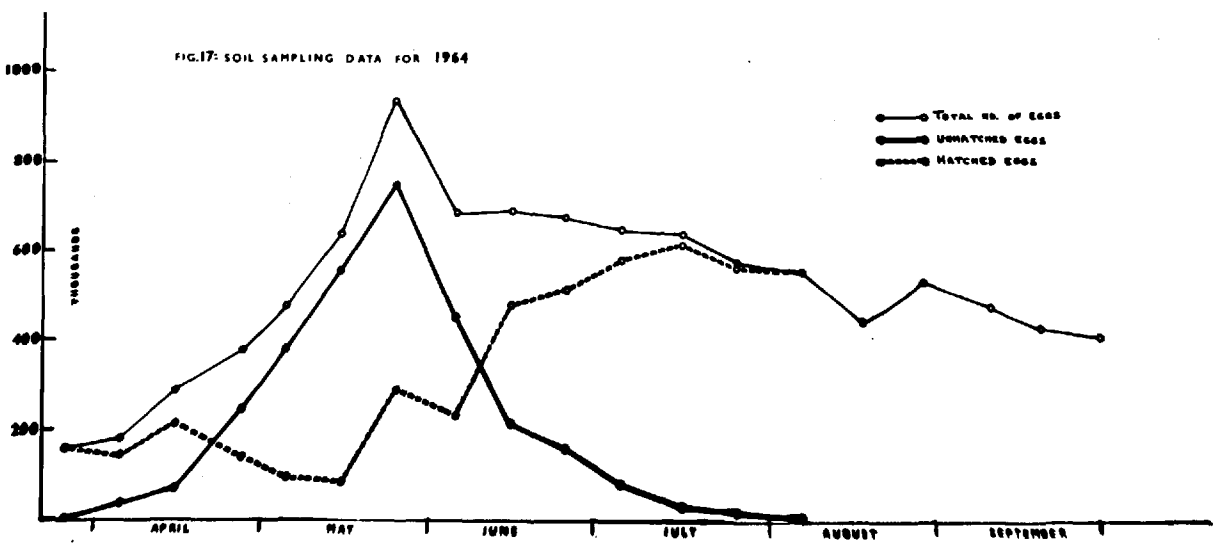


FIG. 18

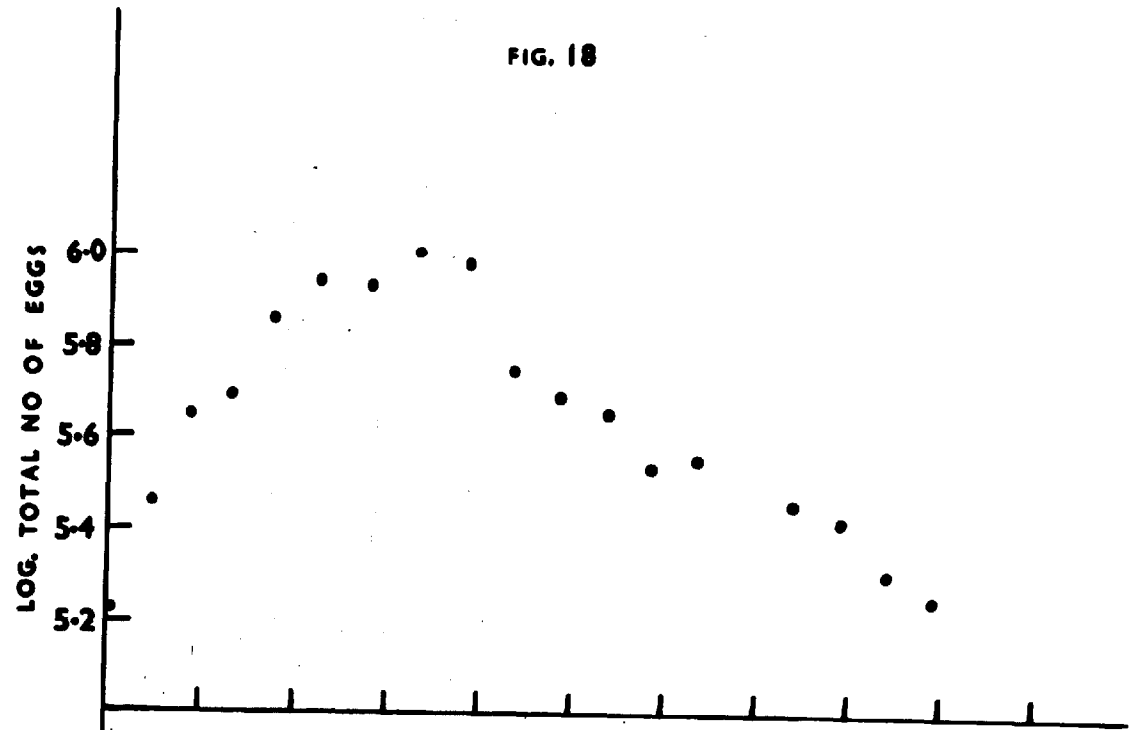

LOG. TOTAL NO OF EGGS
6·0
5·8
5·6
5·4
5·2

FIG. 19

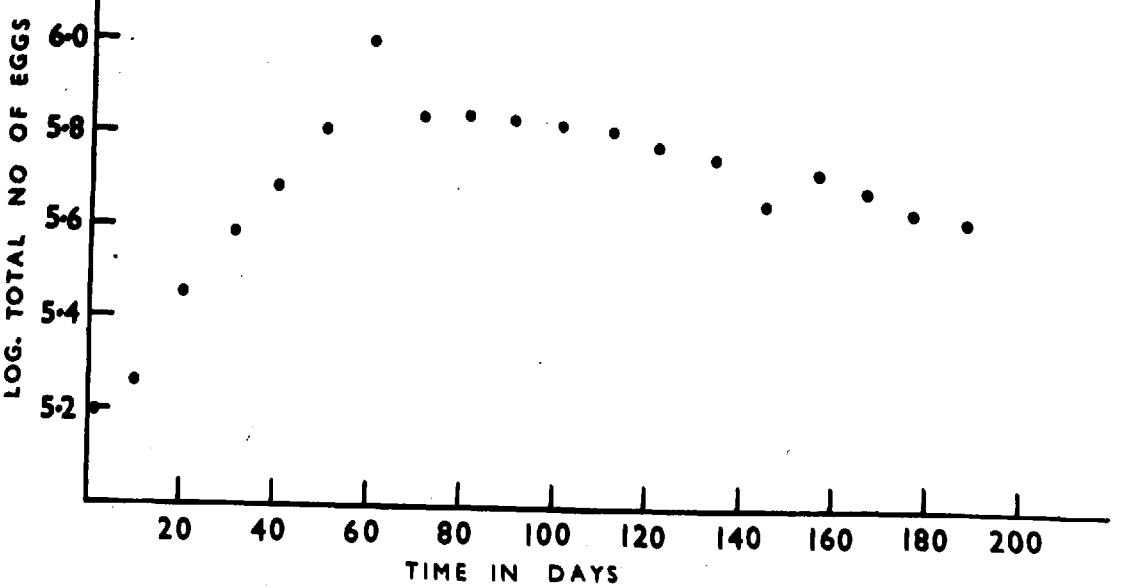

LOG. TOTAL NO OF EGGS
6·0
5·8
5·6
5·4
5·2
20
40
60
80
100
120
140
160
180
200
TIME IN DAYS

FIG. 20: RELATONSHIP BETWEEN THE NO. OF EGGS IN SOIL & OVIPOSITION RATE -1963

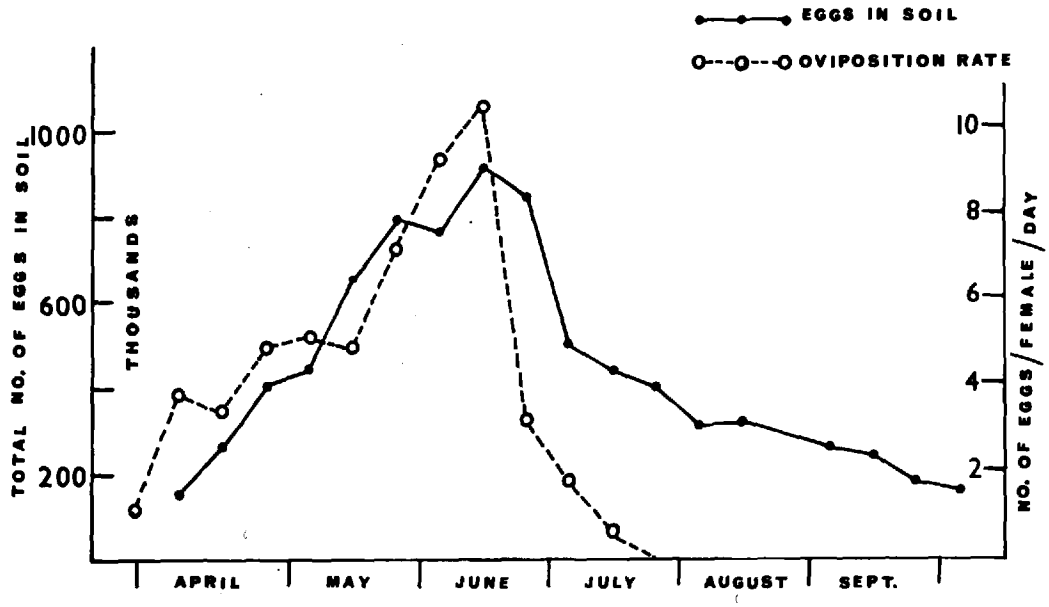



FIG. 21: RELATIONSHIP BETWEEN THE NO. OF EGG IN SOIL & THE OVIPOSITION RATE - 1964

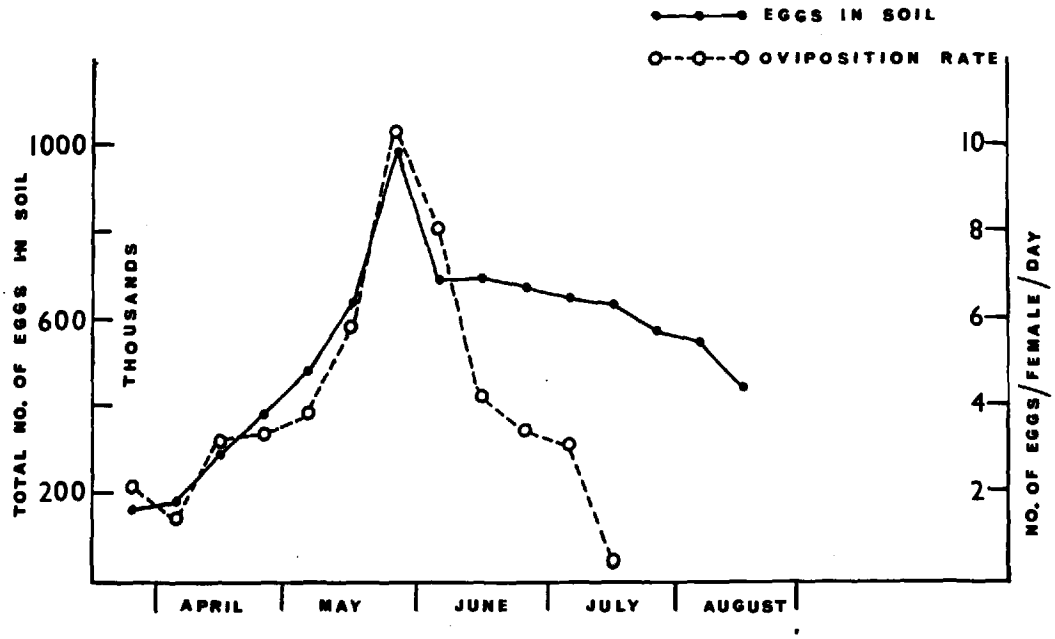


Fig. 22: Soil sampling data for 1963.

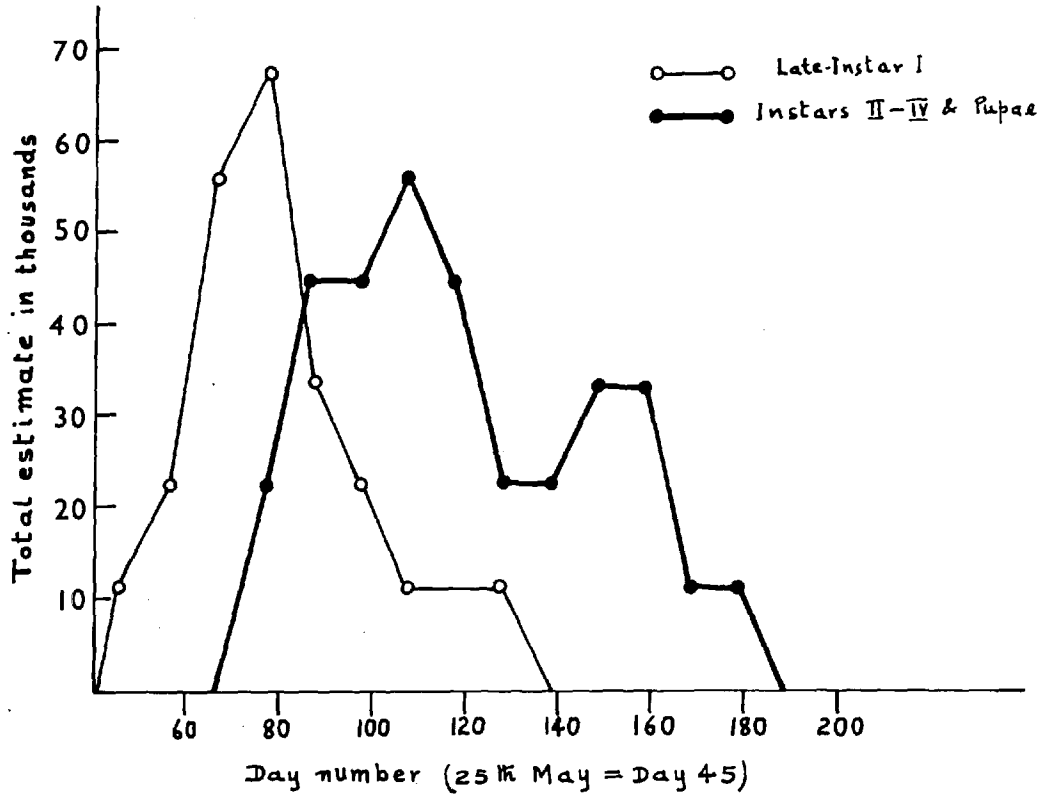
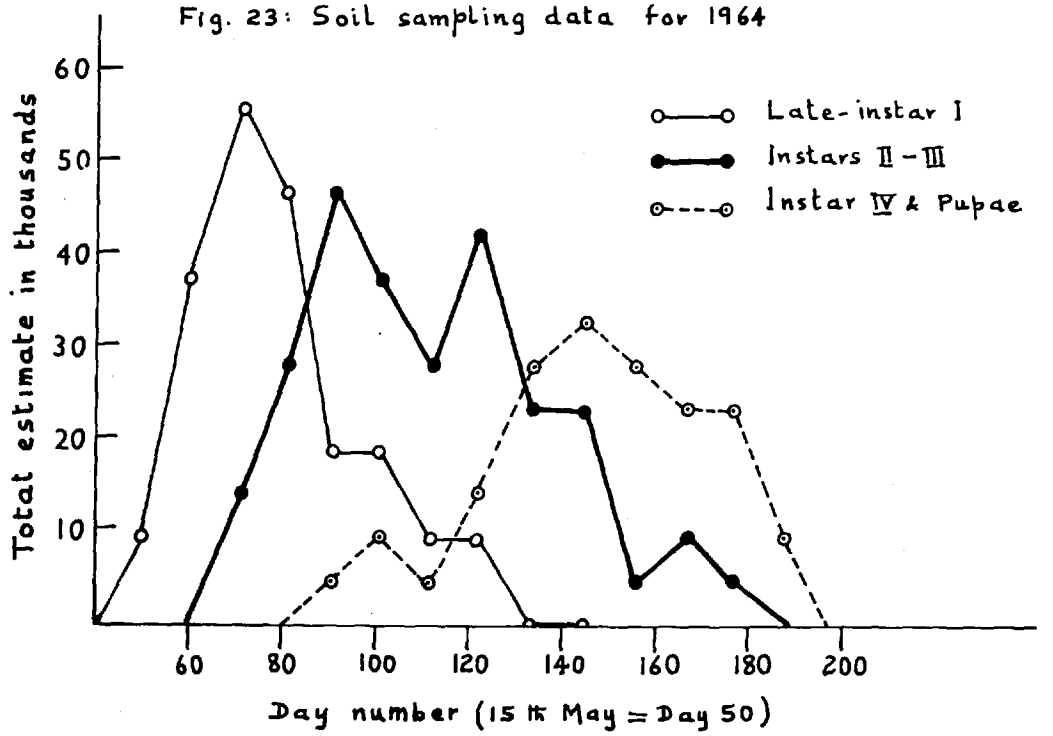



Fig. 23: Soil sampling data for 1964



by sampling errors. Figs. 20 and 21 also indicate that there was a fast disappearance of eggs soon after they were laid. The values for the rate of egg laying in the field have been obtained from the daily records from the method 4 in which caged females were used. This oviposition rate represents the number of eggs laid by an average female during the interval between two sampling days. The close association between the number of eggs in soil and the rate of oviposition also provides some evidence of reliability of the soil sampling and processing method.

A regression calculation based on the total egg numbers gives an estimate of the initial number of eggs laid. The numbers of hatched eggs found in soil also attain a peak and then fall off steadily (Figs. 16 and 17). This fall-off is caused by the gradual disintegration of the empty egg shells. A regression calculation based on the numbers of hatched eggs therefore provides an estimate of the initial number of hatched eggs which is also an estimate of the total number recruited into the first instar larvae. The first instar larvae found in soil (Figs. 22 and 23) should provide another estimate of the initial recruitment to this stage, but these were far below the numbers given by hatched eggs. This may be attributed to two causes: the small first instar larvae being lost during soil processing or they may suffer high mortality soon after hatching. It is unlikely that such large numbers of larvae were lost during soil processing as the method is almost full-proof if carried out with due care. The efficiency of both Salt and Hollick and centrifugal floatation methods is undisputable (see Raw, 1962; Muller, 1962); moreover, the extraction

technique was tested several times by introducing known numbers of eggs and larvae and the results showed over 80% recovery. These facts lead to the assumption of high initial mortality of first instar larvae immediately after hatching. On account of this, the larvae have been divided into two groups, the early-first instar derived from hatched eggs and the late-first instar derived from the numbers found in soil.

The numbers of second, third, fourth instar larvae and pupae found in the soil samples were low because of high mortality during the egg stage and the first instar. The time available did not permit handling of larger samples, although this was desirable. Another factor that had to be considered was the possible damage to the habitat; as parts of the roots are taken away with soil cores, extensive sampling from a small area over a long period could lead to death of bushes. This was found to be true for several bushes in the habitat. In the treatment of 1963 data, the second to fourth instar larvae and pupae had to be considered as one stage because the numbers of each stage were not consistent and big enough to be analysed separately. A regression calculation based on the accumulated totals of second to fourth instar larvae and pupae gave an estimate of the number that entered the second instar. The difference between this value and the initial number of the late-first instar larvae gives mortality during the latter stage. The mortality of second to fourth instar larvae and pupae is obtained from the difference between the initial number of second instar larvae and the estimate of the total number of adults that emerged, as given by the emergence trap data.

As sampling in 1964 was done with the 2-inch sampler, the sample size was larger than in 1963. Consequently larvae were found in bigger numbers and this enabled the analysis to be done with more detail; the second and third instar larvae have been treated as one unit and fourth instar larvae and pupae together as another. The results which are shown in Fig. 23 illustrates the estimates of numbers of the 3 stages found during the whole developmental period. Estimates of the numbers of various stages obtained for the two years are shown below (Tables 8 and 9).

Table 8. Estimated number of each stage of development in 1963.

| Stage | Estimated number |
|---|---|
| Egg | 2,250,500 |
| Early-instar I | 855,800 |
| Late-instar I | 104,810 |
| Instars II-IV & Pupae | 47,896 |
| Adult | 15,001 |

Table 9. Estimated number of each stage of development in 1964.

| Stage | Estimated number |
|---|---|
| Egg | 1,367,400 |
| Early-instar I | 746,970 |
| Late-instar I | 85,292 |
| Instars II & III | 49,613 |
| Instar IV & Pupae | 36,627 |
| Adult | 16,740 |

Some of the hatched eggs from one year remains in soil until the following year; as a result, when the first soil samples are taken each year, a number of hatched eggs are already present in soil before hatching really begins. To account for this, the number of hatched eggs present in soil on the sampling occasion just prior to the day when hatching is first indicated is subtracted from the total egg number given above. This eliminates the error involved so that a better estimate of the total egg number laid in that season is obtained. The numbers of hatched eggs already present in the two years were:-

1963 - 145,265

1964 - 158,338

therefore the estimate of the real number of eggs laid in the two seasons are:

1963 - 2,105,235

1964 - 1,209,062.

7.3. The Sex Ratio.

The best estimate of the sex ratio is that obtained from the autumn emergence trap data, which provides the sex ratio of the new generation, that is not biassed by any difference in the behaviour of males and females over the seasons. The sex ratios obtained by this means for the two years are as follows:

1963 Autumn: 55.4 ♂: 44.6 ♀

1964 Autumn: 52.5 ♂: 47.5 ♀

The numbers of males and females caught in the traps at 3-day intervals are

FIG. 24 NUMBERS CAUGHT IN EMERGENCE TRAPS IN AUTUMN 1963

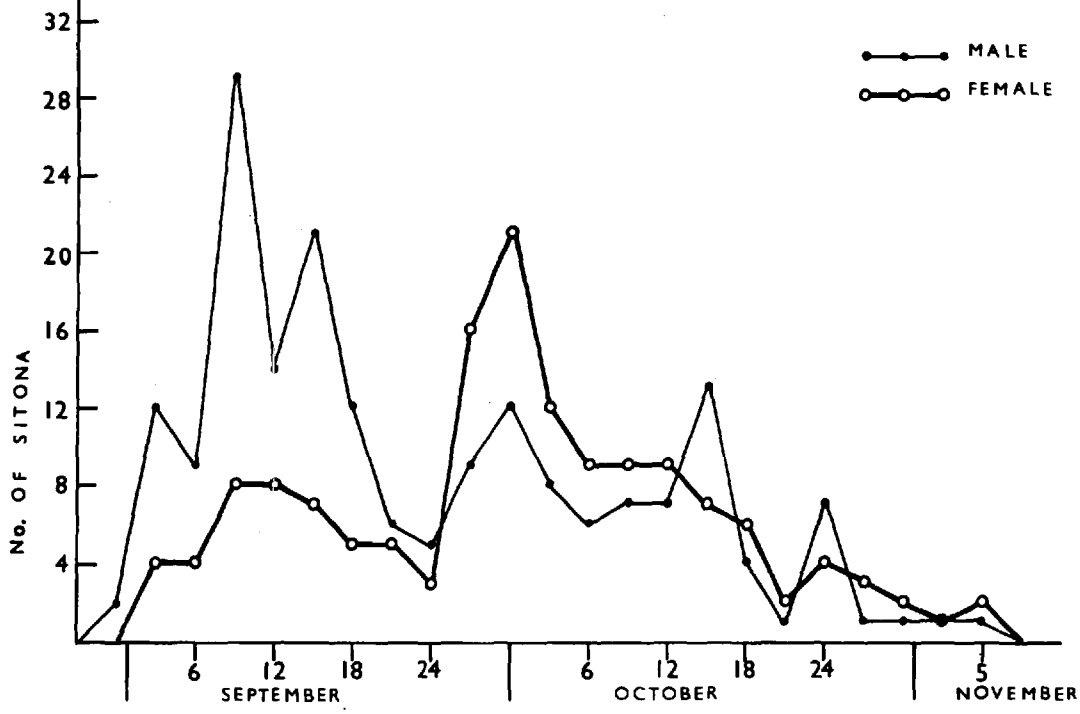



FIG. 25 NUMBERS CAUGHT IN EMERGENCE TRAPS IN AUTUMN 1964

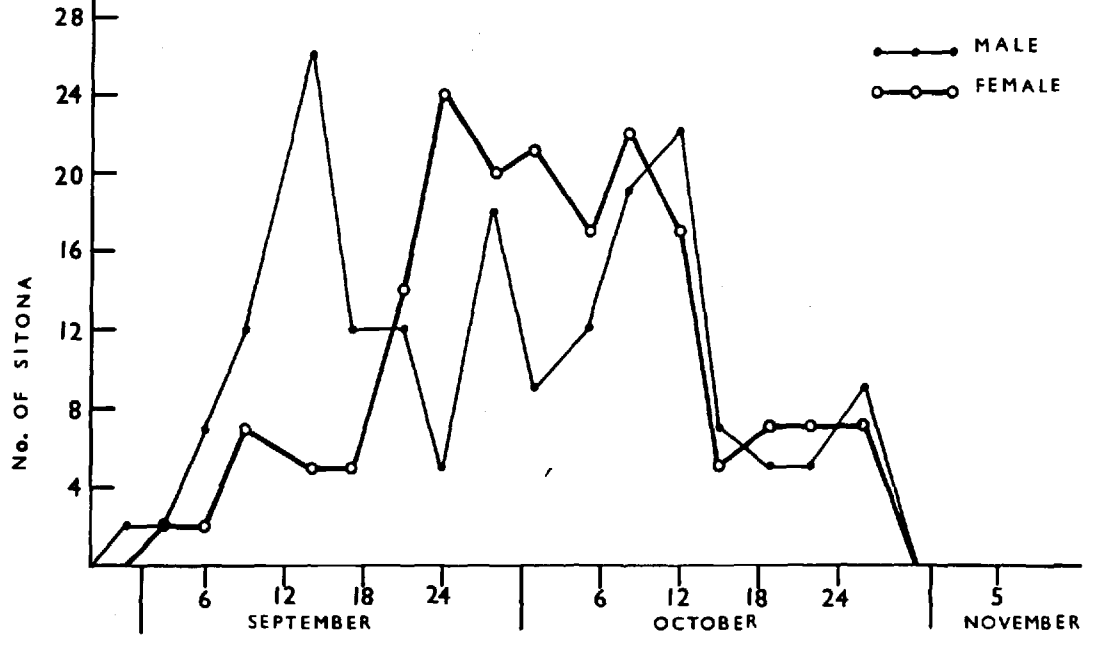
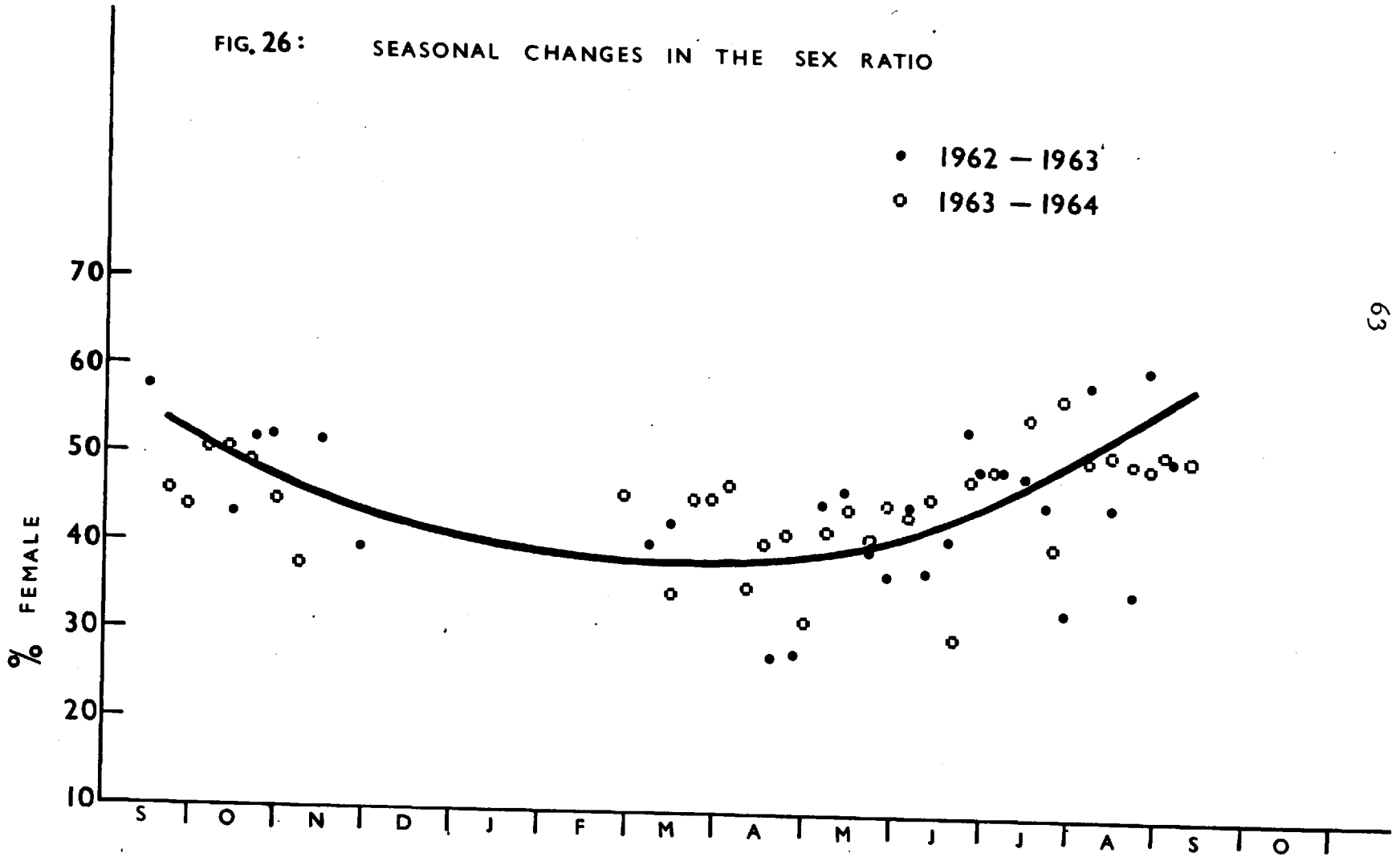


FIG. 26: SEASONAL CHANGES IN THE SEX RATIO



shown in Figs. 24 and 25. As is common to many insects, a good proportion of males emerge from pupation before the females. This indicates that the male and female larvae and pupae develop at different rates, and may be associated with the fact that the average female is bigger in size than the average male.

The seasonal changes in sex ratios (represented by percentages of females) obtained from routine sampling by beating is shown in Fig. 26. There is much fluctuation in the sex ratio, which may reflect sampling errors, but one may trace a path through the points which indicates changes in the sex ratio through seasons. It will be noticed that values for both years follow the same pattern, and a curve has been drawn in by eye to represent the nature of changes in the sex ratio with time. The gap from December to March is the hibernation period during which the beetles stay in the soil.

7.4. Fecundity.

Estimates of average fecundity have been obtained by three methods. The first estimate is derived from the soil sampling data, from which it is calculated as follows:-

$$\text{Fecundity} = \frac{\text{Estimate of the total number of eggs in soil}}{\text{Initial number of spring females}} \quad \text{eggs per female}$$

Thus for 1963, the fecundity was equal to $\frac{2,105,235}{3045}$ or 691 eggs per female, and the fecundity for 1964 was $\frac{1,209,062}{4620}$ or 262 eggs per female. The initial number of spring females was obtained from the survivorship curve

Table 10. Weekly egg laying in the field in 1963.

| Date | Female Population in Field | Rate of Egg Laying. No. of eggs per week/♀. | Total Number of Eggs Laid | % Sterility | Total No. of Sterile Eggs |
|---|---|---|---|---|---|
| 23.3.63 | 3045 | 12.4 | 37758 | 16 | 6041 |
| 30.3.63 | 3042 | 23.8 | 72399 | 19 | 13756 |
| 5.4.63 | 3002 | 43.0 | 129086 | 15 | 19363 |
| 12.4.63 | 2927 | 30.5 | 89274 | 12 | 10713 |
| 19.4.63 | 2819 | 60.3 | 169986 | 17 | 28897 |
| 26.4.63 | 2682 | 57.1 | 153142 | 8 | 12251 |
| 3.5.63 | 2521 | 45.5 | 114706 | 9 | 10323 |
| 10.5.63 | 2341 | 50.1 | 117284 | 12 | 14074 |
| 17.5.63 | 2146 | 65.1 | 139705 | 9 | 12573 |
| 24.5.63 | 1945 | 96.4 | 187498 | 9 | 16875 |
| 31.5.63 | 1740 | 94.4 | 164256 | 12 | 19711 |
| 7.6.63 | 1538 | 116.1 | 178562 | 31 | 55354 |
| 14.6.63 | 1344 | 37.1 | 49862 | 32 | 15956 |
| 21.6.63 | 1160 | 23.7 | 27492 | 49 | 13471 |
| 28.6.63 | 988 | 15.5 | 15314 | 46 | 7044 |
| 5.7.63 | 832 | 3.0 | 2496 | 56 | 1398 |
| | | Total | 1643820 | | 257800 |

Table 11. Weekly egg laying in the field in 1964.

| Date | Female Population in Field | Rate of Egg Laying. No. of eggs per week/♀. | Total Number of Eggs Laid | % Sterility | Total No. of Sterile Eggs |
|---|---|---|---|---|---|
| 13.3.64 | 4620 | 7.1 | 32802 | 28 | 9184 |
| 20.3.64 | 4597 | 18.5 | 85044 | 9 | 7654 |
| 27.3.64 | 4467 | 7.5 | 33503 | 10 | 3350 |
| 4.4.64 | 4296 | 21.0 | 90216 | 13 | 11728 |
| 11.4.64 | 4036 | 31.7 | 127941 | 7 | 8956 |
| 18.4.64 | 3718 | 21.2 | 78822 | 6 | 4729 |
| 25.4.64 | 3360 | 31.4 | 105504 | 5 | 5275 |
| 2.5.64 | 2980 | 45.9 | 136782 | 6 | 8206 |
| 9.5.64 | 2591 | 46.9 | 121518 | 6 | 7291 |
| 16.5.64 | 2211 | 49.3 | 109002 | 4 | 4360 |
| 23.5.64 | 1850 | 124.5 | 230325 | 6 | 13819 |
| 30.5.64 | 1519 | 36.1 | 54836 | 5 | 2742 |
| 6.6.64 | 1223 | 79.6 | 97351 | 5 | 4867 |
| 13.6.64 | 967 | 33.5 | 32394 | 22 | 7127 |
| 20.6.64 | 750 | 27.6 | 20700 | 32 | 6624 |
| 27.6.64 | 569 | 21.4 | 12177 | 48 | 5845 |
| 4.7.64 | 425 | 1.2 | 510 | 53 | 270 |
| | | Total | 1369427 | | 112027 |

The second estimate of the fecundity was derived from the daily egg laying records in the field (see Method 4, page 42) in which caged females were used. The daily number of eggs per female is converted to a weekly figure by adding up the totals for the seven successive days. This multiplied by the mean number of females in the field throughout that week (obtained from the survivorship curve) gave the total number of eggs laid by the field population during the oviposition period (Tables 10 and 11). The sum of weekly totals provides an estimate of the total number of eggs laid through the season and the fecundity can be then estimated as follows:-

$$\text{Fecundity} = \frac{\text{Estimate of the total number of eggs (method 4)}}{\text{Initial number of spring females}}$$

Thus the fecundity for 1963 = $\frac{1648820}{3045}$ or 542 eggs per female, and the fecundity for 1964 = $\frac{1369427}{4620}$ or 296 eggs per female. The third estimate of fecundity was derived from the egg laying data of a known number of laboratory females (26 in 1963 and 33 in 1964). The beetles were kept in pairs (a male and a female) in separate cages made from plastic petri dishes. Food and water were provided regularly. In contrast to method 4, the beetles were not changed, so that the information is based only on one set of individuals. A separate account of the laboratory observations on oviposition is given later (page 153). From the egg laying records of these beetles, the average laboratory fecundity was obtained as follows:

$$\text{Laboratory fecundity} = \frac{\text{Total number of eggs laid}}{\text{Number of laboratory females}} \quad \pm \text{ 95\% fiducial limits}$$

Thus the laboratory fecundity for 1963 was equal to $\frac{11373}{26}$ or 437 $\pm$ 325 eggs per female, and that for 1964 was $\frac{6526}{33}$ or 197 $\pm$ 46 eggs per female. The results from the three methods are presented in Table 12 for comparison.

Table 12. Comparison of egg laying data for 1963 and 1964.

| Method | Initial No. of Spring ♀♀ | | Estimate of the total no. of eggs laid during the season | | Fecundity per ♀ | |
|---|---|---|---|---|---|---|
| | 1963 | 1964 | 1963 | 1964 | 1963 | 1964 |
| Soil Sampling. | 3045 | 4620 | 2,105,235 | 1,209,062 | 691 | 262 |
| Field egg laying (Method 4) | do. | do. | 1,648,820 | 1,369,427 | 542 | 296 |
| Laboratory egg laying. | do. | do. | 1,330,665 | 910,140 | 437 | 197 |

The estimates of the total egg number and the average fecundity obtained by the three methods tend to support one another, especially in 1964. Compared to the other two methods, soil sampling appears to give a slight overestimate in 1963. As the laboratory estimates are based on the same set of individuals, kept in a C.T. room at 25°C., the values given by the two field methods can be considered to be more reliable. Table 12 shows that the outstanding difference is not between the methods, but between the two years. All the three estimates point to the fact that fecundity was halved in 1964 as compared with that in 1963.

This difference in fecundity can be studied a step further by considering the factors that influence the rate of oviposition, as it is

this that determines the ultimate fecundity. Generally, in insects, temperature has a marked effect on oviposition, but it is complicated by the effects of the age of the females; in spite of the rise in temperature, the numbers of eggs per female rise to a peak and then decline with time (Fig. 27). From the field data, regression equations relating the number of eggs laid in 24 hours (y) with the temperature during the period in °C. ($X_1$) and the age of the females in days ($X_2$), where $X_2 = 1$ on the first day of oviposition, have been calculated for both years. These equations take the following form:

1963 $y = 1.9741\ X_1 - 0.1707\ X_2 - 5.4719$

1964 $y = 0.9645\ X_1 - 0.0694\ X_2 - 1.5234$

| | Significance | | Standard Errors | |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_1$ | $X_2$ |
| 1963 | $P < 0.001$ | $P < 0.001$ | $\pm$ 0.1638 | $\pm$ 0.00098 |
| 1964 | $P < 0.001$ | $P < 0.01$ | $\pm$ 0.1628 | $\pm$ 0.02086 |

Since the difference between the regression coefficients of $X_1$ for the two years exceed twice the sum of their standard errors, they differ significantly at 5% level. In the same way the coefficient of $X_2$ in 1963 differs from that of 1964 significantly at 5% level.

By substituting the values for days and for temperatures in the equations, the probable oviposition of the females can be calculated. This is given in Table 13. The regression equations as well as Table 13 derived from them show that the influence of both temperature and age were markedly

Table 13. Probable oviposition for 1963 and 1964 calculated from the regression equations.

| Day | 10°C. | | 15°C. | | 20°C. | |
|---|---|---|---|---|---|---|
| | 1963 | 1964 | 1963 | 1964 | 1963 | 1964 |
| 1 | 14.1 | 8.1 | 24.0 | 12.9 | 33.8 | 17.7 |
| 10 | 12.5 | 7.4 | 22.4 | 12.2 | 32.3 | 17.0 |
| 20 | 10.8 | 6.7 | 20.7 | 11.5 | 30.6 | 16.3 |
| 30 | 9.1 | 6.0 | 18.7 | 10.8 | 28.6 | 15.6 |

different in the two years. Hence it seems likely that these two factors contributed to the differences in fecundity in 1963 and 1964 to some extent.

How much the age and temperature contributed towards the total variation in the rate of oviposition can also be obtained from the regression analysis. The amount of variability brought about by these two factors is expressed as the percentage of the total variation in Table 14. The reasons for the residual variation are not known because only the temperature and age were examined.

Table 14. Showing the amount of variation brought about by age and temperature.

| Year | 1963 | 1964 |
|---|---|---|
| Temperature | 19.4% | 21.4% |
| Age | 33.8% | 5.9% |
| Unknown causes | 46.8% | 72.7% |

From these figures it appears that a greater part of the variation in the rate of oviposition had resulted from unknown causes in 1963.

Among the factors other than temperature and age that may have influenced the fecundity of Sitona, one may include variation in size (weight) of individuals and nutrition. Waloff and Richards (1958) and Donia (1958) have shown that in Phytodecta olivacea which feeds on the leaves, twigs and flowers of broom, the fecundity was higher when the beetles were feeding on the young broom shoots than when they were given older shoots or flowers. Experiments on similar lines were not carried out on Sitona, but they may provide interesting results. Donia also showed that a relationship exists between the weight of the mature Phytodecta and the total number of eggs laid. In Sitona, the weights of 33 laboratory females (1964) did not show a significant correlation with fecundity ($r = 0.2347$; $P > 10\%$).

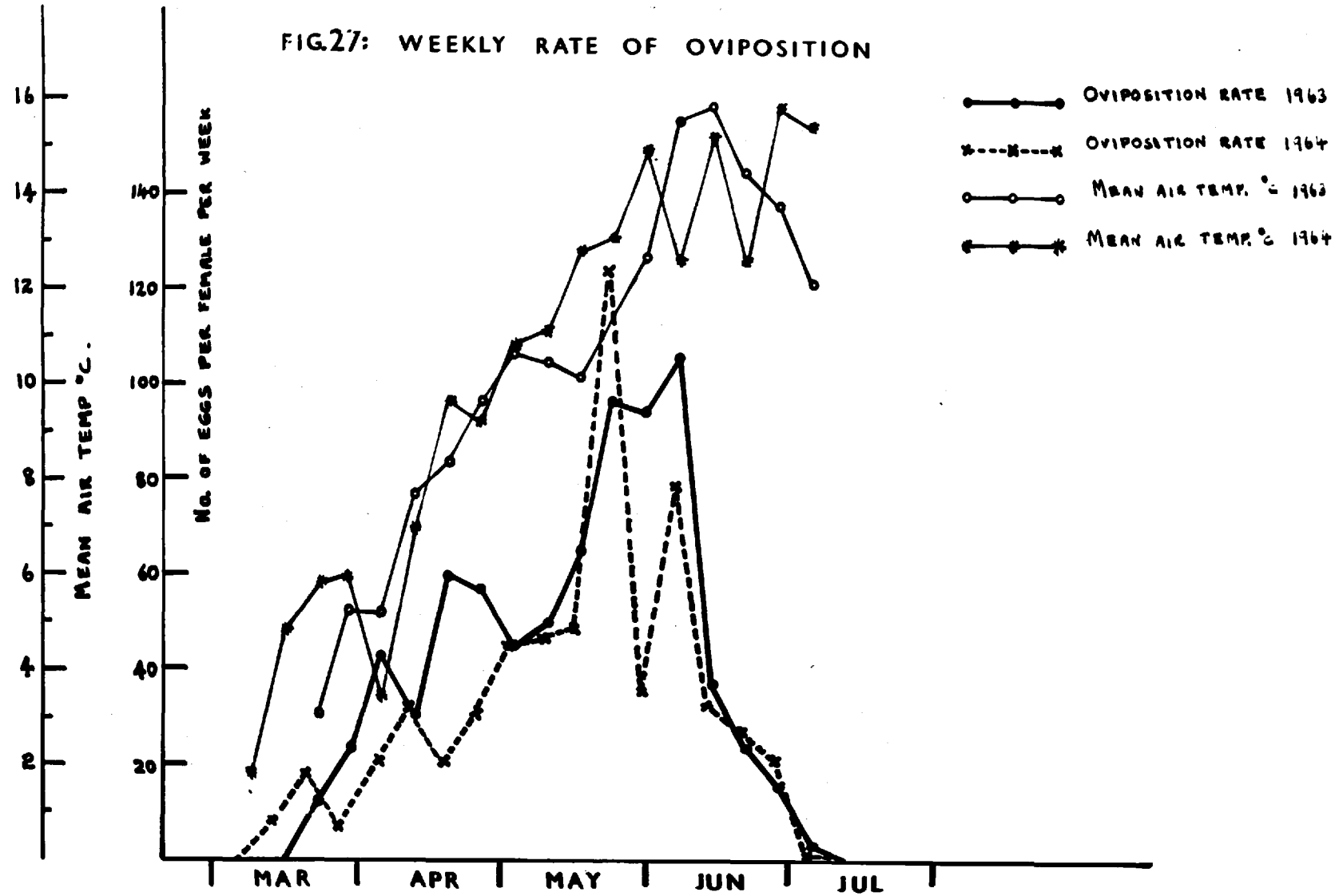
One other factor which may have influenced the fecundity of Sitona is diapause which is broken by exposure to low temperature. It will be shown later that the length of cold treatment influences the length of the pre-oviposition period. In this connection it is interesting to note that the winter of 1962 - 1963 was much more severe than that of 1963 - 1964. Some idea of the conditions in the two winters can be obtained from the following table:

Number of days with the mean temperature 2°C. or below.

| | November | December | January | February | March | Total |
|---|---|---|---|---|---|---|
| 1962-63 | 6 | 19 | 31 | 28 | 7 | 91 |
| 1963-64 | 2 | 19 | 11 | 9 | 8 | 49 |

Finally, the weekly oviposition rates for 1963 and 1964 are compared in Fig. 27. The peak oviposition periods of the two years appear to be about two weeks apart, so that the majority of the eggs were laid earlier in 1964. It is also apparent that the peak period of oviposition was distinctly shorter in 1964 than in 1963, showing that the high rate of egg laying was maintained for a longer period in 1963. Although temperature and aging have been shown to have caused differences in fecundities in the two years to some extent, Table 14 shows that unknown factors had played a more important part, especially in 1964. It is likely that these unknown causes were either nutrition (state of the food plant) or the intensity of diapause or both. The nutritional factor could also explain the lower rate of survival shown earlier, for 1964.

FIG.27: WEEKLY RATE OF OVIPOSITION



## 8. MORTALITY FACTORS

### 8.1. Causes of mortality in the adult beetles.

#### 8.11. Parasitism.

A definite cause of mortality of adult Sitona is a parasitic wasp of the family Braconidae. This was identified by Professor O.W. Richards as Centistes lituratus Hal. (= Leiphron lituratus Hal.). Some specimens were sent to Dr. C.C. Loan of the Canada Department of Agriculture, Belleville, Ontario to be compared with those that have been reported from Sitona scissifrons Say. (Loan, 1963, 1964) and were found to be identical. Dr. W.R.M. Mason of the Entomology Research Institute, Ontario has just completed a world revision of the genus Centistes and according to this, C. lituratus is now called C. excrucians Haliday. Centistes excrucians is a parthenogenetic, thelytokous species with a holarctic distribution. It is an internal parasite in its larval stages on adults of Sitona. There are two generations of the parasite a year, a first generation that emerges in May-June and a second generation that emerges in September-October. The parasites of the first generation lay their eggs in the declining spring generation of adult Sitona, the highest parasitism occurring after the beetles had laid most of their eggs. These parasite eggs develop during the summer to give rise to the second generation of the wasp which lays its eggs in the newly emerging second generation adults of Sitona. The resulting parasite larvae overwinter in the first instar stage within the hibernating hosts, but continue to develop and emerge earlier than in field if warm conditions are provided in

the laboratory. Thus Centistes can be said to be bivoltine with a facultative diapause in the first instar stage in winter. It should be noted that in contrast, the host is univoltine and has an obligatory diapause.

The second generation of the parasite is well synchronised with the newly emerging autumn adult beetles, but since the first generation parasites depend on a declining old population of Sitona, the wasp numbers are not kept high. Moreover, it appears that many of the parasitc larvae of the first generation die in summer prematurely when their hosts die. For these reasons, it cannot be said that the parasite is a successful one as far as its influence on the beetle numbers are concerned. The parasitised host dies when the parasite larva in its fourth instar emerges through an opening close to the anal region of the beetle. The larva descends into the soil where it spins a cocoon before pupation. At this stage only the numbers of Sitona killed by the parasite are considered, as a fuller account of observations on the parasite are given later (see page 171).

The degree of parasitism was determined by weekly dissections of 30 males and 30 females collected during routine sampling. On dissection the parasite larvae are found within the abdomen or thorax, but because of the minute size of the eggs of Centistes, parasitism cannot be detected accurately during its egg stage. Tables 15 - 17 summarise the results of dissections carried out during the period of study. These results are based only on dissections made during the period from the first instar stage of the parasite to the third instar stage so as to avoid any error caused by undetected eggs or by emerged mature larvae. Though a greater number of

Table 15. Parasitism of Sitona adults by Centistes excrucians based on dissections of weekly samples, for the period 1962 - 1963.

| 1 | Generation | First Generation | | Second Generation |
|---|---|---|---|---|
| 2 | Period. (% parasitism is based on dissections done during this period) | Autumn, 1962 13th Nov. - 18th Dec. | Spring, 1963 8th March - 13th May | Summer, 1963 15th July - 22nd August |
| 3 | No. of beetles in the field | 13,837 | 6,799 | 4,062 |
| 4 | No. dissected | 240 | 420 | 360 |
| 5 | % Parasitism | 5.9 | 9.3 | 51.5 |
| 6 | Calculated no. of parasitised hosts | 927 | 632 | 2,092 |
| 7 | Estimated no. of Sitona killed by parasite | | 2,724 | |

Table 16. Parasitism of Sitona adults by Centistes excrucians based on dissections of weekly samples, for the period 1963 - 1964.

| 1 | Generation | First Generation | | Second Generation |
|---|---|---|---|---|
| 2 | Period. (% parasitism is based on dissections done during this period) | Autumn, 1963 1st Nov. - 14th Dec. | Spring, 1964 3rd Jan. - 17th April | Summer, 1964 24th July - 14th August |
| 3 | No. of beetles in the field | 16,414 | 9,785 | 1,980 |
| 4 | No. dissected | 360 | 960 | 180 |
| 5 | % Parasitism | 8.3 | 11.6 | 49.6 |
| 6 | Calculated no. of parasitised hosts | 1,362 | 1,135 | 982 |
| 7 | Estimated no. of Sitona killed by parasite | | 2,117 | |

Table 17. Parasitism of Sitona adults by Centistes excrucians based on dissections of weekly samples, for the period 1964 - 1965.

| 1 | Generation | First Generation | |
|---|---|---|---|
| 2 | Period. (% parasitism is based on dissections done during this period) | Autumn, 1964 23rd Oct. - 13th Nov. | Spring, 1965 8th Jan. - 9th April |
| 3 | No. of beetles in the field | 17,177 | 10,305 |
| 4 | No. dissected | 240 | 660 |
| 5 | % Parasitism | 3.4 | 4.2 |
| 6 | Calculated no. of parasitised hosts | 584 | 433 |

parasitised hosts are shown by the autumn figures, some of them die during the winter and consequently the number of Sitona actually killed by the parasite is the sum of the number of spring beetles parasitised by the first generation of Centistes and the number of summer beetles parasitised by the second generation of Centistes.

Tables 15 - 17 also show that 632 first generation parasites of 1963 had 4062 hosts available to them in summer and of these, 2092 were parasitised whereas 1135 first generation parasites of 1964 had only 1980 hosts available to them and consequently the number of parasitised hosts was reduced to 982. This in turn brought about the low level of parasitism in the autumn of 1964. It therefore appears that the parasite density depends on the number of hosts available to the second generation of the parasite, that is, the maintenance of parasite numbers depends on the survival of Sitona in summer. The fact that the high rate of parasitism by the second generation of the parasite cuts down the number of old beetles that can live and lay eggs for a second season by about 50% indicates a regulatory effect on the population. However, this regulatory effect did not influence the size of Sitona populations during the study period as the numbers of Sitona that survived for a second season were small.

8.12. Fungal disease.

A second cause of adult death is a fungal disease caused by Beauveria bassiana which is one of the hyphomycetous Fungi Imperfecti (Deuteromycetes) not usually associated in nature with a perfect stage.

Hyphomycetes are mycelial fungi reproducing by means of conidia which are generally produced on free or aggregated conidiophores on the surface of their substratum. The fungus from specimens of dead Sitona regensteinensis was identified by Dr. M.F. Madelin of the Department of Botany, University of Bristol as Beauveria bassiana which is the causal agent of the famous muscardine disease of the silk worm, Bombyx mori and which also parasitizes numerous other insects. Sussman (1952) lists 63 species of insects parasitized by the fungus and MacLeod (1954) has made a thorough study of the genus Beauveria. Among other members of the Sitona beetles, Beauveria has been reported on S. lineata (Jackson, 1920; Hans, 1959), S. hispidula (Jackson, 1922) and on S. cylindricollis (Bird, 1947). In Silwood Park, the fungus has been shown to be present in populations of the broom beetle Phytodecta olivacea (Richards and Waloff, 1961). Literature on Beauveria is reviewed in books on insect pathology by Steinhaus (1947, 1949) and more recently by Madelin (1963).

Beauveria cannot be detected in Sitona during its early stages, but if the beetles are kept in the laboratory for a period of about two weeks or more under warm and humid conditions, the infected beetles become ensheathed by a soft white mycelium which later turns white and chalky. These white masses take the form of small blunt protuberances from the mouth, anus and at the joints of various parts of the body. Beauveria was not detected in 1962 and in the autumns of 1963 and 1964, the infections were 4.2% and 4.8% respectively. Madelin (1963) citing many examples from the literature, points out that a humid atmosphere usually in excess of 80%

relative humidity is essential for Beauveria infections which are also influenced by temperature. The infectivity of the fungus appears to be greater under warm conditions, for instance, against the larvae of Leptinotarsa decemlineata, B. bassiana declined in infectivity below 6°C. (Schaerffenberg, 1957). Müller-Kögler (1942) similarly found that B. bassiana failed to infect larvae of Bupalus piniarius (Linnaeus) at 0.5°C. whereas it succeeded, though slowly, at 8°C. Any infection detected in Sitona regensteinensis, therefore, is likely to have begun in autumn, probably during the teneral period of adults in soil.

Dr. Madelin also isolated another pathogenic fungus that killed a small percentage of the beetles in the autumn of 1963. This was identified as a species of Paecilomyces which has been also known as Spicaria. The number of Sitona infected by this fungus was very low and appeared to be less than 1%. Paecilomyces is considered to be one of the widely encountered genera in nature pathogenic to insects and belongs to the same group of fungi as Beauveria (Madelin, 1963). In Silwood Park, Paecilomyces has been reported from a laboratory culture of the Lygaeid bug Stygnocoris fuligineus (Eyles, 1962) and also from eggs of Conomelus anceps (Homoptera, Delphacidae) collected in the field (Rothschild, 1962).

8.13. Winter disappearance.

Autumn and spring population estimates of adult beetles show that the peak levels reached in autumn are not maintained during the winter (Fig. 11). The numbers of beetles before and after the winter for three

seasons are as follows:

Table 18.

| | 1962 | 1963 | 1964 |
|---|---|---|---|
| No. of Sitona in autumn | 13,837 | 16,414 | 17,177 |
| No. of Sitona in spring of the following year | 6,799 | 9,785 | 10,305 |
| No. that died of Beauveria | - | 689 | 825 |
| Winter disappearances | 7,038 | 5,940 | 6,047 |

In the previous sections, the amount of mortality brought about by parasitism and fungal disease has been established, but it is not easy to show precisely what caused the disappearance of so many individuals in winter. However, a plausible explanation could be arrived at by the process of elimination of various possible mortality factors. A factor which has not yet been discussed is the weather, which could be important in this respect because the disappearance occurs during the hibernation period in winter. Any mortality brought about by weather in winter must depend on two factors, the coldhardness of Sitona and the duration and extent of low temperatures. Investigations on the coldhardness of Sitona were outside the scope of this work, as the amount of work involved in such a study would make a problem on its own. Nevertheless, fortunately there is some information available from work that is being carried out at present on coldhardness of the beetle by Miss J. Smith at Silwood Park and she has kindly permitted me to use some of her findings here. She measured the 'cold-death

point' (the temperature below which exposure is lethal to the insect) or the undercooling point of beetles brought from the field at different times of the year, using an apparatus which incorporates a thermoelectrical device known as 'Frigister' (Luff, 1964). The undercooling points (mean of 20 replicates) determined in this way during the 1964 - 1965 winter are shown in Table 19.

Table 19. Showing the undercooling points from October 1964 to April 1965. (Results obtained by Miss J. Smith).

| Date | Undercooling Point in Degrees Centigrade | | |
|---|---|---|---|
| | Mean of 20 Readings | Range | |
| | | Lowest | Highest |
| 23.10.64 | - 3.3 | - 10.1 | - 0.1 |
| 20.11.64 | - 3.2 | - 10.2 | - 0.9 |
| 4.12.64 | - 5.9 | - 9.0 | - 1.0 |
| 18.12.64 | - 3.8 | - 6.7 | - 1.1 |
| 1. 1.65 | - 2.5 | - 5.9 | - 0.6 |
| 18. 1.65 | - 4.8 | - 9.4 | - 0.3 |
| 1. 2.65 | - 3.3 | - 9.0 | - 0.2 |
| 19. 2.65 | - 4.8 | - 9.3 | - 0.8 |
| 12. 3.65 | - 6.8 | - 10.8 | - 1.6 |
| 30. 3.65 | - 2.1 | - 5.8 | - 0.2 |

These results indicate that if the winter temperatures fall below this range (-0.1 to -10.8°C.), then death occurs. As hibernation takes place in litter under the broom, I recorded the litter temperature during winter with a mercury-in-steel electrothermograph. The electrothermograph was placed about 2 inches below the ground surface under a broom plant in the study area. These records show that the litter temperatures did not fall below zero at any stage during the winter. This was so even during the very severe 1962 - 1963 winter when the lowest temperature recorded was 0.5°C. Thus it seems that litter temperatures hardly ever reach sub-zero levels. This coupled with the fact that Sitona is sufficiently coldhardy to withstand temperatures of 2 to 3 degrees below zero indicates that freezing can be ruled out as a cause of mortality in winter.

On the 29th of September, 1964, 210 newly emerged beetles were brought to the laboratory for observation. These were kept in 15 cages made from plastic petri-dishes in a constant temperature room at 20°C. The cages contained moist cotton wool to provide adequate humidity and pieces of broom as food. Food and water were replenished as needed and the cages were cleaned twice a week. It was observed that in spite of the 'ideal' conditions not all beetles survived by the end of March, 1965. A count taken on the 25th of March showed that 42% of the individuals had died. Post-mortem examinations carried out on the dead insects showed that 79 had died without showing obvious pathological reasons. Eight beetles showed the presence of Beauveria and one individual showed signs of a protozoan infection in the gut and in the malpighian tubules. Dr. E.U. Canning

classed this either as a Schizogregarine infection or a coccidial infection. No identification beyond this was possible as the organisms had begun sporogony. Since 79 of these beetles (i.e. 37.6% of the total) died for no apparent reason, it appears that the deaths were natural and occurred in some beetles, probably because they were short-lived. If it is accepted that the same process occurs in the field, then the unaccounted winter disappearances may partly result from the short life span of some individuals. One other reason for the winter disappearance is the tendency shown by some brachypterous individuals to leave the habitat (emigrate) during the time of spring emergence, by walking. Experimental evidence, which will be discussed later, suggests that this is a type of short-range dispersal that brings about colonization of other broom plants in the vicinity; it was found that the tendency to occupy new bushes by the dispersing beetles falls off with distance (see section 12.1).

8.14. Numbers killed by dissection.

When all mortality factors are evaluated, the numbers taken to the laboratory for dissection must also be considered. During the period 9.10.62 to 30.9.63, 2130 beetles were dissected and of these 339 were parasitised by Centistes and from 1.10.63 to 30.9.64, 3090 beetles were dissected out of which 411 were found to be parasitised. As parasitism has already been accounted for, the number parasitized must be subtracted from the number dissected to obtain the number of Sitona adults killed by dissection.

8.15. Old age.

Finally, a certain number of deaths are brought about by the ageing of the population. Deaths that result from old age occur usually at the end of the oviposition period. A small number of beetles survive to live and to lay eggs for a second season; in the laboratory this was 23.1% in 1963 and 9.1% in 1964. In the field, the number that survived for the second season was determined by dissection. The amount of mortality caused by ageing can be assessed by subtracting the sum of the numbers killed by other factors, the number which migrated and the number surviving from the initial population. All unaccounted deaths have been attributed to 'old age' with the assumption that any error brought about by such factors as predation or emigration were low or nil. The likelihood of any predation of adults by predators such as birds and emigration after the initial spring dispersal did not appear to be great.

The mortality data of adults discussed so far are summarised in Tables 20 and 21 in which they are presented in the form of a balance sheet. The loss to the population caused by migratory flights was obtained from the knowledge of the percentage of the macropterous form before and after flight had occurred (see section 12.2, page 135). The winter disappearances is a compilation of winter deaths and of individuals which emigrated during spring emergence. It must be pointed out that although all the known causes of mortality have been considered, some of them overlap. For example, in the case of the numbers taken to the laboratory, the total parasitized was subtracted to obtain the actual number killed by dissection.

Table 20. Survival of Sitona regensteinensis adults: 1962 - 1963.

| | | | |
|---|---|---|---|
| Population in Autumn, 1962..... | 13,837 | | |
| Winter disappearance..... | | 7038 | 50.8% |
| No. killed by Centistes excrucians.... | | 2724 | 19.7% |
| No. migrated by flight..... | | 524 | 3.8% |
| No. taken to the laboratory..... | | 1791 | 12.9% |
| Deaths caused by ageing(and unknown causes)..... | | 347 | 2.6% |
| No. survived to Autumn, 1963..... | | 1413 | 10.2% |
| TOTAL..... | 13,837 | 13,837 | 100.0% |

Table 21. Survival of Sitona regensteinensis adults: 1963 - 1964.

| | | | |
|---|---|---|---|
| Survivors from Spring, 1963..... | 1,413 | | |
| Newly emerged adults in Autumn,1963... | 15,001 | | |
| No. killed by Beauveria bassiana..... | | 689 | 4.2% |
| Winter disappearance..... | | 5,940 | 36.2% |
| No. killed by Centistes excrucians.... | | 2,117 | 12.9% |
| No. migrated by flight..... | | 724 | 4.4% |
| No. taken to the laboratory..... | | 2,679 | 16.3% |
| Deaths caused by ageing(and unknown causes)..... | | 3,828 | 23.3% |
| No. survived to Autumn, 1964..... | | 437 | 2.7% |
| TOTAL..... | 16,414 | 16,414 | 100.0% |

No attempt was made to remove the interaction of parasitism and deaths due to 'old age' as the available information on ageing is uncertain. Thus the number said to be killed by parasitism may include some insects that were destined to die of 'old age'. Since the deaths caused by Beauveria infection were low, any interaction of this with other mortality factors has been overlooked with the assumption that this would not cause a significant change in the general trend of events shown by Tables 20 and 21.

When the tables for the two years are compared, the biggest difference seen is in the number of deaths caused by aging and consequently the numbers that survived to live for a second season also differ considerably. These facts agree well with the difference in the rate of survival shown in section 7.1 (page 49 ).

8.2. Causes of Mortality in the Immature Stages.

8.21. Mortality of eggs.

Three factors can be said to be responsible for the mortality of eggs, namely sterility, parasitism and predation. A fourth could be desiccation, but there is no evidence for this as neither totally nor partially dried up eggs were found in the soil samples.

Sterility.

As pointed out previously (section 6.24, page 42 ), sterility of eggs was determined by the colour change shown by the eggs collected in the field-egg-laying apparatus. The total number of sterile eggs laid during the season in the field is calculated as in Tables 10 and 11 where the weekly

numbers of eggs laid and the weekly percentage of sterile eggs are given. From the total number of eggs laid and from the total number sterile, the percentage sterility for the two seasons, that is for 1963 and 1964, have been worked out as 15.63% and 8.18% respectively. Since it is the estimate of the total number of eggs laid denoted from the soil sampling method which is used in the construction of life-tables, the number of sterile eggs should be based on the soil sampling data as given below.

| Year | Total No. of eggs by soil sampling | % Sterile | Total Sterile |
|---|---|---|---|
| 1963 | 2,105,235 | 15.63 | 329,048 |
| 1964 | 1,209,062 | 8.18 | 98,901 |

It must also be noted that Tables 10 and 11 show that the amount of sterility is not constant throughout the period of oviposition and this was not found to be associated with the presence or absence of sperm in the spermathecae.

Parasitism.

A hymenopterous parasite belonging to Mymaridae was found in the eggs of Sitona. The specimens that emerged from the eggs were provisionally identified by Professor O.W. Richards as Anaphes gauthiori Debauche with the description of which it agreed closely. No egg parasites of Sitona spp. have been recorded in Britain before, although Anaphes sp. has been reported from several species of Sitona (excluding S. regensteinensis) in Russia (Kurdyumov, 1917; Grossheim, 1928).

In the field, the Mymarid was found during the first half of June, a time when Sitona eggs are abundant. The fact that Anaphes was found only in eggs from a single set of soil samples each year suggests that it spends only one generation in the eggs of Sitona regensteinensis from which it probably passes on to other hosts. The extent of parasitism by Anaphes was low and details are given below:

| Date | No. of Sitona eggs in the soil sample | No. of Anaphes emerged | % Parasitism | Total no. of unhatched eggs on this day | Total no. parasitised |
|---|---|---|---|---|---|
| 15.6.63 | 57 | 2 | 3.5 | 625,856 | 21,904 |
| 5.6.64 | 97 | 3 | 3.1 | 451,729 | 14,003 |

Predation.

In the field, the incubation period of Sitona eggs is a long one. Two attempts were made in 1963 to determine the exact incubation period by placing eggs in petri-dishes among litter, under broom. The petri-dishes were lined with moist filter paper and the eggs were examined daily to see when the hatching began. The results are as follows:

| Egg Laying | | Hatching | Incubation Period. Number of Days. |
|---|---|---|---|
| 27.3.63 | - | 30.5.63 | 64 |
| 8.4.63 | - | 3.6.63 | 55 |

Thus, under field conditions, Sitona spends a long period in the egg stage and it was not surprising that natural enemies, particularly predators caused

mortality during that time. A simple laboratory observation was made by confining eggs with a suspected predator in a small plastic box 2" in diameter. Of the many species of Staphylinidae tested, Staphylinus compressus, Staphylinus stercorarius, Ocypus sp., Oxypoda elongatula, Stenus compressus, Stenus clavicornis and Tachinus rufipes fed on the eggs provided. The commonest Carabidae of the habitat are Pterostichus madidus and Abax ater, but these did not feed on the eggs. The predatory mite Anystis agilis Banks which is common on broom and known to feed on the eggs of broom Psyllids (Watmough, 1963) and Phytodecta (Dempster, 1960) also fed on Sitona eggs. Though extremely common on the broom plant itself, Anystis was not recovered from the soil samples. Further observations were made by exposing eggs on petri-dish lids in the field, under the broom plants. These were always invaded by mites and on one occasion, a Parasitid mite was captured while sucking on an egg which was being carried around on its proboscis during the process of feeding. This mite was subsequently identified as Pergamasus crassipes L. by Mr. W.O. Steel. Among other egg predators, Thysanoptera too could be included as Grossheim (1928) found that in Russia, eggs of Sitona spp. are sucked dry by the larvae of Aeolothrips fasciatus L.

In section 7.2 (page 51) estimates were made of the total number of eggs laid and the total number of eggs hatched (empty egg shells). The difference between these two is the estimate of the number of eggs that is completely missing which undoubtedly comprise of the sterile eggs and the eggs that had been eaten by predators. The eggs that had been parasitized

by Anaphes cannot be separated from the normally hatched eggs as the Mymarid emerges in the same manner as the Sitona larva. Therefore, while the difference between the total number of viable eggs and the number of hatched eggs gives the number consumed by the predators, those that had been parasitised by Anaphes must be subtracted from the number hatched to obtain the number of eggs that finally gave rise to Sitona larvae. The egg mortality data obtained in this manner can be tabulated as follows:

Table 22.

| | 1963 | | 1964 | |
|---|---|---|---|---|
| Total no. of eggs laid | 2,105,235 | | 1,209,062 | |
| No. of sterile eggs | 329,048 | 15.63% | 98,901 | 8.18% |
| No. of viable eggs | 1,776,187 | 84.37% | 1,110,161 | 91.82% |
| No of eggs parasitized by Anaphes | 21,904 | 1.04% | 14,003 | 1.16% |
| No. of eggs destroyed by predation | 1,043,748 | 49.58% | 507,526 | 41.98% |
| No. that finally hatched | 710,535 | 33.75% | 588,632 | 48.68% |

As in the case of adults, there is some overlapping of the mortality factors given in Table 22. Of the total estimate of the sterile eggs, some may have been destroyed by the action of predators, but no adjustment was made since the primary cause of death was sterility. The number of eggs parasitized by Anaphes was too small to consider its interaction with either sterility or predation.

8.22. Mortality of the larvae and pupae.

As with eggs, there is considerable mortality in the larval and pupal stages. The first instar larva has to find its way to the root nodules from the top layer of soil and this makes it vulnerable to many hazards. Once the first instar larva arrives at a nodule it feeds on it from the inside, after making an entrance hole, and shifts to a new one when the contents of the first nodule are fully consumed. The second instar as well as some of the third instar larvae also feed on the nodules from within, and are protected by the 'skin' of the nodule. A great majority of the larvae of the third and the fourth instars however, have been found to feed from the outside, probably as the nodules are not large enough to accommodate them. It therefore seems likely that one of the main causes of death of these stages and the pupae was predation. During the two seasons of study, only a single larva was found mummified by Beauveria and the fact that the fungus was not detected in the adults in summer suggests that Beauveria may not be a serious cause of death of the larval and pupal stages.

The predator fauna in soil in the habitat is a large one, comprising of numerous Staphylinids, Carabids, Thrips, Ants, Phalangids, Spiders, Mites and Centipedes. To these could also be added small mammals, as numerous excavations by shrews are frequent in the habitat; for instance, in the summer of 1963 five shrews were caught in the pit-fall traps set out for arthropod predators.

Predation of the immature stages of Sitona in the field was

studied by means of the precipitin test. This test is based on the interaction of Sitona material in the gut of a predator with antibodies in the blood serum of rabbits which have been inoculated with an extract of Sitona. The preparation of the antigen and the antiserum was done by Dr. Dempster according to the method described in his paper (Dempster, 1960).

For the preparation of the antigen or the cell-free extract of Sitona required for injection into rabbits, a large number of beetles (about 20 g. in weight) was collected from another locality. They were then starved for 24 hours to remove broom from the guts, killed in a cyanide bottle and then crushed in a pestle and mortar with 10 ml. of saline (0.9% NaCl). This was kept for 24 hours at 4°C., centrifuged and sterilized by passing through a Seitz E.K. sterilizing filter pad. The clear, sterile antigen was freeze-dried and stored until injection into rabbits.

For the production of the antiserum, the antigen was reconstituted with distilled water and the soluble proteins precipitated with 0.4% potassium alum. The pH of the resulting suspension was adjusted to 6.8 and 2.5 ml. was injected intramuscularly into each hind leg of a rabbit. Ten to 14 days after inoculation the rabbit was bled from the ear, and its serum tested against a standard Sitona extract. Further injections were given to increase the sensitivity of the serum. The production of antibodies varies somewhat between rabbits, but usually reaches a peak after the fourth and the fifth injection. Fifty ml. of blood was taken from each rabbit at this time and all sera which were sufficiently sensitive were pooled, sterilized, freeze-dried and stored in ampoules. Lipoids were

removed from the serum before freeze-drying to prevent the serum turning opaque on reconstitution with distilled water. The lipoid extraction was done with ether at a temperature below -25°C.

A satisfactory antiserum should react with a dilution of between 1 : 1000 and 1 : 4000 of the original antigen used for injection. The pooled sera collected on 30.12.63 were tested for sensitivity against Sitona as well as for some other Coleoptera found in the habitat and the following reactions were obtained by Dr. Dempster:-

| | |
|---|---|
| Sitona regensteinensis | +++ $\frac{1}{2000}$ ; + $\frac{1}{4000}$ |
| Apion sp. (Curculionidae) | + $\frac{1}{20}$ |
| Phytodecta olivacea (Chrysomelidae) | + $\frac{1}{8}$ |
| Coccinellid | + $\frac{1}{4}$ |
| Elaterid | + $\frac{1}{4}$ |
| Staphylinid | no reaction |
| Carabid | no reaction |

The symbols +, ++ and +++ denotes sensitive, strongly sensitive and very strongly sensitive respectively. Thus the antiserum though strongly sensitive to Sitona, showed some reaction with other beetles except Staphylinidae and Carabidae which were the suspected predators. Since wireworms and Apion are comparatively rare in the study area and the larval stages of Coccinellids and Apion are not found in soil, the likelihood of

confusion with these forms was small. On the other hand, the fourth instar larva of Phytodecta descends to the ground to pupate (Waloff and Richards, 1958) and this occurs at a time when the Sitona larvae and pupae are abundant. Moreover, it has been shown serologically that Carabidae prey on the pupal stage of Phytodecta (Dempster, Richards and Waloff, 1959). Therefore the reaction to Phytodecta had to be removed by a process known as 'absorption' (see Dempster, 1960). This was done by adding just sufficient Phytodecta material to precipitate the antibodies to it. The sera collected on 10 and 13.1.64 gave a reaction similar to the previous one, but the sensitivity to Phytodecta was stronger than before. All sera were pooled and after absorption of the Phytodecta reaction, the sensitivity to Sitona was ++ $\frac{1}{2000}$ and trace $\frac{1}{4000}$ and that for Phytodecta was ++ $\frac{1}{32}$.

Samples of possible predators were collected in the study area by soil sampling and pit-fall trapping. Pit-fall trapping was done by sinking ten wide-mouthed bottles (4" high and 4" in diameter) in the soil up to their necks under the broom bushes. Each morning, the trapped predators were collected, identified and smears were made on filter paper of the whole animal if they were small or only of the gut and its contents in the case of large Carabids and Staphylinids. These smears were labelled, dried rapidly over $P_2O_5$ and stored until testing. During the winter, saline extracts of these smears were tested against the antiserum. 0.02 ml. of the extract was drawn into a capillary tube followed by an equal volume of serum. With care little mixing took place and since the serum was denser than the extract, an interphase was clearly visible between the two liquids. After two hours of incubation, the presence of Sitona material, if present,

was shown by a white ring of precipitate at the junction of the two liquids.

In the summers of 1963 and 1964, a total of 1853 gut smears of predators were made during the period when eggs, larvae and pupae of Sitona are present in soil. These were tested for the presence of Sitona material and the results are given in Tables 23 and 24 where Staphylinids and Carabids have been treated separately. In addition to these, 274 mites, 42 phalangids and 72 centipedes were tested, but showed no positive reaction.

Table 23. Showing the predation of immature stages of Sitona by Carabids as given by the precipitin test.

| Predator | No. Tested | No. Reacting | % Reacting |
|---|---|---|---|
| CARABIDAE | | | |
| Pterostichus madidus | 776 | 44 | 5.7 |
| Abax ater | 91 | 5 | 5.5 |
| Pterostichus niger | 54 | 1 | 1.9 |
| Laemosthenes spp. | 30 | - | - |
| Leistus terminatus<br>Leistus ferrugineus<br>Leistus sp. | 21 | 1 | 4.8 |
| Carabus violaceus | 18 | - | - |
| Cychrus rostratus | 8 | - | - |
| Badister bipustulatus | 7 | - | - |
| Amara sp. | 2 | - | - |
| Total no. of Carabidae | 1007 | 51 | 5.1 |

Table 24. Showing predation of immature stages of Sitona by Staphylinidae as given by the precipitin test.

| Predator | No. Tested | No. Reacting | % Reacting |
|---|---|---|---|
| Tachinus rufipes | 228 | 16 | 7.0 |
| Myrmedonia limbatus | 55 | - | - |
| Ilyobates nigricollis | 35 | 2 | 5.7 |
| Staphylinus stercorarius<br>Staphylinus compressus<br>Staphylinus aeneocephelus | 31 | 1 | 3.2 |
| Tachyporus pallidus<br>Tachyporus hypnorum | 24 | - | - |
| Oxypoda elongatula | 18 | 1 | 5.5 |
| Quedius picipes<br>Quedius sp. | 16 | - | - |
| Ocypus spp. | 12 | - | - |
| Philonthus acneus | 12 | 1 | 8.3 |
| Stenus sp,<br>Stenus clavicornis<br>Stenus impressus | 12 | 1 | 8.3 |
| Gyrohypnus punctulatus<br>Gyrohypnus myrmecophilus | 6 | - | - |
| Xantholinus linearis | 3 | 1 | 33.3 |
| Conosomus pubescens | 3 | - | - |
| Bryocharis analis | 1 | - | - |
| Semiris rigidicornis | 1 | - | - |
| Total no. of Staphylinidae | 458 | 23 | 5.0 |

Although some of these predators have already been shown to prey on the eggs and larvae of Sitona in the laboratory, the results of the precipitin test show that these predators do feed on Sitona under natural conditions in the field. Furthermore, the test helps to identify the important predators that are likely to have some control of the abundance of the beetle. From the data of Tables 23 and 24 it would seem that Pterostichus madidus (Carabidae) and Tachinus rufipes (Staphylinidae) are the most important of the predatory insects. The time of occurrence of the two predators appears to synchronise well with abundance of the fourth instar larvae and pupae of Sitona in soil. This point is made clear in Table 25 where the monthly peak number of fourth instar larvae and pupae (as given by soil sampling) and the monthly pit-fall trap counts of Pterostichus and Tachinus are compared with the frequency of positive reaction (by these two predators only) to the precipitin test.

Table 25. Comparison of peak numbers of fourth instar larvae and pupae of Sitona, and pit-fall trap counts of Pterostichus madidus and Tachinus rufipes with frequency of positive reaction to the precipitin test. Data for 1964.

| Month | Estimated No.of Sitona larvae and pupae | No.of P.madidus caught in traps | No.of T. rufipes caught in traps | Frequency of positive reaction to precipitin test |
|---|---|---|---|---|
| April | - | - | - | - |
| May | - | 14 | 7 | 1 |
| June | 9314 | 10 | 12 | 1 |
| July | 27942 | 46 | 56 | 4 |
| Aug. | 32599 | 132 | 46 | 16 |
| Sept. | 23285 | 30 | 13 | 11 |
| Oct. | 4657 | 18 | 8 | 5 |

From this it would seem that the mature larvae and pupae of Sitona are more vulnerable to predation by Pterostichus and Tachinus than any other stage. Among Carabidae, others that are proved to be predatory are Abax ater, Pterostichus niger and Leistus spp. Of the Staphylinids, a variety of species such as Ilyobates nigricollis, Staphylinus spp., Oxypoda elongatula, Philonthus aeneus, Stenus spp. and Xantholinus linearis are shown to be predatory on Sitona by the precipitin test. Tables 23 and 24 also list all other Carabids and Staphylinids that were caught in the study area in 1963 and 1964; all these identifications were done by Mr. W.O. Steel, Dr. R.C. Welch and Dr. M.L. Luff.

The length of time a Sitona meal remains detectable in the gut of Pterostichus madidus was investigated by testing specimens which were fed with a pupa at a known time at subsequent intervals. These tests revealed that Sitona material remains generally detectable up to 36 hours, and in some cases up to 48 hours after a meal. Pterostichus also feeds on Sitona adults when offered so that it is likely that larvae, pupae as well as teneral adults are preyed upon by the Carabid.

In another series of experiments, a Sitona egg or a first instar larva was fed to a number of Tachinus rufipes and tested as before. The serum did not show any sensitivity unless the smear was made immediately after Tachinus consumed the egg or the larva. The mite Anystis agilis too gave similar results. A possible reason for this could be the small size of the meal. One other factor that must be taken into consideration is that predators such as mites which are likely to be the main egg predat-

ors occur in astronomical numbers in soil so that very large numbers may have to be tested before obtaining any positive results in the precipitin test. Thus it is not surprising why the test did not show any predation by mites in the field even though this was observed to happen.

It was not possible to obtain any quantitative information on the causes of larval and pupal mortalities. Though it is evident that an important cause of death of these stages was predation by Staphylinids, Carabids and mites, the effect of other mortality factors are not known. Some indirect evidence to show that larval mortality could also be governed by the root nodule content in soil is given later (section 10, page 106).

## 9. LIFE-TABLES

The life-tables (Tables 26 and 27) presented in this section are based on the design of that given by Richards and Waloff (1961) for Phytodecta. They consider that tables of this nature might better be called 'budgets' since the population changes are not mainly due to changes in the age distribution which is relatively unimportant in a short lived animal. As these tables show variation in reproduction as well as variation in survival, Solomon (1964) prefers to call them life- and fertility-tables.

Tables 26 and 27 show successive mortalities through the life-cycle as shown by the data described and analysed in sections 7 and 8. These mortalities are expressed as percentages of the total number of eggs laid and also as percentages of the number entering the stage whenever possible. The greatest amount of death is seen to occur in the egg and the early-first instar stage, before the larvae could find or arrive at the feeding sites. The total percentage of mortality of these two stages for 1963 and 1964 amounts to 95.02 and 92.94 respectively (see Table 28).

If the sexes occur in equal numbers (this is approximately true of Sitona which had a sex ratio of 1 ♀ : 1.2 ♂ in 1963 and 1 ♀ : 1.1 ♂ in 1964) then a species laying a 100 eggs would have a mortality equal to 98% for the population to remain stable. Since the fecundities (as given by the soil sampling method) for 1963 and 1964 were 691 and 262 respectively, mortalities equivalent to 99.71% and 99.24% would have resulted in stability

Table 26. Life-Table for 1963.

| Stage | No. entering | No. died | % of that stage which died | Mortality as % of total egg no. | Accumulated mortalities. % of total egg no. |
|---|---|---|---|---|---|
| Adults in Autumn, 1962 | 13,837 | 7,038 | 50.86 | | |
| Survivors of preceding adults to Spring, 1963 | 6,799 | | | | |
| Eggs | 2,105,235 | 1,394,700 | 66.25 | 66.25 | 66.25 |
| Early-first inster | 710,535 | 605,725 | 85.25 | 28.77 | 95.02 |
| Late-first instar | 104,810 | 56,914 | 54.30 | 2.70 | 97.72 |
| Larval instars II to IV and pupae | 47,896 | 32,895 | 68.68 | 1.56 | 99.28 |
| New adults, Autumn, 1963 | 15,001 | 6,057* | 40.38 | 0.29 | 99.57 |
| Survivors of Spring adults of 1963 to Autumn, 1963 | 1,413 | 572* | 40.38 | | |
| Total adults that over-wintered in 1963 | 16,414 | 6,629* | 40.38 | | |
| Total adults in Spring, 1964 | 9,785 | | | | |

* Includes early-spring emigrants.

Table 27. Life-Table for 1964.

| Stage | No. entering | No. died | % of that stage which died | Mortality as % of total egg no. | Accumulated mortalities. % of total egg no. |
|---|---|---|---|---|---|
| Adults in Spring, 1964 | 9,785 | | | | |
| Eggs | 1,209,062 | 620,430 | 51.31 | 51.31 | 51.31 |
| Early-first instar | 588,632 | 503,340 | 85.51 | 41.63 | 92.94 |
| Late-first instar | 85,292 | 35,679 | 41.83 | 2.95 | 95.89 |
| Larval instars II and III | 49,613 | 12,986 | 26.17 | 1.07 | 96.96 |
| Larval instar IV and pupae | 36,627 | 19,887 | 54.30 | 1.64 | 98.60 |
| New adults in Autumn, 1964 | 16,740 | 6,697* | 40.00 | 0.55 | 99.15 |
| Survivors of spring adults of 1964 to Autumn, 1964 | 437 | 175* | 40.00 | | |
| Total adults that over-wintered in 1964 | 17,177 | 6,872* | 40.00 | | |
| Adults in Spring, 1965 | 10,305 | | | | |

* Includes early-spring emigrants.

of the population. Percentages above this would have led to a decrease and those below to an increase in the population. The changes in population level are examined in Table 29 in relation to the Tables 26 and 27 and those which would give stability with each year's fecundity. Because the work was begun only in Autumn, 1962, the age composition of the 1963 spring generation was unknown, and thus the increase from 1963 to 1964 indicated by + 0.14 is a minimum figure which is supported by the increase in the spring generation of 1964. In 1964, the percentage mortality was above that which theoretically would have produced stability in the following year, and this is reflected in the rise in the number of spring generation of 1965.

Table 28. Distribution of mortalities in different stages of Sitona. Mortalities are expressed as percentages of the initial egg no.

| Year | 1963 | 1964 |
|---|---|---|
| Egg | 66.25 | 51.31 |
| Early-first instar | 28.77 | 41.63 |
| Late-first instar, instars II to IV and pupae | 4.26 | 5.66 |
| Adults in soil in winter* | 0.29 | 0.55 |

* This includes a few that emigrated from the habitat at spring emergence (see section 12, page 133).

Table 29. Annual deviation of mortality from those necessary for stability.

| Year | 1963 | 1964 | 1965 |
|---|---|---|---|
| Survivors of previous autumn generation | 6,799 | 8,944 | 10,043 |
| Mortality necessary for stability (%) | 99.71 | 99.24 | |
| 'Actual' mortality (%) | 99.57 | 99.15 | |
| Difference (%) | + 0.14 | + 0.09 | |

## 10. DISTRIBUTION AND FEEDING HABITS OF IMMATURE STAGES

### 10.1. Feeding Habits of the Larva.

Many members of the genus Sitona are well known pests of leguminous crops. All larvae are subterranean feeders and have been shown to feed on the root nodules, roots or both. The literature on the larval feeding habits of Sitona spp. are summarised in Table 30. It will be noticed that many workers are not in agreement on the exact site of feeding or damage. Since many authors have found damaged root nodules, a general statement can be made that the main food of larvae of many species of this genus are the root nodules.

Lean (1961) photographed a S. cylindricollis larva feeding on sweet clover rootlets and Newton (1958) photographed the root system of an alfalfa plant heavily damaged by S. hispidula larvae. It is likely that the roots and rootlets may serve as food in the absence of nodules, particularly in the case of plants grown in the laboratory. Some evidence that S. regensteinensis larvae feed solely on the root nodules is presented in this section.

Firstly, a preliminary observation was made by growing broom seedlings between glass plates held 5 mm apart (Fig. 28) and introducing first instar larvae on to the soil surface. The larvae made their way to the root nodules, and began to feed on these (Fig. 29). In the photograph, there are two larvae feeding on a nodule, but one moved away from light while the photographs were taken. In the field samples, larvae were

FIG. 28

FIG. 29. Larva feeding on root nodule.

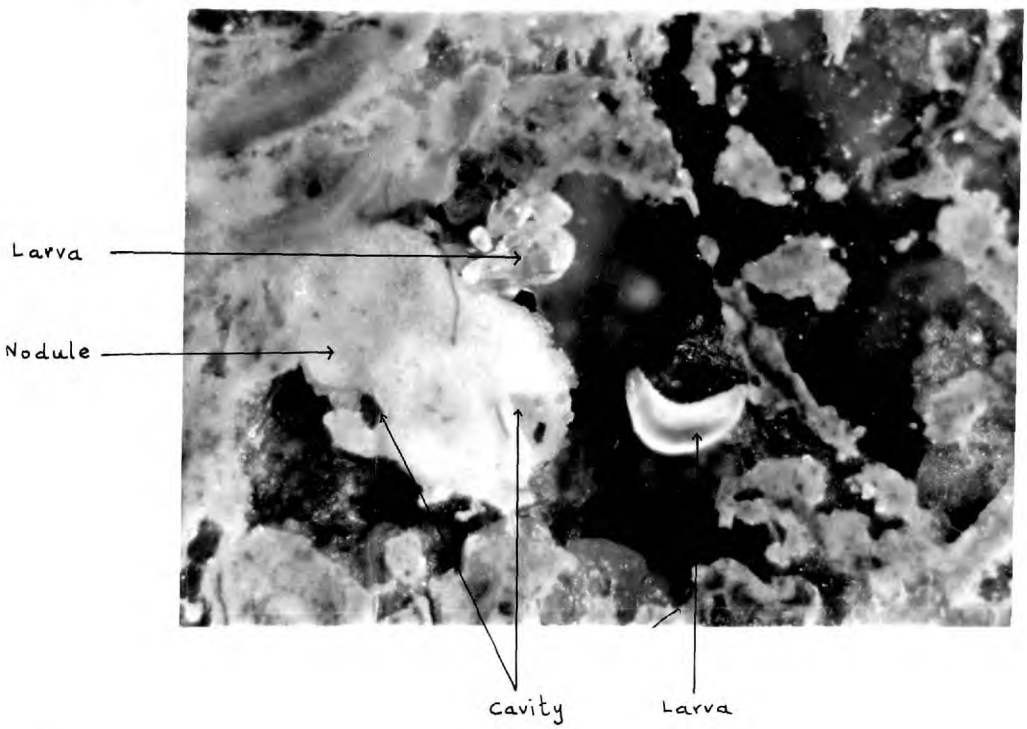


Table 30. Literature on the Larval Feeding Habits of Sitona spp.

| Species | Feeding Habit | Host Plant | Locality | Reference |
|---|---|---|---|---|
| callosus | Root nodules | Alfalfa | Russia | Alimdzhanov, 1941 |
| crinita | Root nodules | Lentils | Russia | Sakharov, Pilyngina & Ginsburg, 1934 |
| " | Roots and nodules | Peas, Clover | Austria | Ripper, 1937 |
| " | Nodules | Vetch, Peas | Russia | Belyaev, 1934 |
| " | Nodules | Alfalfa | Russia | Alimdzhanov, 1941. |
| cylindricollis F. | Small roots and root hairs | Alsike clover | Ontario, Canada | Goble, 1936. |
| " | Nodules | Alfalfa | Russia | Alimdzhanov, 1941. |
| " | Root hairs | Sweet clover | Manitoba, Canada | Munro & Telford, 1942. |
| " | Nodules and root hairs | Sweet clover | " | Bird, 1947. |
| " | Rootlets | " | Montreal, Canada | Munro, Leraas & Nostdahl, 1949. |
| " | Rootlets | " | N.Dakota, U.S.A. | Munro, Bry & Stephenson, 1951. |
| " | Roots only | " | Ohio, U.S. | Herron, 1953. |
| " | Roots and nodules | " | Manitoba, Canada | Loan, 1961. |
| " | Roots and nodules | " | Nebraska, U.S. | Manglitz, Anderson & Gorz, 1963. |
| flavescens | Roots | Red clover | Jersey, U.S. | Lau & Filmer, 1959. |

Table 30 continued.

| Species | Feeding Habit | Host Plant | Locality | Reference |
|---|---|---|---|---|
| fronto | Nodules | Alfalfa | Russia | Alimdzhanov,1941. |
| grossorius | Nodules | Yellow lupin | Portugal | Magalhaes Silva & de Oliveira, 1959. |
| griseus | Nodules | " | " | " |
| hispidula | Nodules and small roots | Clover, Alfalfa | Illinois, U.S. | Bigger, 1930. |
| " | Nodules and roots | Alfalfa | Kansas, U.S. | Marshall & Wilbur, 1934. |
| " | Secondary roots and tap roots. | Clover, Alfalfa | Virginia, U.S. | Underhill, Turner & Henderson, 1955. |
| " | Secondary roots, tap roots and nodules | Alsike clover | Oregon, U.S. | Dickason, Leach & Gross, 1958. |
| " | Roots | Alfalfa | | Newton, 1958. |
| " | Roots | Red clover | Jersey, U.S. | Lau & Filmer, 1959. |
| " | Nodules and roots | Sweet clover | Nebraska, U.S. | Manglitz,Anderson & Gorz, 1963. |
| humeralis | Roots | Alsike clover | Germany | Urban, 1933. |
| " | Nodules | Alfalfa | Russia | Alimdzhanov,1941. |
| lineatus | Nodules | Clover, Peas | Moscow, Russia | Baranov, 1914. |
| " | Nodules | Peas, Beans | Sweden | Kemner, 1917. |

Table 30 continued.

| Species | Feeding Habit | Host Plant | Locality | Reference |
|---|---|---|---|---|
| lineatus | Nodules | Peas, Beans, Lucerne, Vetch | Britain | Jackson, 1920. |
| " | Nodules | Peas | Russia | Belyaev, 1934. |
| " | Roots and nodules | Peas, Beans | Germany | Anderson, 1931. |
| " | Roots and nodules | Peas, Clover | Austria | Ripper, 1937. |
| " | Nodules | Pea | Holland | Mulder, 1948. |
| " | Nodules | Beans | Britain | Masefield, 1952. |
| regensteinensis | Roots | Broom | Germany | Scherf, 1958. |
| scissifrons | Roots and nodules | Vetch | Ontario, Canada | Loan, 1963. |
| tibialis | Nodules | Peas, Vetch | Moscow, Russia | Belyaev, 1934 |
| longulus | Nodules | Alfalfa | Russia | Alimdzhanov, 1941. |
| lineelus | Nodules | " | " | " |

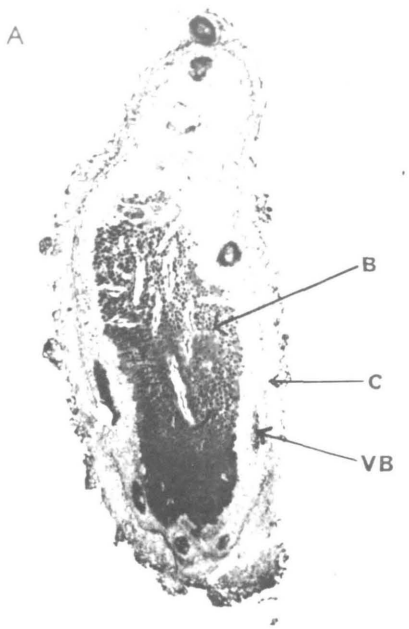
frequently found within the nodules. It appears that they pierce an entrance hole, eat their way through and feed on the contents from the inside. When the contents of a nodule are fully consumed, the empty outer covering is abandoned and the larva shifts to a new one. Some of the third and almost all fourth instar larvae are found free in soil and cannot be associated with

roots or nodules, but their distribution suggests that they too feed on the nodules.

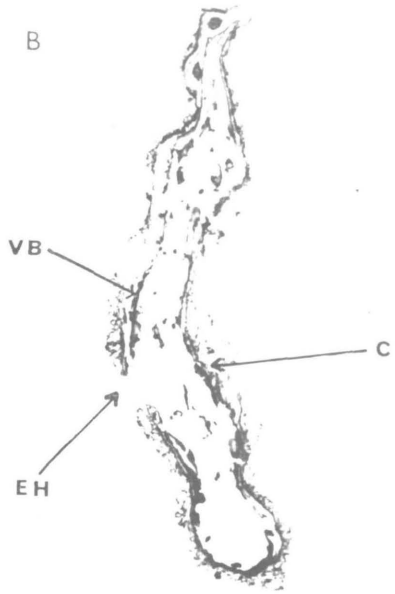
There is no reference in the literature to the nature of damage of the nodules by larvae of any of the species of Sitona. Therefore, a study of this was made with the aid of longitudinal and transverse sections of damaged and undamaged nodules. Broom nodules from the field were doubly embedded in celloidin and paraffin, ten microns thick sections were cut and stained with Carbol-thionin followed by Orange-G. This staining method was used by Stoughton (1930) to demonstrate bacteria in plant tissue; the bacteria are stained deep blue, cellulose walls yellow or green and lignified tissue light blue. Photographs of preparations of a damaged and an undamaged nodule are compared in Fig. 30. A normal undamaged nodule shows an epidermis, a narrow cortical region of parenchymatous cells, an endodermis, a pericycle and then a ring of vascular bundles; the region of the pith is occupied by cells filled with bacteria which are darkly stained; in Fig. 30 the bacteria filled tissue which occupies the greater part of the nodule is clearly distinguishable. The sections of the damaged (and abandoned) nodules reveal that the larvae have a specialized feeding habit in that they consume only the bacteria filled cells; the epidermis, cortex, endodermis and the vascular bundles are not eaten. Thus the parts of a normal root do not seem to be included in the diet of a S. regensteinensis larva. One other reason why roots are unlikely to be the food of Sitona larvae is that in roots of Dicotyledons secondary growth commences shortly behind the root hair region. During this process, more vascular bundles

FIG. 30.

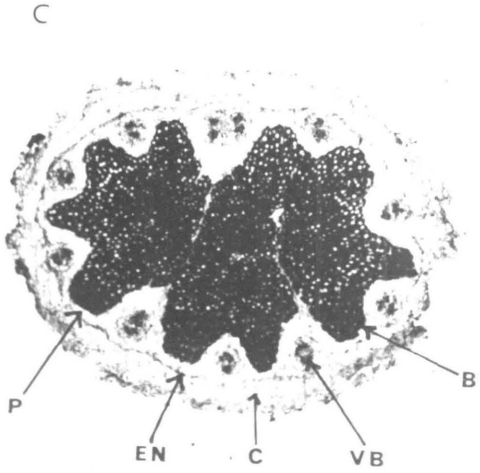
L.S. undamaged nodule.



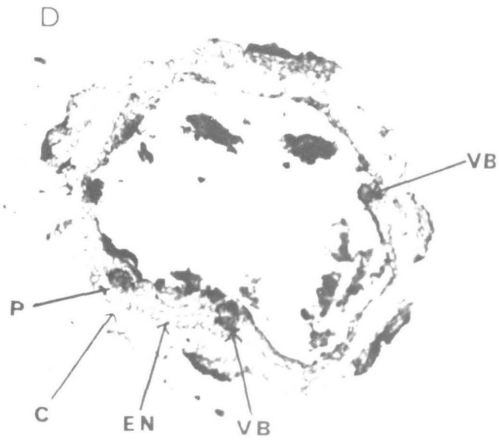

L.S. damaged nodule.




T.S. undamaged nodule.




T.S. damaged nodule.




B = Bacteria containing cells.
C = Cortex.
VB = Vascular bundle.

P = Pericycle.
E = Entrance hole.
EN= Endodermis.

are formed, the endodermis and the cortical tissue die and are replaced by the phallogen which produces cork externally and phelloderm internally. As the cork cells mature, their walls become suberised and they loose their living contents. Thus the parts of the root behind the root hair region becomes pulpy and fibrous and offer hardly anything of nutritive value. The observation of Loan (1961) on S. cylindricollis larvae feeding on the roots of sweet clover agrees with this as the photograph clearly shows that the larvae were feeding on the root hair region. Therefore it seems reasonable to suppose that if the larvae of Sitona spp. feed on roots at all, it is likely that they feed only on the tender parts in the meristematic and root hair regions, and not on tap roots or mature secondary roots.

These observations lead to the question of the nutritive value of root nodules. They contain bacteria, and their function is nitrogen fixation and their importance as a source of protein need not be stressed. A comprehensive account of the biology of bacterial nodules of legumes is given in the book 'Soil Conditions and Plant Growth' by E. Walter Russell (1961). The following interesting points that may be applicable to the present problem were derived from this book. The nitrogen fixing nodules of leguminous plants contain a substance similar to haemoglobin, which is sometimes called leghaemoglobin. The role and mode of haemoglobin is unknown, but it is formed only in cells containing bacteria. In perennial crops new nodules are formed throughout most of the growing season. These nodules are sometimes annual growths shed every autumn or winter, but this is not invariable. Nodules seem to remain on the roots of many leguminous

crops only if the soil is kept moist, and the first effect of the onset of drought is for the crop to shed them. Nodules vary widely in their shape and size. The mean size of 61 broom nodules was 3.5 mm × 1.7 mm with a range of (0.5 × 0.7) mm to (3.5 × 10.2) mm.

10.2. Distribution of Eggs in Soil.

Analysis of soil sampling shows that the numbers of eggs and root nodules found in the samples from the three regions under a broom plant differ markedly. This is evident in the 1963 and 1964 data which are given below (Tables 31, 32).

Table 31. The mean number of eggs and nodules found in each region per set of soil samples in 1963 (20 samples of 1" diameter each).

| | Base | Middle | Periphery |
|---|---|---|---|
| Eggs | 29.9 | 11.3 | 6.4 |
| Nodules | 40.6 | 11.0 | 4,2 |

Table 32. The mean number of eggs and nodules found in each region per set of soil samples in 1964 (12 samples of 2" diameter each).

| | Base | Middle | Periphery |
|---|---|---|---|
| Eggs | 70.1 | 31.5 | 19.4 |
| Nodules | 119.5 | 25.1 | 13.1 |

To study the linear relationship indicated above, a separate set of samples was taken on 2.6.64. For this, 2" diameter and 2" deep samples were taken

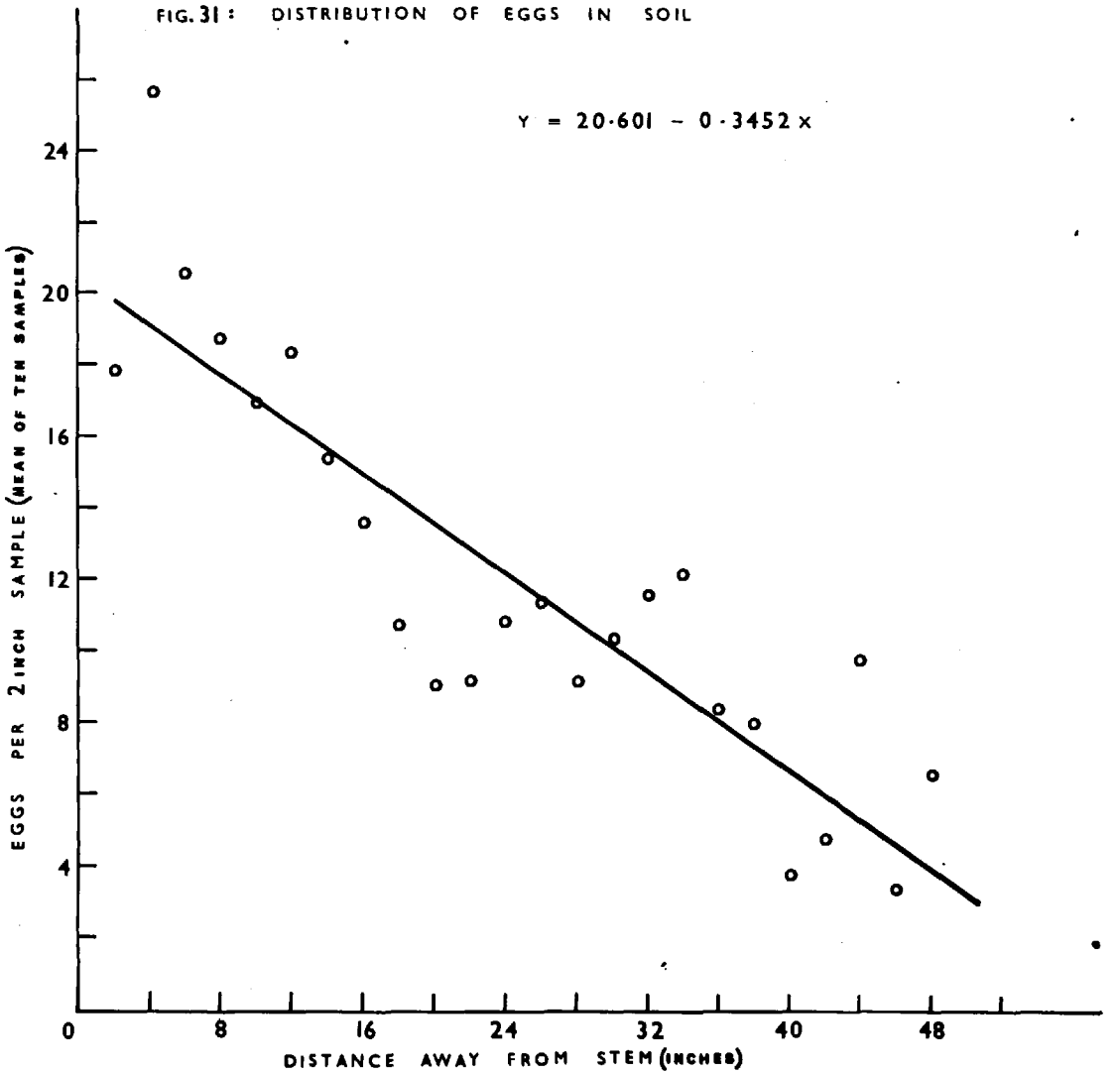
along the radius from the stem in such a way that the first sample was just adjacent to the base of the stem, the second sample adjacent to the first and so on. Twenty four such samples covering a distance of four feet from the stem (the area usually covered by the branches) were taken from each of ten bushes. The eggs were extracted by the method described previously, but the replicates were processed together to reduce the amount of work involved. The results are shown in Fig. 31 where the mean number of eggs per sample is plotted against the distance away from the stem. There is a significant negative correlation between the number of eggs and this distance ($r = -0.8744$; $P < 0.001$). The regression equation is as follows:

$y = 20.601 - 0.3452x$, where x is the distance from the stem in inches and y is the number of eggs.

A closer examination of Fig. 31 shows that there is a distinctly higher number of eggs up to sixteen inches which is equivalent to the basal region and after this the numbers appear to be more or less constant with a marked fall-off towards the edge of the bush.

These observations were followed up by a simple experiment in 1965 to see whether this gradient in the number of eggs is caused by discriminate dropping of eggs from above. This was done by setting up a row of 12 funnels along the radius, with the first funnel near the stem of the broom plant. Each funnel was provided with a (3 × 1)" specimen tube, fitted to the funnel stem by means of a bored cork. The funnels which

FIG. 31 : DISTRIBUTION OF EGGS IN SOIL



have been described previously (section 6.22, page 37b) contained Formalin solution to kill any beetles that fell in. The counts of eggs collected from 18.3.65 to 22.4.65 were as follows:

| Funnel no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distance from the stem (in inches) | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 |
| No. of eggs collected | 52 | 50 | 6 | 22 | 21 | 4 | 14 | - | 14 | 5 | 8 | 7 |

These results show a relationship similar to that obtained in the soil samples. The correlation coefficient of the number of eggs in the funnels and distance is $-0.7107$ ($P < 0.01$). Thus, although females may go down to lay eggs on soil, the distribution of eggs appears to be caused by the position taken up by the beetles on the bush and indicates that they mostly occupy branches towards the centre.

10.3. Distribution of Larval, Adult Stages and Root Nodules in Soil.

To find out whether a relationship exists between the distribution of root nodules and that of the larvae and adults in the soil, samples were taken along the radius as previously, but the sampling was done by a 4 in. diameter wireworm sampler up to a depth of 12 inches. The larvae (instars III and IV) and the nodules were separated by sifting. Altogether ten bushes were sampled between 22.7.64 and 28.7.64. The adult emergence in five bushes was recorded by setting up a series of emergence traps. Each trap consisted of an inverted 8" square and 9" high biscuit tin fitted at the top with a 3" × 1" specimen tube containing a celluloid trap funnel

(Fig. 32). Each plant was provided with a battery of six such traps radiating from the centre (Fig. 33). The beetles that emerged from 28.9.64 to 2.12.64 (i.e. throughout the whole period of emergence) in these traps were recorded.

The soil sampling and the emergence trap results are shown in Fig. 34 where the distributions are expressed as the percentage of the total of each stage. The soil sampling results for eggs from the previous section are also included, but they have been converted to 4" distances. The following conclusions can be drawn from the results: 65.3% of all eggs were laid within the first 24" from the main stem; the adult beetles emerged only from the first three traps, with 61.1% emerging from the first trap alone; the larval distribution and adult emergence closely follow the distribution of root nodules in the soil; the distribution of eggs shows a similar relationship, but not so clearly; assuming that there are no long distance horizontal movements of larvae in soil, it appears that the eggs laid beyond 24" from the stem (i.e. 34.7% of all eggs) do not develop into adults.

Another aspect of distribution in soil is the vertical one. The distribution in relation to depth was studied between 16.7.63 and 17.9.63, when larval, pupal and the adult stages are found in soil. The sampling was done up to a depth of 16", soil being removed at different levels (1 - 2", 2 - 5", 5 - 8", 8 - 12" and 12 - 16") with the 4" diameter wireworm sampler. Each sample consisted of a set of ten taken from the basal region of ten different bushes; the sampling was done on five occasions. Samples of

FIG. 32.

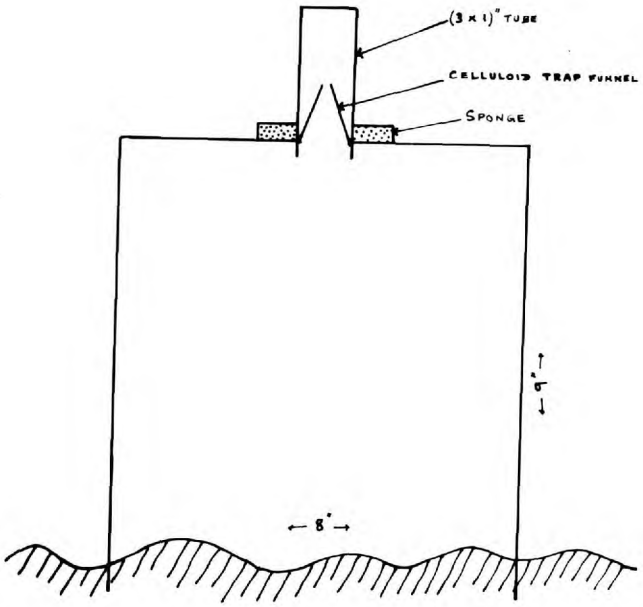



FIG. 32. EMERGENCE TRAP

FIG. 33. The battery of emergence traps.

FIG. 34 : DISTRIBUTION OF IMMATURE STAGES & ROOT NODULES IN SOIL COMPARED WITH ADULT EMERGENCE

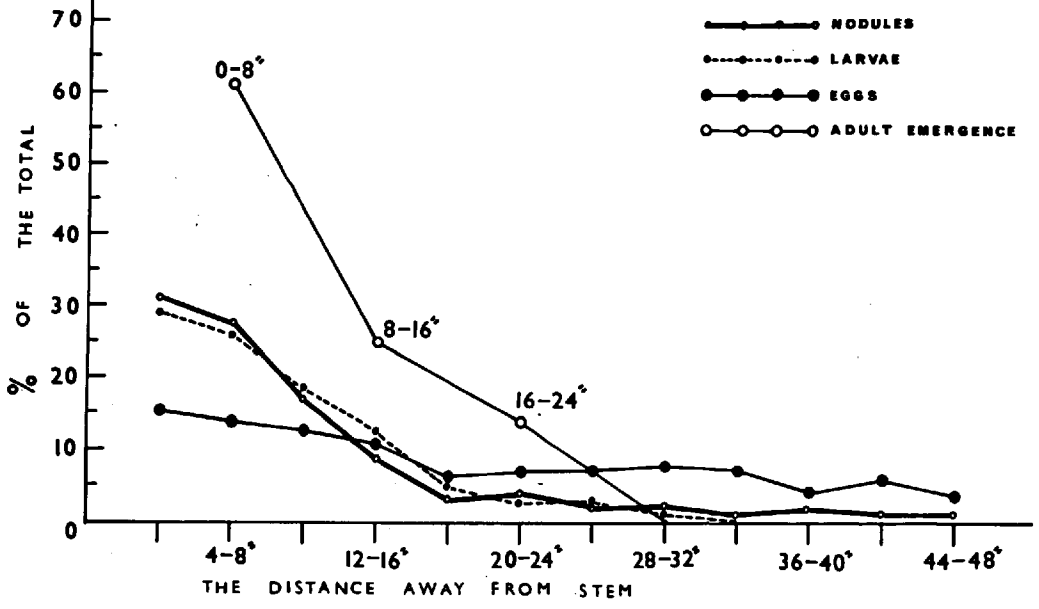
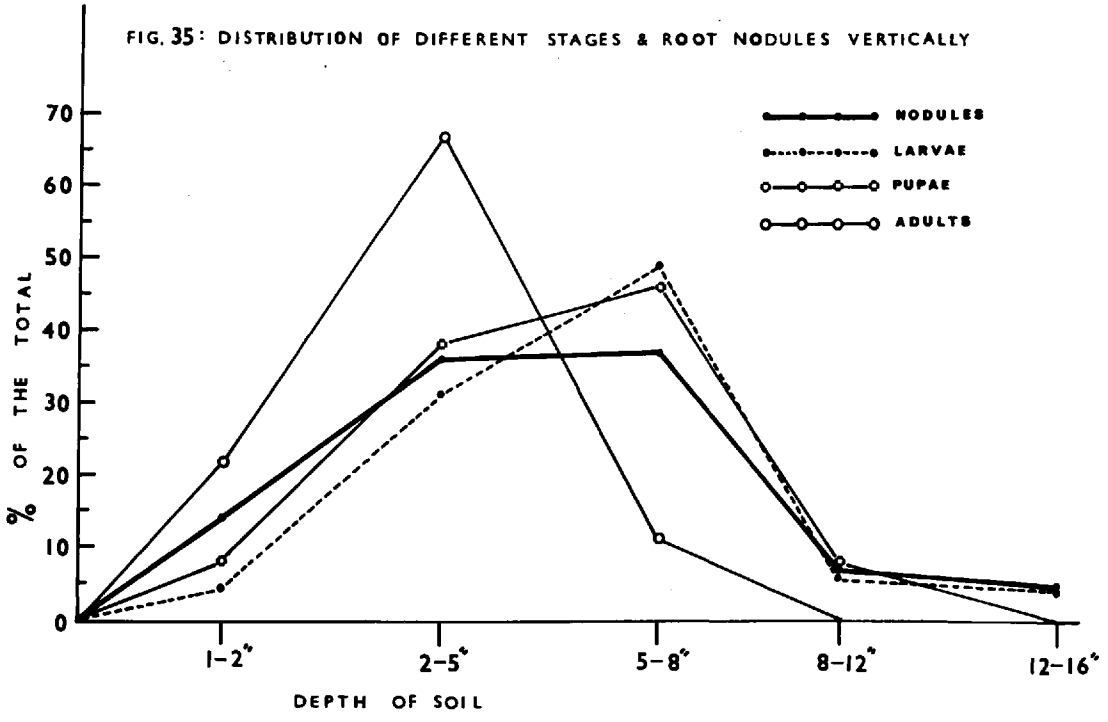



FIG. 35 : DISTRIBUTION OF DIFFERENT STAGES & ROOT NODULES VERTICALLY



16.7,63 and 29.7.63 were subjected to the soil washing - floatation procedure so as to establish the location of eggs. The larvae (instars III and IV), pupae, adults and the root nodules of the other three sets of samples were obtained by sifting. The results are shown in Table 33. The same results are expressed as the percentage of the total of each stage in Fig. 35. Once more, the distribution of larvae follows that of the nodules closely. The distribution of pupae shows that pupation takes place over a wide vertical range, up to 12" in depth. The fact that adults were found at 2 - 5" indicates the movement towards the soil surface takes place during this stage. As expected, the eggs were found in the first layer of soil which is mainly composed of litter and grass roots. Presence of a few eggs in the other layers could be due to contamination while sampling. The fall in the number of nodules beyond a certain depth is believed to result from the reduction in the supply of oxygen since nodulation requires a good oxygen supply.

Evidence given so far shows that the larvae require root nodules as food and consequently their distribution in soil and the emergence of adults closely follow the distribution of root nodules. These observations suggest that a limiting factor in the abundance of Sitona may be found in the ability of the host plant to produce root nodules.

Scherf (1958) gave an account of the larval feeding habits of Sitona regensteinensis, in which he states that the larvae nibble at first on more delicate roots but later attack stronger roots and as they grow older they move along the roots in the direction of the root neck. Scherf's

Table 33. Showing the distribution of different stages of Sitona and root nodules with depth in 10 samples, 4" in diameter.

| Depth / Date | | 1 - 2" | 2 - 5" | 5 - 8" | 8 - 12" | 12 - 16" |
|---|---|---|---|---|---|---|
| 16.7.63 | Eggs | 380 | 40 | 10 | 6 | - |
| | Larvae | - | - | 9 | 1 | 1 |
| | Nodules | 10 | 8 | 108 | 30 | 11 |
| 29.7.63 | Eggs | 344 | 52 | 16 | 8 | - |
| | Larvae | - | 8 | 10 | 4 | - |
| | Nodules | 20 | 56 | 24 | 18 | 21 |
| 14.8.63 | Larvae | 2 | 5 | 8 | 1 | 2 |
| | Pupae | - | 2 | - | - | - |
| | Nodules | 28 | 76 | 210 | 27 | 18 |
| 6.9.63 | Larvae | - | 5 | 1 | 2 | - |
| | Pupae | 2 | 3 | 6 | - | - |
| | Nodules | 108 | 124 | 87 | 15 | 17 |
| 17.9.63 | Larvae | - | - | - | - | - |
| | Pupae | - | 4 | 5 | - | - |
| | Adults | 8 | 2 | 6 | 1 | - |
| | Nodules | 68 | 314 | 164 | 20 | 10 |
| TOTAL | Larvae | 2 | 18 | 28 | 6 | 3 |
| | Pupae | 2 | 9 | 11 | 2 | - |
| | Adults | 8 | 2 | 6 | 1 | - |
| | All individuals | 12 | 29 | 45 | 9 | 3 |
| | Nodules | 224 | 578 | 593 | 110 | 77 |

statements are not based on any experimental evidence, and appears to be erroneous as reasons for the aggregation of larvae towards the root neck are now clearly known.

10.4. Damage caused by Larvae to the Broom Plant.

The damage caused by the larva to the plant was assessed by counting the numbers of damaged and undamaged root nodules contained in the soil samples. Results for the two years are given in Figs. 36, 37 where the total estimate of root nodules and the percentage damage is plotted against time. For comparison, the total numbers of instar II to IV larvae are also shown. A most noticeable feature is that there is an increase in the root nodule content from June to August which is also the time of larval development. A simultaneous increase in the percentage damage which rises to a peak towards the end of July is another point of interest. The maximum damage to the root nodule system seems to be in the region of 70%, but it must be noted that there is a growth of new nodules throughout the season.

An important problem arising from root nodule damage is how much damage does the larva cause to the plant as a whole. This would be especially important in the case of those species of Sitona which attack leguminous crops. It has been shown that the amount of nitrogen fixed by a leguminous crop depends very largely on the longevity of the nodules on its roots. Four factors affect longevity: the physiological condition of the plant, the moisture content of the soil, parasites in the nodules,

and the strain of bacteria forming the nodule (Russell, 1961). 'Parasites in the nodules' referred to here are Sitona larvae, as no other nodule feeders are known. Therefore, it is very likely that the larvae of S. regensteinensis invariably cause damage to the broom plant in affecting its nigrogen nutrition.

FIG. 36: ROOT NODULE DAMAGE - 1963

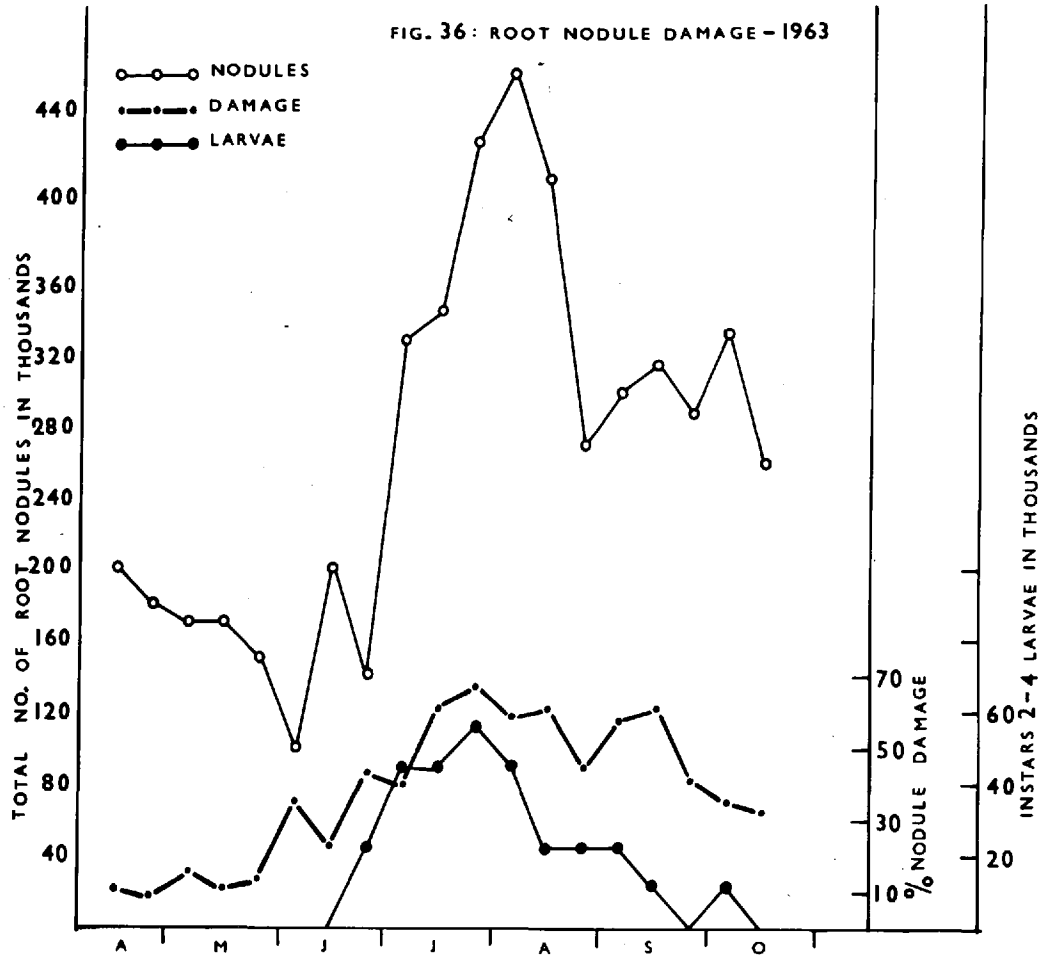
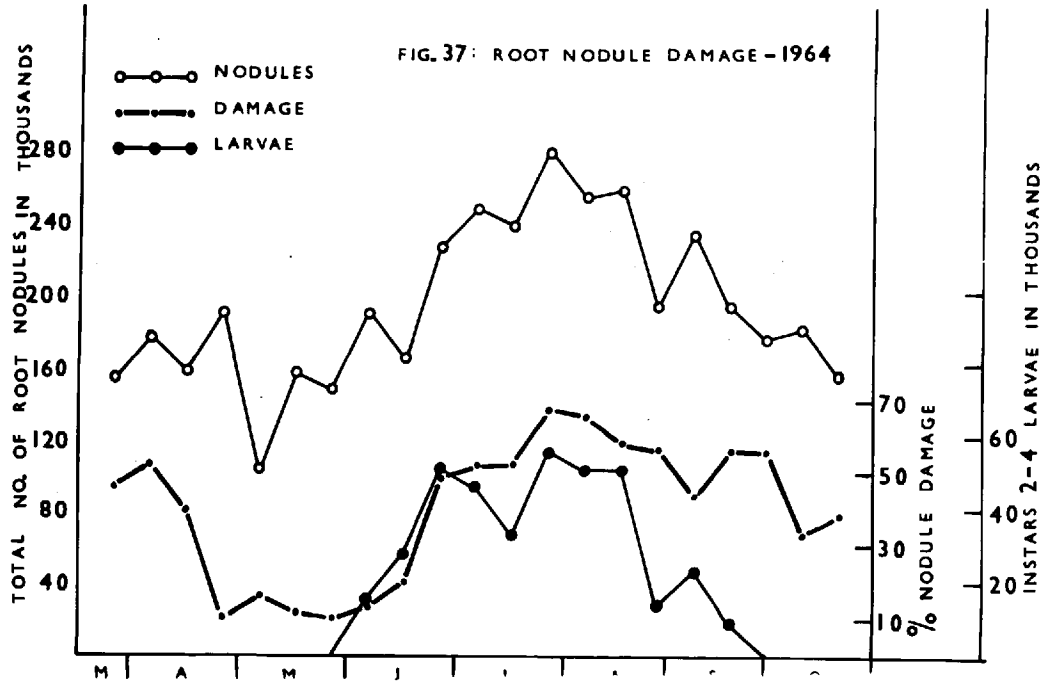



FIG. 37: ROOT NODULE DAMAGE - 1964



## 11. MOVEMENT OF INDIVIDUALS WITHIN THE HABITAT

It was mentioned in section 6.14 (page 27) that the number of Sitona found at any time on the broom branches is greatly influenced by the prevailing weather and consequently unsettled conditions in autumn and spring caused marked fluctuations in the adult population on the plants. An experiment was carried out to study the factors governing these movements of the beetles to and from soil to the foliage.

For this, eight small (2 - 4 year old) isolated broom bushes were selected in the spring of 1964 and these were sampled almost daily in the mornings (between 10 a.m. and 12 noon) by beating whole bushes. Counts of the number of Sitona found on each bush were recorded and the beetles were returned to their particular plants. In this way, each population unit was undisturbed as far as possible and by beating whole bushes any sampling error was reduced. To counteract the possibility of variation caused by mortality, emigration and immigration of the beetles, the experiment was carried out only for a short period and altogether 37 recordings were made. Concurrently the measurement of weather factors were made. They were, the air temperature, soil temperature, relative humidity, rainfall, wetness of soil, cloudiness and hours of sunshine and the radiation. The air temperature was measured in shade by an ordinary mercury thermometer. The soil temperature under broom at a depth of 2 inches was recorded by a mercury-in-steel electrothermograph. Relative humidity was measured by means of a whirling hygrometer. The measurements of rainfall

and radiation were obtained from the Field Station records. The rainfall and radiation measuring instruments are situated 350 yards away from the experimental area. The data collected in this experiment were kindly analysed by Dr. J.N.R. Jeffers, the Statistician of the Forestry Commission Research Station, Farnham, Surrey in an I.C.T. Sirius electronic computer. First, a multiple correlation and regression analysis was carried out of the number of Sitona on the other variables. The details of this analysis are given in Tables 34, 35 and 36.

Table 34. The mean, minimum and maximum measurements of the variables with their standard deviations. No. of sets = 37.

| Variable | | Minimum | Mean | Maximum | S.D. |
|---|---|---|---|---|---|
| A | No. of Sitona on broom | 0.0 | 57.622 | 230.0 | 64.569 |
| B | Air temperature in °C. | 3.0 | 10.459 | 22.0 | 4.477 |
| C | Soil (litter) temperature in °C. | 0.0 | 5.159 | 11.5 | 3.270 |
| D | Relative humidity (%) | 39.0 | 74.162 | 97.0 | 14.601 |
| E | Rainfall in mm. | 0.0 | 0.751 | 8.2 | 1.952 |
| F | Wet or dry soil | 0.0 | 0.865 | 1.0 | 0.347 |
| G | Cloudy or sunny | 0.0 | 0.486 | 1.0 | 0.507 |
| H | Radiation ($MWL/cm^2$) | 3.0 | 159.243 | 521.0 | 130.913 |

Table 35. Multiple correlation coefficients of the variables.

| A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|
| 0.784*** | | | | | | |
| 0.804*** | 0.785*** | | | | | |
| 0.221 | 0.132 | 0.312 | | | | |
| 0.037 | 0.118 | 0.271 | 0.357** | | | |
| -0.038 | -0.209 | -0.091 | 0.284 | 0.154 | | |
| -0.188 | 0.095 | -0.124 | -0.624*** | -0.223 | -0.248 | |
| 0.227 | 0.560** | 0.261 | -0.428** | -0.148 | -0.457*** | 0.548*** |

Level of significance: * = 10%; ** = 5%; *** = 1%.

Table 36. Multiple regression of the number of Sitona on the other variables.

| Variable | Regression Coefficient | Standard Error | Ratio |
|---|---|---|---|
| B | 8.732797 | 2.481086 | 3.52** |
| C | 9.031112 | 2.953132 | 3.06** |
| D | - 0.754693 | 0.544968 | -1.38 |
| E | - 6.077070 | 3.044496 | -2.00* |
| F | 13.107982 | 17.771290 | 0.74 |
| G | -28.164492 | 15.056384 | -1.87* |
| H | - 0.088169 | 0.071100 | -1.24 |

Continued.

Table 36 continued.

| | | |
|---|---|---|
| Total sum of squares | = | 150090.700000 |
| Regression sum of squares | = | 119526.090000 |
| Residual sum of squares | = | 30564.610000 |
| Residual mean square | = | 1053.952100 |
| S.D. from regression | = | 32.464629 |
| R squared | = | 0.7964 |

Regression constant = -3.37339

Level of significance: * = 10%; ** = 5%.

This shows that the number of Sitona on broom was significantly correlated with the atmospheric and soil temperatures, but not with any other recorded variable (Table 35). There were also some correlations between the independent variables, notably between atmospheric and soil temperature, atmospheric temperature and radiation, relative humidity and rainfall, and sunshine and radiation, and significant negative correlations between relative humidity and sunshine, relative humidity and radiation, and wetness of soil and radiation. The regression of numbers of Sitona on all variables accounted for 79.6 per cent. of the variability (Table 36), but only two of the variables, atmospheric and soil temperatures had significant coefficients.

A series of reduced regression equations, the details of which are not given here, showed that there is little to choose between all

those which contain the atmospheric and soil temperature. Therefore a regression of numbers of Sitona was carried out on the atmospheric and soil temperature alone. Table 37 shows that this regression accounted for 70.8 per cent. of the variability in numbers and is clearly a simple and direct predictor of numbers of Sitona on the foliage. The equation may be written as $N = -52.63 + 5.77\ A + 9.67\ S$

where N = number of Sitona,

A = Atmospheric temperature in °C. and

S = Soil temperature in °C.

Since this equation is based on a known population of Sitona (the maximum number recorded was 230), it could be used to predict the movements of individuals in any population.

Table 37. Regression of no. of Sitona on broom on atmospheric and soil temperature. Level of significance: ** = 5%.

| | | |
|---|---|---|
| Total sum of squares | = | 150090.700000 |
| Regression sum of squares | = | 106199.260000 |
| Residual sum of squares | = | 43891.440000 |
| Residual mean square | = | 1290.924700 |
| S.D. from regression | = | 35.929441 |
| R squared | = | 0.7076 |
| | | |
| Regression constant | = | -52.631790 |
| Regression coefficient of air temp. | = | 5.769772** (S.D.=2.157) |
| Regression coefficient of soil temp. | = | 9.672471** (S.D.=2.953) |

The values of the seven independent variables were then subjected to a principal component analysis. In statistical practice, the method of principal component analysis is used to find the linear combinations with large variance. In many extrapolatory studies, the number of variables under consideration is too large to handle. Since it is the deviations in these studies which are of interest, a way of reducing the number of variables to be treated is to discard the linear combinations which have small variances and study only those with large variances. Thus the main advantage of this type of analysis is that it reduces the number of comparisons to a minimum and indicates those which it is necessary to measure if it is intended to carry out more observations on the same lines (see Anderson, 1958; Gardiner and Jeffers, 1962). In the case of Sitona data, four components were found to be of practical significance. The first, accounting for 36 per cent. of the variability measured by the independent variables, was the index of radiation, the second, accounting for 31 per cent., the index of temperature, and the third, accounting for 12 per cent., the index of rainfall. A fourth component, accounting for 10 per cent., was the index of the wetness of the soil. The regression on the number of Sitona on these components also gave the greatest weight to the relationship with temperature. The analysis therefore suggests that the abundance of Sitona on broom can be predicted reasonably well from measurements of atmospheric and soil temperature, with only slight improvement from measurement of other variables.

This experiment has conclusively shown that the movements of

Sitona up and down from soil in bad weather is governed by the soil and air temperatures, and hence explains the fluctuations in adult numbers shown by the beating method in spring. However, the main significance of these observations is that the weather factors (predominantly temperature) regulates the feeding activity of Sitona, at least during early spring and this may probably have some influence on the oviposition of the beetle.

## 12. DISPERSAL OF ADULT SITONA.

Many authors (Andrewartha and Birch, 1954; Kennedy, 1961; Schneider, 1962; Southwood, 1962) recognise that dispersal in insects means a scattering, an increase in the mean distance between individuals and may be used for movements within the population territory as well as for those away from it. In Sitona, there are two types of movements that lead to dispersal. One is by the brachypterous form by walking, at the time of emergence from hibernation, and the other is by flight, a few weeks later by the macropterous form. Following the classification of Southwood (1962), the walking movement of Sitona may be called a 'trivial' movement (except when this brings about a limited amount of dispersal; section 12.1). and flight, a 'migratory' movement. Whether the latter is truly a migratory movement in the modern sense (see Johnson, 1960, 1963, 1965; Kennedy, 1961) will be discussed later.

### 12.1. Dispersal by Walking.

This was assessed by sampling a series of two year old trap broom plants, each about 2.5 ft. high. The trap plants were planted in seven pairs at the end of summer, 1964 (each pair 7 ft. apart) on a logarithmic scale of 2, $2^2$, .....$2^7$, i.e. at a distance of 2 - 128 ft. from the north-east of the study area. These plants were sampled several times in the autumn and winter, but no beetles were collected, since the movements of the new generation beetles in autumn and winter appear to be confined to up and down movements from the broom to soil in the manner described

previously(section 6). There were no indications of other movements, even from one group of bushes to another within the habitat; this was established by marking beetles from several groups of plants with a colour corresponding to each group, releasing and recapturing over a period during the routine weekly sampling.

In the spring of 1965, the bushes that were planted at logarithmic distances were sampled at weekly intervals by shaking each plant on a tray between 10 and 11 in the morning on Fridays, and the Sitona collected were counted and removed. The sampling was done from 19.3.65 to 30.4.65 with only a few beetles caught towards the end of the period. Altogether 84 beetles were caught and of these 53 were taken within the first two weeks. The details are as follows:

| Distance from the habitat in feet | 2 | 4 | 8 | 16 | 32 | 64 | 128 | Total |
|---|---|---|---|---|---|---|---|---|
| No. of Sitona captured | 44 | 18 | 15 | 5 | 2 | 0 | 0 | 84 |

When the total catches for the seven weeks on successive pairs of plants are compared, a tendency for the numbers to fall off with distance from the habitat becomes evident. The relation of the logarithm of the numbers collected on the trap plants to the logarithm of the distance from the habitat is linear. The regression equation takes the form $y = 2.272 - 1.28513x$ ($P = 0.01$) where y is the logarithm of the number of Sitona and x the logarithm of the distance from the study area. This shows that the density of dispersing individuals was inversely proportional to the distance from the source.

This method has been used previously by Kettle (1951a, b) in a study of dispersal of Culicoïdes impunctatus and also by Waloff and Bakker (1963) to study the flight activity of Miridae (Heteroptera) living on broom. They found that two of the five species of Mirids, namely Heterocordylus tibialis and Orthotylus adenocarpi showed a linear relationship between the logarithm of the catch and the distance from the breeding site.

It is very unlikely that walking by the brachypterous beetle could lead to dispersal over large distances. This seems more like a type of short-range dispersal that brings about colonization of other broom plants in the vicinity. The experimental results discussed above supports this view, since the numbers of Sitona that occupied new bushes fell off with distance. Thus dispersal by walking may be considered as a means of finding new or young plants in a natural broomland where plants of various age and state are found, and where expansion of the habitat itself takes place by the growth of new plants at the boundaries.

12.2. Dispersal by Flight.

Three methods were used to study the flight activity: (1) examination of weekly samples of adults to determine the proportion of the macropterous individuals, (2) by means of two 'window traps' (Chapman and Kinghorn, 1955), and (3) by the use of suction traps (Johnson and Taylor, 1955a, b; Johnson, 1950).

12.21. The proportion of the macropterous form.

In Sitona, the migratory flights occur within a few weeks after the emergence from hibernation in spring. There is only a single flight period each year which is distinct and short. It lasts about a week or two, but the great majority of the flyers leave the habitat within the first few days. This is indicated in Fig. 38 where the percentage macropterous form in weekly samples is plotted against time. This shows a sharp drop in the proportion of the macropterous individuals, and makes it possible to divide the life of a macropterous beetle into a pre-flight period and a post-flight period. Difference between the percentage macropterous before and after flight had occurred provides the percentage of adults that dispersed by flight. Information on dispersal obtained by this means is given in Table 38.

Table 38. Showing the proportion of the macropterous form before and after flight.

| Year | % Macropterous | | % | Total no. of Spring Adults | Total no. of Adults migrated by Flight |
|---|---|---|---|---|---|
| | Before flight | After flight | Migrated | | |
| 1963 | 9.8 | 2.1 | 7.7 | 6799 | 524 |
| 1964 | 9.7 | 2.3 | 7.4 | 9785 | 724 |
| 1965 | 9.0 | 2.9 | 6.1 | 10305 | 629 |

FIG. 39. THE WINDOW TRAP.

12.22. Window Traps.

Window traps are based on the principle that beetles fly into a plane of glass and drop into a trough of water. Two traps were set up facing east-west and north-south at the boundary of the study area with the intention of trapping the beetles that come in or go out (Fig. 39). Those that go out were expected to strike the inner wall of the glass plane and fall into that side of the trough of water, and those that come in to strike the outer wall and fall into the outer side of the trough. Unfortunately these traps were not found to be very effective probably because the numbers flying were low. In 1963, only one beetle was caught, two in 1964 and none in 1965. The details are as follows:

| Date | No. caught | Direction | Wind Direction | Maximum Temp.°C. |
|---|---|---|---|---|
| 19.4.63 | 1 female | leaving towards south | Calm | 15.5 |
| 26.4.64 | 1 female | " | South | 17.5 |
| 27.4.64 | 1 male | " | South | 19.3 |

12.23. Suction Traps.

Sampling with suction traps may be considered as the most up-to-date method of measuring aerial populations of insects. But again, only a limited amount of information was obtained because of the low numbers caught in 1963 and 1964. In 1965, an unexpected outburst of warm weather between 22nd March and 4th April caused the flights to occur earlier than usual, before the suction traps were made ready. Consequently no suction

trap data are available for 1965. Two 18 in. propeller type suction traps are operated at the field station each year in the "Silwood Bottom" area (see Fig. 1). These traps are about 330 - 360 ft. south of the 'Gunnes's Hill' broom plantation. One trap samples at a level of 4 ft. from the ground and the other at 30 ft. The results of the suction trap catches for 1963 are given in Tables 39 and 40. Invariably Sitona was caught in the traps on sunny days.

Table 39. No. of Sitona regensteinensis caught in 18" suction trap at a level of 4 ft. from ground in 1963.

| Date | No.of males | No.of females | Total | Maximum Temperature °C. |
|---|---|---|---|---|
| 22.4.63 | 1 | 1 | 2 | 15.0 |
| 23.4.63 | - | 1 | 1 | 17.0 |
| 26-28.4.63 | 3 | 3 | 6 | 20.0 |
| 3- 5.5.63 | 2 | - | 2 | 15.5 |
| Total | 6 | 5 | 11 | |

Table 40. No. of Sitona regensteinensis caught in 18" suction trap at a level of 30 ft. from ground in 1963.

| Date | No.of males | No.of females | Total | Maximum Temperature °C. |
|---|---|---|---|---|
| 19-21.4.63 | - | 3 | 3 | 15.5 |
| 26-28.4.63 | 1 | - | 1 | 20.0 |
| 3- 5.5.63 | 1 | - | 1 | 15.5 |
| 6.5.63 | 1 | - | 1 | 15.0 |
| Total | 3 | 3 | 6 | |

In 1964, for some unknown reason, no beetles were caught in these two traps; but a single female was caught on 26th April, in a 9 in. Vent-axia trap placed in the broom plantation itself. This trap contained a disc dropping mechanism to separate hourly catches; the beetle was caught between 3 p.m. and 4 p.m. when the air temperature was 18.2°C. All specimens caught in flight showed on dissection large fat bodies; in the females the ovaries appeared to be partly matured with oocytes in the vitellaria, but there were no fully formed eggs in the oviducts.

12.24. Laboratory observations on flight.

In the laboratory, it is difficult to make the beetle fly. Attempts to induce flight on fixed mounts or flight mills were not successful. Beetles collected in late autumn, winter or spring can be made to fly for short periods by warming up under a 60 watt bulb. A Sitona warmed in this way to about 30°C. becomes restless, preparatory attempts to fly are made by opening the elytra and unfolding the wings several times. Then it walks up to the highest point of the container (usually a beaker), faces upwards with the first pair of legs in the air and takes off. As soon as it leaves heat and light it ceases to fly and settles down, but will fly again when brought back to "favourable" conditions. On alighting after flight, the elytra are closed and the wings extended to their full length project from their apices. Then they are gradually withdrawn beneath the elytra. On warm days in the spring, beetles with the wings protruding under the elytra are often seen on the beating tray.

12.25. Seasonal changes in the flight muscles.

A factor associated with the flight ability of the beetle is the state of the flight muscles. There is a distinct build up of flight muscles during the pre-flight period followed by their antolysis soon after flight is completed. Such flight muscle degeneration is known for some insects such as ant and termite queens, aphids, mosquitoes and scolytids (see Chapman, 1956). During 1963 and 1964 an attempt was made to study the flight muscle changes of Sitona quantitatively by measuring the flight muscle area in longitudinal sections. These observations were mainly concerned with the changes in the two prominent indirect muscles that control the up and down strokes of the wing. These are namely, the dorsal longitudinal muscle which runs from the prephragma to the postphragma and the dorsoventral muscle which runs from the tergum to the sternum on either side of the gut.

Macropterous beetles collected in weekly samples were anaesthetised in chloroform and fixed in alcoholic-Bouin's after the removal of elytra. They were then embedded in celloidin and paraffin for sectioning. Londitudinal sections were cut to the thickness of 22 microns as this was found to be the ideal thickness to obtain satisfactory sections. The sections were then double stained in Heidenhein's Iron Haematoxylin and Eosin and permanent preparations made in Euperal. The muscle areas were measured under a low-power microscope (10 × 6X) with the aid of a 0.5 mm squared eye-piece.

The seasonal changes in the size of the two muscles are shown in

Fig. 40 and a representative series of photographed sections are shown in Figs. 41 and 42. There are only traces of the two muscles at the time of emergence. These increase in size during autumn and winter to attain a maximum size. The size of the dorso-ventral muscle is in the region of 0.55 sq. mm. and that of the dorsal longitudinal muscle 0.25 sq. mm. There is no difference in the seasonal changes in the muscles between the two sexes except when degeneration takes place in May which seems to be slightly faster in the female. This agrees with the observation that males continue to fly for a short time after the females have stopped (see suction trap data, Tables 39 and 40). The muscles of the male are marginally smaller than those of the female; this appears to be associated with the slightly smaller size of the male. Other changes that may be connected with those of the flight muscles are seen in the reproductive organs of the female. In spring, the enlarging ovaries project forward gradually into space normally occupied by these muscles and at the height of ovary development, the muscles are partly replaced by the germarium (Figs. 41, 42). In both sexes, the dilated portion of the mid-gut fills part of the space created by muscle autolysis.

The flight muscle area measurements in Fig. 40 are based on the mean muscle area of 1 to 6 beetles on each date. In many instances, although more than one beetle was sectioned to begin with, some suffered destruction during the process of sectioning. Thus out of 156 beetles sectioned, only 102 gave good sections. The results can be taken as reliable because there is not a great deal of variation in muscle size

FIG. 38 : SEASONAL CHANGES IN THE PROPORTION OF MACROPTEROUS SITONA

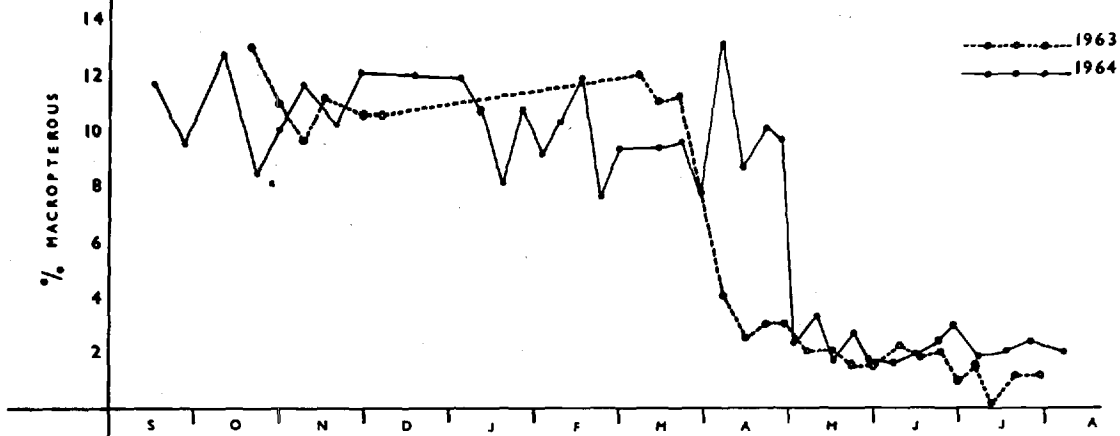



FIG. 40 : SEASONAL CHANGES IN THE QUANTITY OF FLIGHT MUSCLE

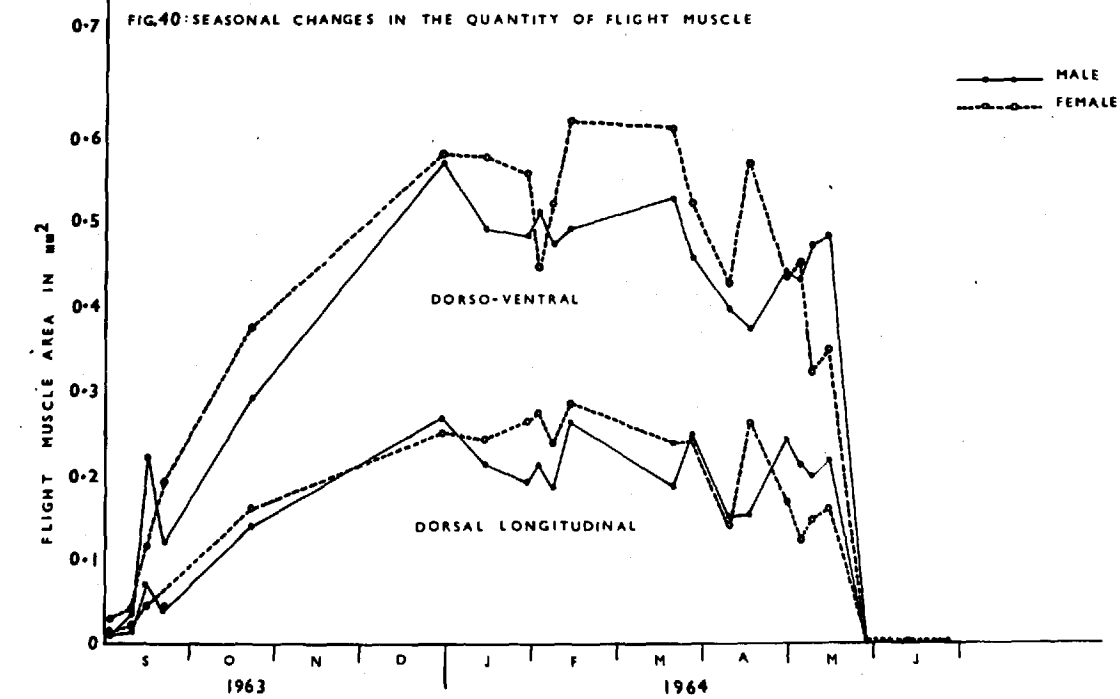
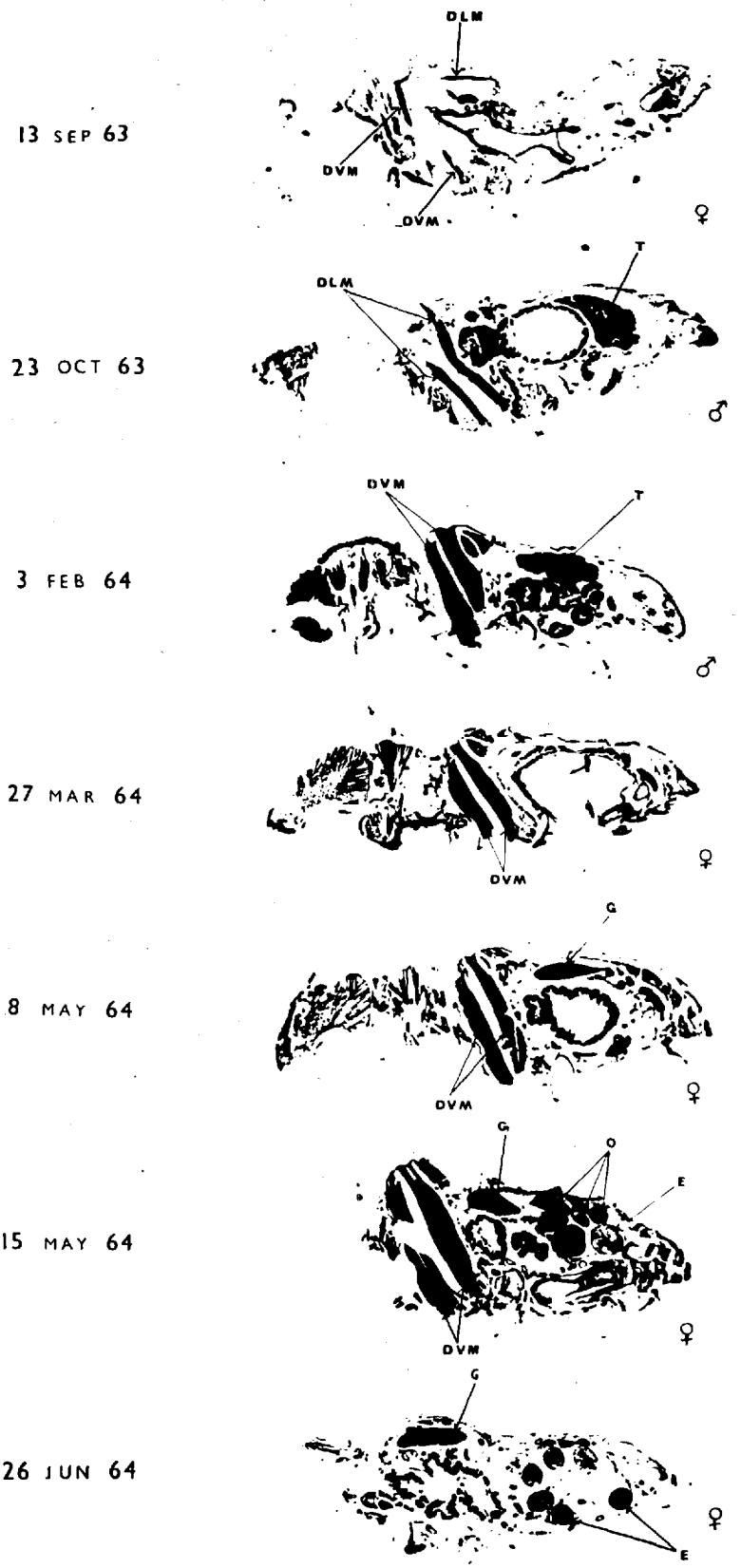
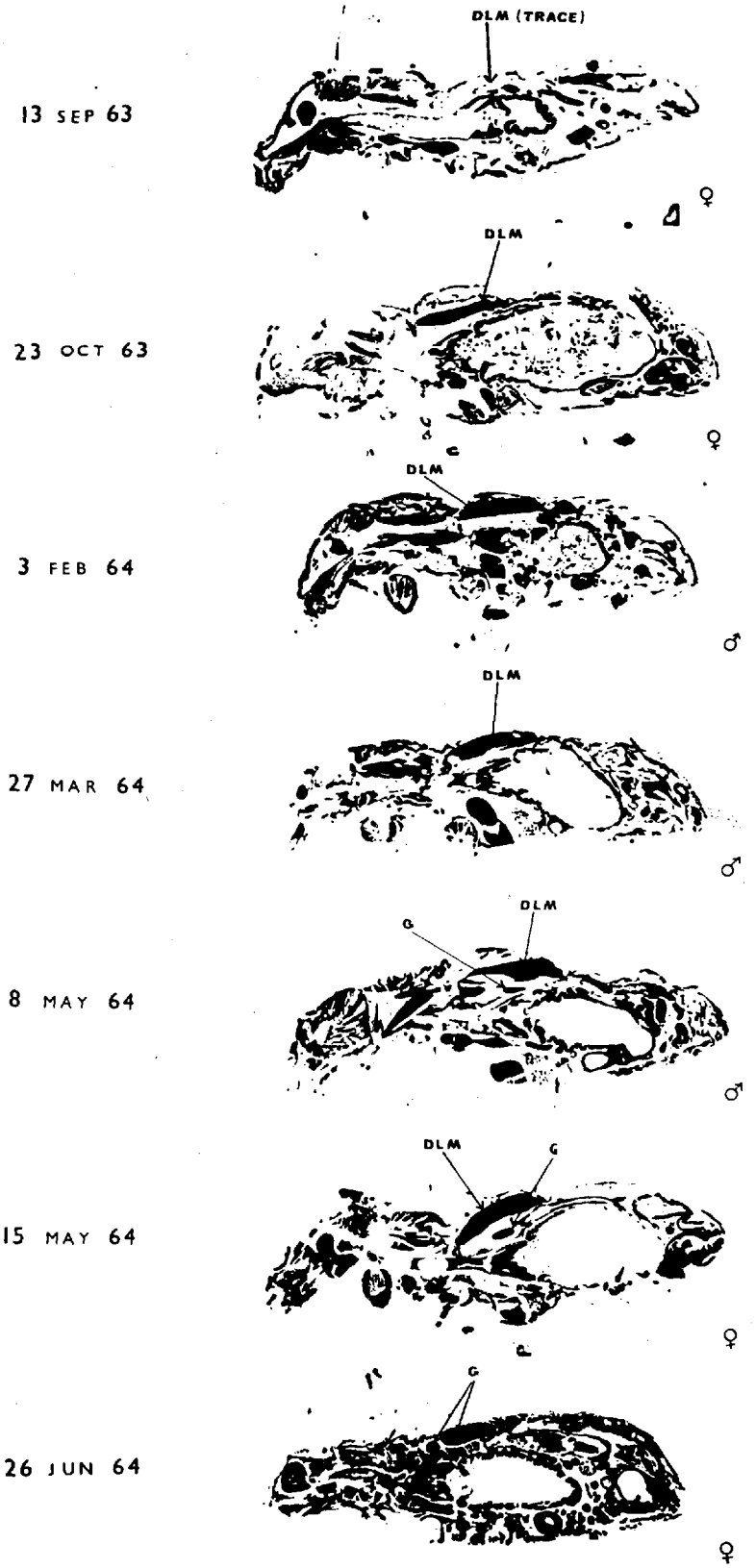


FIG. 41 : L.S. of macropterous Sitona showing dorsoventral muscle.




DVM = Dorsoventral muscle; G = Germarium; E = Eggs.
DLM = Dorsal longitudinal M.; T = Testis; O = Oocytes.

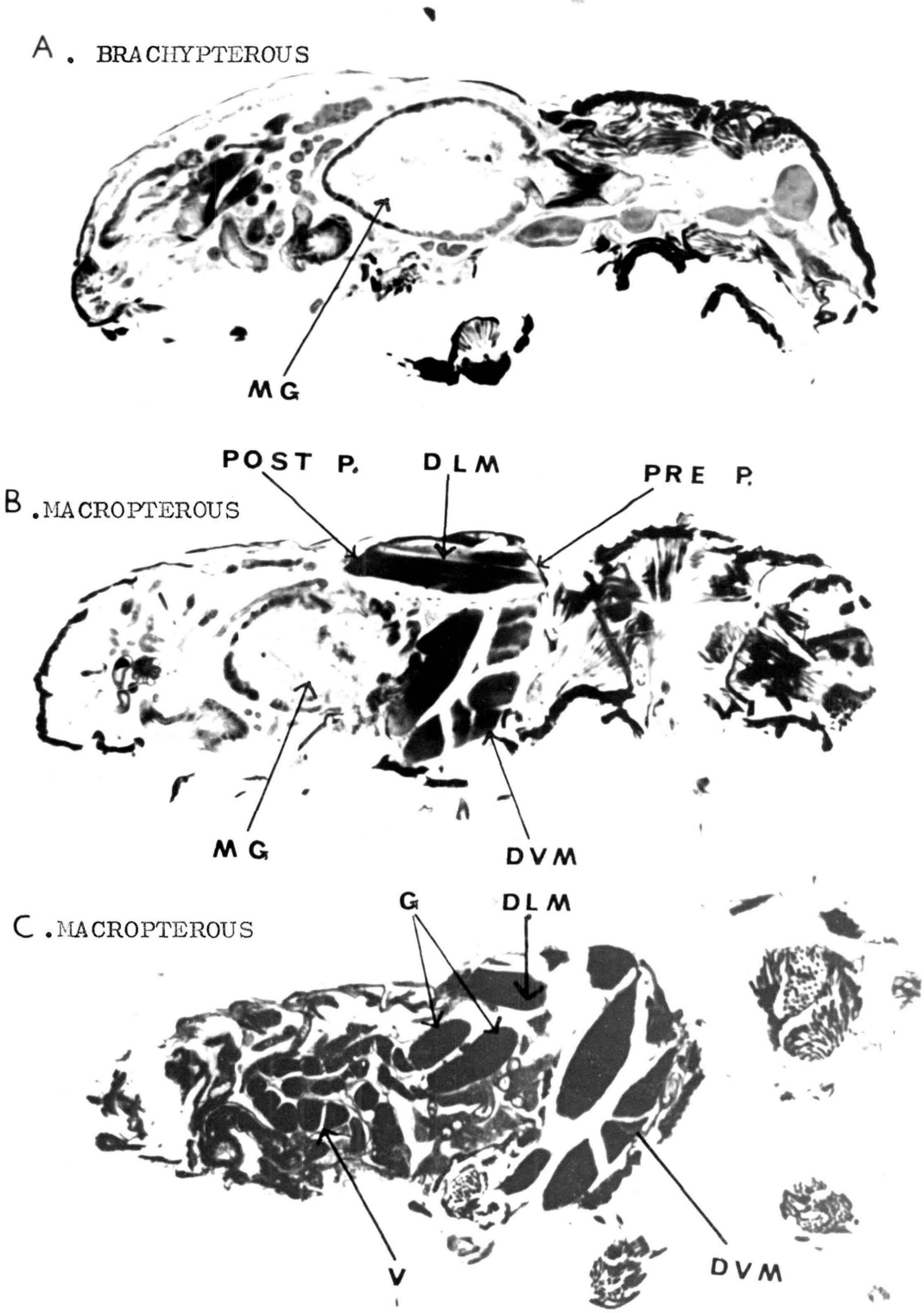
FIG. 42: L.S. of macropterous Sitona showing dorsal longitudinal muscle




DLM = Dorsal longitudinal muscle.

G = Germarium.

FIG. 43




DVM = Dorsoventral muscle; PRE P. = Prephragma.
DLM = Dorsal longitudinal M.; POST P. = Postphragma.
V = Vitellarium; G = Germarium; MG = Mid-gut.

except towards the end of May when muscle autolysis is not simultaneous in all. Some idea of the reliability of these flight muscle measurements can be obtained from Tables 41 and 42.

Table 41. Reliability of flight muscle measurements: dorsal longitudinal muscle.

| Date | No. of Sitona sectioned | Mean area of dorsal longitudinal muscle sq. mm. | Standard Error | Standard error as % of mean |
|---|---|---|---|---|
| 17.4.64 | 5 ♂ | 0.1509 | 0.0137 | 9.1 |
| 8.5.64 | 5 ♀ | 0.1459 | 0.0446 | 30.9 |
| 15.5.64 | 6 ♀ | 0.1574 | 0.0322 | 20.4 |

Table 42. Reliability of flight muscle measurements: dorso-ventral muscle.

| Date | No. of Sitona sectioned | Mean area of dorso-ventral muscle (sq.mm.) | Standard Error | Standard error as % of mean |
|---|---|---|---|---|
| 17.4.64 | 5 ♂ | 0.3695 | 0.0269 | 8.0 |
| 8.5.64 | 5 ♀ | 0.3183 | 0.0479 | 15.1 |
| 15.5.64 | 6 ♀ | 0.3472 | 0.0759 | 21.8 |

In Fig. 43 B, C are shown sections of a female captured during flight on 26.4.64; these are compared with a section of a brachypterous beetle (Fig. 43 A). Points to be noted are the feebly chitinized abdominal and thoracic terga of the brachypterous form, absence of phragmata and muscles and the position of the mudgut. The dorsal longitudinal muscle

of the macropterous beetle (Fig. 43 B, C) measured 0.2491 sq. mm. and the dorso-ventral muscle measured 0.3806 sq. mm. These values can be compared with those of Fig. 36 to obtain some idea of the flight capabilities at different times of the year.

## 13. SOME OBSERVATIONS ON DIAPAUSE.

Sitona regensteinensis is a univoltine species with an obligatory diapause which is terminated during the hibernation period in winter. During this period no copulation or oviposition was observed although the beetles continued to feed whenever the weather permitted. As in many other adult insects the characteristic feature in the diapause in Sitona is the arrest of the development of the reproductive organs. Hence, the first indication of the termination of diapause is sexual maturity. In the field, copulation which does not occur in autumn and early winter may begin as early as the beginning of February provided the weather permits activity. For instance, large numbers of beetles in copula were seen on broom on 29th January, 1964, a day when the temperature was above normal (9.2°C. at the time of sampling) and all females dissected after this date were found to contain sperm in the spermathecae. Other indications of the termination of diapause are seen on dissection. In the female, there is a movement of oocytes from the germarium to the vitellarium; the oocytes become large and conspicuous. In the male, sperm bundles are already present in the testes of a teneral adult at the time of emergence from soil. When a testis from such a male is crushed under a cover-slip with a drop of Ringer's solution and examined, the sperm bundles and individual spermatozoa are seen motionless. This condition prevails throughout the period of diapause. With sexual maturity (termination of diapause) a similar testis preparation shows actively moving spermatozoa; the intact sperm

bundles exhibit 'wave motion'.

Temperature and photoperiod are two factors known to influence diapause in many insects that hibernate in winter. An attempt was made in the autumn of 1964 to determine the effect of these two factors on the diapause of Sitona. A large number of new adults were brought from the field on 10th September. In the laboratory, the insects were divided into groups of 30 (15 males and 15 females) and treated as follows:

(1) One group was kept in a 20°C. constant temperature room under fluorescent light at a 16 hour photoperiod;

(2) Another group was kept in the same room, but within a 8½ hour photoperiod chamber;

(3) The remaining beetles were placed in plastic boxes in the lower compartment of a refrigerator at 4 - 5°C.

Once a week, the containers were filled with fresh twigs of broom as food, because the beetles were always feeding even at low temperatures (unfed teneral adults from emergence traps did not survive low temperatures without food). At the end of each week, 30 males and 30 females were taken from the cold-treatment and kept under the conditions described in (1) and (2). In this way, cold treatments of 0, 1, 2, 3 ..... 12 weeks were given to different batches before subjecting to the 20°C. temperature and 16 and 8½ hour photoperiods.

The length of pre-oviposition period (from the time of cessation of cold treatment) was regarded as a measure of the termination of diapause. Since no gonad investigations were done in this experiment, the time of

male maturation was not established. The effectiveness of the different treatments on the termination of diapause is shown in Table 43.

Table 43. Showing the relationship between the length of cold treatment and the length of pre-oviposition period.

| The length of cold treatment in weeks | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-oviposition period at 16 hour photoperiod | - | - | - | - | - | 161* | - | - | - | 73* | 24 | 14 | 32 |
| Pre-oviposition period at 8½ hour photoperiod | - | - | 189* | 173* | - | 173* | - | - | - | 84* | 19 | 23 | 36 |

*. Eggs laid only by 1 to 4 females.

The results show that the pre-oviposition periods of those specimens treated with cold treatments less than nine weeks were erratic. Although the beetles that were kept in the cold for 9, 5, 3 and 2 weeks are shown in Table 43 to have laid eggs, dissections showed that only a small fraction of the females had mature ovaries, and the eggs laid by these individuals were of poor quality (smaller in size and translucent). None of the females that were given 8, 7, 6, 4, 1 and 0 week cold treatments did lay any eggs even after 224 days; some of these died during this period and the survivors showed no signs of ovary maturation on dissection. It appears that cold treatments of less than ten weeks do not terminate the diapause effectively. There was no notable difference in the pre-oviposition periods of the beetles kept in the two regimes of light indicat-

ing that photoperiod has no effect on diapause in this species. In contrast, Hans (1961) showed that diapause in Sitona cylindricollis is influenced by both temperature and photoperiod and these two factors can be interchangeable in effect on the termination of diapause. It could be concluded that in Sitona regensteinensis, the termination of diapause can be brought about in the laboratory by a cold treatment of 4 - 5°C. for ten weeks followed by exposure to normal day lengths at 20°C. It is not possible to state definitely whether photoperiodism has any effect or not on the induction and termination of diapause without further experimentation.

## 14. STUDIES ON REPRODUCTION.

### 14.1. Laboratory Observations.

Results obtained on fecundity from laboratory observations were partly discussed in section 7.4 (page 64). The type of cage used was a plastic petri-dish divided into a small and a large compartment by means of a strip of celluloid (Fig. 44). The small compartment contained two pieces of moist cotton wool. A small twig of broom in the large compartment passed through a hole in the celluloid strip to rest between the pieces of cotton wool. The moist cotton wool maintained a high humidity and the piece of broom served as food. The cages were cleaned twice a week and food and water renewed. The Sitona used in all oviposition observations in the laboratory were kept in these cages, in pairs (a male and a female). In 1963, thirty male and thirty female Sitona were brought from the field in spring before oviposition began and kept in cages in a 25°C. constant temperature room. The counts of eggs laid were made daily between 9 and 10 in the morning. Of the 30 females, four died soon, without laying any eggs as they were parasitized by Centistes.

The length of the pre-oviposition period was 5.2 days and its limits 4 - 10 days. In 1964, 35 females were used and two died of parasitism and unlike 1963, no daily egg laying records were made. Instead, the eggs were counted twice a week. The results for the two years can be summarised in Table 44.


FIG. 44. Laboratory egg-laying cage.

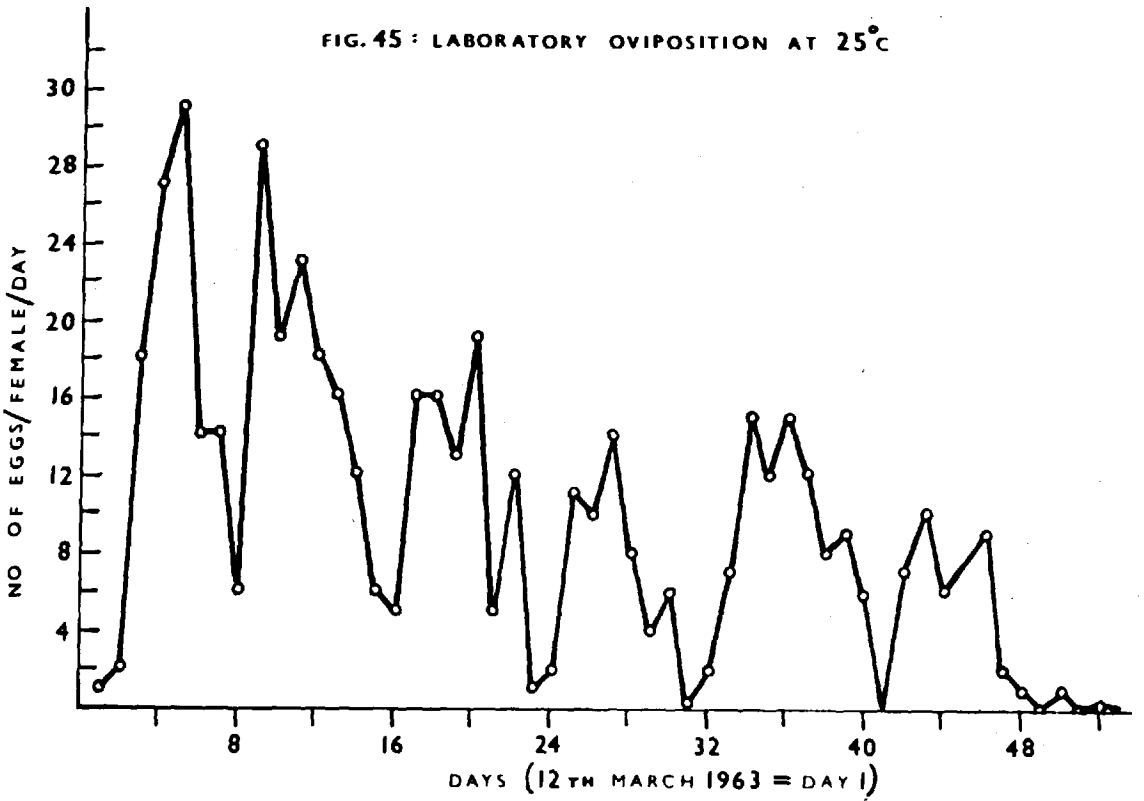



Fig. 47. Periodicity of oviposition of ten females at six hourly intervals.

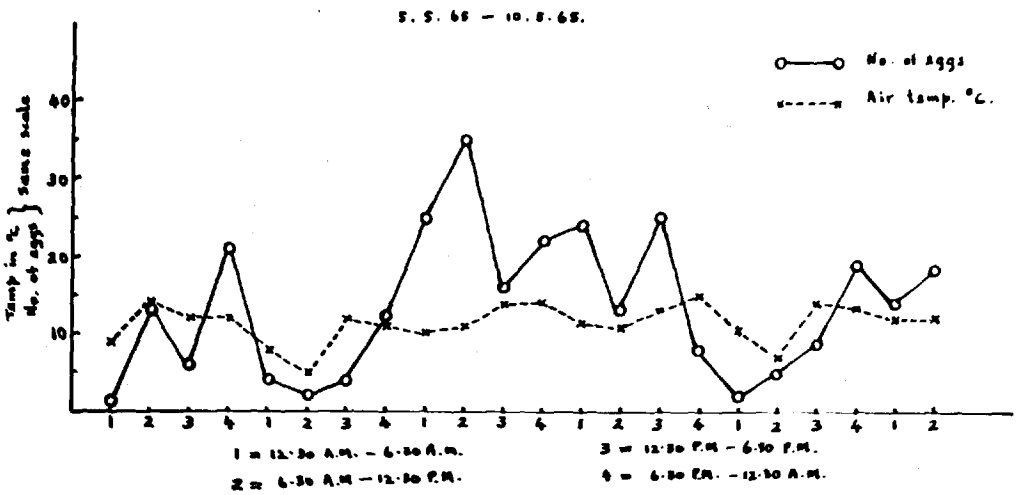


FIG 46 THE OVIPOSITION RECORDS OF FIVE FEMALES

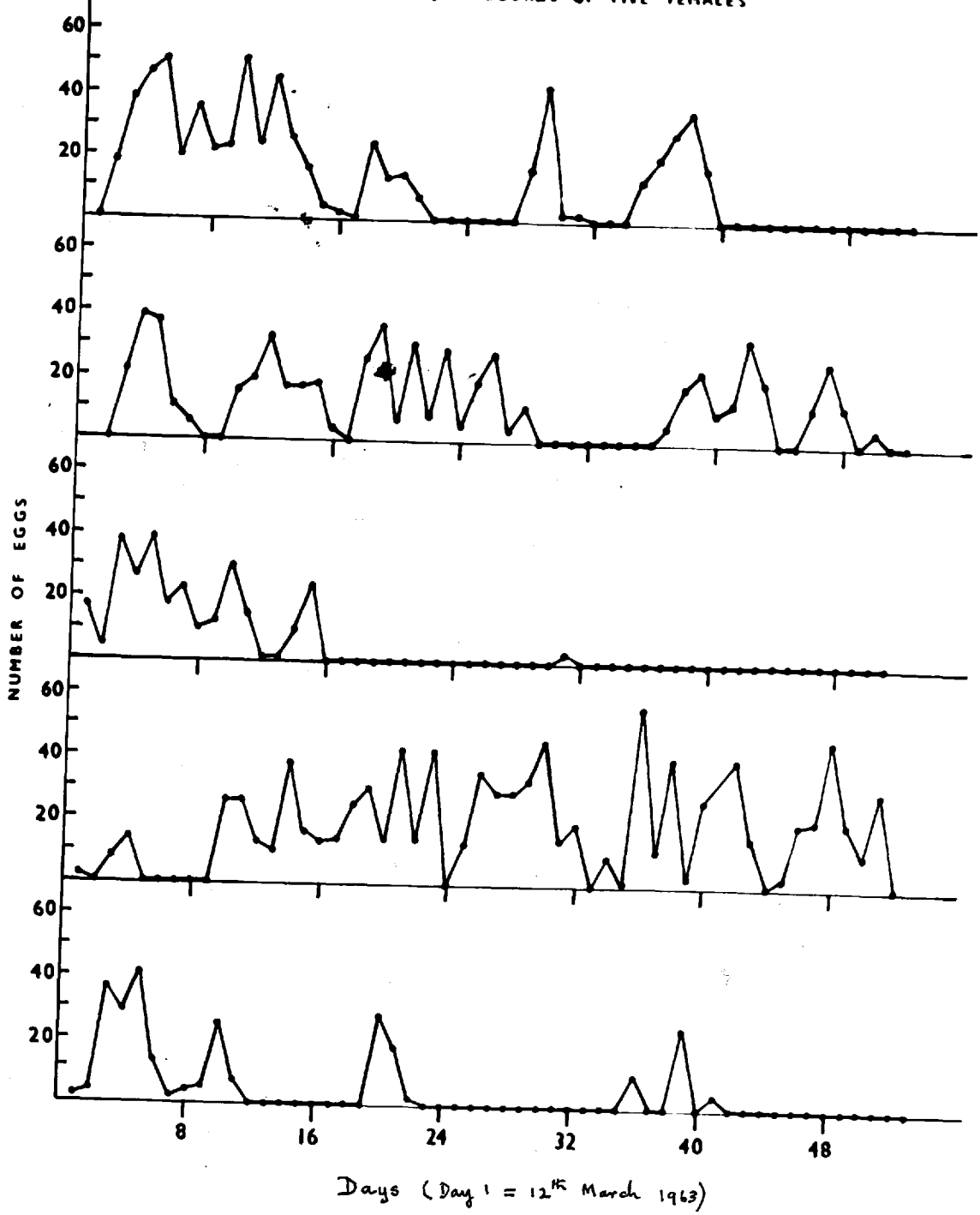


Table 44. Oviposition in 1963 and 1964 at 25°C.

| Year | No. of Laboratory Females | No. of eggs per female | |
|---|---|---|---|
| | | Limits | Mean |
| 1963 | 26 | 156 - 1258 | 437 |
| 1964 | 33 | 2 - 475 | 197 |

When the mean number of eggs per female per day is plotted against time for the whole period of oviposition, a marked periodicity in the rate of egg laying is seen (Fig. 45). Each egg laying period lasts about 4 - 5 days followed by a two-day period of rest when a low number of eggs are laid. Although it may seem surprising that average values from 26 females show such a periodicity, the same is indicated when the oviposition records of each female is examined. To illustrate this, the egg laying records of five females selected at random are shown in Fig. 46. This can be considered to be an indication that feeding and maturation processes occur simultaneously in all adults of the same population. In many females, laying is continuous throughout the period of oviposition, and in some there are breaks lasting up to two weeks (see Fig. 46). The usual number of eggs laid in a 24 hour period lies between sixteen and twenty, while the greatest number laid was 65 (see Table 45).

During the period of oviposition, Sitona lays almost continuously throughout the 24 hours although more eggs are laid at higher temperatures. Above 10°C., the temperature does not seem to have much effect on oviposit-

Table 45. The frequency of the numbers of eggs laid in 24 hours by 26 laboratory females, 1963 at 25°C.

| No. of eggs: | 0 | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 |
|---|---|---|---|---|---|---|---|---|---|
| No. of records: | 470 | 68 | 24 | 67 | 74 | 46 | 43 | 21 | 30 |

| 46-50 | 51-55 | 56-60 | 61-65 |
|---|---|---|---|
| 11 | 12 | 6 | 2 |

ion rate, but below 10°C. there is a marked reduction (Fig. 47). Some of the females survive the second winter and oviposit for the second time. In 1963, six laboratory females survived and of these four oviposited while the other two died without laying. In 1964 three laboratory females survived of which two died without laying. The third did not lay and was dissected on 17.5.65; the ovary appeared shrunk and showed no signs of egg development. The total fecundity of the four 1963 females that laid in the second season is given in Table 46.

Table 46. Fecundity of 4 females ovipositing in two successive seasons.

| | | | | |
|---|---|---|---|---|
| Total fecundity in 1st season | 538 | 261 | 255 | 984 |
| Total fecundity in 2nd season | 32 | 196 | 174 | 680 |
| Total | 570 | 457 | 429 | 1664 |

14.2. Changes in the Reproductive Organs of the Field Adults.

Changes in the reproductive organs were studied by dissection of weekly samples of 30 males and 30 females. In making dissections, the insects were anaesthetized with ether and placed on a slide with a few drops of Ringer's solution; the elytra were removed and the thin terga of the pterothorax and the abdomen peeled off with a fine pair of forceps. The ovaries were then teased free.

14.21. The female reproductive organs.

Sitona has bilateral ovaries, each consisting of two ovarioles, the number of ovarioles being characteristic for Curculionoidea (Robertson, 1961). On one occasion (8.5.64), a single female was seen to have three ovarioles in each ovary, which can be taken as an abnormal condition. As in many other Coleoptera, the ovariole is meroistic and telotrophic. Each comprises a terminal filament, germarium, vitallarium and a short pedicel (see Fig. 48). The pedicel of each pair of ovarioles opens into a lateral oviduct. The two lateral oviducts join to form the common oviduct. The bursa copulatrix is absent, the vagina is short, stout, ovoid and without a sclerotised tubular part.

In the insects collected in autumn, there are no oocytes in the vitellarium, which is markedly shorter than in the mature beetles. As the beetles become older, the young oocytes begin to move from the lower portion of the germarium through the pre-follicular tissue and into the vitellarium. This may occur as early as the last week of November in

some individuals. As the oocytes migrate to the vitellarium, some of the pre-follicular cells form an envelope around them. With the increase in size of the oocyte, the follicular cells form into a single layer of columnar cells and give rise to the follicular epithelium. As the egg or the fully developed oocyte enclosed in its chorion leaves the vitellarium, the follicular cells are left behind at the junction of the ovariole and the lateral oviduct. This tissue, the corpus luteum is clearly discernible towards the later part of the oviposition period and at the termination of oviposition. The corpora lutea are seen as nodule-like structures formed of clumps of brown cells. They stay in this condition for some weeks before being resorbed.

Females can be broadly classified into four age groups based on the progressive stages of ovary development:-

Stage 1. Immature ovary with no oocytes in the vitellarium (presumably diapause or pre-diapause ovary). Fig. 48 A.

Stage 2. Numerous oocytes in the vitellarium, but incomplete maturation indicated by the absence of chorion. Fig. 48 B.

Stage 3. Fully formed eggs in the lower part of the vitellarium or in oviduct or in both. Oocytes in various stages of development and corpora lutea may be present. Fig. 48 C.

Stage 4. No fully formed eggs in the vitellarium or oviducts. Corpora lutea present, large and distinct. Resorption of oocytes indicated in the vitellarium. (The female may be called senescent). Fig. 48 D.

The seasonal percentage distribution of the females belonging to these four categories in 1963 - 1964 is shown in Fig. 49. It will be noticed that stage 2 is short and thus appears in low proportions and is probably a transitory one. Fig. 49 shows that Sitona spends about six months in stages one and two, about four months in stage three and two months in stage four, before the arrival of new adults. Death occurs in stage four and many beetles do not survive the whole two month period.

The length of the ovaries of the dissected females were measured from the apex of the germarium to the end of the lateral oviduct. These measurements are shown in Fig. 50. In Fig. 51, the total number of eggs found on dissection is shown. The ovary lengths and egg counts of the brachypterous form are based on 25 - 30 dissections and those of the macropterous form on five. The length of the ovary at the time of emergence is from 1.2 to 1.5 mm. This increases at a slow rate to 3 - 4 mm. during the autumn and winter. The growth accelerates in spring and summer. The ovary reaches its maximum length of 8 - 9 mm. in May - June, which is the peak oviposition period. Towards the end of oviposition, the ovaries rapidly shrink back to about 4mm. A significant difference in the rate of ovary growth of the two dimorphic forms is indicated in Fig. 50. The ovaries of the brachypterous female grow faster than that of the macropterous female and in latter the egg formation was delayed; the time lag appears to be more than a month. In 1964, the first brachypterous female with fully developed eggs was seen on the 14th of February whereas in the macropterous form, the eggs were first seen on the 17th of April. Similarly, in 1963, the first

FIG. 48.

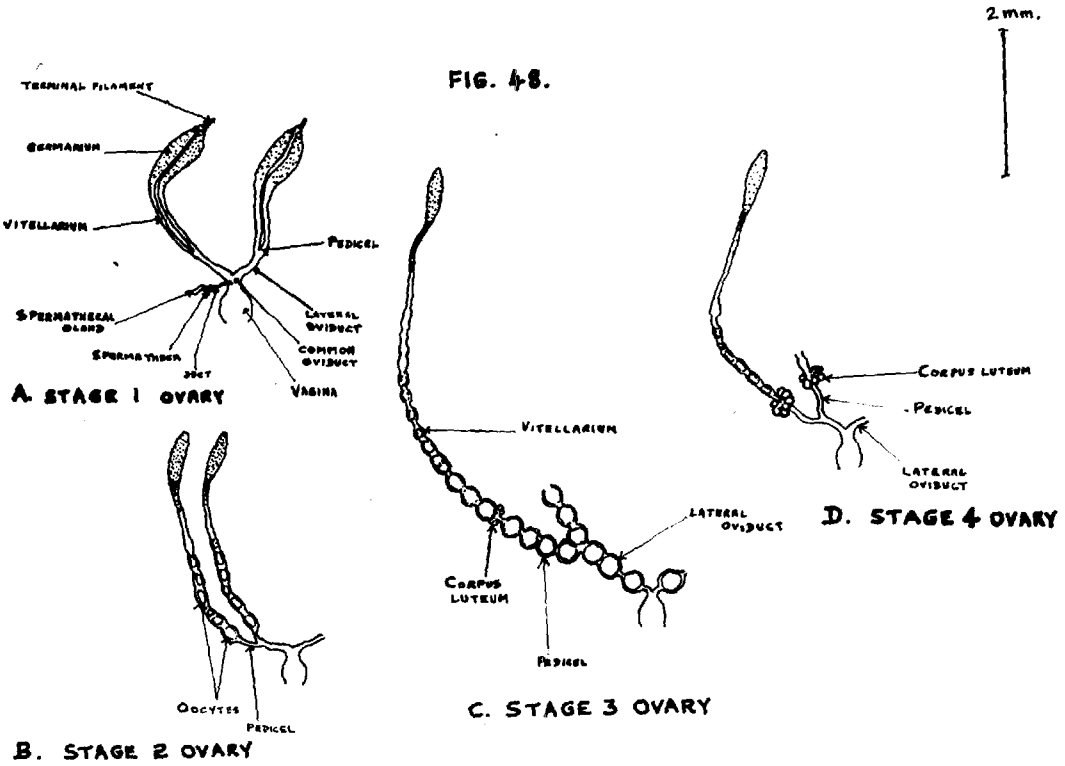



FIG. 49

FIG. 49. PERCENTAGE OCCURENCE OF OVARIES OF DIFFERENT STAGES

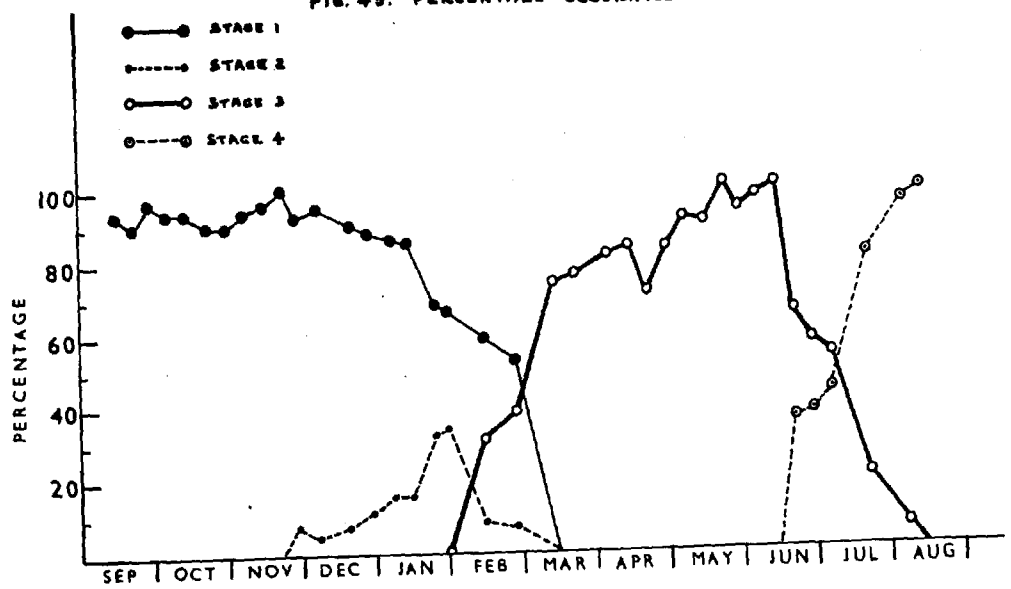


FIG:50 SEASONAL CHANGES IN THE OVARY LENGTHS OF DIMORPHIC FORMS

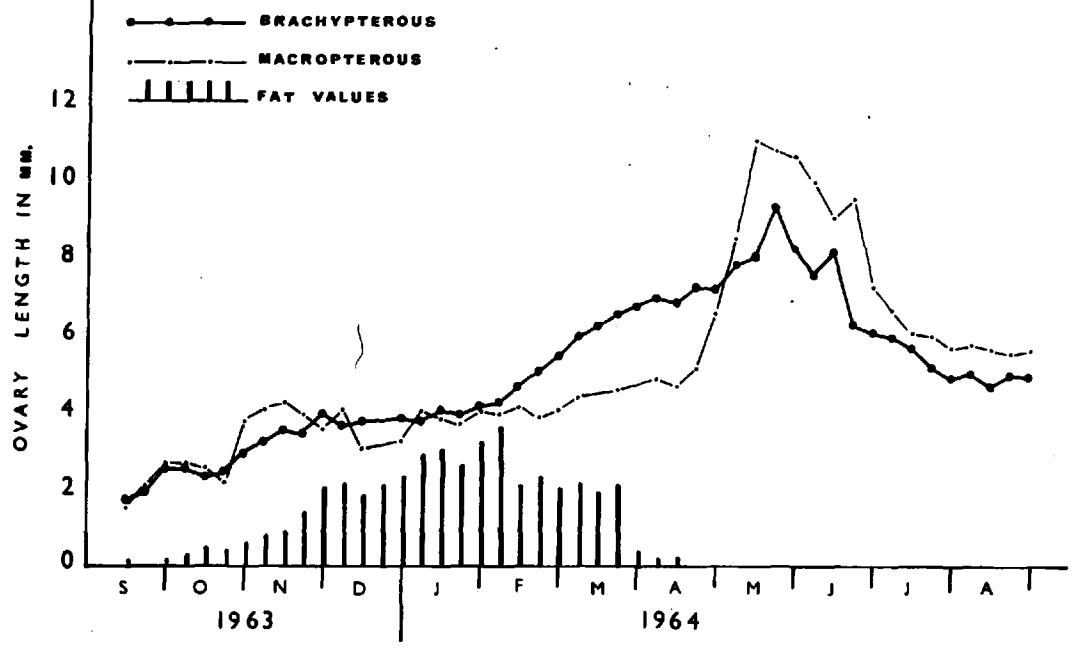



FIG:51 COMPARISON OF EGG No. IN THE DIMORPHIC FORMS

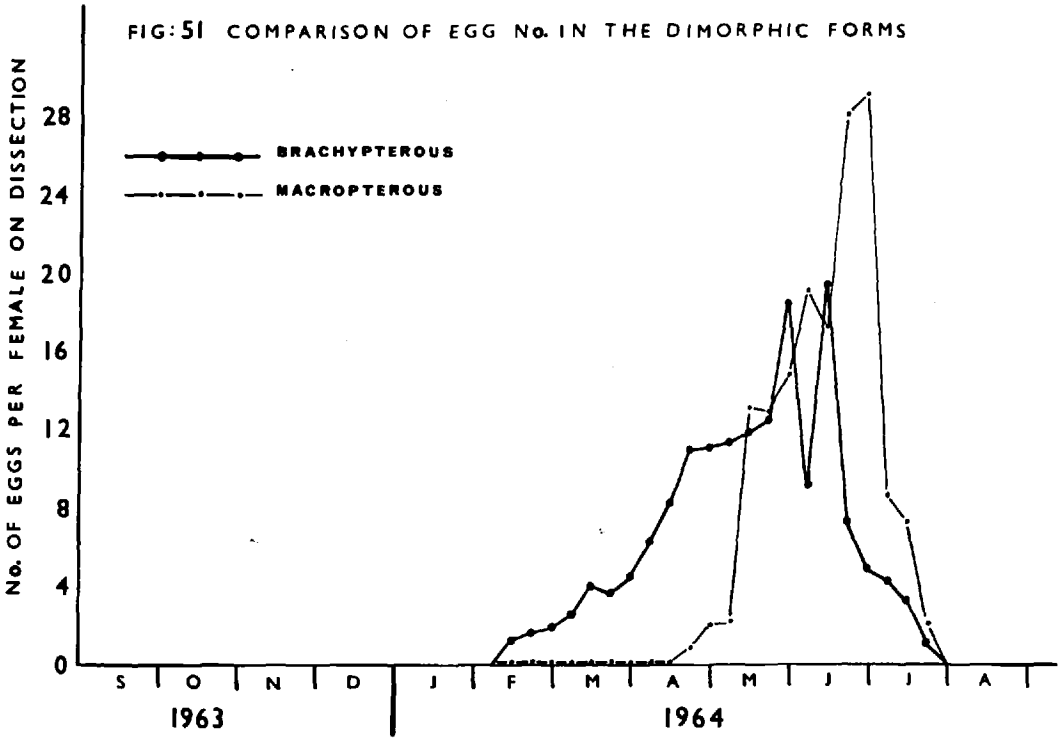
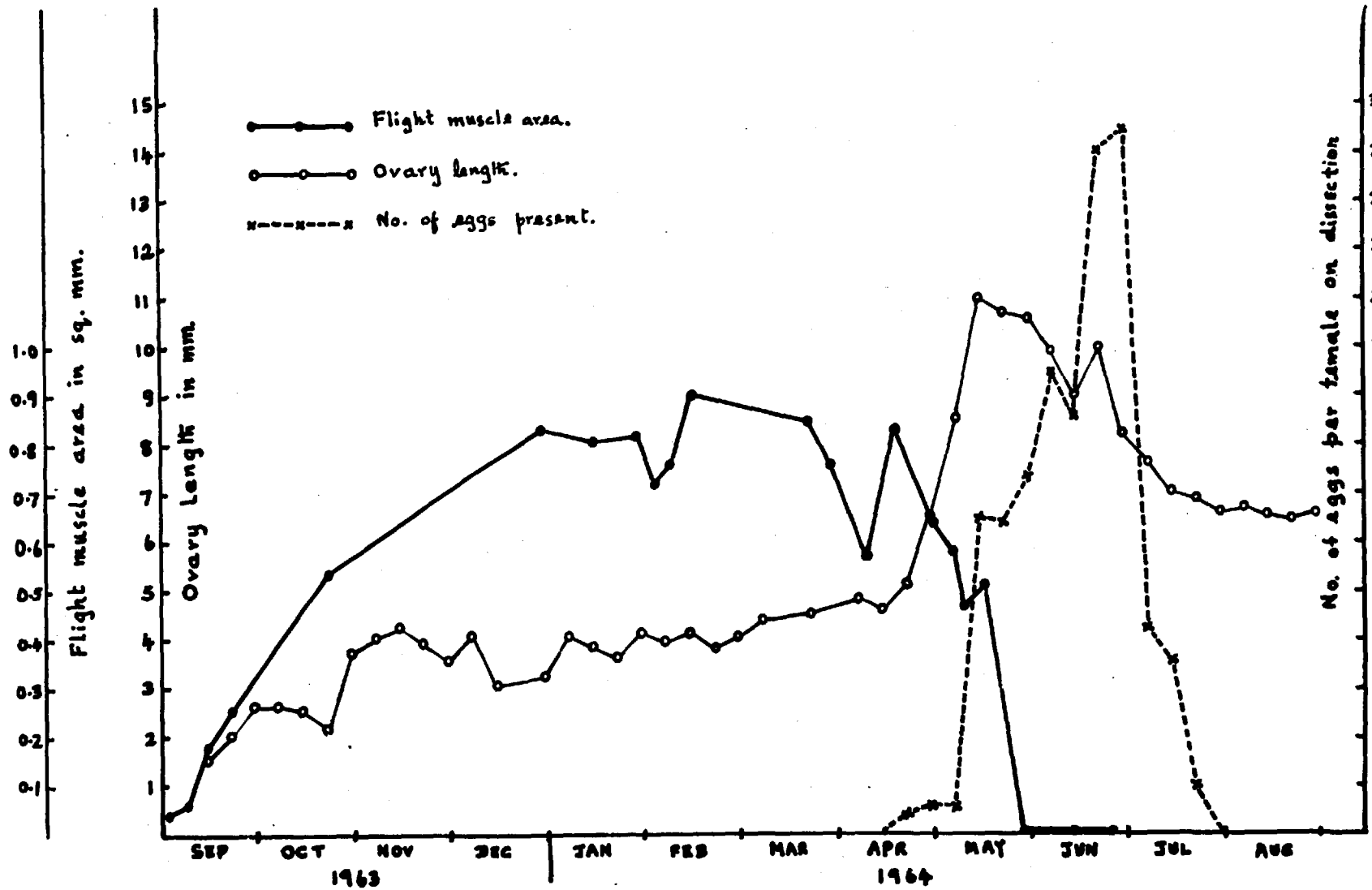


Fig. 53. Flight Muscle Growth & Ovary Development.



brachypterous female to contain eggs was seen on the 8th of March and the first gravid macropterous female was detected on the 6th of May. Thus it appears that the growth of eggs is delayed by over a month in the migratory form and during this period of delay, flight occurs. These observations are also supported by the suction trap catches, since the trapped females did not possess any fully formed eggs, but had stage-2 ovaries.

14.22. Interrelation of migration and reproduction.

The timing of migration and reproduction of the macropterous beetle shown above can be explained on the basis of a hypothesis put forward by Johnson (1963). Johnson states that "Neurophysiological processes cause the characteristic prolonged and undistracted migratory flight, but ecological factors determine when it occurs. Because migratory species, or genotypical migrants, migrate with immature ovaries, factors that control ovary development probably control migration". Evidence in favour of this hypothesis has been taken from many diverse groups of insects and is fully discussed in his paper. Sitona regensteinensis provides a unique example where a difference in the physiology of the migratory and the non-migratory forms of the same species can be seen under natural conditions.

Although migratory flights occur just prior to oviposition, the beetles collected in late autumn and winter can be induced to fly in the laboratory for very short periods, when light and high temperature are provided. The flight muscle 'curve' itself shows that there is sufficient

muscle capacity from December to May. Since it has been noticed that flights occur only on sunny and warm days, the ecological factors that determine the time of flight seem to be light and temperature.

In both sexes, the muscles autolyse soon after migration; the beetles captured after the middle of June do not contain muscles. In the female, autolysis of the flight muscles is synchronized with the development of the ovaries, and no muscles remain at the height of ovary development (Fig. 53). The fact that autolysis also occurs in the male suggests that ovary development is unlikely to be the cause of this autolysis, although some substances may be utilised by the developing eggs.

14.23. Changes in the fat body.

The fat body is not noticeable in the newly emerged insects, but increases in size during autumn feeding and persists without much change during the winter. With the commencement of oviposition, the fat content is abruptly reduced. To describe the seasonal changes in the size of the fat body, arbitrary values were given to the amounts of fat in dissected females. These values were: 4, denoting a fat body filling all the available space of the haemocoele; 3, large; 2, small; 1, trace of fat and 0, fat altogether absent. The results are shown in Fig. 50. In addition to fat, large quantities of food and water in a semi-fluid form accumulate in the mid-gut, prior to hibernation. The fat body of a new adult appears creamy white in colour and that of an old beetle dense yellow. This combined with the condition of the gonads helps to separate the old

from the new insects. The ovaries of old females are slightly larger and possess corpora lutea or their traces. In the young male, the testes are white and the adeagus brown, and in the old male the testes are yellow and the adeagus brownish-black or black.

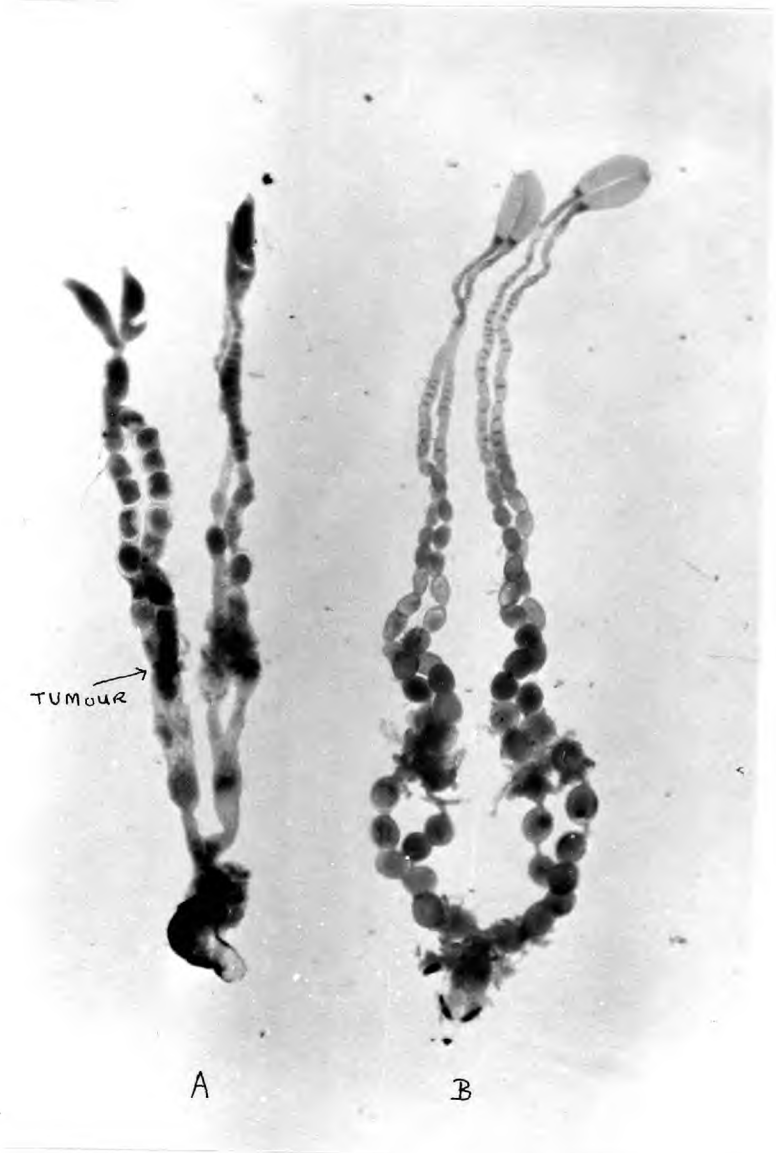
14.24. Factors that inhibit ovary development.

There are two factors that prevent or suppress the development of the ovary and egg formation. These are: (1) parasitism by Centistes and (2) the formation of tumour-like structures on the body organs.

Parasitism by Centistes disrupts the normal functioning of the reproductive organs of Sitona. This phenomenon has been termed 'parasitic castration' by Wheeler (see Doutt, 1963). When the new autumn generation females of Sitona are parasitized, the ovaries of the host cease to mature and remain in the original size without any noticeable growth. The summer parasitized hosts have well developed ovaries with eggs at the time of parasitism, but within a few days a progressive atrophy occurs. The notable feature of this ovary degeneration is the disappearance of yolk granules from the oocytes and eggs and later the oviducts become devoid of eggs. This indirect effect of parasitism is considered to have a nutritional basis by many who have studied parasitism of Coleoptera by Euphorine Braconids (see Doutt, 1963).

Changes in the structure of the ovary almost identical to that described above were observed in many specimens of Sitona which had tumour-like structures. The literature on insect tumours has been reviewed recently by Harker (1963). The structures seen in Sitona closely resemble those

described in this review. In specimens examined, these structures appeared as 'growths' or swellings in the fat body, on the gut wall, testes or on the ovary; these consisted of masses of tissue with a black core, presumably formed of melanized cells. The production of melanin is sometimes regarded as a method of disposing of toxic phenols arising as breakdown products in metabolism. Harker (1963) states that all the evidence suggests that benign, or pseudo tumours are due to an aggregation of haemocytes, either as a group or around specific tissues, and that this in turn stimulates the haemocytic reaction of melanization. A photograph of an ovary containing a tumour is shown in Fig. 54; for comparison a healthy ovary taken on the same date (8.5.64) is shown by the side. Points to be noted are the change in the colour of the two ovaries, particularly in the germaria and traces of egg-atrophy in the oviduct; the black mass is the core of the tumour. The incidence of these tumour-like structures appeared to be about 3 - 5 per cent. It was noticed that the presence of tumours in any part of the body caused the same reaction in the female reproductive organs. In males, although the tumours were present, no changes were visible in the testes.




A. Ovary with tumour.

B. Healthy ovary.

## 15. OBSERVATIONS ON THE BIOLOGY OF CENTISTES EXCRUCIANS HAL.

Some aspects of parasitism by Centistes were described in section 8.11 (page 73). Accounts of the biology of Centistes excrucians as a parasite of Sitona scissifrons Say. in Canada have been already published by Loan (1963, 1964). The observations described here may provide additional information to that given in the above papers.

The amount of parasitism of Sitona regensteinensis by Centistes was recorded during the weekly dissections of 60 beetles. The seasonal changes in the percentage parasitism are shown in Fig. 55, which also indicates the time of larval emergence (that is, when percentage parasitism was zero). Although the percentage parasitism during the first generation of the parasite appears to be low, the total parasite population is greater than in the second generation (see section 8.11). The greater degree of parasitism of the second generation of Centistes is the result of low host numbers available to the parasite at that time of the year (see Figs. 11, 14, 15).

In the spring of 1964, five hundred beetles were brought from the field to obtain parasites. They were kept in an outdoor insectary, in large glass jars with broom cuttings. The floors of the jars were lined with a mixture of sand and dead leaves which was watered at intervals. A second group of insects were kept in plastic petridish cages to observe the period of emergence of parasite larvae. The larval emergence extended from the 13th to the 20th of May. The emergence of the wasps in jars began on the 7th of June and continued until the 13th of June. The pupation period is

thus approximately three weeks. The details of the emergence of adult Centistes in jars are as follows:

| Date (June, 1964) | 7th | 8th | 9th | 10th | 11th | 12th | 13th |
|---|---|---|---|---|---|---|---|
| No. of Centistes emerged | 6 | 8 | 14 | 6 | 3 | 1 | 1 |

This period of emergence agrees with that indicated by the dissections (Fig. 55).

The newly emerged wasps feed on diluted honey immediately after leaving the cocoons, and there is no pre-oviposition period. Oviposition is done by inserting the sub-exerted ovipositor through the membrane between the base of the coxa and the coxal cavity of any of the legs. For this, the parasite grabs the beetle on the side across the thorax, curves its abdomen under the thorax of the beetle and piercing movements are made until the coxal cavity is found. The oviposition itself lasts only a few seconds. In the presence of more than one Sitona, Centistes oviposits without any discrimination between parasitized and non-parasitized beetles so that it may oviposit in the same insect over and over again. Centistes becomes agitated in the presence of the beetle and the same amount of agitation was shown when it was exposed to other weevils such as Otiorhychus, Apion or Strophosomus and attempts were made to oviposit in these weevils, but without success; probably the method of oviposition needs the right shape of the host body since Centistes is able to parasitize other species of Sitona.

Under laboratory conditions, the wasp can be made to lay a large number of eggs within a single host. Yet only a single egg succeeds in

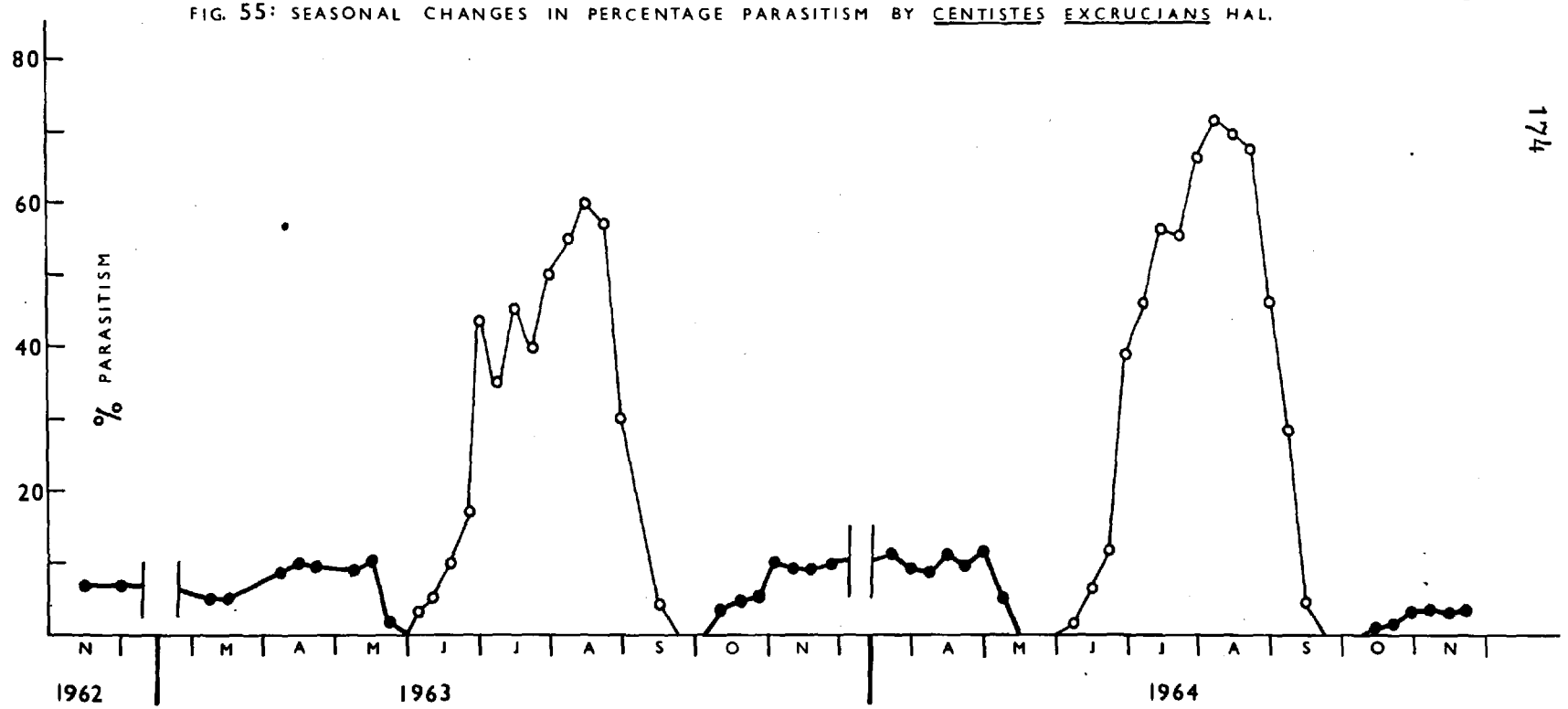
developing beyond the first instar larval stage. The supernumeries die soon after hatching and in dissections, are seen in malformed or partly dissociated condition with or without black areas (presumably of melanin) on some parts of the body (Fig. 56). In nature, usually only a single egg is laid in each beetle. This is specially so in the autumn when large numbers of Sitona are available to the parasite. In summer, however, beetles with more than one parasite larva are found more commonly (Table 46).

Table 46. Number of Centistes excrucians larvae per host beetle. Dissections 1962 - 1965.

| No. of Centistes larvae per host. | Frequency of occurrence in Autumn | Frequency of occurrence in Summer |
|---|---|---|
| 1 | 219 | 338 |
| 2 | 4 | 36 |
| 3 | - | 1 |
| 4 | - | - |
| 5 | - | - |
| 6 | - | 1 |

In the laboratory, the parasite lives only for 3 - 5 days. Ten wasps laid an average of 28.2 eggs ranging from 18 to 41 eggs before they died.

FIG. 55: SEASONAL CHANGES IN PERCENTAGE PARASITISM BY CENTISTES EXCRUCIANS HAL.



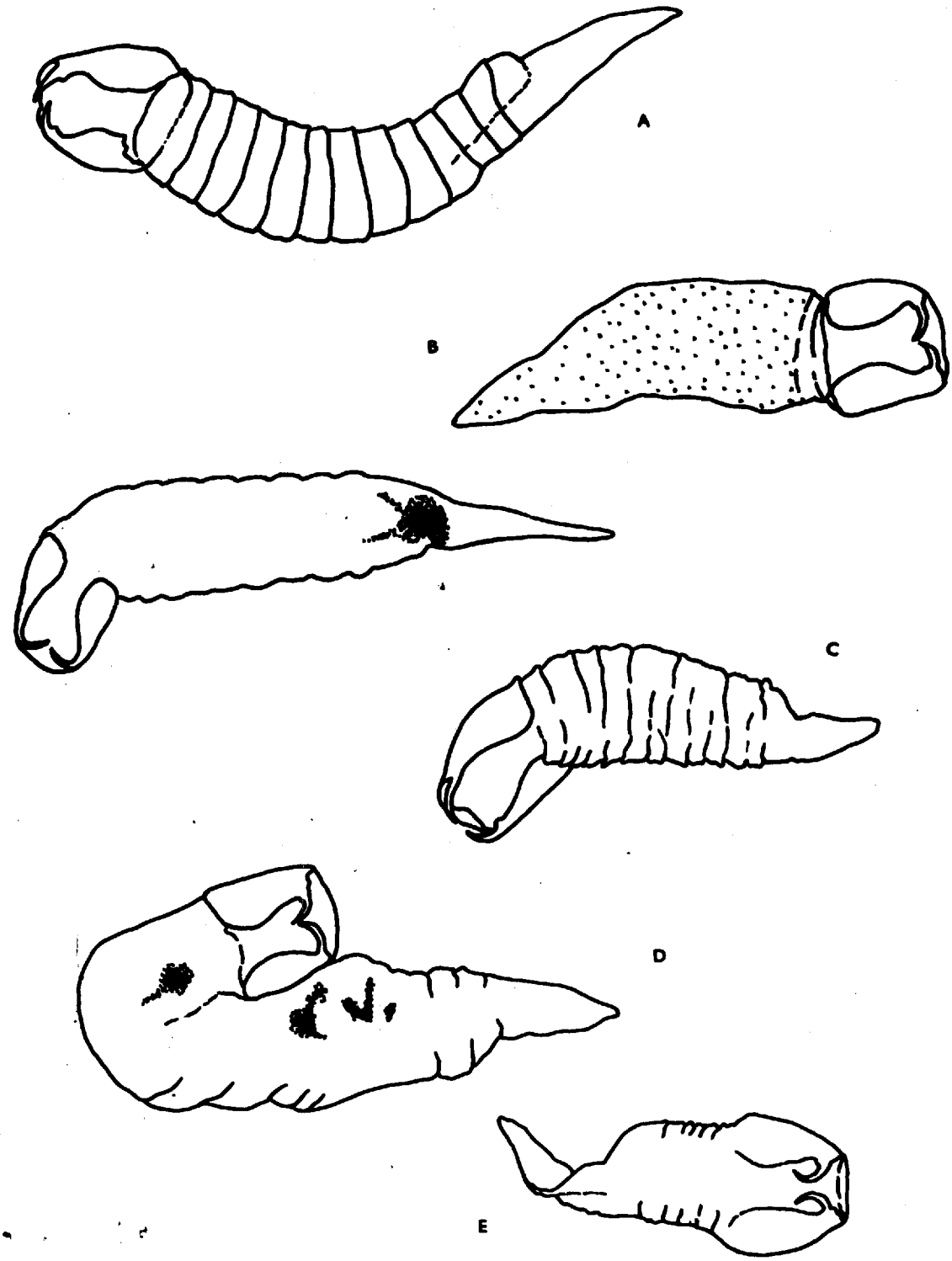



FIG. 56: FIRST INSTAR LARVAE OF CENTISTES
A. NORMAL; B–E. MALFORMED

x 131

## 16. DISCUSSION.

The factors likely to be involved in population changes in insects may fall into several groups which are rarely independent of one another. These are: the climatic (or weather) factors, factors of the habitat, the food or the food plant, the effects of other organisms and intraspecific factors. Collectively, these can be called environmental factors provided one defines the environment with respect to the individual. According to Milne (1962), if the individual is regarded as fulfilling itself when it fully realizes its maximum potentialities for speed of development, amount of reproduction, and length of survival, the effective environment is then everything else in the universe which helps or hinders fulfilment of the individual.

It is intended here to discuss how these components of the environment influenced the population changes of Sitona during the period of study and then to attempt to make some general conclusions. A broader aspect of the problem would lead to a discussion on "how the populations of Sitona regensteinensis are regulated in nature", but although this would be the ultimate aim of a study of population dynamics, such a discussion cannot be based on a study which has lasted only two and a half years.

It is generally accepted that the range of distribution of animal species can be determined through the operation of climatic factors (see Allen et al., 1949; Birch, 1947; Richards, 1961). Climatic factors usually influence populations by reducing or increasing the reproductive rate, and

this with other factors such as emigration, immigration and mortality determines the abundance of a species in a particular region. Climate (or the weather) influenced Sitona mainly by the way of temperature. Temperature was seen to influence fecundity, diapause, movement of the adult within the habitat as well as migration. The rate of oviposition depended on the temperature; the amount of variation caused by temperature was in the region of 20% of the total variability in the rate of oviposition (Table 14, page 70). Since temperature regulated the movements of the beetle from soil to the foliage and vice versa, it determined the length of time the beetle remained above the ground; this indirectly controlled the amount of feeding and hence fecundity. Thus an early warm spring could advance the commencement of oviposition and in this way increase fecundity. In 1964, an early oviposition (compared to 1963) was seen, but there was no apparent increase in fecundity because temperature was not the only factor that influenced the oviposition rate.

A major impact on the Sitona population was made by several other organisms in the habitat. The most noticeable of these were: the parasite of the adult, Centistes excrucians Hal., the predators belonging to the Acarina, Staphylinidae and Carabidae and the parasitic fungus Beauveria bassiana.

Centistes excrucians Hal. by itself did not have a great effect on the size of the population of its host. In other words, parasitism by Centistes is probably not a major population regulatory factor. The second generation of the parasite depends on a declining host population and this

keeps the parasite numbers low. Thus when the host numbers are at a maximum, the degree of parasitism is low and the converse is true when the host population is low. It seems therefore that the Centistes population is dependent on the density of Sitona rather than the host density on the parasite. This relationship may be called a density-inverse process (c.f. Holling, 1961; Solomon, 1964). Many natural enemies are said to behave as inverse factors under certain environmental conditions, or when the ratio of enemies to prey is low (Solomon, 1964). In this instance the reason for the inverse effect appears to be that the parasite is not well adapted to the life cycle of the host; for Sitona is univoltine and Centistes bivoltine.

On the other hand, the degree of parasitism in the Sitona population that would live for a second year is high and reached approximately 50% in both years. This shows that the parasite is capable of reducing the population of the old beetles by half so that parasitism by Centistes in this respect influences the number of Sitona that will live for a second year. This could be important since some beetles are able to live and oviposit for two years. Therefore, Centistes excrucians can be considered as a parasite that has little influence on the Sitona population as a whole, but has the ability to act as a 'safety valve' which diminishes the numbers of survivors of the old population.

Of the predators, mites and Staphylinids were shown to be predatory on eggs and reduced the total laid by about 40 per cent. The predatory mites found in the soil samples solely belonged to the family Parasitidae. Although only Pergamasus crassipes L. was proved to be predatory, it is almost certain that other species such as P. septentrionalis Ond. and Parasitus

spp. too were responsible for the destruction of eggs since Parasitid mites are well known predators of insect eggs, larvae and pupae (Hughes, 1959). Bhattacharyya (1963) states that the members of the genus Pergamasus Berl. s. lat. are amongst the most important acarine predators in Palaearctic soils.

Staphylinids and carabids were shown to be the main larval and pupal predators by the serological technique. The time of maximum occurrence of the commonest of these, Pterostichus madidus (Carabidae) and Tachinus rufipes (Staphylinidae) is synchronized with that of Sitona larvae and pupae. The frequency of positive reaction to the precipitin test by these two species at different times of the year indicated that predation may be largely restricted to the final larval instar and the pupal stage. The feeding behaviour of the early larval instars, in a way, can be considered to be a defence mechanism against predation since these larvae are protected by the 'skin' or the outer layer of the nodule.

Food, as a component of environment, may influence an animal's chance to survive and multiply by modifying its fecundity, longevity or speed of development. Evidence in favour of this statement has been amply provided by Andrewartha and Birch (1954). The comparative results of oviposition for the two years showed a reduction in fecundity by approximately a half in 1964. It is possible that the quality of food may have caused this large reduction in fecundity as well as the higher death rate observed in that year (see section 7.4, page 64). The habitat of Sitona is closely connected with food as an environmental factor, since the food plant itself

forms a part of the habitat. When living on Sarothamnus, Sitona is monophagous and therefore its habitat is simple. The life of the beetle from egg to the pupa is spent in soil. Apart from hibernation among litter in the winter, the rest of the life of the adult is spent on the host plant. The adult beetle was shown to avoid unfavourable climatic conditions by moving from one part of the habitat (foliage) to another (litter).

The main difficulty imposed by the habitat on Sitona is that after hatching, the larva has to find its way to the feeding sites. Apart from the egg stage, the highest mortality in the life cycle occurs during the early first instar period and about 85 per cent. of the larvae failed to survive beyond this stage in both years. This difficulty encountered by the first stage larva is partly solved by oviposition behaviour of adult. Study of oviposition and feeding habits revealed that although the eggs are dropped from above, they are distributed in such a way that the majority accumulate in the region where root nodules are predominantly formed. Thus, oviposition behaviour appears to be a useful adaptation.

It is often argued that intraspecific competition for food and space are the factors which could ultimately set the upper limit to the size of a population (e.g. Nicholson, 1948, 1954; Milne, 1961, 1962; Klomp, 1962; Solomon, 1964). There was no indication that competition for food would occur among the adult Sitona since the amount of broom foliage was far in excess of its food requirements. On the other hand, competition for food is likely to occur among the larvae as the numbers of available root nodules is relatively small, and the production of nodules itself is dependent on a

number of factors. Although that type of competition was not noticed during the short period of this study, the proportion of nodules eaten was very large.

The fact that root nodules are the main source of larval food and that the number of nodules eaten was as large as seventy per cent. indicated that the ability of the host plant to produce the nodules could influence the abundance of the beetle. In this connection, it is of interest to note that Bird (1947) in a study of Sitona cylindricollis found that more beetles were produced in clover grown in plots containing sandy soil than in clay-loam soil. Bird did not assess the root nodule content in the two types of plots, but it is known that legumes grown in sandy soils produce more nodules than those in clay-loam soils (see Masefield, 1952; Nutman, 1958).

The incidence of the parasitic fungus, Beauveria bassiana, was very low in the adult population and was almost absent from the larval stages. Madelin (1963) has shown that the fungus needs high humidity and warmth to cause infection. Since the beetle tends to shift from soil (where the fungus is usually found) to the foliage when it is warm, it can be assumed that the adult incidentally avoids heavy fungal infection. However, the fact that Beauveria is capable of causing considerable damage to insect populations must not be ignored. Among the destructive insects known to be susceptible to Beauveria bassiana are the European corn borer (Ostrinia nubilalis Hbn.), the codling moth (Enarmonia pomonella L.) and the chinch bug (Blissus leucopterus Say.). The chinch bug is subject to

natural outbreaks of muscardine (Beauveria) that may markedly reduce destructive populations (Steinhaus, 1964).

In addition to the mortality factors discussed so far, a noticeable loss to the population was caused by winter deaths and emigration in spring. These two factors were together called 'winter disappearance' in the life tables, as they were not studied separately. No definite cause for the winter deaths could be found; freezing was eliminated as a reason for these deaths because in litter, where the beetles hibernate in winter, the temperatures remained above zero. Laboratory observations suggested that these deaths were unlikely to have been caused by parasitism or by any obviously pathological condition. It was concluded that this may be due to the short life-span of some individuals (section 8.13, page 78 ). If this is so, the causes for winter deaths can be considered to be physiological.

Another instance of physiological death was seen when beetles died of old age at the termination of oviposition. Those that died of old age can be said probably to have completed their physiological longevity. Physiological longevity represents the capacities of individuals of a species to live out their life span (Allee et al., 1949). The old age deaths in 1963 and 1964 were dissimilar. This was indicated by the higher death rate and the reduction in the number of adults that lived for a second year in 1964. It is not impossible that as the broom plants get older, they provide a less suitable diet and nutritive factors may influence survival. Indeed the condition of the broom plants in the habitat did show signs of deterioration with age; this was indicated by a reduction of the amount of new

foliage and flowering in 1965 when compared with the previous years.

Finally, dispersal accounted for a part of the changes in the adult population. Dispersal occurred in two ways: (1) by walking at the time of spring emergence by the brachypterous forms and (2) by flight in the case of macropterous forms in spring. The reasons for the emigration of brachypterous forms cannot be similar to that of macropterous forms since it is very unlikely that colonization of new habitats could ever be rapidly achieved by walking as a form of locomotion. A probable reason for this emigration in spring by walking is the likelihood of beetles to leave a broom plant when it gets old and becomes unattractive. One feature of the habitat where this study was carried out is that the individual plants are of the same age and consequently with their deterioration the insects are faced with a situation similar to "being stranded". It is doubtful whether an emigration of this type would occur in a natural broomland where plants of different ages are more likely to be found, as beetles could shift from old to young bushes in the same habitat. A clue to this problem may be found if some information is available on the relative abundance of Sitona on broom plants of different age groups within the same habitat.

A loss to the population also occurred when the macropterous forms dispersed by flight in spring. Dispersal of the macropterous beetle by flight is purely a migratory one resulting in colonizing new habitats. Today, migration of insects is regarded as an important adaptation which ensures dispersal; it is not a sudden behavioural response by adults to adverse changes in the environment such as crowding or food shortage, but is a part of the chronological development of the individuals of genotypical

migrants (Johnson, 1960 , 1963, 1965). It has been shown that migratory movements have several striking characteristics. One of these is the collective simultaneity (Johnson, 1965). Another is that the vegetative reactions (feeding, mating and breeding) are inhibited (Kennedy, 1961). This study revealed that in Sitona, the flight is simultaneous and occurs just prior to oviposition which is inhibited until the ecological factors (light and temperature) become suitable for flight. Thus the flight movement in Sitona is truly a migratory one.

Being dimorphic, with an obligatory migrant and a non-migrant, Sitona maintains a variable level of migratory movement. Southwood (1962) states that in polymorphic species with obligatory migrants and non-migrants, the proportions in which these two forms occur will be geared, by natural selection, to the frequency of change of the habitat: the more temporary the habitat, the more obligatory migratory individuals and vice versa. He further states that all cases of completely genetically controlled wing polymorphism are examples of this. According to this interpretation, the limited amount of migration (approximately 4% of the total adult population) seen in Sitona has been evolved to suit the frequency of change of the habitat. It must be recalled that areas of broom were regarded as relatively temporary habitats (section 2, page 4 ).

Johnson (1963, 1965) suggests that neurophysiological processes are responsible for the characteristic migratory flight of insects because the factors which prolong sexual maturity appear to evoke and prolong migration in females. Johnson's hypothesis explains why Sitona migrates only

during the pre-oviposition period.

Another interesting feature of the migration of Sitona is that it appears at the end of the period of diapause. Kennedy (1961) has suggested that migratory behaviour itself is an example of diapause since migrating insects show a delay in the onset of reproduction and a reduction in the number of eggs laid or young produced, when compared with non-migrating forms of the same species. The delay in egg production as well as ovary growth of the macropterous Sitona (Figs. 50, 51; pp. 163 ) indeed supports such a view. Thus, the macropterous form seems to have an extended diapause when compared with the brachypterous one.

The essential features of the population dynamics of Sitona were given in the life-tables or the 'budgets' (Tables 26 and 27, pp. 102, 103). The most notable difference seen in the two years is that the number of eggs laid in 1964 was approximately halved. In spite of this, the total number of adults produced in autumn, that survived until the spring of the following year was maintained at a steady level with a slight increase from one year to the next. A comparison of the life tables of the two years reveals that the fall in fecundity in 1964 was compensated by a reduction in egg mortality. The mortality during the other stages does not appear to have contributed to this; on the contrary other causes of mortality were slightly greater in 1964.

Now, turning to the factors which caused the egg mortality, reference to Table 22 (page 89 ) would indicate that sterility and predation were the factors responsible. The following data obtained from Table 22 shows that, of the two factors, it is predation which contributed more to the levelling

off of the difference caused by the differences in fecundity.

| Year | 1963 | 1964 |
|---|---|---|
| Total no. of eggs | 2,105,235 | 1,209,062 |
| No. sterile | 329,048 (15.63%) | 98,901 (8.18%) |
| No. destroyed by predation | 1,043,748 (49.58%) | 507,526 (41.98%) |

It is tempting to think that the above results indicate that sterility and predation act in density dependent manner, but results deduced from two sets of data are far from being conclusive.

To sum up, the population of Sitona regensteinensis under investigation remained more or less steady during 1962 - 1965. This appeared to have been achieved by variations in fecundity and egg mortality. The latter was caused by sterility and predation. It is not known how fecundity and egg mortality would maintain a population balance in the long run. Hence, the other mortality factors are still regarded as important.

Of these, predation of larvae and the pupae appears to be important and needs further study since no quantitative investigation on predation of these stages was made. The total number of prey destroyed by predators is the product of the number killed per predator and the number of predators that are present. This twofold nature of predation has been described as the functional response, concerning prey consumption, and the numerical response concerning the density of predators (Solomon, 1949; Holling, 1961). A study of population dynamics is incomplete if these two responses are not

studied; for Richards (1961) has shown that any attempt to make detailed deductions from even several consecutive years of life tables will involve study of the biology of the more important parasites and predators in some detail. In this respect, the information obtained on the parasite Centistes excrucians during this study can be said to be more or less complete. Among the predators, those that are most likely to have some influence over the abundance of Sitona are: the Carabid, Pterostichus madidus and the Staphylinid, Tachinus rufipes (section 8.22, page 90). A study of the numbers of these two predators in the habitat in conjunction with the precipitin test would provide quantitative estimates of the numbers of larvae and pupae lost by predation. Such assessments on the predation of Phytodecta olivacea have been done by Dempster, Richards and Waloff (1959) and Dempster (1960).

One other mortality factor that needs further study is the winter deaths, the effects of which could not be separated from spring emigration. For this, the seasonal changes of Sitona numbers have to be measured during the hibernation period by taking soil samples, a few inches deep. Although it may be difficult to assess the exact amount of annual emigration of brachypterous forms in spring, trap plants planted in the manner described in section 12.1 (page 133) may help to obtain some idea of the degree of emigration in different years.

At this stage it is intended to discuss, or rather speculate on the physiological processes that are likely to be involved in two of the topics discussed so far, i.e. diapause and migration. In section 13 (page

149 ) some observations were made regarding adult diapause and it was concluded that it can be terminated in the laboratory by subjecting the beetles to a cold treatment of 4 - 5°C. for ten weeks followed by exposure to normal day lengths at 20°C. It is generally accepted that adult insects in diapause have some fundamental features such as (1) standstill of morphogenesis, (2) strongly reduced basal metabolism and (3) low water content and high fat content (see Wilde, 1954; Lees, 1955, 1956 and Danilevskii, 1965).

Davey (1956) showed that in Sitona cylindricollis, diapause is characterized by a drop in oxygen consumption, a cessation of development in the reproductive organs, and a slight rise in the fat content; and that the termination of dormancy is characterized by a reversal of these conditions.

Wilde (1960, 1961, 1962) has demonstrated that in the adult Colorado beetle, Leptinotarsa decemlineata Say., the complete syndrome of diapause is produced upon surgical removal of the corpora allata. This includes standstill of reproduction, lowering of oxygen consumption to as little as 20% of the normal value, and a change in behaviour from feeding to burying. Reimplantation of active corpora allata restores the reproductive condition. Wilde (1961) and Wilde and Boer (1961) have also shown that as in many other insects (c.f. Davey, 1965), the deposition of yolk in Leptinotarsa is controlled by corpora allata. The work on Sitona cylindricollis and Leptinotarsa suggests that the physiology of diapause in Sitona regensteinensis may be similar.

In his hypothesis on migration of insects, Johnson (1963) states that "Migratory flight can be prolonged by a lengthened pre-oviposition period and obliterated by a short one. Therefore the factors which prolong sexual immaturity also probably evoke and prolong migration in females. These factors are crowding, too little food or food of the wrong kind, a short day and high temperature acting through the corpus allatum and associated endocrines, especially during pre-adult development. Extended photoperiod, lack of crowding and enough of the right food would have an opposite effect, and tend to shorten or suppress migratory flight". Thus it seems possible that the neurophysiological processes involved in both migration and diapause in Sitona regensteinensis are the same.

Finally, one is inclined to see how far Sitona is adapted to the conditions of a temperate climate. Oviposition and development takes place in the spring and summer; in winter, the adult beetle avoids death by cold by hibernating among the litter where the temperature remains above that required for survival. The imaginal diapause is obligatory and enables synchronization of the life cycle with the weather so that the immature stages are present only when the conditions are favourable for growth. The long incubation period of the egg ensures that the larval stages will occur when food is abundant; for it was shown that the quantity of root nodules produced by the broom plant changes with time and the peak number of larvae and that of nodules occur at the same time (Figs. 36, 37; pp. 125 ). The ability of some adults to survive and breed for a year is another feature of the life history that appears to be adaptive. This

process was shown to happen in Phytodecta olivacea by Richards and Waloff (1961) and they are of the opinion that in effect this is a method of surviving unusually severe weather or heavy predation which may occur on rare occasions.

In conclusion, it is possible to state that this investigation provided details of the population changes that took place in 1963 and 1964. For reasons given above this does not enable prediction of future changes. Nevertheless, the degree and the causes of mortality of various stages are known and should be useful in planning any future work on this or any other species of Sitona.

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## APPENDIX I.

Daily Egg Laying for 1963 (Method 4); 24th March = Day 1.

| Age (Days) | No. of Eggs per Female | Mean air temp. °C. |
|---|---|---|
| 1 | 0.2 | 2.2 |
| 2 | 0.4 | 4.5 |
| 3 | 2.4 | 8.2 |
| 4 | 2.6 | 6.1 |
| 5 | 1.2 | 6.0 |
| 6 | 4.6 | 6.0 |
| 7 | 1.0 | 5.0 |
| 8 | 2.5 | 4.5 |
| 9 | 2.9 | 4.5 |
| 10 | 3.2 | 4.5 |
| 11 | 4.2 | 8.4 |
| 12 | 4.6 | 6.8 |
| 13 | 3.1 | 4.5 |
| 14 | 3.3 | 4.0 |
| 15 | 4.2 | 7.0 |
| 16 | 6.8 | 6.8 |
| 17 | 7.2 | 8.0 |
| 18 | 6.5 | 9.4 |
| 19 | 6.3 | 9.0 |
| 20 | 8.0 | 9.0 |
| 21 | 4.0 | 5.0 |
| 22 | 2.4 | 5.0 |
| 23 | 2.3 | 8.0 |
| 24 | 5.5 | 10.0 |
| 25 | 5.7 | 10.0 |
| 26 | 5.4 | 8.0 |
| 27 | 4.5 | 8.4 |
| 28 | 4.7 | 9.0 |
| 29 | 4.9 | 8.4 |
| 30 | 11.7 | 9.4 |
| 31 | 8.1 | 10.5 |

| Age (Days) | No. of Eggs per Female | Mean air temp. °C. |
|---|---|---|
| 32 | 7.5 | 11.0 |
| 33 | 7.2 | 9.4 |
| 34 | 10.5 | 9.4 |
| 35 | 10.4 | 10.0 |
| 36 | 14.5 | 12.2 |
| 37 | 10.5 | 14.5 |
| 38 | 6.0 | 12.5 |
| 39 | 5.7 | 10.5 |
| 40 | 7.0 | 9.0 |
| 41 | 8.0 | 7.2 |
| 42 | 5.4 | 10.0 |
| 43 | 5.7 | 9.4 |
| 44 | 6.2 | 10.5 |
| 45 | 5.8 | 10.0 |
| 46 | 8.8 | 12.2 |
| 47 | 5.9 | 11.2 |
| 48 | 6.7 | 10.5 |
| 49 | 6.4 | 10.0 |
| 50 | 8.0 | 10.5 |
| 51 | 4.5 | 10.0 |
| 52 | 2.7 | 9.0 |
| 53 | 2.6 | 8.4 |
| 54 | 4.2 | 11.2 |
| 55 | 14.9 | 12.2 |
| 56 | 13.2 | 10.0 |
| 57 | 12.9 | 10.0 |
| 58 | 8.2 | 9.4 |
| 59 | 9.5 | 9.4 |
| 60 | 10.5 | 10.5 |
| 61 | 9.1 | 10.0 |
| 62 | 2.7 | 9.0 |

Continued

Continued.

| Age (Days) | No. of Eggs per Female | Mean Air temp. °C. |
|---|---|---|
| 63 | 12.2 | 10.0 |
| 64 | 17.5 | 11.2 |
| 65 | 15.2 | 12.8 |
| 66 | 15.8 | 12.8 |
| 67 | 3.9 | 10.5 |
| 68 | 2.8 | 9.4 |
| 69 | 12.5 | 15.0 |
| 70 | 28.7 | 17.2 |
| 71 | 18.2 | 17.2 |
| 72 | 11.5 | 15.6 |
| 73 | 9.5 | 14.5 |
| 74 | 10.0 | 15.0 |
| 75 | 7.4 | 13.3 |
| 76 | 14.0 | 16.6 |
| 77 | 23.8 | 17.2 |
| 78 | 25.0 | 17.7 |
| 79 | 18.0 | 17.7 |
| 80 | 18.5 | 17.7 |
| 81 | 21.2 | 16.1 |
| 82 | 17.2 | 16.1 |
| 83 | 10.7 | 13.8 |
| 84 | 5.7 | 12.1 |
| 85 | 5.7 | 14.5 |
| 86 | 5.7 | 13.0 |
| 87 | 9.5 | 15.5 |
| 88 | 6.3 | 14.5 |
| 89 | 1.4 | 12.8 |
| 90 | 3.6 | 15.5 |
| 91 | 4.9 | 16.1 |
| 92 | 4.7 | 15.5 |
| 93 | 3.7 | 13.3 |
| 94 | 3.7 | 13.3 |

| Age (Days) | No. of Eggs per Female | Mean Air temp. °C. |
|---|---|---|
| 95 | 2.4 | 13.3 |
| 96 | 2.9 | 13.8 |
| 97 | 3.7 | 14.5 |
| 98 | 2.6 | 12.8 |
| 99 | 2.9 | 13.3 |
| 100 | 1.6 | 13.3 |
| 101 | 3.5 | 15.0 |
| 102 | 2.6 | 14.5 |
| 103 | 2.4 | 15.0 |
| 104 | 1.2 | 13.8 |
| 105 | 1.3 | 15.8 |
| 106 | 0.6 | 13.0 |
| 107 | 0.1 | 14.0 |
| 108 | 1.1 | 14.0 |
| 109 | 0.6 | 14.4 |
| 110 | 0.3 | 14.4 |
| 111 | 0.3 | 14.2 |

## APPENDIX II.

Daily Egg Laying for 1964 (Method 4). 14th March = Day 1.

| Age (Days) | No. of Eggs per Female | Mean Air Temp. °C. |
|---|---|---|
| 1 | 0.4 | 8.9 |
| 2 | 0.8 | 2.8 |
| 3 | 0.5 | 1.1 |
| 4 | 1.3 | 2.3 |
| 5 | 0.9 | 2.1 |
| 6 | 1.1 | 5.0 |
| 7 | 2.1 | 7.7 |
| 8 | 2.0 | 8.2 |
| 9 | 2.8 | 8.5 |
| 10 | 4.9 | 7.0 |
| 11 | 3.4 | 8.7 |
| 12 | 2.8 | 7.2 |
| 13 | 1.6 | 6.0 |
| 14 | 1.0 | 4.5 |
| 15 | 1.1 | 5.5 |
| 16 | 0.7 | 4.1 |
| 17 | 0.6 | 3.2 |
| 18 | 0.4 | 3.1 |
| 19 | 1.1 | 3.3 |
| 20 | 1.7 | 3.2 |
| 21 | 1.5 | 2.9 |
| 22 | 1.1 | 2.2 |
| 23 | 2.9 | 4.5 |
| 24 | 2.6 | 2.6 |
| 25 | 3.4 | 3.8 |
| 26 | 3.6 | 8.2 |
| 27 | 2.9 | 8.2 |
| 28 | 4.5 | 7.9 |
| 29 | 4.8 | 8.3 |
| 30 | 5.6 | 9.9 |
| 31 | 3.8 | 8.6 |

| Age (Days) | No. of Eggs per Female | Mean Air Temp. °C. |
|---|---|---|
| 32 | 3.8 | 8.8 |
| 33 | 3.3 | 9.9 |
| 34 | 5.1 | 10.7 |
| 35 | 5.3 | 11.3 |
| 36 | 4.7 | 9.7 |
| 37 | 3.6 | 9.0 |
| 38 | 3.0 | 8.7 |
| 39 | 4.1 | 8.2 |
| 40 | 2.1 | 9.2 |
| 41 | 1.3 | 9.2 |
| 42 | 2.4 | 8.8 |
| 43 | 2.6 | 8.8 |
| 44 | 2.7 | 12.2 |
| 45 | 5.5 | 12.7 |
| 46 | 3.7 | 10.6 |
| 47 | 4.8 | 10.1 |
| 48 | 6.3 | 9.9 |
| 49 | 5.8 | 9.7 |
| 50 | 6.6 | 11.0 |
| 51 | 8.0 | 12.1 |
| 52 | 6.0 | 11.1 |
| 53 | 6.1 | 10.7 |
| 54 | 4.2 | 10.9 |
| 55 | 9.2 | 12.1 |
| 56 | 5.8 | 11.0 |
| 57 | 4.5 | 10.7 |
| 58 | 4.0 | 11.7 |
| 59 | 5.3 | 12.6 |
| 60 | 10.6 | 15.2 |
| 61 | 9.2 | 13.0 |
| 62 | 6.2 | 10.8 |

Continued.

Continued.

| Age (Days) | No. of Eggs per Female | Mean Air Temp. °C. |
|---|---|---|
| 63 | 7.1 | 11.1 |
| 64 | 8.1 | 12.5 |
| 65 | 9.9 | 15.5 |
| 66 | 10.7 | 16.0 |
| 67 | 6.0 | 11.8 |
| 68 | 5.3 | 11.6 |
| 69 | 4.4 | 11.5 |
| 70 | 4.9 | 11.8 |
| 71 | 10.7 | 15.2 |
| 72 | 31.3 | 14.3 |
| 73 | 24.4 | 15.3 |
| 74 | 15.7 | 13.9 |
| 75 | 18.4 | 14.6 |
| 76 | 15.0 | 14.6 |
| 77 | 9.0 | 14.8 |
| 78 | 10.3 | 17.1 |
| 79 | 10.9 | 15.4 |
| 80 | 4.2 | 9.9 |
| 81 | 2.4 | 9.1 |
| 82 | 3.7 | 11.3 |
| 83 | 0.8 | 14.4 |
| 84 | 3.8 | 14.4 |
| 85 | 4.7 | 14.6 |
| 86 | 6.1 | 14.6 |
| 87 | 4.9 | 14.1 |
| 88 | 9.8 | 15.4 |
| 89 | 6.3 | 17.1 |
| 90 | 4.1 | 14.2 |
| 91 | 7.6 | 17.0 |
| 92 | 3.0 | 15.8 |
| 93 | 2.7 | 13.7 |

| Age (Days) | No. of Eggs per Female | Mean Air Temp. °C. |
|---|---|---|
| 94 | 4.1 | 14.1 |
| 95 | 5.9 | 14.1 |
| 96 | 6.5 | 13.9 |
| 97 | 6.4 | 13.9 |
| 98 | 4.9 | 12.1 |
| 99 | 2.6 | 9.1 |
| 100 | 3.2 | 11.5 |
| 101 | 1.6 | 12.2 |
| 102 | 2.0 | 11.7 |
| 103 | 4.6 | 15.1 |
| 104 | 6.2 | 17.5 |
| 105 | 7.4 | 18.2 |
| 106 | 8.8 | 18.4 |
| 107 | 8.0 | 17.5 |
| 108 | 1.8 | 15.8 |
| 109 | 1.0 | 16.1 |
| 110 | 0.6 | 17.1 |
| 111 | 0.7 | 17.1 |
| 112 | 0.5 | 15.1 |
| 113 | 0.9 | 13.6 |
| 114 | 0.3 | 14.2 |