I.

THE STRUCTURE AND PERMEABILITY OF
PROTECTIVE COVERINGS SECRETED BY
CERTAIN INSECT SPECIES.

by

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A thesis submitted for the Degree of Doctor of
Philosophy in the Faculty of Science, London
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Imperial College Field Station,   September 1965.
Sunninghill,
Berkshire.

ABSTRACT

The structure and permeability of the protective coverings secreted by four insect species, chosen to represent the major types of insect protective coverings, have been investigated.

Different techniques were used to study the structure of the four coverings. Histochemical methods employed to investigate the chemical structure of the spittle covering of Philaenus spumarius nymphs are described. A method for cutting thin sections of the scale covering of Saissetia hemisphaerica is reported. Acid hydrolysis of the protective coverings of P.spumarius, S.hemisphaerica and the pupal silk cocoon of Plodia interpunctella showed that protein was present.

Apparatus designed and adapted to study permeability includes a Spray Tower used to spray Cnaphalodes strobilobius on Larch trees and P. spumarius on detached vegetation with insecticide and other solutions. A spray mist could be produced by removal of the larger spray droplets on shutters.

Permeability was measured indirectly by assessment of mortality caused by an insecticide, -BHC, applied to insects. Special exposure chambers to study the penetration of saturated vapour of BHC are described. The vapour phase of radio-active -BHC was used to penetrate the covering of C.strobilobius, and the method of penetration has been

discussed.

Precipitation of metallic salts from aqueous and oil solutions was used to study the permeability of the scale covering of _S.hemisphaerica_ immersed in the solutions.

The destruction of spittle masses of _P.spumarius_ by a number of groups of compounds, used both in test tubes and as sprays, was found to be directly related to the chemical structure of the spittle material.

The value of the protection afforded to the insects by the protective coverings studied has been considered in the general discussion.

ACKNOWLEDGEMENTS

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INTRODUCTION

Many species of animals, particularly among the invertebrates, secrete coverings to protect themselves from adverse surroundings. The protective coverings secreted by members of the Arachnida and Insecta, although closely associated with the animals, are usually secreted as separate structures while in the Mollusca, which are mainly aquatic, the protective covering forms an integral part of the animal. In general, where protective coverings are employed, they are secreted by developmental stages which are more vulnerable to unfavourable climatic conditions and to attack by predators and parasites, usually because of restricted mobility.

The protective coverings secreted by insects vary in their efficiency and in the type of protection they afford, and it is the structure and permeability of the coverings secreted by four selected species of insects which are investigated in the present work.

Classification of Insect Protective Coverings.

Any classification of insect protective coverings is difficult because of their diversity, but they may be arbitrarily divided into two main types viz:-

1. those which make use of secretions in some form, with or without the incorporation of extraneous materials, and

2. those which result from adaptation of existing substrates, without the use of secretions.

Insects using these methods of protection may be classed as "actively" and "passively" protected respectively.

Passive Protection.

Passive protection is conferred upon an insect by virtue of its position within a host, either plant or animal. Some examples of passive protection in plant hosts are gall formation and the excavations of leaf miners and stem borers; in animal hosts many species of parasitic insects are passively protected.

Passively protected insects are not considered in this work.

Active Protection.

Actively protected insects are defined as those which secrete at least a part of their protective covering. The most commonly occurring secreted materials found in insect protective coverings are silk and wax. Other materials are used but they are more specialised and restricted to a few species. (See below).

Silk

Silk production is mainly associated with a limited number of insect orders, for example the Lepidoptera, Trichoptera and Hymenoptera but members of other insect orders do utilize this material. The silk may be used as protection for a single insect or for a communal group of insects. The silk employed may be formed in many different parts of the body, for example,

the labial glands of larval Lepidoptera and Trichoptera, the malpighian tubules in Neuroptera Planipennia, and from dermal glands in the fore tarsi of Embioptera.

The best known use of silk is in the pupal cocoon of Lepidoptera, which vary from the extremely dense and perfect formations of the family Saturniidae to the small pad of silk formed by some Papilionoidea representing the last vestige of a cocoon. Other larval Lepidoptera, for example, the Phycitidae, live in silken tunnels in their food medium and pupate subsequently in freshly spun cocoons.

Communal silk cocoons are spun by larvae of Dielocerus (Hymenoptera, Argidae) and certain parasitic Braconidae (Hymenoptera), a number of which may emerge simultaneously from a host.

Silk is occasionally used as a protection for the adult insect. The Embioptera live gregariously in silken tunnels, which are used as a retreat for protection from predaceous insects and for moisture conservation. Many Psocoptera are also gregarious and live under a fine silken canopy; in this case the eggs are also often protected against dislodgement from the leaf surface.

The larvae of many species of the Lepidopteran families Tortricidae, Gelechiidae and Drepanidae, obtain protection by binding the leaves of their host plants together with silk

threads.

The incorporation of extraneous materials into protective silk coverings is restricted to the larval and occasionally the pupal stages of certain insects. Many larval Trichoptera construct cases of silk upon which are placed fragments of vegetation. In some families, for example the Phryganeidae, these fragments are arranged in a specific pattern, while in others, for example the Limnephilidae, they are arranged in a random manner. Some larvae spin a silk wall across both ends of the case before pupation, often incorporating small stones or other materials in these walls. An analogous type of protective structure is formed by the larvae of the bagworms (Lepidoptera, Psychidae); in this instance the insects are terrestrial.

In the Hymenoptera, some larval Siricidae construct silk cocoons, including gnawed wood fragments, within the larval galleries excavated in the tree.

Wax

Insects secreting wax belong almost exclusively to the Hemiptera-Homoptera. The wax may be secreted in the form of a powdery covering, as filamentous threads or as lamellae. Wax secretion is particularly evident in the Coccoidea where it is a powdery covering or thread-like in some Pseudococcoidea, as lamellae of wax on the integument of some Coccidae, or as a hard waxy scale in some Diaspididae.

In other Homopteran families, filamentous waxy materials secreted for protection are found, for example, in the nymphs of some Flatidae, the adults of some generations of Adelgidae and the 'pupae' of Aleyrodidae.

Among other orders the occurrences of wax secreted for protective purposes are few. Two examples are the larvae of some Coccinellidae (Coleoptera) and a species of Selandria (Hymenoptera, Tenthredinidae).

Other Secretions

The developmental stages of some insect species construct protective coverings which are specific and highly characteristic.

The nymphs of many Cercopidae (Homoptera) cover themselves with a frothy secretion, the well known spittle, composed largely of water derived from the host plant, and secretions from the malpighian tubules and certain dermal glands.

Lac is secreted by certain Coccoidea (Lacciferidae) and is basically a resinous substance containing wax and other materials. The constitution of the protective coverings varies and depends largely on the amount of resin present in the host plant.

The secretion of the aphid, Eriosoma lanigerum (Hausm), although similar in appearance to the powdery and filamentous

wax secretions of certain Coccoidea, is composed mainly of fats.

The pupal cocoon of Dicranura (Lepidoptera, Notodontidae) is constructed from silk and gnawed fragments of wood . agglutinated by a thoracic gland secretion reported by Latter (1897) to be formic acid (c.f. Hymenoptera, Siricidae). The cocoon of Euplectrus (Hymenoptera, Eulophidae) is formed from products of the malpighian tubules modified in the hind gut; in some Curculionidae and Scarabaeidae (Coleoptera), malpighian tubule products are modified in the posterior caecum.

The examples presented above, although not exhaustive, show clearly the diversity of methods and materials employed by insects to secure protection from their surroundings.

MATERIALS AND METHODS

CHOICE OF INSECT MATERIAL

In this work the following species of insect were chosen to provide examples of different types of protective coverings.

HEMIPTERA — HOMOPTERA

APHIDOIDEA — ADELGIDAE

Cnaphalodes

strobilobius (Kaltenbach 1843)

Type of protective covering — flocculent wax-wool of adults of colonici generation.

CICADOIDEA — CERCOPIDAE

Philaenus

spumarius (Fallén 1826)

Type of protective covering — foam-like spittle mass of nymphs.

COCCOIDEA — COCCIDAE

Saissetia

hemisphaerica (Targioni-Tozzetti 1867)

Type of protective covering — shield-like scale of adults.

LEPIDOPTERA

PYRALIDOIDEA — PHYCITIDAE

Plodia

interpunctella (Huebner 1810/3)

Type of protective covering — silk cocoon of pupae.

These species of insects were selected for the following reasons.

1. They provide representatives of the major types of protection described above.

2. The case of obtaining experimental material was of prime importance in view of the limited time available. Apart from Plodia interpunctella (Hb) it was not possible to rear the insects in the laboratory, and they had, therefore, to be available in quantity in the field.

3. As far as possible the insects were selected as representatives of species possessing protective coverings of some economic importance.

CULTURE OF INSECTS

Cnaphalodes strobilobius (Kalt)

Two hundred Japanese larch seedlings (Larix leptolepis Murr.), obtained from the Forestry Commission, were planted in eight inch pots placed in rows on the surface of an experimental plot. To conserve moisture in hot weather, a

number of pots were sunk in the ground, but the trees in these pots tended to produce abnormal flattened crowns (Figure 1.A.). This method of water conservation was later abandoned, and instead a muslin screen was erected over the experimental plot. This provided shade for the trees during hot weather, and also protected the nymphs used to infest the trees, (Varty 1956).

Young sistens and progrediens nymphs of the colonici generation were collected in large numbers from infested Japanese and European larches (Larix europaea D.C.), and transferred to the experimental trees. Two methods of infestation were tried. In the first, glass tubes containing nymphs were placed over the ends of branches, but this method failed in hot weather because condensation within the tubes trapped the insects. A better method was to place small, heavily infested twigs from the original trees among the foliage of the experimental trees (Crooke 1952). As a result of artificial infestations carried out over a two year period a population was established on the experimental trees. (Figure 2.).

At various times the trees showed symptoms of magnesium and boron deficiencies. In order that these deficiencies would not be confused with phytotoxicity caused by sprays, these conditions were treated. Magnesium was supplied by the

A

Figure 1. Japanese Larch trees showing
    flattened crown (tree A) produced
    after pots were buried for water
    conservation.

Figure 2. Part of Larch tree with a
    heavy infestation of Cnaphalodes
    strobilobius (Kalt).

application of a mixture of sand, dolomitic limestone and magnesium sulphate to the soil while boron deficiency was overcome by the addition of a small quantity of borax.

Philaenus spumarius (F).

As spittle was required in quantity for experimental purposes, an attempt was made to produce it in the laboratory and so avoid field collecting as far as possible. Nymphs from plants collected in the field were placed on plants of the same species growing in pots in the glasshouse. Observations over a long period showed that although spittle masses were occasionally produced, this method was impractical in view of the relatively short developmental period of the insect. All experimental material was, therefore, collected in the field.

To obtain intact spittle masses it was important to prevent contact with any surface during collection because of the viscous nature of the spittle. The best method found was to support the piece of vegetation bearing the spittle mass in a tube fitted with a cork, through a hole in which the stem projected.

Saissetia hemisphaerica (Targioni-Tozzetti)

This insect was cultured in a heated conservatory, on species of ornamental fern (Terris spp) and also on Asparagus sprengeri and was collected when required. The temperature

range in the conservatory was 55°F to 80°F.

Plodia interpunctella (Hb.)

All laboratory stocks of stored products insects
originate from wild stock infestations, bred under controlled
conditions (Parkin 1951). The existing stock of Plodia
interpunctella (Hb.) at Ashurst Lodge, was used to build up new
cultures. The insects were bred in wide mouthed glass jars
with a muslin cover. To exclude mites from the cultures, the
jars were placed on enamel trays containing one-quarter inch
of kerosene oil. Controlled conditions were maintained, the
temperature at 25°C ± 0.5°C and the relative humidity at 70%
± 5%. Light conditions varied, but generally a 16 hour day
length was maintained. A variable temperature regime during
larval development is known to induce larval diapause in
Plodia. This effect is enhanced by overcrowding and short
day-length conditions. (Tsuji 1958, 1959, 1960;
Tzanakakis 1959).

Most culture methods for rearing Plodia depend on the use
of grain products in some form. However Russell (1961), used
heavily salted pistachio nuts as a culture medium at a
relative humidity of 30% — 40%. The excess salt absorbed
moisture to keep the kernels moist, and reduced the development
of mould. This method was only successful for rearing small

numbers of insects for a short time. More recently Williams
(1964) has described the life history of _Plodia_ on a wide
variety of foodstuffs in a British warehouse, and found that
the following materials were used in decreasing order of
suitability, wheat, milo, sultanas, maize and groundnuts, and
maize meal.

The food medium used was a mixture of bran and yeast
("Yestamin"), moistened with glycerol, in the following
proportions:—

        Bran   600 gms.

        Yeast   30 gms.

        Glycerol 100 ml.   (de Almeida 1962)

The jars were filled to a depth of two inches, and twenty
freshly emerged moths were placed in each jar, each group of
moths containing a number of actively mating pairs.

The production of Standardised Pupae.

The duration of the egg, larval and adult stages of
Plodia were not accurately determined, but a standard procedure
was adopted for selecting candidate larvae for pupation.
De Almeida (1962) states that under the controlled conditions
previously mentioned, the larval stage lasts from 15 to 18 days.
After several observations, only larvae which were visibly
migrating for two days were selected for the production of
experimental pupae.

The susceptibility of test insects to insecticides is known to change if culture containers are shaken or agitated to obtain samples, and their resistance may not return to the normal value for several hours. This procedure also increases the heterogeneity of any sample taken (Parkin 1951). To minimise this effect, the culture jars were examined every six hours when the larvae had begun to migrate from the culture medium seeking pupation sites. When migration had been in progress for about 48 hours, the jars were uncovered and all larvae that crawled out of the jars were placed in batches of ten in 4 inch plastic petri dishes to pupate. A roll of corrugated cardboard placed in the culture jars provided pupation sites, but this method failed to produce pupae which could be removed with perfectly formed, undamaged cocoons. The removal of larvae to clean containers also allowed the production of cocoons uncontaminated with particles of culture medium and frass.

Table 1 and Graph 1 show the percentage mortalities of larvae, removed to petri dishes at one day intervals after the start of migration. The mortality of both larvae and pupae was lowest when larvae were removed on the second day after the start of migration. For all experiments, therefore, larvae were removed at this time, as control mortality was then expected to be lowest. Mortality of larvae and pupae was

Graph 1.

Mortality of migrating larvae, and pupae of
P. interpunctella.

● – Mortality-Larvae+Pupae.
X – Mortality-Larvae.
O – Mortality-Pupae.

% Mortality

Days after start of larval migration
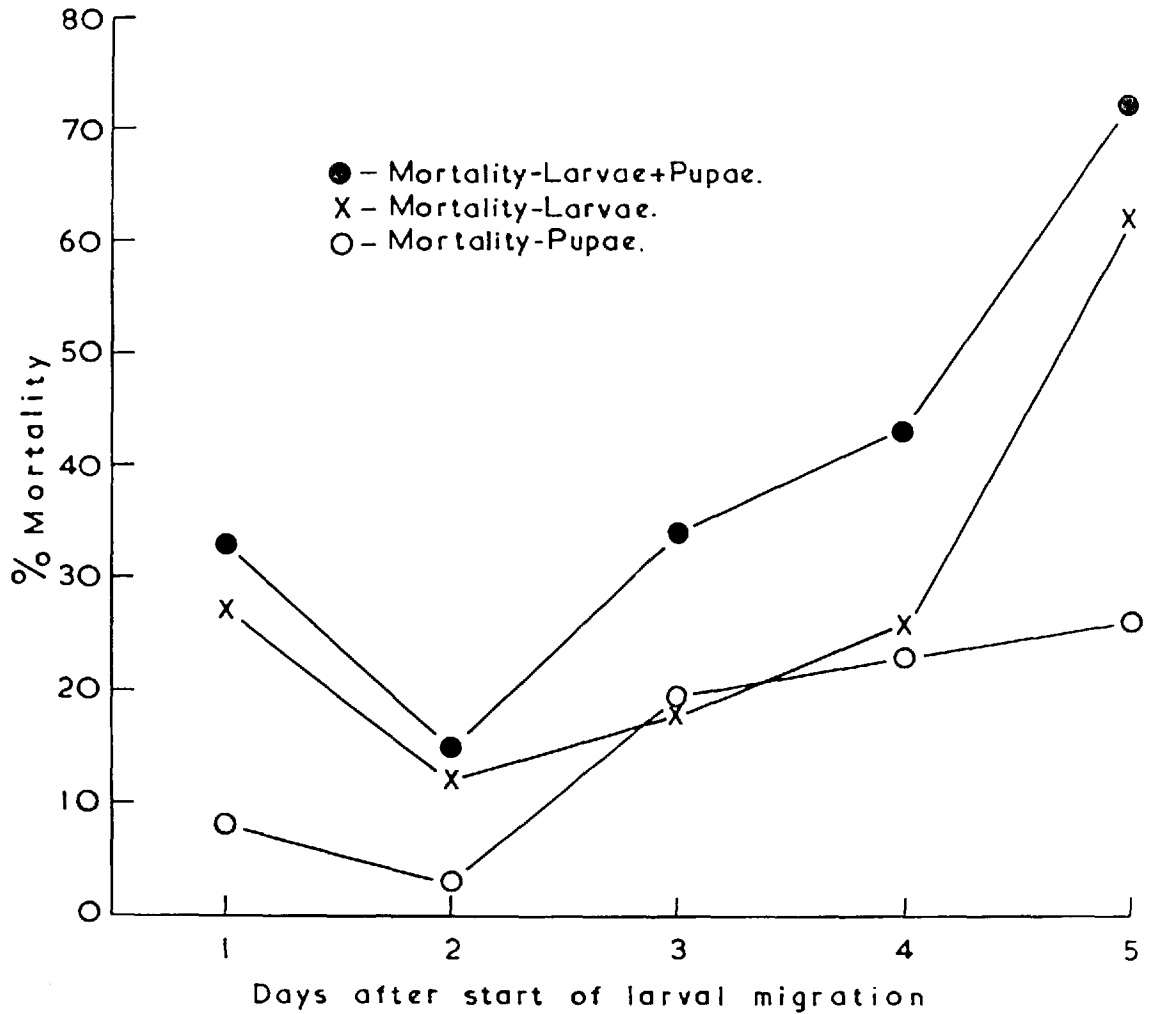
caused almost entirely by cannibalism.

Migrating larvae removed to petri dishes searched for a crevice in which to pupate, but eventually settled on the lid or floor of the petri dish, or in the angle between either flat surface and the side of the dish. For experimental purposes, only those pupae which had formed cocoons on a flat surface were selected, as cocoons formed in other positions were irregular in shape. By gripping one extreme end of the cocoon with f rceps, it was possible to peel off the cocoon containing the pupa, completely undamaged. Some larvae in the petri dishes pupated without spinning a protective cocoon, and it was thought at first that this might be a manifestation of excess handling or change in surroundings. However, as similar pupae were also found on the surface of the culture medium in the jars, the non-production of a cocoon was not regarded as an abnormal occurrence. Potter (1938) stated that removal of pupae from their cocoons made them less resistant than normal pupae to insecticides, but that the differences in resistance did not appear to be very great.

A series of observations was conducted to see whether sufficient numbers of pupae with intact cocoons could be obtained in this manner; the results in Table 2 show that this method was satisfactory. Further observations confirmed that ten larvae in one dish was a suitable number for

production of pupae with cocoons.   (Table 3).

ASSESSMENT OF PERMEABILITY

In some of the experimental work, direct estimation of the degree of permeability of the protective coverings was possible for example, the comparison of colour intensities of solutions containing recovered fractions of dye solutions sprayed on to insects, and the measurement of activity in extracts of insects and protective coverings treated with C-14 labelled insecticide.   In other experiments, permeability was estimated indirectly by using mortality caused by insecticide as a correlate of permeability.   The amount of insecticide applied, either topically or as a spray, was adjusted so that mortality between 0% and 100% would occur.   Using these methods permeability was estimated by using insects, as far as possible, in protected'and 'unprotected' states, that is with their protective coverings intact and with their protective coverings removed respectively.

THE INSECTICIDE

The insecticide selected for the work was the gamma (γ) isomer of 1,2,3,4,5,6 — hexachlorocyclohexane, γ-BHC. A sample of the pure isomer was obtained from Imperical Chemical Industries Ltd. and was not less than 99.6% pure. This insecticide was chosen because of its contact toxicity and

and fumigant action, the latter resulting from its high vapour pressure. Pure γ-BHC has a melting point not lower than $112^{\circ}C_{\gamma}$ and its vapour pressure is 9.4×10^{-6} mm. Hg.. Before use the insecticide was recrystallised twice from analytical grade methanol.

The properties of the C-14 labelled sample of γ-BHC used in some of the penetration experiments are described in the section dealing with the permeability of the wax—wool covering of Cnaphalodes strobilobius (Kalt.)

APPARATUS

Apart from the spray tower, all items of specially constructed apparatus used are described in the appropriate sections, and are only listed here.

VAPOUR PENETRATION CHAMBERS

These chambers were designed to study the penetration of an insecticide, γ—benzene hexachloride (γ-BHC), through the protective coverings of Plodia interpunctella (Hb) pupae, and the adults of the colonici generation of Cnaphalodes strobilobius. (Kalt).

CHAMBERS FOR USE WITH C-14 LABELLED INSECTICIDE

These special small chambers were required because factors such as humidity, temperature and vapour distribution within the chamber had to be controlled to a high degree of

accuracy, and also because of the limited amount of radioactive (labelled) material available.

THE SPRAY TOWER

A spray tower was required in which it was possible to produce an evenly distributed and easily replicated spray mist to settle on small flat objects, such as open petri dishes, and also on larger objects, particularly small plants infested with nymphs of Philaenus spumarius (F), and small larch trees infested with Cnaphalodes strobilobius (Kalt). A comparatively large structure was, therefore, required and the spray tower described had to be specially designed.

Development of Laboratory Spray Towers

Since Tattersfield and Morris (1924) built an apparatus able to deliver accurate and reproducible deposits of insecticide or other solutions, many workers have designed and adapted similar pieces of apparatus. These have been of two main types, those which spray solutions directly on to a test surface, and those in which the larger spray droplets are removed before the remaining mist is allowed to settle. The method of spraying has varied from vertically downwards, (Tattersfield and Morris, 1924; Nelson, 1937; O'Kane et al. 1941; Bottimer, 1945; ten Houten and Kraak, 1949; Subba Rao and Pollard, 1951; Potter, 1941 and 1952;) to vertically

upwards, (Stultz, 1939; McGovran and Mayer, 1943; Way, 1949; Ebeling, 1951), and to horizontal spraying by means of air currents, usually fan assisted, (Hoskins and Caldwell, 1947; Roan and Kearns, 1948; Dorman and Hall, 1953). The settling mist method has been employed by Waters, (1937); Campbell and Sullivan, (1938); Petty, (1946); Webb, (1947) and Dean (1947 and 1951).

Other methods of obtaining accurate and repeatable deposits have included the vibrating rod principle (Rayner and Hurtig 1952), where a rod oscillating at a constant rate removes droplets from the end of a fine pipette, and the spinning disc principle (Parr and Busvine 1948), where liquid is fed at a constant rate on to a disc spinning at constant speed, and droplets of standard size are deposited at a given distance, measured horizontally, along the trajectory.

The most complete record of results obtained in a vertical spray tower are those of Potter (1952), who described and analysed the effects of nozzle sizes and settings, solution viscosity, humidity, temperature, air pressure, turbulence and electrostatic charge on droplets.

The essential features of any apparatus of this type are an atomising nozzle, a cylinder or cage to contain the spray and assist in the production of turbulence, and a receptacle upon which the spray or settling mist is deposited.

ATOMIZING NOZZLE

Atomizing nozzles which reduce liquids to a fine spray may be of several types, but the choice becomes more limited when for research purposes, a regular and repeatable spray pattern with the minimum variation in droplet size is required.

The most important contributions to nozzle design for experimental spray towers have been those of Hewlett, (1946), ten Houten and Kraak, (1949) and Potter, (1952). In all these nozzles a central liquid jet is surrounded by an annular orifice through which a current of air passes, removing small droplets of liquid to form the spray.

The nozzle described by Potter (1952) is the most versatile of those mentioned, as it is supplied with mechanisms for projecting and retracting the liquid jet, and for centering this jet in the air orifice. This nozzle was selected for preliminary tests in the tower, and proving successful with a range of spray solutions, was retained. The air cap venturi length was 0.04 in. The nozzle is described in detail by Potter, (1952).

A second type of nozzle was considered for the production of smaller droplets. This was the Aeraspray Ultra-Fanex G.5 spray gun. Supporting struts fitted to the tower lid (Figure 5) held the gun which was assembled to spray vertically downwards. The needle valve was retracted from

the liquid orifice by securing the trigger to the body of the gun. Substantially greater air pressures were used to reduce the droplet size, but no reproducible results were obtained for spray distribution or replication. The gun was, therefore, regarded as unsatisfactory for the present purpose.

In view of the variation in size of the objects to be sprayed it was decided to spray directly down the tower, removing the larger spray droplets when necessary by means of shutters.

DESCRIPTION OF TOWER

The tower (Figures 3 and 4) consists of two main parts, the aluminium cylinders which form the main bulk of the apparatus, and the supports and frameworks which separate the cylinders.

The cylinder sections are made from separate sheets of hardened aluminium. The upper section (US) is the largest, 29 inches high and 23 inches in diameter. Upon this section the lid (L) fits tightly, overlapping round the top to a depth of 2 inches. This tight fit ensured the complete rigidity necessary for the nozzle mounting. The middle section (MS) is of the same diameter and 29 inches high. One side of this cylinder is modified by having a 14 inch by 21 inch section removed to facilitate the introduction and removal
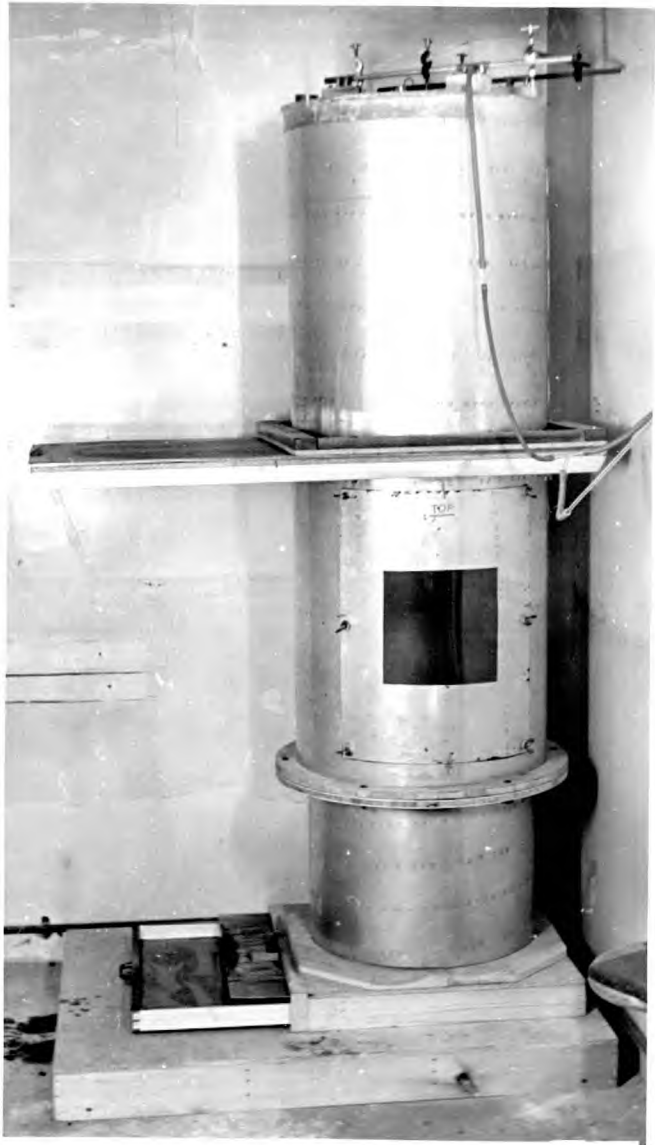
Figure 3. Vertical Spraying Tower.

Figure 4.

VERTICAL SPRAYING TOWER

KEY   1 foot
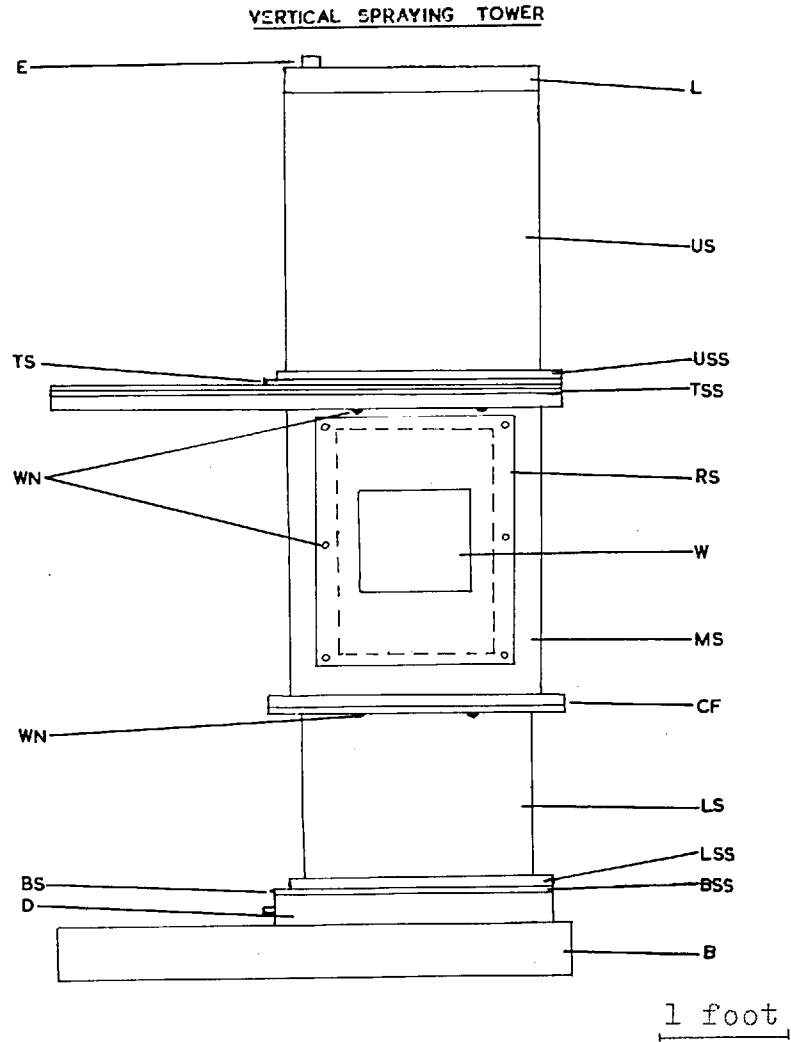
B   Base
BS   Lower shutter   MS   Middle section
BSS   Lower shutter support   RS   Removeable section
CF   TS   Upper shutter
D   Spray drawer   TSS   Upper shutter support
E   Exhaust outlet   US   Upper section
L   Lid   USS   Upper section support
LS   Lower section   W   Window
LSS   Lower section support   WN   Bolts

of trees for spraying. The aperture is covered when spraying
is in progress by a curved rectangular plate (RS), 18 inches
by 23 inches, which is bolted in place. In the centre of the
removable section is a 10 inch square window (W). The lower
section (LS) is 15 inches high by 21 inches in diameter, and
was made smaller in diameter to limit the amount of turbulence
in the spray mist directly above the spray drawer.

Although spray solutions were not sprayed to the point of
run—off, the inner surface of the lower section was cleaned
regularly to prevent build up of liquid, and the resultant
possibility of this liquid running into the spray drawer
during exposure of test material to the spray mist. The
overlap of the middle section over the lower section retained
any excess spray liquid collecting on the walls of the middle
and upper cylinders. (Figures 3 and 4).

To overcome any possible build up of electrostatic
charge on the spray tower (Potter, 1941) the three metal
cylinders were connected together and earthed.

Including the base the overall height of the tower is
87 inches. The base (B) is a wooden construction strengthened
by internal braces, and large enough (4 feet long, 3 feet wide,
5 inches deep) to give complete stability. Towards one end
of the base is fixed a wooden framework (BSS) incorporating

the spray drawer (D) and lower shutter (BS), and upon this is set the support for the lower section (LSS). Between the lower and middle sections is a framework (CF) consisting of twohhalf
_ inch thick circles of composition board, one attached to each cylinder and the two bolted together externally. The upper section support incorporates the upper shutter (TS) and is made in two parts, the lower and larger upper shutter support (TSS) being a sheet of board supported below by two wooden strips 4 feet long and $1\frac{1}{2}$ inches thick. Two very thin wooden strips above the board serve as guide runners for the upper shutter. The second part of the upper section support (USS) fits closely around the cylinder, and is strengthened by four wooden braces, one along each side running tangential to the cylinder itself. The whole framework is bolted together by six large bolts (WN). The upper shutter (TS) is of polished hardboard, guided into position by the runners mentioned above.

The spray drawer (D) is made of wood with a hardboard base. Before spraying the drawer base was covered with absorbent paper to prevent its contamination with spray materials. The lower shutter (BS) is held about $\frac{1}{2}$ inch above the drawer, and is made of aluminium slightly dished in the centre to collect excess spray material.

When firmly bolted together the whole structure was rigid
and too large to be levelled with adjustable screw supports.
It was, therefore, levelled with metal wedges beneath the base
and the use of a spirit level checked that the apparatus
remained level during spraying.

The whole tower was easily dismantled for cleaning.

THE NOZZLE MOUNTING

The nozzle is mounted (Figure 5) in a 6 inch diameter
hole in the centre of the lid (L). Three mild steel rods are
screwed into the body of the nozzle and project at angles of
120 degrees to each other. These rods are located distally in
the terminals of three levelling screws located in turn in
one inch radial slots in the tower lid. By means of these
screws it is possible to centre the nozzle and to align it to
spray vertically down the tower. The use of a plummet
(Tattersfield and Morris 1924), ensures that the nozzle is
mounted directly above the centre of the spray drawer.

An exhaust outlet (E) is provided in the lid but this
proved unnecessary when an extraction fan was fitted in the room
wall near the top of the tower. In view of the large size of
the tower it was considered necessary for the operator to wear
a mask while spraying was in progress.

The nozzle described by Potter (1952) was used without

35.
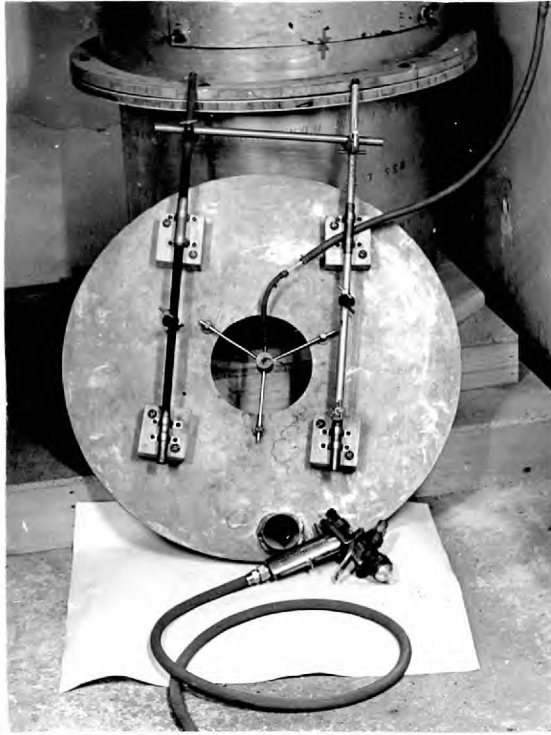
Figure 5. Lid of Spray Tower with
nozzle and nozzle mounting and
showing supporting struts fitted
for Spray gun.

modification (as mentioned above).

To obtain an accurately measured dose, liquid to be sprayed is pipetted into a reservoir supported next to the nozzle. The liquid is drawn into the nozzle through a feed pipe by air passing through the annular orifice surrounding the liquid jet, and is thus atomized.

LOCATION AND USE OF TOWER

The tower was used in a tall narrow room maintained at $22^{\circ}C \pm 2^{\circ}C$ by means of a combined heater and fan unit. All spray liquids were allowed to attain temperature equilibrium in the room before use. Humidity control was not practical.

Compressed air was first passed through a reservoir to reduce air-flow fluctuations and then through two pressure reduction valves. A mercury manometer and an air-flow meter in the circuit monitored the air flow rate. At the air pressure used, (76 cm. Hg), the nozzle gave good results for all liquids sprayed.

SPRAYING PROCEDURE

Ten ml. of liquid were sprayed down the tower irrespective of delivery time, which varied with the viscosity of the liquid. Dean (1951) relied on a solenoid switch in the air system to allow delivery of spray for an accurately measured time, but ten Houten and Kraak (1949) preferred to spray a known volume

of liquid, to overcome factors such as unnoticed partial blockage of the nozzle.

Before use, the nozzle was flushed out with a small quantity of acetone sprayed under operating air pressure, a procedure advocated by Roan and Kearns (1948), Allen, Dicke and Brooks (1943), Bottimer (1945), and Subba Rao and Pollard (1951). The liquid to be sprayed was used as a priming dose (Webb 1947) until the atmosphere within the tower was saturated.

The lower shutter was used to remove large droplets produced during spraying; the shutter was removed 5 seconds after spraying was completed and replaced after 3 minutes. The upper shutter was not used during spraying of Larch trees because, when in use, loss of spray mist occurred even when using reduced pressure and less spray fluid. Standardisation of tree size, and the distribution of spray produced with and without the upper shutter are described later.

The effect of turbulence created in the tower is known to assist in the production of an even spray deposit (Roan and Kearns 1948, Potter 1952). As the amount of turbulence can vary between spraying operations regular checks were made by weighing deposits delivered on to glass slides placed in standardised positions in the spray drawer.

TOWER PERFORMANCE

Three liquids — acetone, water and Risella oil (R.17) were selected to examine the performance of the tower and provide details of the distribution and replication of deposits from liquids of widely differing physical properties (Potter, 1952). The liquids were sprayed on to glass slides arranged in the spray drawer; these slides were first oven dried at 100°C, cooled in a dessicator, weighed and returned to the dessicator in the spray room.

Acetone

10 mls. of a 5% solution of the insecticide γ-BHC in acetone were sprayed on to five 3 inch by 1 inch glass microscope slides, four arranged round the periphery of a 12 inch diameter circle in the spray drawer with the fifth slide in the centre. After spraying the acetone was allowed to evaporate and the amount of insecticide deposited on each slide was weighed. Ten sprayings were carried out (Table 4).

Water

10 mls. of a 1% solution of the dye methylene blue in distilled water were sprayed on to glass slides arranged in the same positions as above. The deposit on each slide was washed into a volumetric flask and the volume was made up to 25 ml. with distilled water. The colour intensities of the solutions were measured with a Hilger "Spekker"

photoabsorptiometer and the weights of dye deposited were estimated by comparing with a standard calibration curve. Ten sprayings were carried out (Table 5).

Risella oil (R.17)

The quantities of oil deposited on the glass slides from the 10 mls. sprayed were weighed directly. Six sprayings were carried out (Table 6). The physical properties of Risella oil (R.17) are given in Table 7.

DISCUSSION OF TOWER PERFORMANCE

For all the sprays the mean deposit on the centre slide was slightly lower than the mean deposits on the four peripheral slides, but in the three experiments using the different spray liquids there was no significant departure from evenness of deposit at one time of spraying over the area tested, using the F test (Fisher 1936) at the 5% significance level. The same test showed that there were significant differences of deposit between trials. These differences, though significant, are nevertheless relatively small. (Tables 4, 5 and 6).

Hewlett (1946) states that in work on insecticidal sprays, the standard deviation of deposit is usually a better measure of performance than the standard error because, in general, the variation between individual deposits is more

important than the difference between the means of sets of deposits. For all spray results the standard deviation of a single deposit is given, together with the standard errors of the means of deposits for positions and trials.

From the figures obtained it was concluded that the tower performance was satisfactory for each of the spray liquids used. The effect of the differences between trials was taken into account by checking the deposits obtained at intervals during spraying.

The results for distribution and replication in the tower were better than expected for an apparatus of this size. It has already been mentioned that turbulence is an important factor in the production of an even deposit, and the results obtained might be explained in terms of this effect. The large volume of air in the tower is relatively static before spraying is started. The impact of the cone of spray liquid from the nozzle on the air mass may tend to produce turbulence near the top of the tower, as there is considerable room for the expansion of the spray as compared with smaller apparatus of this type. The turbulence would continue down the tower cylinder until the whole air mass was moving. The smaller diameter of the lower section of the tower immediately above the spray drawer would tend to lessen the amount of turbulence slightly, allowing a more stable mist to settle on the objects in the spray drawer.

DROPLET SIZE

Droplet size may be measured in several ways; by measuring crater marks produced on coated slides (May, 1945, Hoskins and Caldwell 1947, Roan and Kearns 1948); by the measurement of spherical droplets deposited in non-miscible, viscous materials (Campbell and Sullivan 1938, Parr and Busvine 1948) and by the preparation of microphotographs of sprays (ten Houten and Kraak 1949).

In the present work it was intended to investigate the penetration of spray droplets in two size ranges, coarse droplets above 100 μ in diameter, and fine droplets below 25μ diameter. With aqueous sprays, measurements were made using the technique of May (1945), that is measuring the diameter of craters formed by the impact of droplets on slides coated with magnesium oxide, and the technique of Parr and Busvine (1948) measuring the diameter of spheres formed from the spray droplets in a non-miscible medium, a heavy mineral oil.

The larger spray droplets were removed by the lower shutter for 5 seconds after spraying finished, and the spray mist was allowed to settle for 3 minutes.

The diameter of the spray droplets measured ranged from 40 μ to 260 μ. These droplets were obtained within the first three minutes after settling commenced and were considered as coarse droplets. Attempts to produce smaller droplets

in sufficient quantity with the apparatus were unsuccessful.
If the shutter remained closed until all the coarse droplets
had settled finer droplets were obtained (David 1946), but not
in sufficient quantity due to the size of the apparatus.
Increasing the air pressure to obtain finer droplets
(Campbell and Sullivan 1938 and Roan and Kearns 1948)
resulted in very uneven distribution and was, therefore,
unsatisfactory.

THE STRUCTURE AND PERMEABILITY OF THE WAX-WOOL COVERING SECRETED BY CNAPHALODES STROBILOBIUS (KALT).

INTRODUCTION

Cnaphalodes strobilobius (Kalt) belongs to the family Adelgidae (Homoptera, Aphidoidea) which is separated from the closely related family Aphididae mainly by the fact that all females, sexual and parthenogenetic are oviparous while all parthenogenetic females of the Aphididae are viviparous.

The life history of many species of Adelgidae has been intensively studied; all species are obligate pests of conifers and most species normally require a primary and a secondary host on which to complete the life cycle. The primary host is invariably a species of Spruce (Picea) and the secondary host either Larch (Larix), Douglas Fir (Pseudotsuga), Pine (Pinus), Fir (Abies), or Western Hemlock (Tsuga). The life cycle takes two years to complete through five generations and in all species has proved difficult to elucidate because of its complexity. Many species are difficult to distinguish anatomically and there has been considerable confusion over terminology.

Of the two very similar species known to infest Larch, Adelges (Chermes) viridis (Ratz) and Cnaphalodes strobilobius (Kalt), the latter species was studied in the present work.

Identification of this species was based on the following
observations:-

1. Cnaphalodes infests the twigs and leaves of Larch (Steven
   1917) contrasting with Adelges which is primarily a bark
   feeder.

2. Cnaphalodes is black in colour (Burdon 1908b); Adelges
   is more green than black.

3. The apterous progrediens (see life cycle) of Cnaphalodes
   produce eggs under a copious wax-wool secretion on the
   leaves (Figure 6),differing in this respect from Adelges
   (Burdon 1908b).

4. The head and prothoracie plates of the first stage
   colonici sistens larvae of Cnaphalodes are fused into a
   homogeneous shield,while in Adelges the plates are
   separate (Steven 1917).

LIFE CYCLE

The basic details of the life cycle of this species
were worked out over a period of many years by such
authorities as Blochmann, Börner and Cholodkovsky. The
publications of these workers are numerous and reference
is made in the Bibliography to a single publication only.
Burdon (1908a) and Steven (1917) have described the life cycle
in Britain. The last mentioned author was the first to record

Figure 6. Secretion of wax—wool by
Cnaphalodes strobilobius. X c.7

A   E   C   B   D

Figure 8. Phytotoxicity of Oil emulsion
sprays to larch trees.

that Larix leptolepis Murr (Japanese Larch) is an
intermediate host for this species.

Crooke (1952) and more recently Steffan (1962) have shown
that Adelges viridis (Ratz) (Sacchiphantes—complex of the
latter author) can break the normally obligatory alternation
of hosts and maintain themselves wholly on the secondary host
by parthenogenetic reproduction, at least for a number of
seasons.

The life history of Cnaphalodes strobilobius (Kalt)
is complex and it was important to understand it as far as
possible to ensure that the same developmental stage was used
for experimental work. Alate adults were captured in traps
during the establishment of the experimental culture
(Figure 2) and provided evidence that the normal life cycle
was operating. Whether a reduced parthenogenetic cycle on Larch
alone provided a further source of infestation is not known.
Figure 7 is a diagrammatic representation of the life cycle
of Cnaphalodes strobilobius (Kalt) in Great Britain compiled
from several sources (Burdon 1908a, 1908b, Steven 1917,
Speyer 1919, and Crooke 1952) and shows the normal life cycle
with inset, the possible reduced parthenogenetic cycle on
Larch. The authors of the terminologies used for the
different generations are referred to in Figure 7.

Diagrammatic representation of life cycle of <u>CNAPHALODES STROBILOBIUS</u> (KALT)
compiled from various sources

SEXUPARA (Dreyfus 1889)
IV

SEXUALIS
V
(Lichtenstein 1878)

<u>PICEA</u>
Primary host

<u>LARIX</u>
Secondary host

Progrediens alata

Sistens

Progrediens
aptera

FUNDATRIX
I
(Lichtenstein 1878)

COLONICI (Steven 1917)
III

Gallicola non-migrans

Gallicola migrans

GALLICOLA (Steven 1917)
II
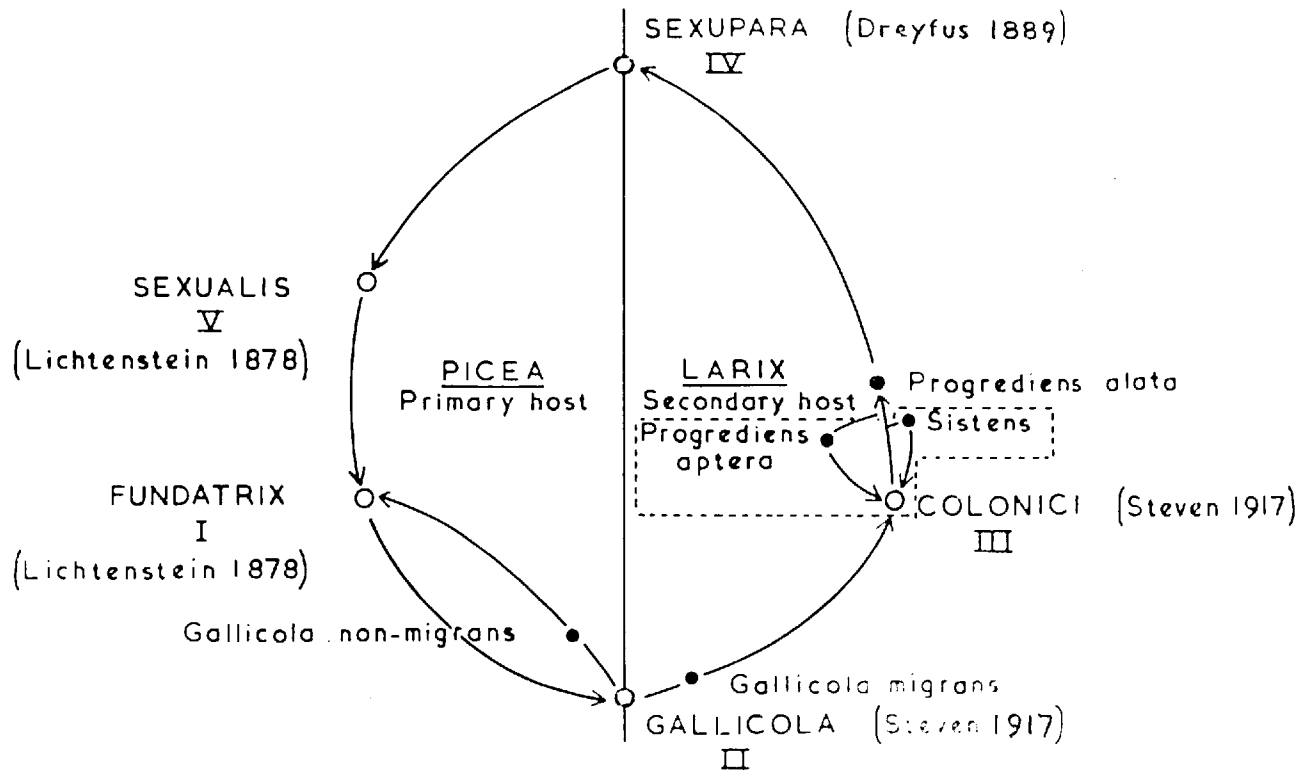
Inset represents Reduced
Parthenogenetic Cycle.

Figure 7.

47.

Three of the five generations are completed on <u>Picea</u>,
one sexual (Figure 7, V) and two parthenogenetic (Figure 7,
I and II). The fundatrix (stem-mother) lays many eggs which
develop into first stage gallicolae larvae, and these together
with the fundatrix hibernate in a gall produced from a weak
spruce bud. This is an example of "passive" protection
referred to earlier. The following spring two types of larvae
are produced, alate gallicolae migrans which carry the
infestation to the secondary host <u>Larix</u> and apterous
gallicolae non-migrans which help to build up the infestation
on <u>Picea</u>. The colonici generation on <u>Larix</u>,derived from the
gallicolae migrans lays many eggs. These hatch as two types
of larvae, sistens and progrediens. The sistens are all
apterous; some serve to build up the population after
hibernation while others continue feeding (impulse feeding)
on the Larch needles, moult and eventually die. After the
third moult progrediens larvae develop into two types, the
first becoming apterous adults which lay many eggs, under
copious wax-wool, while the second becomes the alate sexupara
generation which migrates to <u>Picea</u>. Eggs laid by this
generation develop into the sexualis, or sexual, generation.
The fertilised female sexualis lays one egg from which the
fundatrix develops to renew the cycle. The cycle takes two

years to complete.

In the present work the apterous, colonici progrediens generation which secretes a wax-wool covering was studied (Figure 7, III).

CHEMICAL CONTROL OF ADELGIDAE

All species of Adelgidae are pests on conifers but their economic importance is generally slight. Infestations are certainly unsightly, an important factor on ornamental trees, and their attack may render trees more liable to secondary fungal infection. It is also known that attack can lead to the formation of compression sapwood (rotholz) thus rendering timber unsuitable for use (Varty, 1956). Whether the insects reduce vigour or rate of growth of their hosts is not certain, but they can cause considerable damage in seed orchards where the trees are particularly valuable (Crooke, 1952).

Originally some control was obtained by spraying infested trees with paraffin and soap solutions (Burdon 1908a). Modern control measures rely on chlorinated hydrocarbon and organo-phosphorus insecticides. The high fecundity of the insects and the fact that they secrete themselves in crevices in the bark make it necessary to treat infested trees every two or three years (Schneider-Orelli, 1950). Several insecticides were examined as sprays by Flamant (1962) and as sprays and

dusts by Petersen (1962). The latter author found that diazinon and γ-BHC were satisfactory, when used alone, for control of Chermes nordmannianae Eckstein on Silver Fir, but the best material was Midol 556 (3% DDT., 2% γ-BHC and 1.5% chlorbenside). Dusts were less effective than sprays. Flamant (1962) found that a single winter spray of γ-BHC or parathion caused high mortality of Chermes tardus on Spruce. Wettable powder sprays (75% DDT and 25% γ-BHC) were effective against Cnaphalodes strobilobius (Kalt) on Larch (Kerr, 1953).

The wax—wool secretion of certain generations of the insect is an effective barrier to sprays, but Lightly and Faulkner (1964) state that control of light infestations of a number of species on many types of conifers in nurseries, is satisfactorily achieved . by fumigation with γ-BHC. Heavier infestations are better controlled by mist applications of malathion.

STRUCTURE OF WAX—WOOL SECRETED BY CNAPHALODES STROBILOBIUS (KALT).

Physical

The terms 'wool' and 'wax—wool' used by many authors to describe the material secreted by certain generations of species of Adelgidae, relate both to its general appearance and chemical nature. The wax—wool of Cnaphalodes strobilobius

(Kalt) is composed of long brittle filaments of waxy material interspersed with shorter fluffier masses of filaments, the whole structure completely covering the insect. Burdon (1908b) described a number of characters useful for identification of species of Adelgidae found in Britain; one of these characters was based on the type of wool produced, which was short and 'frizzy' in Adelges (Chermes) viridis (Ratz) and long and straight in Cnaphalodes (Chermes) strobilobius (Kalt).

In Cnaphalodes the material is secreted through pores located in glandular plates on the dorsal surface of the insect (Burdon 1908b; Steven 1917) and is distributed around and over the whole body. The eggs laid by the colonici generation are sometimes also protected by the secretion.

A number of hypotheses dealing with the secretion of wax by insects are mentioned by Locke (in Rockstein 1964). The wax may be conveyed to the body surface in a solvent which later evaporates,as in the cockroach (Beament 1955) or may be secreted in a water soluble form in combination with a protein as in the eggs of ticks (Lees and Beament 1948). Pore canals in the cuticle may allow passage of wax to the exterior; in a number of insects final synthesis of wax by an esterase in the pore canals and epicuticle has been suggested (Locke 1961). Rogojanu (1934) found no pore canals in the 'coccid' Eriosoma and concluded that the wax escaped in a liquid state. In Orthezia he observed discharge of

wax through the apex of hollow hairs on the body.

The wax-wool secretion of Adelgidae is extruded in a filamentous form through wax pores in the cuticle, but the source and method of production of the secretion is not known.

Chemical

The researches of Chibnall et al. (1934a, 1934b, 1934c) on the chemical composition of waxes suggested that all waxes are essentially of the same general type, containing primary alcohols, normal fatty acids and paraffin hydrocarbons. Waxes differ in chemical composition only in the proportions in which these products occur; the physical properties are determined not only by the amount and respective chain length of paraffins (C_{25-37}), free primary alcohols (C_{24-36}), free normal fatty acids (C_{24-34}) and true wax esters present, but also by the chain length of the two compounds of the wax esters (Chibnall et al. 1934a). However, Baker et al (1960) estimated the percentages of the constituents present in the epicuticular wax of the Mormon cricket, Anabrus simplex Hald, as 48-58 per cent hydrocarbons, 15-18 per cent free acids, 9-11 per cent esters, 2-3 per cent free alcohols and 12-14 per cent acidic resin polymers. Gilby and Cox (1963) found the grease of the cockroach, Periplaneta americana (L), to contain 75-77 per cent hydrocarbons (C_{25-27}), 7-11 per cent

fatty acids (mostly C_{18}), 8-9 per cent aldehydes, 3-5 per cent esters and underl per cent sterols. Warth (1956) stated that the free alcohol content of beeswax was very low. These results contrast to those obtained by Chibnall et al. (1938a) who claimed that high quantities of free alcohols were present in waxes.

Blount, Chibnalland el Mangouri (1937) found that the wax of White pine Chermes, Adelges strobi Börner was very similar to that of Chinese insect wax or cochineal wax obtained from Coccus cacti.

They identified the components as a ketonic alcohol (17-keto-n-hexatriacontanol) esterfied by a ketonic acid (11-keto-n-triacontanoic acid) with a negligible amount of n-fatty acid. These compounds all possess the common group $CH_3(CH_2)_{18}CO-$ and fit neatly into the scheme of metabolism of waxes proposed by Chibnall and Piper (1934c).

The chain lengths of the constituents were foretold by X-ray analysis and the constitution was determined by putting the oxines of the respective ketonic acids through a Beckmann transformation and hydrolysing the resulting mixed amides. (Blount et al. 1937).

In view of the time available and the complicated chemical nature reported for the wax of Adelges strobi no

attempt was made to identify the components of the wax-wool
of <u>Cnaphalodes strobilobius</u>, but it is reasonable to assume
that the compounds present may be similar to those of
<u>Adelges strobi</u> wax.

THE PERMEABILITY OF THE WAX-WOOL SECRETION OF
CNAPHALODES STROBILOBIUS (KALT.)

A number of methods for assessing the permeability of
the protective covering of this insect were tried but very
few were successful. Topical aplication of solutions of dyes
in water and oils gave little indication of the degree of
permeability, due mainly to the small size of the insects and
the difficulty of recovering sufficient dye for colorimetric
estimation. High control mortality was obtained after topical
application of γ-BHC in oils, odourless kerosene and hexane,
and it was considered unsatisfactory to apply small volumes
of emulsions containing the insecticide.

The work of Greenslade (1934) and Ebeling (1939), who
investigated the penetration of insecticide solutions con-
taining different amounts of emulsifiers into the waxy
coverings of Eriosoma lanigerum (Hausm) and Aonidiella
aurantii (Mask) respectively, suggested that it might be
of interest to study the penetration of oil and water
emulsions containing different proportions of emulsifier and
γ-BHC. Insects, in situ on Larch trees, were, therefore,
sprayed with two such emulsions.

The permeability of the protective covering of
C. strobilobius was also investigated by exposing protected
insects to saturated γ-BHC vapour; the insecticide was used
as the normal γ-isomer and as carbon 14 labelled γ-BHC,

to study the rate of uptake of the insecticide and the possible method of penetration through the wax—wool covering.

SPRAYING WITH EMULSIONS CONTAINING γ-BHC.

Young Larch trees infested with <u>Cnaphalodes strobilobius</u> were sprayed with two water—kerosene oil emulsions, containing different amounts of a non—ionic surface active agent, Lissapol NX.

The use of oil—containing sprays gave rise to the problem of possible phytotoxic effects on the trees (referred to earlier in "Culture of Insects"); γ-BHC has also been shown to have phytotoxic effects. Gast and Early (1956) suggested that phytotoxicity of sprays is often caused by the emulsifier used rather than by the solvent. Stoker (1948) showed that the use of γ-BHC sprays below 0.04 per cent concentration avoids direct foliage damage to most plants, while Rohwer (1949) stated that plant safety would be ensured if the major part of the α and δ isomers of BHC were eliminated. Simkover and Shenefelt (1952) applied BHC to twenty species of Pine, via the soil, at rates of one pound per acre, and found that γ-BHC caused root malformation in all species.

It was mentioned earlier that the upper shutter was not used during spraying of Larch trees. With the upper shutter in the closed position, spray mist was lost from the tower even when reduced pressure and less spray fluid were used.

Consideration of the dimensions of the trees suggested that a regular and repeatable spray could not be expected. Measurements of the amount of spray deposited in different positions on several trees, standardised as far as possible for height and general shape, showed this to be the case. It was, therefore, decided for this section of the work to spray directly and to take a far larger representative sample of insects for mortality counts. 150 insects were taken at one and three day intervals after spraying for each mortality count. The same number of control insects were taken from trees sprayed with the emulsions alone.

As far as possible, all trees sprayed were not more than 4 feet high, and the spread of the lateral branches was not more than 2 feet 6 inches.

The following emulsions were screened for phytotoxicity. All emulsions contained 1.0 per cent γ-BHC.

| Emulsion | Parts by volume | | | |
	Kerosene oil	Decane	Water	Lissapol NX
A	——	——	8	2
B	——	6	3	1
C	——	3	6	1
D	6	——	3	1
E	3	——	6	1

5 mls. of each emulsion were sprayed directly onto the trees in the spray tower, and the trees were removed from the tower three minutes after spraying finished. The pots and soil were covered during spraying to prevent soil contamination.

Figure 8 shows the typical results of these spray tests fifteen days after spraying. Emulsions B and D caused heavy defoliation of the trees within this period; emulsions C and E caused slight leaf scorch, less severe with emulsion E. No phytotoxic effects were observed after spraying with solution A.

Emulsion E was selected to investigate the permeability of the wax-wool secretion of C. strobilobius, but the emulsion was modified by the addition of different quantities of insecticide and surface active agent.

Experimental Procedure

Larch trees, heavily infested with adult colonici progrediens of C. strobilobius, were collected from the experimental plot and placed in the spray room for 12 hours at $22^{\circ}C$ before spraying. The trees were introduced into the tower through the hole in the middle section (Figure 3) and the removable section was bolted in position. The two emulsions sprayed contained five different concentrations of δ-BHC, 1,000, 100, 10, 1.0 and 0.1 mgs. per cent., and 0.2 and 2.0 parts by volume of Lissapol NX respectively.

The trees were removed from the spray tower 3 minutes after spraying finished, and were replaced in the experimental plot. 150 insects were taken from each tree one and three days later and mortality counts were made. The sampling procedure was standardised, 50 insects being taken at random at approximately 1, 2 and 3 feet levels measured from the lowest branch.

Results and Discussion

The results are shown in Tables 8 and 9 . After 24 hours, the LD.50 values for the insects sprayed with the two emulsions differed significantly at the 5 per cent probability level. It appears that the emulsion containing the larger amount of surface active agent was able to penetrate the wax-wool covering of the insects more effect-ively. The LD.50 values after 72 hours were again significantly different although the mortality in both cases was similar to the mortality after 24 hours (Tables 8 and 9).

The mortality of the insects sprayed with the emulsion containing the larger quantity of surface active agent was significantly greater after three days (Table 9) than after one day.

Ebeling (1939) found that mortality of the red scale (Aonidiella aurantii (Mask)) caused by a given toxicant, correlated with the effectiveness and concentration of

the spreader used; the more effective and higher concentrations resulted in greater insecticidal efficiency, due to an increased rate of penetration through the mass of **waxy** threads under the bodies of the insects.

Greenslade (1934) found that <u>Eriosoma lanigerum</u> (Hausm.), a woolly aphid, was killed by a 0.025 per cent concentration of nicotine if the liquid actually reached the insect body, but he found that it was difficult to penetrate and wet the mass of wax threads covering each insect. The percentage kill was increased from 1 to 96.6 by the same concentration of insecticide, as the percentage concentration of emulsifier (a soft soap solution) was increased from 0.1 to 3.

It appears from these results that the amount of emulsifier present in the emulsions sprayed, considerably influenced the rate and degree of penetration of the wax-wool covering of <u>C. strobilobius</u>.

EXPOSURE OF CNAPHALODES STROBILOBIUS TO SATURATED VAPOUR OF γ-BHC

These experiments were carried out in two ways. The first series of experiments investigated the rate of action of saturated γ-BHC vapour, by exposure of insects protected by their wax-wool covering. Subsequently a further series of experiments using the saturated vapour of carbon-14 labelled γ-BHC investigated the rate of uptake of the insecticide by the wax-wool covering and by the insect itself.

Apparatus

To maintain an insecticide saturated environment special fumigation chambers were designed (Figure 9)

The body of the chambers were 2 cm. lengths of brass tubing, 6.3 cm. internal diameter with a flat base soldered to one end. At 120 degree intervals round the outer circumference, hexagonal studs were soldered flush with the top of the chamber, of sufficient length to accommodate a screw thread firmly. The circular lids were of perspex, 7.9 cm. in diameter and 0.3 cm. thick; three holes drilled through the lid corresponded in position to the screw threads fixed to the chamber body. A rubber ring and two circles of

Figure 9. Chambers used for exposure
of Plodia interpunctella pupae and
Cnaphalodes strobilobius to satur-
ated vapour of γ-BHC.

polythene placed between the lid and the top rim of the
chamber acted as leak-proof gaskets. The lids were clamped
tightly to the chamber bodies by 4 B.A. nuts.

To test for air tightness the chamber bodies were heated
to dryness in an oven, the gaskets and lid were dried in a
dessicator over solid potassium hydroxide, and crystals of
anhydrous copper sulphate were introduced. The state of the
crystals remained unaltered over a period of ten days in a
humid atmosphere.

About 0.5 gm. of solid γ-BHC crystals were placed in a
small petri dish covered with a circle of metal gauze, which
provided a surface for the insects and also prevented the
test insects falling into the insecticide container.

The atmosphere within the chambers was allowed to become
saturated with insecticide vapour for 12 hours at $25^{\circ}C$ before
the insects were introduced. A small container of saturated
sodium chloride solution in the chamber maintained the
relative humidity at 75 per cent.

Procedure

25 adult insects, completely covered with wax-wool
secretion, were placed on the gauze circle, without detaching
them from their leaves. The exposure periods ranged from

6 hours, increasing by 6 hour intervals, to 48 hours. At the
end of the exposure periods the chambers were opened and a
mortality count was made. All dead insects had withdrawn
their sylets from the leaf but the criterion of death used
was inability to move any of the appendages after stimulation
with a needle.

Results

The results are shown in Table 10 , and show that
the rate of action of ϒ-BHC vapour is fairly rapid. During
the initial stages of exposure little mortality occurred
but after about 18 hours exposure mortality increased
rapidly. Over half the insects were dead after 30 hours
exposure, but mortality was not complete even after 48 hours
exposure.

The discussion of these results is included in the
next section.

EXPOSURE OF CNAPHALODES STROBILOBIUS (KALT) TO VAPOUR OF CARBON-14 LABELLED γ-BHC

INTRODUCTION

The quantitative measurement of very small amounts of insecticides picked up by insects has been made possible by the development of radioactive tracer techniques.

Lewis (1963) used iodine-131 to investigate the spread of oil over the cuticle of _Phormia terraenovae_ R-D. Tritium-labelled oils and carbon-14 labelled dieldrin were used in a second series of experiments to study the penetration of both an oily solvent and insecticide through the cuticle of _Tribolium castaneum_ Herbst. Both these series of experiments used active insects crawling over treated filter papers.

Gösswald et al (1963) studied the pickup of sulphur-35 labelled thiodan by the surface of the integument of _Sitophilus (Calandra) granaria_ L. and stated that the vapour phase of the insecticide was advantageous because test insects are exposed to pure insecticide in molecular form.

In the present work _Cnaphalodes strobilobius_ (Kalt) was exposed to the vapour phase of carbon-14 labelled γ-BHC, to investigate the relative adsorption, penetration and assimilation of the insecticide of and by the wax-wool covering of the insect and by the insect itself. The vapour phase was used as these insects are sessile on Larch leaves.

MATERIALS AND METHODS

Radioactive material

The sample of Carbon-14 labelled γ-BHC, obtained from the Radiochemical Centre, Amersham, had a specific activity of 9.4 mc/mM (millicuries/milliMole) and its radiochemical purity was 95 per cent. The insecticide was supplied in benzene solution in a vacuum sealed ampoule which was opened by cutting a deep groove in the neck and applying a white hot glass rod to the groove; the sample was poured into a carefully cleaned glass tube and stored in a deep freeze refrigerator.

Insect material

The insects selected for experimental study were mature, adult colonici progrediens which had just started oviposition and which were completely covered with wax-wool secretion. All visible eggs were removed without disturbing the secretion. The insects were allowed to attain temperature equilibrium at the temperature of exposure (25°C) for four hours before use. Ten insects, each on a length of their original leaf, were used for each exposure.

Exposure chambers

The special exposure chambers (Figure 10) for studying the fumigant action of C-14 labelled γ-BHC were modified from an apparatus used in the Department for measuring uptake of aldrin by _Tribolium._ Each chamber consisted of a brass
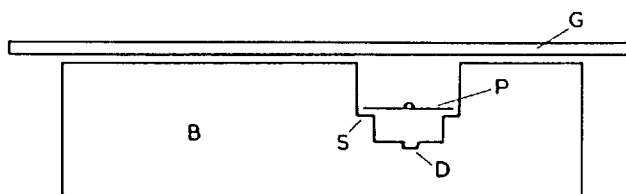
B — Brass block.
D — Depression for sodium chloride solution.
G — Glass cover.
P — Rocking platform.
S — Shelf.

Scale.
⊢————⊣
1 cm.

Figure 10. Diagram of chamber for exposure of C.strobilobius to saturated vapour of C—14 labelled ɣ- BHC.
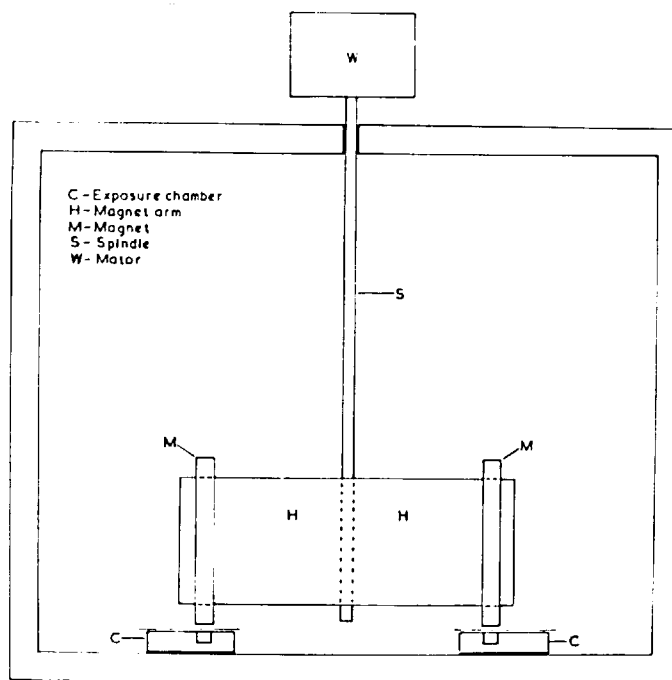
C — Exposure chamber
H — Magnet arm
M — Magnet
S — Spindle
W — Motor

Scale ———
2 inches

Figure 11. Diagram of magnetic stirring mechanism for use with exposure chambers (Figure 10).

block (B), 8.0 cm. by 4.5 cm. by 2.0 cm. deep, covered by a glass microscope slide (G) held in position by clips. A circular well drilled in two stages formed the actual exposure chamber. The upper well, 1.5 cm. across and 0.8 cm. deep contained the insect material; the insecticide was placed in the lower well, 1.1 cm. across and 0.4 cm. deep.

A thin perforated circular plate of aluminium (P), pivoted about a diameter on a thin steel rod supported in a short length of capillary tubing attached to the plate, formed a rocking platform. The ends of the steel rod rested in grooves cut in the shelf (S). Two small pieces of soft iron, secured on opposite sides of the top surface of the plate, were attracted in turn by a magnet passed over the chamber, thus rocking the platform. This rocking movement, assisted by the perforations in the plate, ensured even distribution of the insecticide vapour within the chamber. Two six inch long bar magnets, (Figure 11 , M) were secured vertically to either end of a 12 inch length of hardboard (H) which was attached at its mid point to the vertical spindle (S) of a motor-car windscreen wiper motor (W). The whole stirring apparatus was arranged so that the magnets oscillated just above the glass covers over the chambers (C) continually rocking the aluminium platform. The oscillation time of the magnets was 5.5 seconds.

Gösswald et al (1963) found that pickup of S-35 labelled thiodan by _Sitophilus (Calandra) granaria_ L. was considerably influenced by relative humidity. At high humidity a thin film of water covered the epicuticle reducing the solubility of the insecticide. At very low humidities, the rate of transpiration and loss of water through the cuticle acted against the deposition and pickup of thiodan molecules. The fastest rate of pickup was shown to be at the middle range of humidity where both of these factors were minimised. The relative humidity within the chamber was controlled at 75 per cent in the present work, by placing a small aluminium container of saturated sodium chloride solution in a small depression (Figure 10, D) in the floor of the exposure chamber.

All experiments were conducted at a temperature of $25^{o}C$ $\pm 0.3^{o}C$. To minimise temperature fluctuations, particularly those due to heat produced by the motor, all exposures were carried out in a constant temperature box (Figure 11) constructed from 1 inch thick sheets of polystyrene. The internal dimensions of the box were 20 inches high, 24 inches long and 19 inches deep. The motor was mounted outside this box with the spindle passing through the roof to support the magnet arm (H). A number of exposures could be carried out at the same time as the combined sweep of the two magnets covered a complete circle.

EXPERIMENTAL PROCEDURE

A series of trial tests were carried out using a range of μl. quantities of radio-active material in the chambers, to find the quantity required to maintain a constant concentration of insecticide vapour within the chamber for the exposure periods used. 25 μl. of the original benzene solution was selected as satisfactory.

All component parts of the exposure chambers were cleaned by rapid immersion in concentrated nitric acid followed by rinsing in glass-distilled water, and then dried in an oven at 110°C.

25 μl. of the radioactive solution were introduced on to the base of the chamber from a micro-capillary tube. The benzene was allowed to evaporate, the shelf and pivot were placed in position and the glass cover fixed on, and the whole apparatus was placed in the constant temperature box to equilibrate for 12 hours. To expose the insects the chambers were carefully opened (in 25°C surroundings) and the insects quickly placed in position on the aluminium shelf. A mark on the glass cover allowed it to be replaced in exactly the same position. The chambers were returned to the constant temperature box and the stirring apparatus was started. After checking that the pivot mechanism was working smoothly the constant temperature box was closed and the apparatus left for

the required exposure time. Regular checks were made on the pivot mechanism.

Insects were exposed for periods of time ranging from $\frac{1}{2}$ hour to 48 hours in order that the rate of uptake and penetration of the insecticide by and through the wax—wool secretion and the rate of assimilation by the insect could be determined.

Wax—wool extract

The insects were taken singly from the chamber, detached from the leaf fragment and the wax—wool carefully removed beneath a binocular microscope. The coverings were placed in a glass tube and rinsed with a small quantity of hexane which dissolved any deposited γ-BHC. Several separate rinses were made, not amounting to more than 5 ml., and these were combined in a separate tube. The residue,after hexane extraction,was checked for residual activity with a thin—end—window Geiger—Müller counter.

Insect surface extract

After removal of their coverings the insects were rinsed with several aliquots of hexane to a total of 5 ml. This dissolved the insecticide present in the epicuticular wax (Lewis 1963). The possibility of insecticide assimilated by the insects leaching out into the external washes was

considered. However, as the immersion periods in hexane were short any leaching which might occur was considered negligible.

Insect internal extract

This extract recovered insecticide that had been assimilated by the insects. The washed insects were placed in a hard glass tube with a small quantity of hexane and also anhydrous sodium sulphite to remove water, and crushed. A further quantity of hexane was added and the crushed insects were extracted in boiling hexane for two hours. When cool the hexane was pipetted off into a fresh tube; two washes of the crushed insects and the tube were combined with the original extract.

Water-soluble metabolites

The crushed insect material was also extracted with boiling water to dissolve any water-soluble derivatives of γ-BHC which might have been formed within the insects. No evidence of water-soluble metabolites of γ-BHC was found in the extracts.

Measurement of activity in the extracts

The amount of radioactive material in the extracts was measured in a scintillation counter, an Isotope Development Ltd. (IDL) Scaler 1700. The scintillation head was from Nuclear Enterprises (G.B.) Ltd. The machine was switched to

the working voltage thirty minutes before readings were taken,
and was set to accept all emissions at all energy levels. A
satisfactory working voltage for the machine was 900 volts,
and it was found to be necessary to avoid times of day when the
mains voltage was known to fluctuate.

Specially constructed tubes with clear quartz bases were
used to contain the standard C-14 solution and the samples.
Five readings for the C-14 standard were taken before and
after the sample readings to check the working efficiency
of the counter for each sample (Table 12).

The samples were added to 5 ml. of liquid scintillator;
the scintillation liquids, obtained already formulated from
Nuclear Enterprises (G.B.) Ltd., were NE 213 based on xylene
for the hexane extracts and NE 220 based on dioxane for the
water extracts. Before addition of the sample to the
scintillator five background counts were taken for each tube.
The samples were added to the scintillator together with a
wash of the sample tube and five counts were taken. The C-14
standard and background emissions were recorded as counts per
second (cps); the activity of the samples was recorded as
the number of seconds needed for 400 or 4000 counts. After
counting the sample was poured into a special container and
the counting tube cleaned.

The results obtained from these experiments are shown in Table 12 and Graph 2 . Table 11 is given as an example to show the method of recording and calculation of results.

Results and Discussion

The curves in Graph 2 show that in the initial stages of exposure, the rate of uptake of C-14 labelled γ-BHC by the wax-wool covering and the insect surface is similar and is fairly rapid. It appears that the build-up of γ-BHC on the wax-wool covering begins to level off after about 8 hours exposure, while at the same time the rate of uptake by the insect surface is still increasing.

Gösswald et al. (1963) found that S-35 labelled thiodan is to some extent soluble in the epicuticular lipoids of Sitophilus (Calandra) granaria. As γ-BHC, like thiodan, is a lipophilic material it seems possible that at first the build-up of γ-BHC on the wax-wool is due to surface adsorption; at a later stage the rate of uptake may decrease as the surface of the wax-wool covering becomes progressively more saturated with the insecticide. If this is the case, then after about 8 hours exposure the atmosphere contained within the wax-wool coverings would be saturated with γ-BHC vapour and a state of dynamic equilibrium would result,

Graph 2.

Exposure of Colonici progrediens of <u>Cnaphalodes strobilobius</u> (Kalt)
to vapour of C^{14}-labelled γ-BHC.

O — Insect surface extract.
● — Wax wool extract.
△ — Insect internal extract.
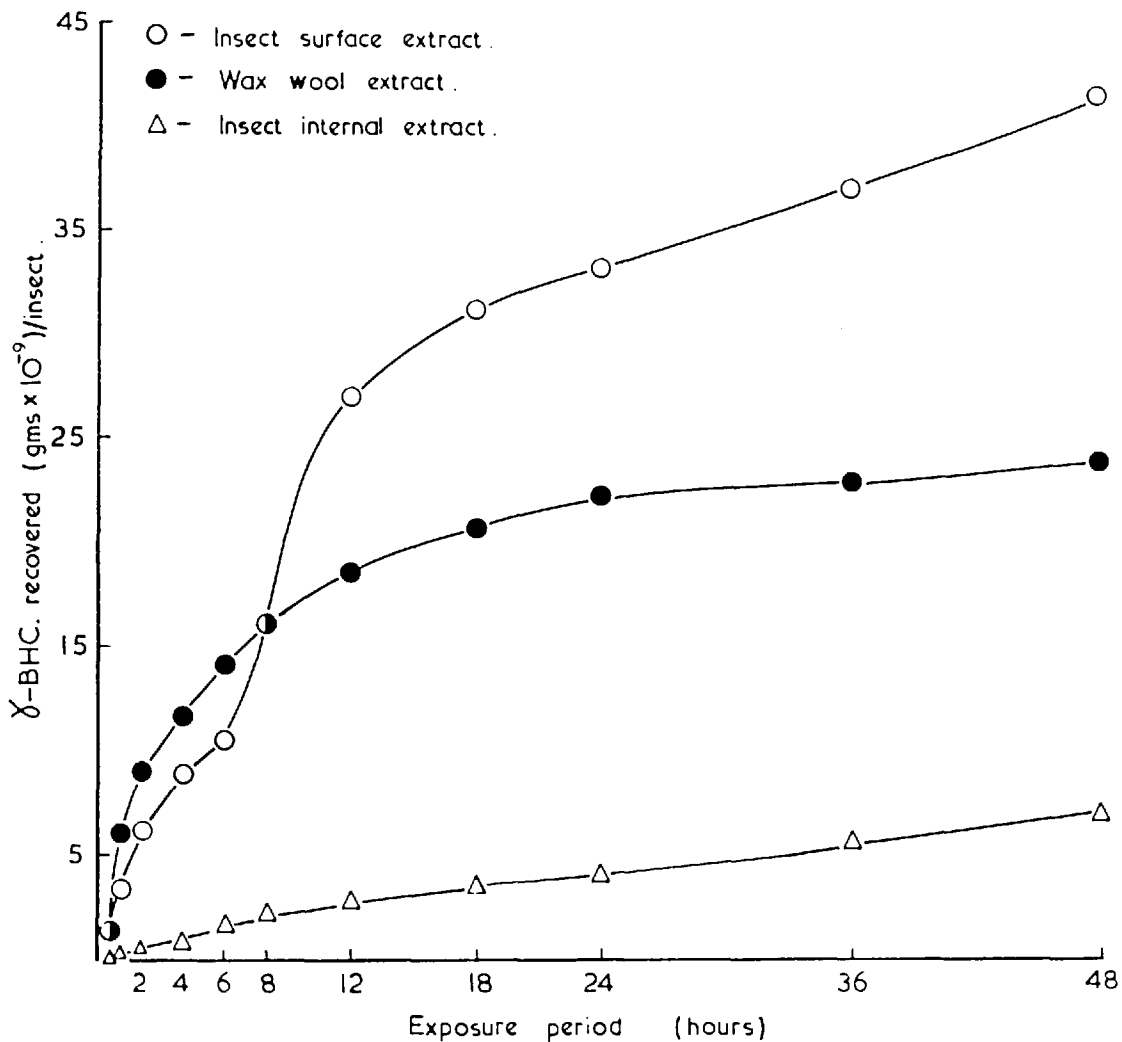
molecules of insecticide leaving and returning to the
wax-wool in equal numbers in unit time. The access of the
insecticide molecules to the body surface would, therefore,
be facilitated and may account for the increase noted in the
rate of uptake by the insect surface.

Gösswald et al. (1963) found that the pickup of S-35
labelled thiodan by freshly killed Sitophilus (Calandra)
showed a linear increase. In the present work, the curve
showing the amount of insecticide in the insect surface
extract increases linearly after 18 hours exposure, suggest-
ing that mortality of the insects increased fairly rapidly
after this time. Table 10 shows that 50 per cent mortality
of C. strobilobius exposed to saturated γ-BHC vapour (at the
same temperature, 25°C) would be expected to occur between
24 and 31 hours exposure.

The same authors separated the responses of Sitophilus
(Calandra) to thiodan at 25°C into five phases, and found
that during the third, or strong excitation phase, the rate
of pickup of the insecticide by the insect surface fell, due
to a physiological reaction of the insects. Armstrong et
al. (1951), investigating the penetration of different BHC
isomers into Sitophilus (Calandra), found that between 12 and
22 hours the amount of γ-BHC on the insect surface fell, and
concluded that this was due to the relative immobility of

the insects as almost complete mortality occurred after 22 hours.

The curve for the insect surface extract (Graph 2) shows a slight fall in the rate of pickup of γ-BHC between the 4 and 12 hour exposure periods. Although this fall is not marked, it may indicate a decrease in the rate of pickup of insecticide as a result of strong excitation, which in a small, sessile insect would possibly be less intense than in an active, crawling insect. A greater heterogeneity within the sample batches of insects exposed at the 4 to 12 hour periods, and possible fluctuations of mains voltage during counting of the samples, are considered less likely explanations as several observations are affected.

The amount of γ-BHC recovered in the internal extracts increases with time, indicating that penetration of the insects by the insecticide occurred. Armstrong et al. (1951) showed that γ-BHC penetrates dead Sitophilus (Calandra) freely, the rate of penetration remaining constant or increasing. The rate of penetration of C. strobilobius by C—14 labelled γ-BHC (Graph 2) increased linearly with time of exposure.

As was shown in the earlier fumigation experiment with γ-BHC, the wax—wool covering of C. strobilobius appears to have a protective function for a relatively short time only. The results of the radio—active γ-BHC experiments indicate

how the protective function of the covering might fail under treatment with a lipophilic insecticide.

It would be of great interest to compare the behaviour of other insecticides, both lipophilic and non-lipophilic, in the vapour phase under similar conditions.

THE STRUCTURE AND PERMEABILITY OF THE PROTECTIVE COVERING SECRETED BY NYMPHS OF PHILAENUS SPUMARIUS (F)

INTRODUCTION

Of the few palaerctic genera of Cercopidae (Homoptera) only four, Cercopis, Aphrophora, Neophilaenus, and Philaenus are found in Britain. The nymphs of the last three named genera are known as 'froghoppers', which at certain times of the year, May to early July, settle on vegetation and surround themselves with a frothy secretion, the well known 'cuckoo-spit' or spittle. This material is believed to act mainly as protection against varying climatic conditions, for example for prevention of dessication under strong sun (Kirkaldy 1906). It has been generally thought that the nymphs are protected in this way from predaceous insects and other Arthropods, but they are not infrequently seized from their spittle coverings by fossorial Hymenoptera, for example Gorytes spp. (Sphecidae). (in Imms, revised edition Richards and Davies 1957).

In the work described below the structure and permeability of the protective covering secreted by nymphs of Philaenus spumarius (F) was investigated.

IDENTIFICATION OF NYMPHS

A key to the adult Cercopidae of the British Isles

was prepared by Edwards (1896) but there is no key for identification of the nymphal stages. Correct identification was important in this work because naturally occurring material, that is not produced in the laboratory, was used for experimental purposes. Nymphs of three common spittle producing Cercopidae, _Philaenus spumarius_ (F), _Neophilaenus lineatus_ (L) and _Aphrophora_ sp. were found, the last named easily identified from their habitat, small shrubs and trees, and thus excluded from further consideration. The nymphs of the fourth British genus, _Cercopis_, are subterranean.

A number of characters which distinguish between the nymphs of _Philaenus spumarius_ (F) and _Neophilaenus lineatus_ (L) were studied and are summarised below, using genetic names only.

(a) _Host vegetation_ (Table 13)

Philaenus is found almost exclusively on plants other than grasses. _Neophilaenus_ is restricted exclusively to grasses. The type of host vegetation is of major importance to the survival of the nymphs. Osborn (1916) transferred nymphs of _Philaenus_ to grasses and found that they produced no spittle. Very few survived for any length of time although not all transferred nymphs died. Nymphs of _Neophilaenus_ removed from grasses to clover (_Trifolium_ sp.) all died.

(b) Position on plant

Philaenus nymphs form spittle masses in the axil of a
leaf petiole or inflorescence (Figure 12) towards the tip of
the plant. When supporting vegetation is removed the spittle
mass remains intact over the nymphs, being less liquid in
structure than that of Neophilaenus. The spittle masses of
Neophilaenus nymphs are formed low down on grass stems and
they are prevented from running down to the ground by the
proximity of other grass stems. If the grass is parted the
spittle mass runs to the ground often leaving the nymph
partially exposed.

(c) Colour

The nymphs of Philaenus have a pale to dark green thorax
with a yellow to orange abdomen. Neophilaenus nymphs are
generally pale yellowish green with occasional pale green
areas.

(d) Appearance of gonads

The developing gonads of Philaenus nymphs are not easily
visible through the abdominal wall until late in development.
In Neophilaenus nymphs, however, the developing gonads are
visible as red patches through the side of abdominal segments
two to five in the second and subsequent instars.

Figure 12. Spittle masses of nymphs
of Philaenus spumarius.

(e) Rostrum

The rostrum of Philaenus nymphs reaches at its tip to
the level of the metacoxae. Neophilaenus nymphs have a shorter
rostrum reaching at most slightly beyond the mesocoxae.

(f) Eyes and ocelli

The eyes of Philaenus nymphs are more purple than red and
the ocelli are not set in darker areas. Neophilaenus nymphs
have redder eyes and the ocelli are often set in darker areas.

(g) Morphometrics

Fewkes (1960) found that measurements of the head and
antennae of nymphs of Aeneolamia varia saccharina (Dist) could
be placed in five distinct groups, corresponding with the five
nymphal instars. Comparative measurements of various parts
of the head and body of nymphs of both the species under
consideration here, such as head width, frons width, rostrum
length, length of wing pads, varied too much over the number
of measurements taken to be of immediate value in this work.

The above mentioned characters were sufficient to
establish the identity of the species of nymphs found.
Edwards (1896) described many intra-specific varieties of the
Philaenus spumarius complex. Only three of his varieties were
found locally, viz:-
var. spumaria Auct., var. populi Fab., and var. lateralis
Linn. Of these varieties the former was most widely found,

but no attempt was made to distinguish the varieties of nymphs.

CHEMICAL CONTROL OF PEST SPECIES OF CERCOPIDAE

The nymphs of some species of Cercopidae are important crop pests, particularly on sugar cane and forage crops, and are controlled by spray and dust formulations of insecticides. Most control measures appear to avoid the problem of spittle penetration (c.f. Howden and Marshall 1961) and rely rather on the natural movements of the nymphs over the insecticide treated vegetation.

The meadow spittle bug, Philaenus leucophthalamus (L) has been controlled for many years by γ-BHC. Marshall and Gyrisco (1951) tested several insecticides against this insect and concluded that γ-BHC and dieldrin as sprays were the most effective. γ - BHC sprays at 0.4 lbs. per acre (App and Weaver 1953), 0.25 lbs. per acre (Weaver 1958) and 0.2 lbs. per acre (Fahey et al. 1960) have all been reported to give good control. The action of γ-BHC as a spray was investigated by Koehler and Gyrisco (1957) and the insecticide was found to be highly specific against Philaenus leucophthalamus (L) nymphs in the sap of lucerne. The mode of action of the insecticide appeared to be partly systemic.

After some years of treatment, populations of the sugar—

cane froghopper, <u>Aeneolania varia saccharina</u> Dist. have acquired resistance to chlorinated hydrocarbon insecticides. Subsequently after several years treatment with other types of insecticide, nymphs have been found to be susceptible to DDT again. Vlitos and Merry (1962) suggested that combination of DDT with faster acting compounds such as carbaryl or malathion, also rotation with compounds of different chemical structure, might be of use in the sense that resistance would not develop so rapidly.

Fewkes (1961a) showed that at any time up to 30 per cent of these nymphs may be underground. In a personal communication Fawkes (1961b) stated that penetration of spittle by insecticides is not considered important as excellent practical control is obtained with insecticides having a long residual action. Application of granular formulations controls the underground nymphs when they emerge from the soil. Howden and Marshall (1961) sprayed spittle masses of Philaenus spumarius (F) and Neophilaenus sp. with various concentrations of sulphuric acid containing 0.06 per cent dieldrin in an attempt to destroy the spittle and kill the exposed nymphs. 48 hours after spraying with a normal concentration of acid 95 per cent of spittle masses were destroyed.

A less economically important species, Aphrophora
saratogensis (Fitch), a pest on Red Pine is controlled by
DDT—oil emulsions sprayed from the air (Ewan 1961). The
most successful control follows insecticide spraying of the
young adults before oviposition.

STRUCTURE OF THE PROTECTIVE COVERING
THE MECHANISM OF SPITTLE PRODUCTION

The mechanism of spittle production has been described
in detail by Sulč (1911) and Gahan (1918). The nymphs of
spittle producing Cercopidae possess certain anatomical
characteristics which enable them to produce the material
rapidly and with a minimum of visible effort or movement.

The third to ninth abdominal spiracles in these nymphs
are located in a cavity formed by the sterites and laterally
expanded tergites and pleurites which meet in the mid ventral
line (Sulč 1911). This cavity is closed anteriorly and air
admitted through the enclosed abdominal spiracles passes along
the "air—channel" to be expelled through a posterior V—shaped
valve or slit. The spittle is produced as a result of a
viscid fluid secreted through the anus forming a film over
the valve and being blown into bubbles by the air expelled
from the valve. The spittle is distributed by the insect
stretching its abdomen downwards and gradually closing the

lips of the valve until a bubble is freed. The bubbles are deposited alternately to right and left and pressed forwards along each side until the nymph is completely covered (Gahan 1918). Figures 13 and 14 show two stages in the secretion and distribution of spittle by an early third instar nymph of Philaenus spumarius (F) in the laboratory. The time interval between the two photographic observations was twelve minutes. If the nymph is disturbed whilst producing spittle it moves rapidly away, leaving its partially formed mass. Unless it maintains contact with the original mass the nymph eventually begins to form a fresh one. The whole nymphal stage is passed within this protective covering, several of which are produced during development. Spittle is a very durable material; even when dehydrated it does not lose its foamy nature (Ziegler and Ziegler 1958), but once water is lost the mass is never reconstituted.

Figure 13. The start of spittle prod-
uction by a third instar nymph of
Philaenus spumarius.   X c.6
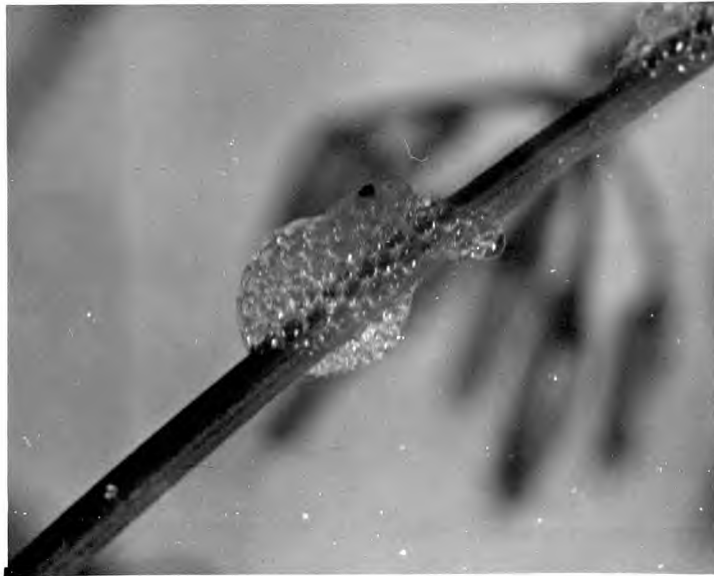
Figure 14. The same nymph twelve
minutes later.   X c.6

CHEMICAL STRUCTURE OF SPITTLE

This section is dealt with in two parts, (a) the water content of spittle and (b) the nature of the remaining solid material.

(a) WATER CONTENT

Values given in the literature for the percentage of water by weight in spittle are all of the same order of magnitude. Gruner (1901) stated that the water content of Aphrophora sp. spittle was 99.48 per cent, while Ziegler and Ziegler (1958) give the water content of Philaenus spumarius spittle as 99.3 to 99.75 per cent; the mean of twelve determinations was 99.67 per cent. Neither of these reports specified the instar from which the spittle was obtained.

Under warm, humid conditions spittle masses are often seen with drops of water suspended from them. These drops do not form part of the spittle mass but are excess liquid secreted by the insect under these conditions. To determine the water content fresh spittle was collected early in the day under as similar conditions as possible and placed on clean, weighed glass slides. The slides were dried at 105°C and stored in a dessicator until required. The slides plus spittle were weighed, dried at 105°C for 24 hours, cooled in a dessicator and reweighed. Twenty five determinations were made, five for each instar. The average water content varied

very little in different instars and was 99.2 per cent,
instar 1; 99.0 per cent, instar 2; 99.4 per cent, instar 3;
99.5 per cent, instar 4; 99.4 per cent, instar 5.

When development is completed the insects cease to
produce fresh spittle, the last inhabited mass partially
drying out without losing its foamy nature (Ziegler and
Ziegler 1958). The young adults remain for a while within
a cavity in the spittle mass, often several adults collecting
within one mass. The difference in texture and appearance of
the partially dried spittle masses appears to be due solely
to water loss; determinations of the water content of this
naturally dried spittle resulted in an average value of
46.7 per cent.

OTHER MATERIALS IN SPITTLE

SPITTLE STABILISATION THEORIES

A number of workers have attempted to identify the
substance or substances necessary for maintenance of the
coherent frothy structure of spittle bearing in mind that
spittle contains over 99 per cent water.

The large quantity of liquid necessary to form spittle
is obtained from the host plant and is passed directly through
the modified alimentary system (Licent 1912). To maintain the
froth the presence of a 'viscous substance' has been suggested
by many authors. Wilson and Dorsey (1954) state that the

formation of bubbles in aqueous solution depends on the presence of a suitable surface tension depressant. The origin and nature of this substance or secretion has been described in the following ways.

Gland Secretion Theories

Porta (1901) thought that the secretion originated in glands distributed over the general body surface, particularly in the anal region, and was waxy in nature. Other workers suggested that the secretion was produced in special hypodermal glands first described by Batelli (1891) and named after him. Batelli identified these glands on the last two abdominal segments while Gruner (1900) and Berlese (1909) reported their presence on the 7th and 8th, and 8th and 9th abdominal pleurites respectively. Guilbeau—Braxton (1908) agreed with Gruner's finding, and also found that if air was blown into liquid produced by nymphs in which these glands had been cauterised no stable bubble formation resulted. Liquid from nymphs with normal glands produced bubbles when treated in the same manner. By placing live nymphs in weak alcohol solutions Osborn (1916) found that a viscid substance was secreted from lateral glands on the 7th and 8th abdominal segments. Kato (1958) thought that a fatty acid produced after enzyme decomposition of fatty substances, probably wax

in the secretion from Batelli's glands, was present in the
anal secretion. The acid combined with ammonia or cholesterol
to form the stabilising substance. Kato also thought that a
protein secreted by Batelli's glands helped to stabilise the
spittle. Sulč (1911) explained the change of the liquid into
a frothy substance by the action of an enzyme, cerotinase,
which hydrolysed the wax secreted by Batelli's glands.
Fatty acids produced by this hydrolysis in conjunction with
an alkaline material in the "liquide anal" formed a soap
 like substance (also Weber 1930).

Malpighian Tubule Theories

Further theories explaining the formation of a stable
material result from investigations of the malpighian tubules
and most of the authors suggest the presence of a
proteinaceous material.

Licent (1912) obtained a positive Millon reaction with
spittle indicating the presence of an albuminoid substance
and concluded that this was the "viscous substance". He
suggested that a secretion from the glandular proximal
section of the malpighian tubules, similar to the sericin
covering of the silk fibres secreted by Bombyx mori L.,
was passed into the liquid resulting in stable bubble
formation. Kershaw (1914) suggested that the spittle was

probably stabilised by a mucin secreted by the proximal segments of the malpighian tubules. The results of dissections of nymphs in Ringers solution by Pesson (1956) showed that a substance was present in the glandular secretion of the malpighian tubules which swelled after prolonged immersion in water; his conclusion was that a substance similar to a mucin was present. Using staining techniques Lison (1953) supported the presence of an acid polysaccharide secreted by the malpighian tubules. Pesson (1956) obtained positive Mucicarmine and Toluidine blue reactions with the malpighian tubules but an inconclusive reaction with the Periodic acid—Schiff reaction. Comparison of results obtained by Licent (1912) and Day (1949) led Pesson to conclude that a muco— or glyco—protein was present in the malpighian tubule secretion.

Other Theories

Cecil (1930) was unable to locate any Batelli's glands in Philaenus leucophthalamus (L) and concluded that the anal fluid alone was sufficient for spittle stabilisation. The salivary re—imbibition theory of Gruner (1901) suggested that a ptyalin—like substance was produced by the salivary glands, re—imbibed and passed through the gut to form the 'viscous substance'. Wilson and Dorsey (1954) thought that a surface tension depressant may be derived from saponin in the plant

sap or from the synthesis of polysaccharides by bacteria present in spittle. Chromatographic analysis showed that in three out of four samples, hydrolysed spittle contained more sugars than unhydrolysed spittle and they concluded that spittle must contain a polysaccharide. Ziegler and Ziegler (1958), however, found no evidence of either simple or complex saccharides and maintained that protein was the main stabilising agent.

From the evidence presented by these various theories it appears probable that spittle is stablised by a combination of secretions from the glands of Batelli and the malpighian tubules, in the form of a mucocomplex of protein and polysaccharide.

The work described below was attempted to determine the major chemical components present in spittle and to resolve the various opinions on the presence of polysaccharide.

Histochemical investigations (techniques in Gurr, 1958).

The following histochemical investigations into the structure of spittle were carried out. Spittle masses of third instar nymphs were collected, placed on carefully

cleaned glass slides and dried at $90^{\circ}C$. for 48 hours. The techniques used are listed below together with the reactions observed; the interpretation of the results is discussed later.

Periodic Acid — Schiff reaction.

1. Three slides were immersed in distilled water, then
2. placed in a 0.5 per cent aqueous solution of Periodic acid for 10 minutes.
3. The slides were washed in a solution of 1 gm. potassium iodide and 1 gm. sodium thiosulphate in 20 ml. water, to which 30 ml. ethanol and 1 ml. N. hydrochloric acid were added, followed by
4. immersion in Schiff's reagent for 10 minutes.
5. Three washes in freshly prepared 'sulphite rinse', prepared from equal volumes of 1 per cent sodium metabisulphite and 0.1N hydrochloric acid were followed by
6. washing in tap water for 5 minutes.

The spittle mass was stained a red — purple colour indic — ating the presence of carbohydrate. This reaction detects a variety of substances containing 1,2— glycol linkages, which are believed to occur only in carbohydrates and some amino acid.

Gentian violet reaction.

1. Slides taken from distilled water were immersed in a solution of 0.25 ml. of 0.1 per cent aqueous gentian violet in 100 ml. distilled water.

3. After draining the slides were decolourized in a solution of equal volumes of aniline and xylene.

The spittle stained violet in colour suggesting the pres — ence of acid mucopolysaccharide, possibly hyaluronic acid.

Mucicarmine reaction.

1. Slides taken from distilled water were placed in a solution of 1 volume of carmine solution and 9 volumes of 70 per cent alcohol. The carmine solution was prepared from 1 gm. carmine and 0.5 gm. anhydrous aluminium chloride dissolved in 100 ml. of 50 per cent alcohol, boiled and filtered when cool.

2. The slides were then washed in distilled water.

The spittle stained a light red colour suggesting that a mucoid substance is present.

Toluidine blue reaction.

1. Slides taken from distilled water were immersed for 5 min — utes in a 1:100 aqueous dilution of a 1 per cent solution of Toluidine blue, followed by rinsing in distilled water.

2. The slides were placed for 2 minutes in solution of equal volumes of 5 per cent aqueous ammonium molybdate and 1 per cent aqueous potassium ferricyanide and were then washed in distilled water.

The spittle stained a purple—blue colour indicating the possible presence of acid mucopolysaccharide.

Bismarck brown reaction.

1. Slides taken from distilled water were rinsed quickly in acid alcohol.

2. The slides were then immersed in a solution of 7 volumes of

   a. 1.5 gm. bismarck brown in 70 ml. absolute alcohol, and 3 volumes of

   b. 0.5 gm. of ferric chloride in 30 ml. water, for 2 hours.

3. A quick rinse in 70 per cent alcohol followed by immersion in a solution made from equal volumes of the following,

   a. 10 ml. of 10 per cent haemotoxylin and 90 ml. absolute alcohol.

   b. 4 ml. of 30 per cent aqueous ferric chloride, 1 ml. of hydrochloric acid and 95 ml. distilled water,

   for 5 minutes.

4. The slides were finally washed in tap water.

   The spittle stained a light brown colour indicating the possible presence of mucoprotein.

   The results of the tests described above suggest the presence of acid polysaccharide, possibly combined with protein in a mucocomplex. The positive results obtained with the Gentian violet and Toluidine blue reactions suggest that the acid polysaccharide is in fact an acid mucopolysaccharide; the following enzyme digestion of spittle was carried out to determine this point.

Enzyme Digestion of Spittle.

Two series of slides with dried spittle were used for this test. The slides were immersed in distilled water; half of the slides were then incubated for three hours at 37°C. in a solution of 1 per cent hyaluronidase (from bovine testes) in 0.85 per cent aqueous sodium chloride. The second half of the slides were treated in the same manner except that the enzyme was not present in the incubating solution. After washing in distilled water both series of slides were examined for presence of carbohydrate by the Periodic acid — Schiff reaction. A negative reaction was obtained with the spittle that had been digested with hyaluronidase, indicating that acid mucopolysaccharide was originally present.

Acid Hydrolysis of Spittle.

To determine whether protein is present in spittle acid hydrolysis was carried out. About 20mg. of dried spittle was placed in a glass ampoule with 5 ml. of 6N. hydrochloric acid and the ampoule was sealed. Hydrolysis was carried out in a pressure cooker at an extra pressure of 15 pounds per square inch for 6 hours, (Alexander and Block,1960). The hydrolysed material was decanted and centrifuged at 4,500 revolutions per minute for 10 minutes to remove all débris. The liquid hydrolysate was then decolourised with charcoal and the hydrochloric acid was removed by distillation under reduced pressure.

The amino acid hydrochlorides were dissolved in 10 per cent iso-propanol. To determine the presence of amino acids in this solution a small volume was pipetted on to a filter paper, dried and immersed in ninhydrin solution (0.25 gm. ninhydrin in 100 ml. acetone) and heated until the characteristic purple spot developed. The presence of any possible unhydrolysed protein was tested for by placing a drop of the iso-propanol solution on filter paper, drying and immersing in a solution of 0.1 per cent bromophenol blue in absolute ethanol saturated with mer — curic chloride. The paper was then washed under the tap until all the blue colour was washed out. The presence of unhydrol -- ysed protein is indicated by a blue spot. No unhydrolysed protein was found in the spittle hydrolysate.

Two dimensional chromatograms of the amino acids were prepared. 25 μl. of the iso-propanol solution were applied to a marked spot on the intersection of two pencil lines drawn 1 $\frac{1}{2}$ inches from the edge of the paper. The paper used was Whatmann Number 1.

The two solvents used were Butanol — Acetic acid and water saturated phenol. To prepare the Butanol — Acetic acid solvent 60 ml. of glacial acetic acid were added to 500 ml. of a freshly shaken mixture of equal volumes of water and n-butanol. After separation the upper layer was decanted off and used as the moving phase; the lower layer was used in the chamber to maintain a saturated atmosphere.

The phenol solution was prepared by adding distilled water to analytical grade phenol until a clear solution was obtained after gentle warming.

Several papers were supported in a multiple frame in the chamber for each solvent run. The Butanol — Acetic acid run was carried out at 20°C. overnight (Figure 15, direction 1). After removal from the chamber the papers were dried at room temperature and replaced in the chamber for the phenol run.

The second solvent, phenol, was run so that the advancing front was at right angles to that of the first solvent. The chromatograms were allowed to develop overnight at 20°C., and the papers were then removed and dried again at room temperature.

When dry the papers were sprayed with a solution of ninhydrin in acetone and dried at 80°C. until the coloured spots produced by reaction with the amino acids developed. Figure 15 shows a typical chromatogram of hydrolysed spittle.

The amino acids were identified by calculating the R_f values, that is the ratio of the distance moved by the amino acids to the distance moved by the solvent front. This latter distance was taken as 100 so that the R_f values are represented as percentages (Table 14), a method used by Smith (1958). The relative positions of the amino acids on the paper were compared with those obtained by Smith (1958) and certain amino acids were further identified by specific colour reactions.

Glycine gave a green spot when papers were sprayed with a 0.2 per cent solution of o-phthalaldehyde and dried at 50°C. A number of amino acids were identified by dipping papers in a 0.2 per cent solution of isatin in acetone and drying at 70°C. in a water saturated atmosphere. The spot colours obtained with isatin are; glutamic and aspartic acids (dark blue), serine threonine and tyrosine (light brown), proline (bright blue) and glutamine, valine, glycine and alanine (pink). Papers dipped in the above solution to which had been added 4 per cent of acetic acid gave blue to blue-green spots with proline, phenyl-alanine, tyrosine, glutamic acid, arginine, aspartic acid and cystine. These specific reactions are reported in Block et al. (1955).

The amino acids identified in hydrolysed spittle are given in Table 14 and Figure 15.

Any polysaccharides present in spittle will be present as monosaccharides after hydrolysis. A few drops of the iso-prop-anol solution were placed on filter paper and dried. The paper was then dipped in a solution of 0.1ml. saturated aqueous silver nitrate and 5N ammonium hydroxide in equal volumes and dried at 105°C. The brown background colour of the paper after this treatment, due to the production of silver oxide, was removed by washing in water and Kodak 'liquid X-ray fixer' (Benson,1952). Sugars appear as brown spots on the paper (Part-ridge,1948); no such spots were found.
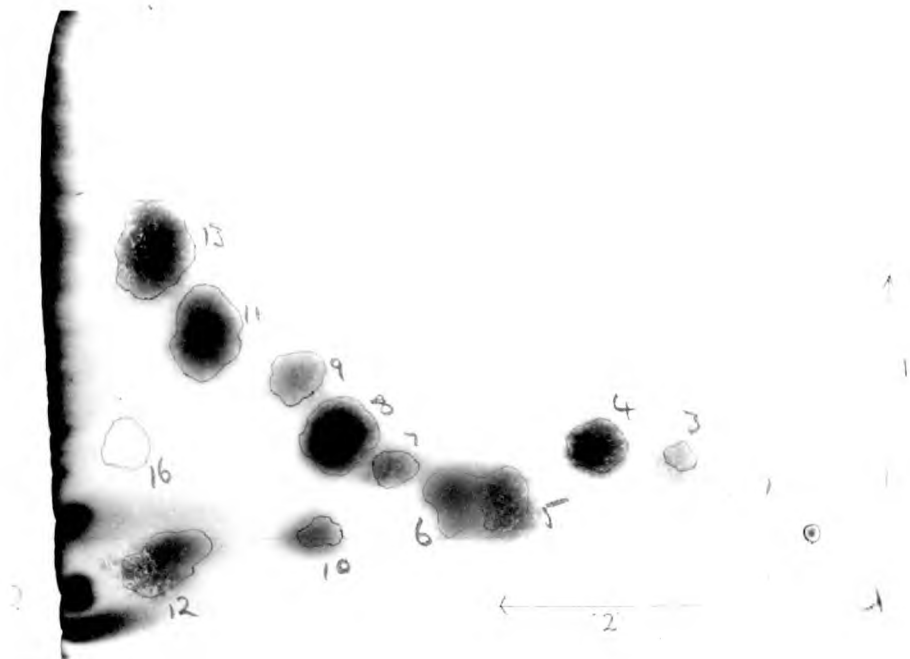
Figure 15. Chromatogram of amino
  acids present in hydrolysed
  spittle of P.spumarius.

See Table 14 for identification.

Direction 1. Butanol—Acetic acid.

Direction 2. Phenol.

Salts

Ziegler and Ziegler (1958) reported the presence of a number of inorganic anions and cations in the spittle of P. spumarius. The major anions found were sulphate and carbonate with small · amounts of chloride and phosphate. The major cations were magnesium, calcium, silicon and potassium with traces of sodium, copper, aluminium, titanium and iron. These results were not verified in the time available.

Conclusions on the structure of Spittle.

The investigations reported above suggest that the solid material of spittle is composed largely of protein, possibly conjugated protein, containing sugars or sugar derivatives. Conjugated, or glucoproteins, contain polysaccharide as the prosthetic group, and are classed as mucoproteins and glyco-proteins if they contain more or less than 4 per cent sugars, respectively. The presence of mucoprotein is suggested by the positive staining reactions obtained with the Mucicarmine and Bismarck brown tests, and the Gentian violet and Toluidine blue reactions indicate that an acid mucopolysaccharide may be present. This was further confirmed when enzyme digestion of spittle destroyed the carbohydrate material. Testicular hyaluronidase destroys both hyaluronic acid and some chondroitin sulphates; the linkages of both of these compounds to protein are similar.

Certain proteins, for example gelatin, form gels with water when present in less than 2 per cent concentrations

(in Haurowitz,1950). A smaller concentration of protein may possibly be sufficient to help maintain stability of spittle in the form of a more fluid gel. The presence of an acid muco-polysaccharide such as hyaluronic acid might also help cohesion by cross linking with salts (in Haurowitz,1950); magnesium and calcium might well be used. The absence of hydolysed sugars after acid hydroysis of spittle suggests that any polysaccharide present is there in very small amounts.

These results prove the presence of protein in spittle and indicate that an acid mucopolysaccharide is also present. The enzyme hyaluronidase is known to occur in pathogenic org-anisms, snake and insect venoms and many animal tissues, in particular testes (Pigman,1957). Stevens (1956) has also dem-onstrated the presence of hyaluronidase in the salivary glands of non—venomous insects. Although the presence of an acid mucopolysaccharide in spittle has not been conclusively proved, if present it would be unique in insects.

THE PERMEABILITY OF SPITTLE MASSES SECRETED BY NYMPHS OF PHILAENUS SPUMARIUS (F).

Dissolution of spittle masses.

Howden and Marshall (1961) described the destruction of spittle masses of Philaenus spumarius and Neophilaenus lineatus by solutions of mineral acids, the rate of destruction appearing to solely depend on the normality of the acid used for a given sized mass. In the present these experiments were extended, and members of a number of other groups of compounds were tested, to investigate the stability and the chemical nature of spittle. The compounds were first screened for their ability to destroy spittle in a test tube in the laboratory; some of the more interesting compounds were subsequently chosen for testing as sprays in the spray tower. This procedure saved time and material and also minimised the amount of possibly corrosive material which had to be passed through the spraying equipment.

Experimental Procedure.

10 mls. of each concentration of the compounds tested were placed in a glass test tube and a spittle mass from a late third or early fourth instar nymph of P. spumarius was introduced on to the liquid surface. The tube was agitated at a uniform rate and the time taken for the spittle mass to break down was recorded. The 'end-point' of dissolution

was taken when, at most, there was a single half ring of small
bubbles around the . meniscus of the liquid. Each concentra-
tion of all compounds was tested five times and the average
time was recorded (Graphs 3 to 9).

The following compounds were tested

Chemical Group	Compound	Effect on Spittle.
Mineral acids	Hydrochloric acid, Nitric acid, Sulphuric acid, o—Phosphoric acid.	Dissolution
Normal aliphatic acids (monobasic)	Formic acid, Acetic acid, Propionic acid	Dissolution
Saturated dibasic acids	Oxalic acid, Malonic acid Succinic acid	Dissolution
Chlorine-substituted acetic acids	Monochloro—, Dichloro— and Trichloroacetic acids	Dissolution Precipitation by Trichloro-acetic acid.
Unsaturated acids		
Monobasic	Glycollic acid,Lactic acid, Malic acid	No effect
Dibasic	Maleic acid, Fumaric acid	No effect
Aliphatic Monohydroxy Alcohols	Methanol, Ethanol, n—Propanol, n—, sec—, iso—, tert—Butanol n—Amyl alcohol.	Dissolution Precipitation
Ketones	Acetone, Methyl ethyl ketone,	Dissolution by acetone.
	Methyl propyl ketone, Iso—butyl ketone	Precipitation by all ketones.

Aldehydes	Formaldehyde	No effect
Aromatic alcohols	Benzyl alcohol	No effect
Ethers	Di-ethyl ether	No effect
Glycols	Ethylene glycol	No effect
Paraffins	n-Hexane, n-Decane, Odourless kerosene	Reduction of surface tension.
Other compounds	p'-Xylene, Benzene, Chloroform, Amyl acetate, Methyl benzoate	Slight reduction of surface tension
Surface active agents	Lissapol NX, Aphrosol FC, Fixanol C.	Reduction of surface tension.
Inorganic hydroxides	Potassium hydroxide, sodium hydroxide.	Dissolution

RESULTS

Mineral acids.

Mineral acids were the only compounds tested by Howden and Marshall (1961) for their ability to destroy spittle masses and formed an obvious starting point in the present work. Four common mineral acids, hydrochloric acid, nitric acid, sulphuric acid and o-phosphoric acid were tested as solutions of the following normalities, 0.001 N, 0.01N, 0.1N, 1.0N and 10.0N. The results are given in Table 15 and Graph 3

The optimum observed normalities for dissolution of spittle are shown in Table 22, and were 0.1N for hydrochloric acid, nitric acid and o-phosphoric acid and 1.0N for sulphuric acid. The molarity of the acid solutions at these

Graph 3.

Dissolution of Cercopid spittle in Mineral Acids

☐ – HCl
○ – HNO$_3$
△ – H$_2$SO$_4$
● – o-H$_3$PO$_4$

Dissolution time (mins) vs Normality of Acid (log scale)
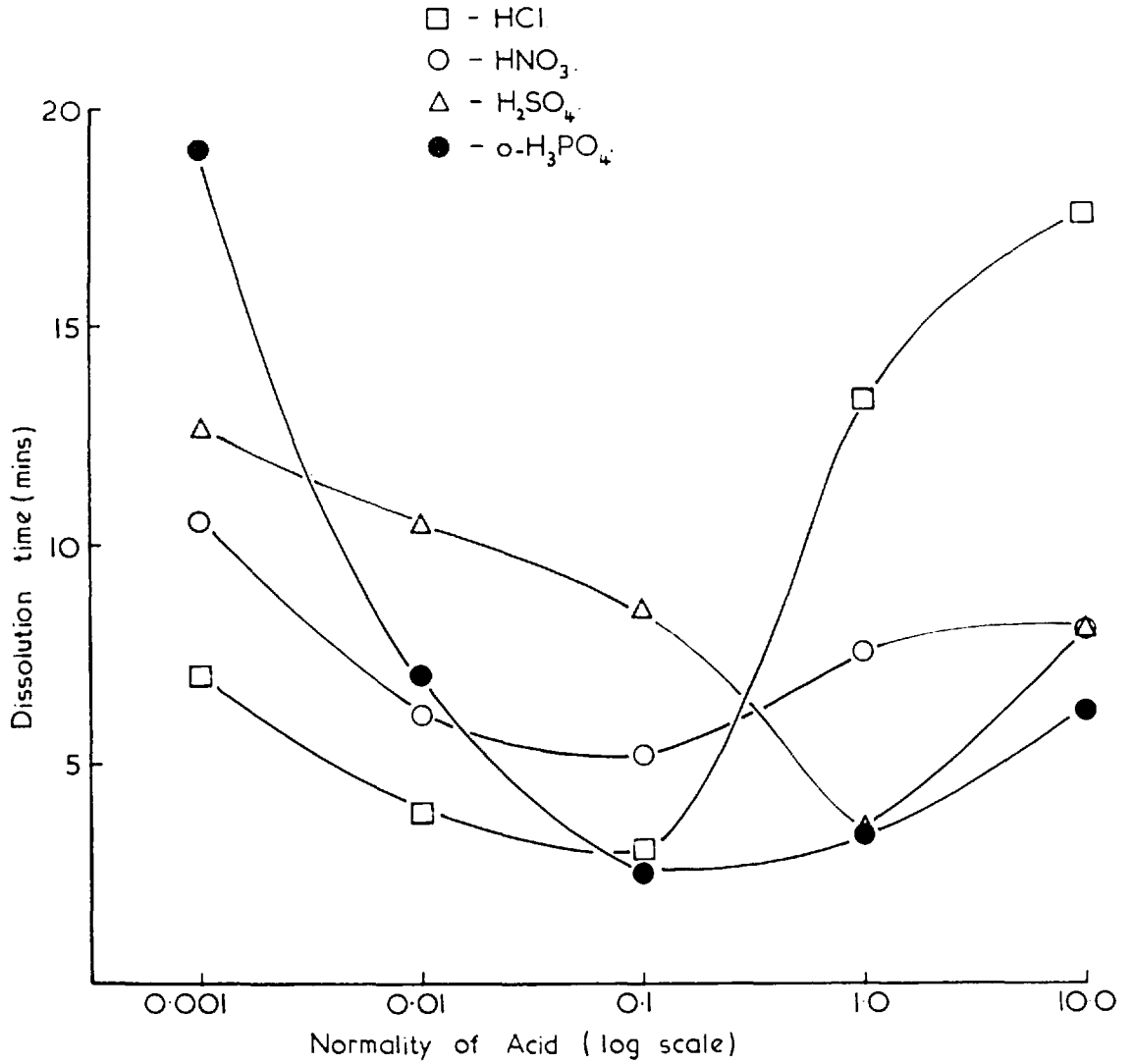
normalities suggest that the rate of dissolution of spittle is not related to the number of molecules of the acids available.

It was expected that as the normality of the acid increased the rate of dissolution of spittle would also increase (Howden and Marshall 1961) but this did not happen. Graph 3 shows that all four acids behaved very differently at the minimum and maximum normalities used (0.001N and 10.0N).

It appears from these results that apart from physical factors such as molarity and degree of dissociation of the acid which might affect spittle stability, the actual composition of the acid itself may be important. pKa values (the negative logarithms of the ionization constants) given in Table 22 do not appear to be correlated with the dissolution rate at the optimum observed normalities. At the 0.001N concentration the two factors do appear to be correlated, the stronger the acid the more rapid the dissolution rate, while the reverse is true at the 10.0N concentration. (Graph 3).

Aliphatic Monobasic acids.

The first three members of this homologous acid series, formic acid, acetic acid and propionic acid were tested at 0.001N, 0.01N, 0.05N, 0.1N, and 1.0N concentrations. The

results are shown in Table 16 and Graph 4 . At all normalities used the rate of dissolution appeared to be inversely related to the pKa value (Table 22), but directly related to the chain length of the acids.

Aliphatic Dibasic acids.

Oxalic acid, malonic acid and succinic acids were tested, at 0.1N, 0.2N, 0.5N, and 1.0N concentration. The results are shown in Table 17 and Graph 5 . The curves are typically U-shaped within the range of normalities tested, and for all the acids the optimum observed normality was 0.5N. As with the Aliphatic monobasic acids, the stronger the acid the slower the rate of dissolution observed, except at the 1.0N concentration. The pattern of the curves in Graph 5 suggests that chain length of the acids may affect the rate of dissolution of spittle.

Chlorine-substituted acetic acids.

The results of the tests made with these acids are shown in Table 18 and Graph 6 . The acids were tested at 0.001N, 0.01N, 0.1N and 1.0N. The curves in Graph 6, indicating the rate of action of these acids compared with that of acetic acid, suggest that substitution of additional chlorine atoms in the acetic acid molecule increases the effectiveness of the acid, particularly at the lower concentrations. In this case the pKa value of the acids appear to be directly related

Graph 4.

Dissolution of Cercopid spittle in Aliphatic Monobasic Acids.

Dissolution time (mins)

□ — Formic acid.
○ — Acetic acid.
△ — Propionic acid.

Normality of Acid (log. scale)
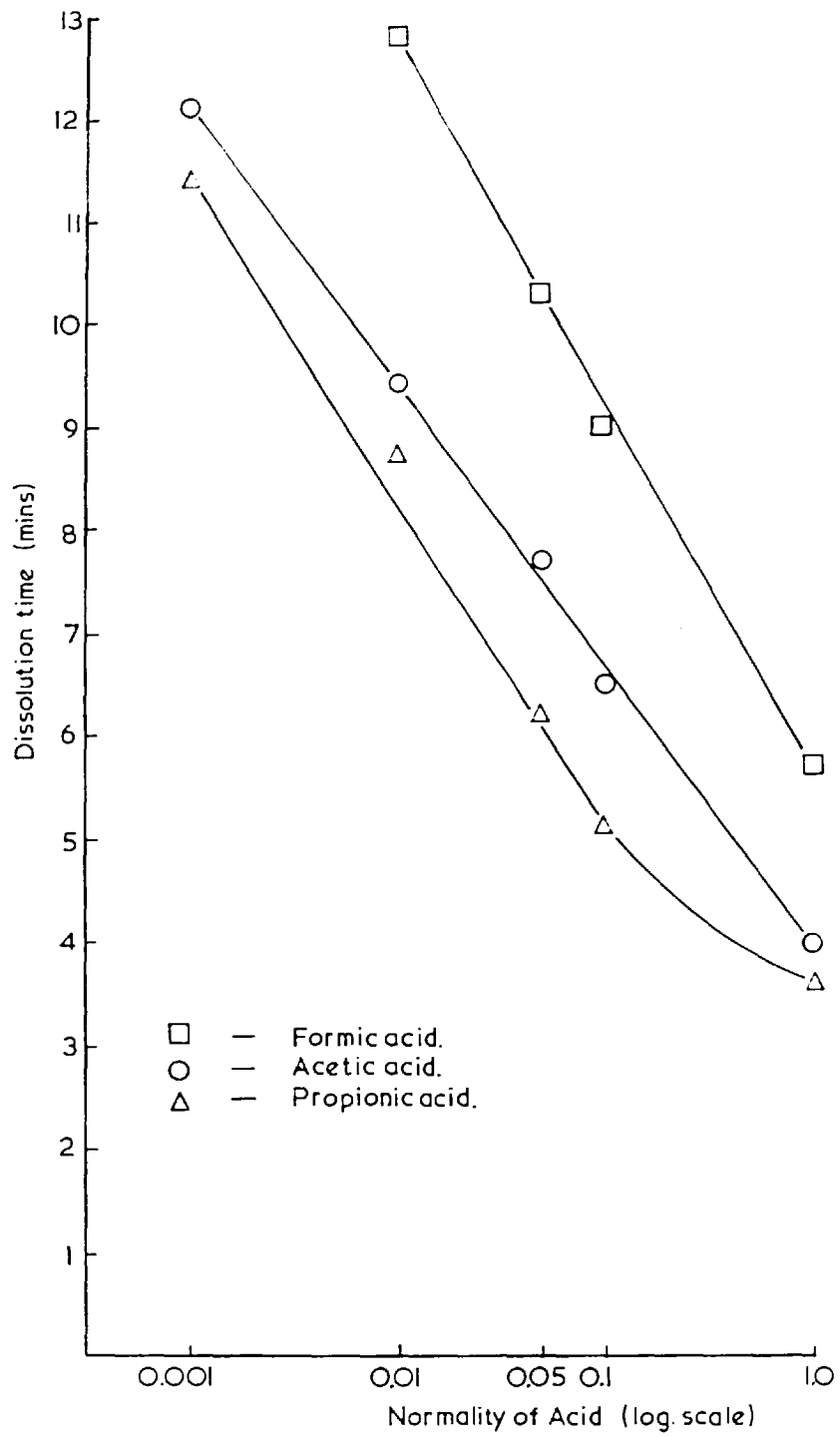
Graph 5.

Dissolution of Cercopid spittle in
Aliphatic Dibasic Acids

□ — Oxalic acid.
○ — Malonic acid.
△ — Succinic acid.

Dissolution time (secs)

Normality of Acid (log. scale)
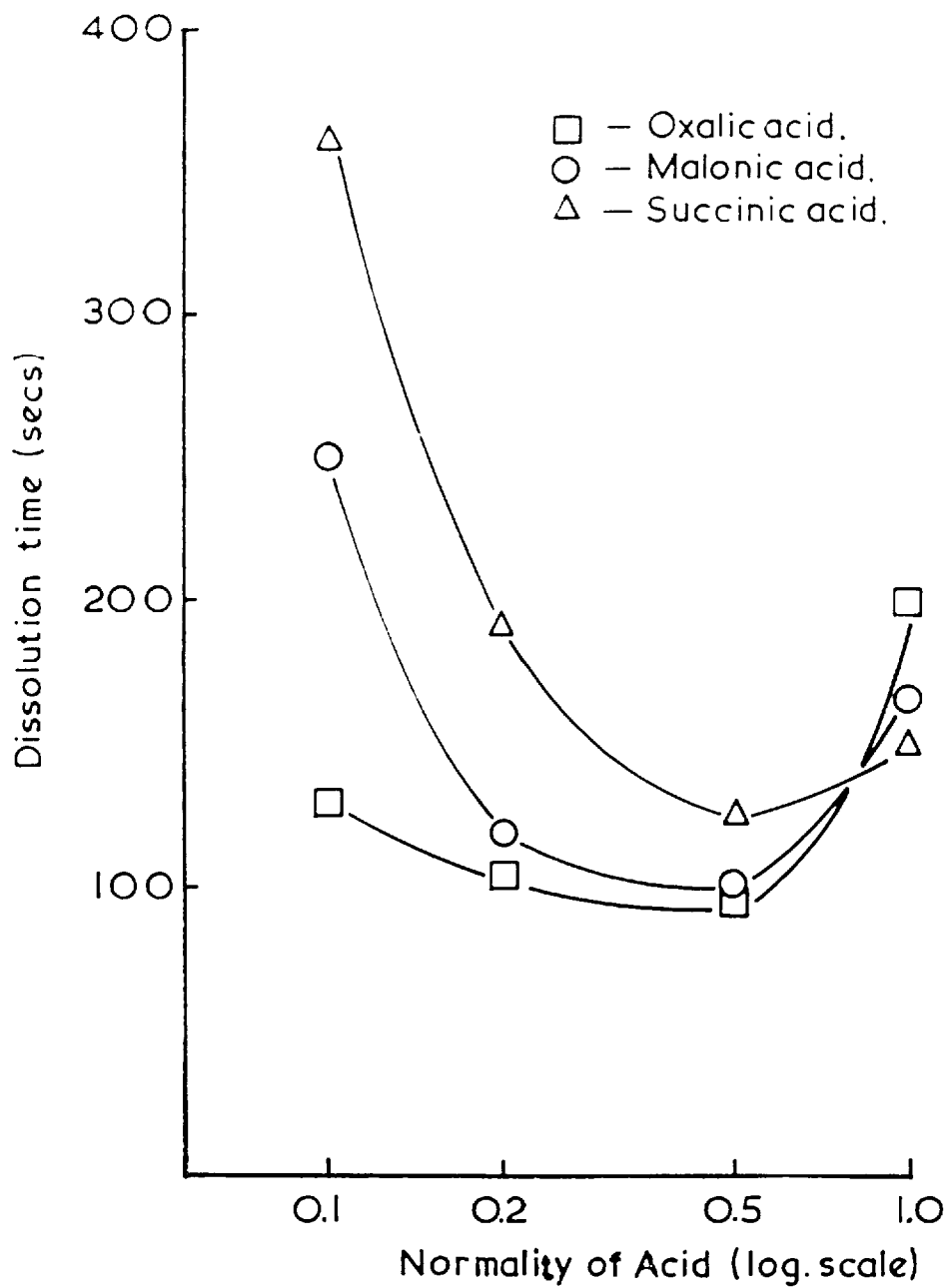
Graph 6.

Dissolution of Cercopid spittle in Chloro-acetic acids

● – Monochloro-acetic acid
△ – Dichloro-acetic acid
☐ – Trichloro-acetic acid
○ – Acetic acid.

Normality of Acid (log. scale)
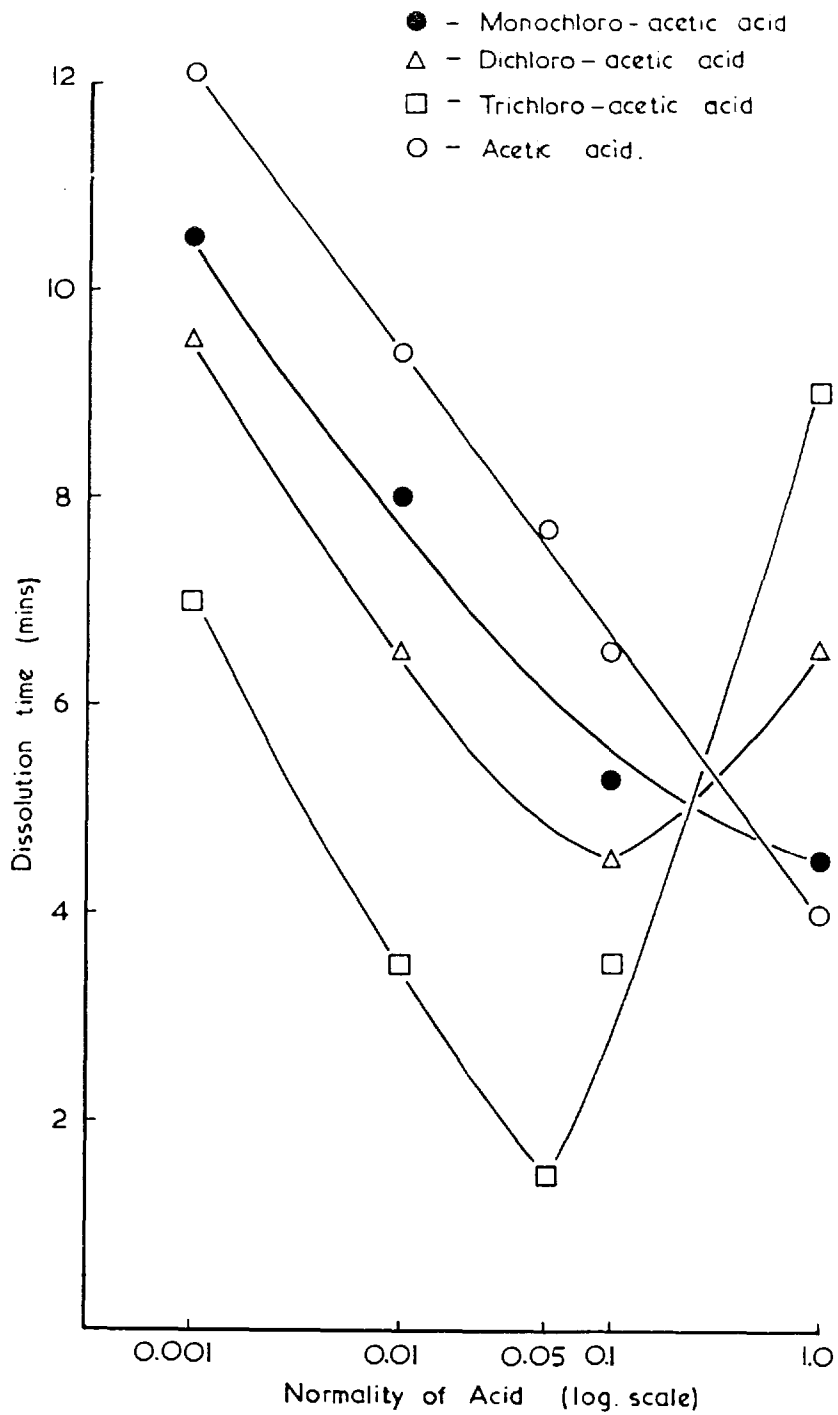
to the rate of dissolution observed, except at the 1.0N
concentration.

Dissolution of spittle in the higher concentrations of
trichloroacetic acid resulted in the formation of a
precipitate, suggesting that protein may have been originally
present in the spittle.

Unsaturated acid series

Attempts to use the lower members of the unsaturated
monobasic and dibasic acid series were abandoned, as no
measureable dissolution of the spittle masses occurred.

Aliphatic monohydroxy alcohols

Methanol, ethanol, n-propanol and tert-butanol were
tested at different per cent concentrations in water. The
results are shown in Table 19 and Graph 7 . At 80 per cent
concentration or higher, the dissolution of spittle was almost
immediate, the rate decreasing as the concentration of
alcohol decreased. The curves in Graph 7 show that the rate
of dissolution of spittle is similar for all the alcohols
tested, but more rapid as the chain length of the alcohols
increases. All the butanol isomers and n-amyl alcohol
dissolved spittle very rapidly as pure compounds. Table 19
shows that a precipitate was formed by the higher concentra-
tions of the alcohols, again suggesting that protein may
be originally present in the spittle.

Graph 7.

Dissolution of Cercopid spittle in Aliphatic Monohydroxy Alcohols.

O – Methanol.
● – Ethanol.
□ – n-Propanol.
△ – tert-Butanol.
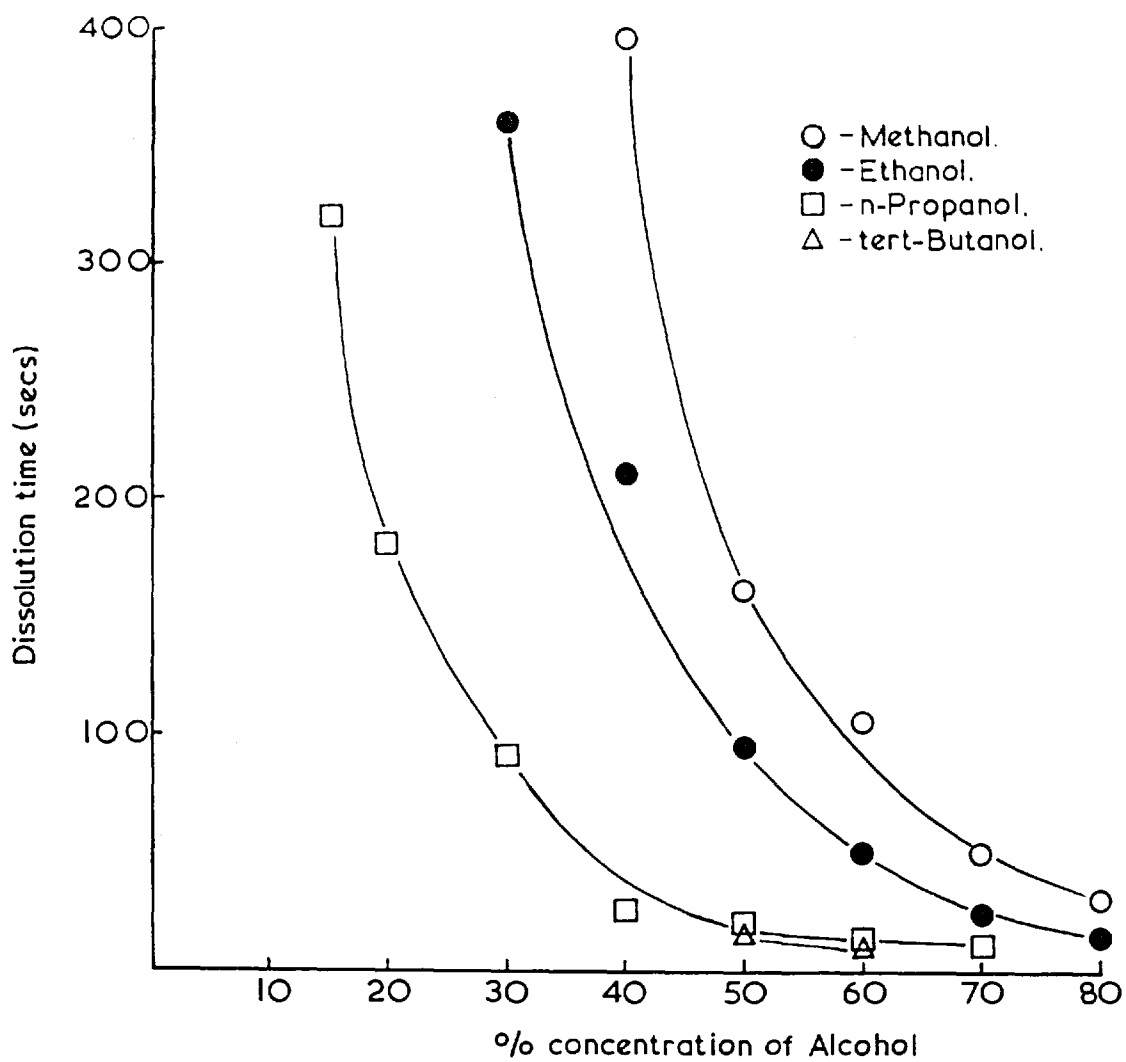
Ketones

Solutions of acetone in water dissolved spittle more
rapidly with increasing concentration (Table 20 and Graph 8).
A precipitate was formed with the higher concentrations of
acetone and a more glutinous precipitate was formed when
spittle masses were treated with methyl ethyl ketone, methyl
propyl ketone and iso-butyl ketone, again demonstrating the
possible presence of protein in the spittle.

Inorganic hydroxides

Sodium and potassium hydroxides were used in these tests
at concentrations of .0.001N, 0.01N, 0.1N, 1.0N and 10.0N.
The results are given in Table 21 and Graph 9 and show that
the rate of action of both compounds was similar at all
concentrations tested.

Other compounds

None of the other compounds tested had any measurable
effect on spittle. Certain compounds such as ethylene glycol,
retained the spittle on the surface as an intact mass; others
such as ether, benzene, chloroform, the paraffin hydrocarbons
and solutions of surface active agents appeared to slightly
reduce the surface tension of the spittle mass but did not
cause any dissolution.

Graph 8.

Dissolution of Cercopid spittle in Acetone

Graph 9.

Dissolution of Cercopid spittle in Inorganic Hydroxides

O-Potassium hydroxide
●-Sodium hydroxide
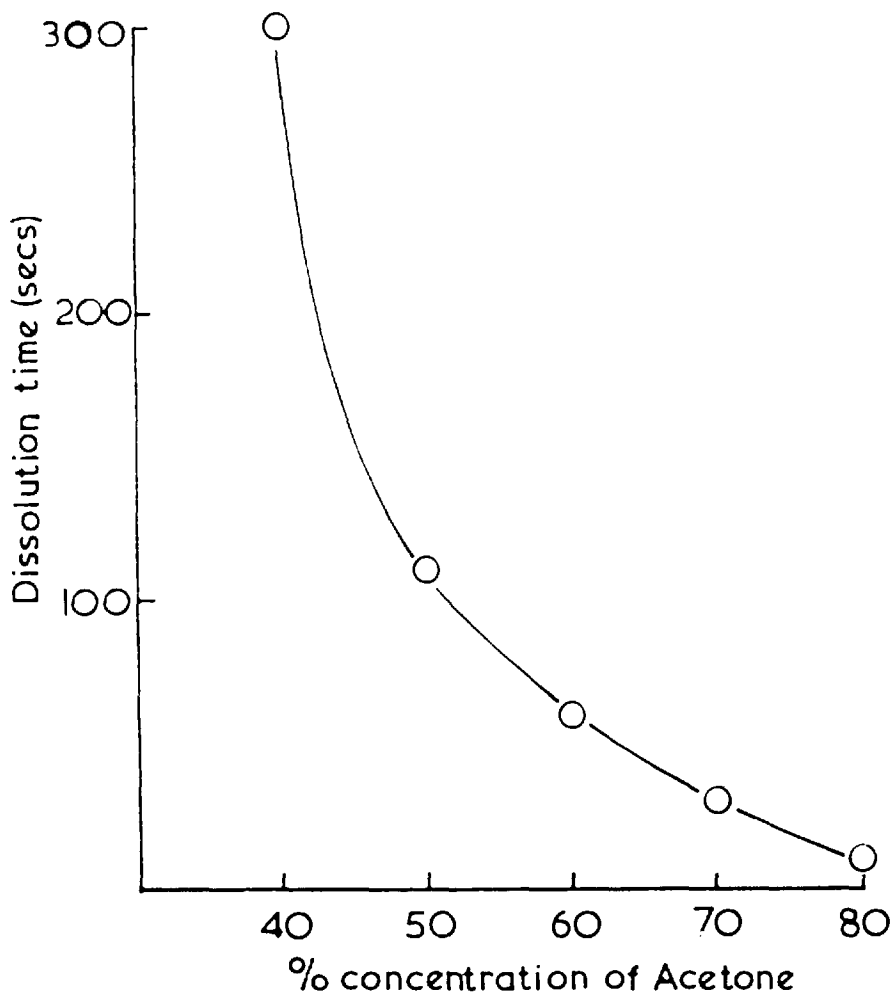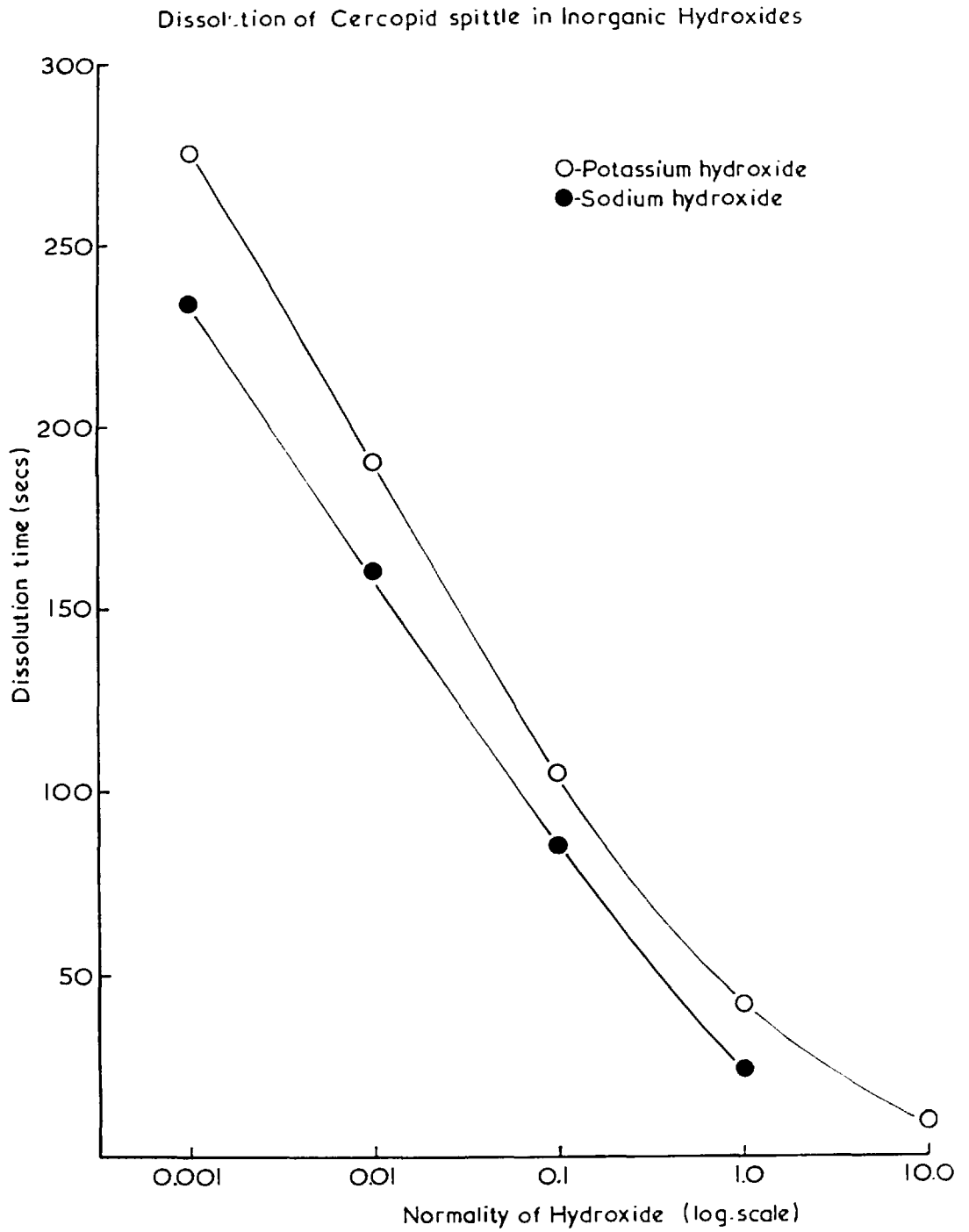
Discussion

Spittle was dissolved by most of the acids tested
and also by inorganic hydroxides, alcohols and ketones (Tables 15
to 21). The dissolution of spittle by these compounds may be
explained by considering the chemical structure of the spittle
material. It has been shown earlier that protein is a major
component of the solid material in spittle, probably present
as part of a mucocomplex.

Dissolution of spittle in acids and alkalis may be due
to the combination of positive and negative ions formed by
these compounds in solution with ions formed by the protein
fraction of the spittle. It is thought that amino acids
in solution consist of an exceptional type of ion, the
"zwitter-ion" (in Karrer 1950). Proteins behave towards
acids and alkalis in much the same way as do amino acids and
they may, in fact, be regarded as very complex amino acids;
they combine both with acids and alkalis and it is probable
that they also form zwitter-ions. Each zwitter-ion has a
polar character, the amino group becoming a charged cation
while the acidic portion becomes a charged anion (in Karrer
1950). At a certain pH value, the isoelectric point, specific
for each protein, a protein solution apparently carries no
charge; this indicates that the protein is in equilibrium
with its neutral zwitter-ions. The addition of acids or

alkalis to protein solutions will convert these to basic
ions and acidic ions respectively, thereby causing further
dissociation and solution of the protein (in Taylor 1957).
The faster rate of action of alkalis may be explained if
the pH value of the isoelectric point is much less than
7, as it is for many proteins, for example, gelatin (4.7)
and egg albumin (4.8). (in Taylor 1957).

The dissolution of spittle by certain other compounds,
with the production of a precipitate, is also explained by the
presence of protein in spittle. Alcohols, ketones and
trichloroacetic acid all precipitate proteins from solution,
and, in the present work these compounds did produce a
precipitate.

From this work it appears that dissolution of spittle
is brought about by chemical reactions of the successful
compounds tested, with the material which composes spittle.
For the homologous series of compounds tested, chain length
and pKa values seem to be connected with dissolution rate,
but for other compounds such as mineral acids, there does not
appear to be any single factor acting, normality of the
acid, the pKa value and possibly the chemical composition of
the acid all appearing to exert some influence.

DESTRUCTION OF SPITTLE BY SPRAYS

Only certain compounds, selected from results in the previous tests, were used as sprays in these experiments. Initially water, both with and without dye in solution, and later odourless kerosene, sulphuric acid, n-decane emulsions and several surface active agents were sprayed on the spittle masses in the spray tower.

Spray method

Pieces of vegetation, each supporting one spittle mass, were held in a vertical position in glass tubes with the stem projecting through a hole in the cork into a small volume of water. Several masses were presented in this manner within a 12 inch diameter circle in the centre of the spray drawer. The height of the vegetation above the base of the drawer was standardised as far as possible at 20 cms; the lower shutter could not, therefore, be used. Investigations of the effect of sprays settling on the test material were made with the upper shutter in the open and closed position. Examination of the spittle masses after spraying showed that the effect of the larger spray droplets was negligible, and all sprayings were, therefore, conducted with the upper shutter in the open position. To avoid the possibility of larger drops of liquid falling from the nozzle on to the spittle masses, no test material was placed in the centre of the spray drawer.

Spittle masses formed by third and fourth instar nymphs were used in these tests. Ten mls. of solution were sprayed in each test and the deposit was allowed to settle for 3 minutes.

Aqueous sprays

These sprays had no effect on spittle, a result which was expected, as spittle masses in the field are not disrupted by the impact of small rain drops. All nymphs emerged from the spittle masses within 10-15 minutes after spraying. This effect was observed in all the spray tests, and it was recognised that the nymphs on pieces of vegetation removed from the growing plant would be unable to maintain the spittle by drawing water from the vascular system. It is also possible that the impact of spray droplets on the spittle may stimulate the insects to leave their protective surroundings.

Spittle masses sprayed with a one per cent aqueous solution of methylene blue stained blue , as dye deposited on the spittle dissolved in the water contained in the spittle. An attempt was made to discover whether the dye penetrated into the spittle mass, via its contained water, by preparing frozen sections. The spittle material was too vacuolated to allow proper sections to be cut; it appeared that the dye did not penetrate the spittle, but remained in the outer region of the spittle mass.

Oil Sprays

Odourless kerosene sprayed on to spittle masses had no effect apart from a small reduction in size of the outer bubbles. The same result was obtained with an n-decane spray. These results substantiated those obtained when attempts to destroy spittle in these compounds in test tubes failed.

Surface active agent sprays.

Five surface active agents were sprayed on to spittle masses at concentrations of 0.1 per cent, 0.5 per cent, 1.0 per cent, 5.0 per cent, and 10.0 per cent in distilled water. The spray time for a fixed volume of spray (10 mls.), increased with concentration. As surface active agents are of three types, non-ionic, anionic and cationic (Barker 1948), at least one of each type was used.

The non-ionic surface active agents used were, Lissapol NX (nonyl phenol ethoxylate), Nonex 52 (polyglycol 600 mono-oleate) and Tween 80 (polyoxyethylene sorbitan mono-oleate). The anionic and cationic surface active agents were Aphrosol FC (sodium alkylnaphthalene sulphonate), and Fixanol C (cetyl pyridinium bromide). The cationic surface active agent was dissolved originally in hot water.

None of the surface active agent sprays had an effect on the spittle apart from reducing the size of the outer layer of bubbles, presumably by lowering the surface tension.

However, all sprays drove the nymphs from the spittle faster
than water alone, within 5 minutes for the cationic surfaceact:
agent and in 5 to 10 minutes for the anionic and non-ionic
surface active agents.

Lissapol NX, is known to produce an increasingly alkaline
solution in water as the concentration increases. The pH
value lies between 7.0 and 9.0 at a concentration of 10' per cen
Solutions of Aphrosol FC are neutral,while Fixanol C produces
an acid solution in water. Although there was no difference
between the effect of the various sprays on spittle, it was
noticed that the nymphs were driven out of their protective
masses sooner by the cationic surface active agent, Fixanol C,
possibly because of the slightly acidic nature of this compound
at the higher concentrations used.

Emulsion sprays

Emulsions were prepared from odourless kerosene, Lissapol
NX and distilled water in the following ml. proportions:

Emulsion	Odourless kerosene (ml)	Lissapol NX(ml)	Water (ml).
A	20	9	71
B	40	9	51
C	60	9	31

At 76 cm. Hg. air pressure the times to spray 10 mls. were,
25, 28 and 34 seconds for emulsions A, B and C respectively.
The shutters were not used and the spray was allowed to settle

for three minutes.

The only visible effect of the emulsion sprays was that the nymphs vacated the spittle masses. However, the activity of the nymphs was impaired after contact with the spray deposit, the length of time needed to recover full mobility depending on the proportion of oil in the spray. The nymphs in contact with emulsion A appeared to recover within about 20 minutes after removal to damp filter paper in a petri dish. Nymphs in contact with emulsions B and C, when treated similarly, seemed to regain full mobility after about two hours. No mortalities were recorded within a 24 hour period after spraying.

A similar set of emulsions was prepared substituting n-decane for odourless kerosene and the most stable of these, 60ml. decane, 9ml. Lissapol NX and 31ml. distilled water (Bard 1961) was selected. No mortality of nymphs was recorded 24 hours after spraying spittle masses with this emulsion.

Emulsion sprays containing γ-BHC

The above emulsion was used to spray the insecticide γ-BHC, dissolved originally in the decane, on to spittle masses. Twenty five spittle masses containing third and fourth instar nymphs were sprayed with the solutions. The emerging nymphs were placed on damp filter paper in petri dishes and maintained at the temperature of the spray room,

$22^{\circ}C \pm 2^{\circ}C$. The mortality of the nymphs was assessed after 24 hours (Table 23).

Mortality of nymphs of both instars increased as the concentration of insecticide present increased; a t test (Finney 1947) showed that there was no significant difference between the LD.50 values obtained for instars three and four (Table 23).

Sulphuric acid sprays followed by γ-BHC sprays

To determine whether the destruction or partial destruction of the spittle mass would alter the susceptibility of the nymphs to γ-BHC, spittle masses containing third and fourth instar nymphs were sprayed with sulphuric acid solutions, which unlike n—decane had been shown to destroy spittle, immediately before spraying with the emulsion containing γ-BHC. Sulphuric acid was selected because of its low vapour pressure (Howden and Marshall 1961) and the consequent reduced risk of complicating fumigant effects.

10 mls. of sulphuric acid solutions of 0.1N, 0.5N, and 1.0N concentration were sprayed on to spittle masses, and each of these sprays was followed by a 10 ml. spray of the decane emulsion containing 10, 50 and 100 mg. per cent γ-BHC. Three minutes elapsed before the second of each pair of sprays was used. Twenty five spittle masses of instars three and four were used for each pair of sprays.

Each of the acid sprays decomposed the spittle to some extent, the most effective being the 1.0N spray. Three minutes after spraying with the decane emulsion, the nymphs were removed to damp filter paper in petri dishes in the spray room and mortality counts were made 24 hours later.

Tables 23 and 24 show that the mortalities caused by the γ-BHC were substantially the same, whether the spittle masses were first sprayed with solutions of sulphuric acid or not.

DISCUSSION OF SPRAY RESULTS

Spittle masses sprayed with a number of different compounds appeared to afford little or no protection to the insect. It was noted that the insects were stimulated to leave their masses faster by compounds which either partially decomposed the spittle mass or which provided an irritant stimulus.

The effects produced on spittle by spraying compounds which had been successful in the laboratory tests showed the same type of action but to a lesser extent.

The mortality caused by γ-BHC sprays (Tables 23 and 24) was similar, regardless of whether previous partial destruction of the spittle mass had taken place. It appears from these results that the presence of an irritant material in the spraying liquid is sufficient to drive the insect from its protection and thus into contact with the deposited insecticide.

Exposure to Saturated γ-BHC vapour.

Protected and unprotected nymphs of P.spumarius were confined in a small chamber saturated with γ-BHC vapour at 18°C. and at a relative humidity of 79 per cent, maintained by saturated ammonium chloride solution, in order that effects due to dessication would be minimised.

25 nymphs of each instar were exposed. The mortalities given in Table 25 are those for originally unprotected nymphs, as protected nymphs were stimulated to leave their spittle masses within a few minutes. The resistance of the nymphs to the insecticide vapour appeared to increase with age, with the exception of the third instar. Nymphs maintained under the same conditions but not exposed to the insecticide vapour were alive after 24 hours.

Spittle as a protection against dessication.

Spittle is of great importance for preventing the rapid dessication of nymphs of Cercopidae (Kirkaldy,1906). To in - vestigate the effects of dessication 25 protected and unprotected nymphs of each instar were exposed over potassium hydroxide at 18°C. Survival times for originally unprotected nymphs are given in Table 26.

The first reaction to dessication was a slowing down of searching movements; later the insects fell on their backs at the bottom of the tube. The criterion of death was taken as

inability to move the fore legs, these being the last append-
ages seen to move. Nymphs maintained on damp filter paper at
the same temperature survived for 24 hours.

The results of these two series of experiments (Tables
25 and 26) show that even under the influence of irritant
stimuli which have no mechanical effect on spittle, as for
example in spraying, nymphs are stimulated to leave their prot-
ective surroundings rapidly. It may be possible that there is
a contact between the outside environment and the nymph enclos-
ed in the spittle which permits stimuli to reach sensory rec-
eptors on the insect. It would be of great interest to locate
any such receptors and to study whether the insect is able to
maintain contact with the external environment.

THE STRUCTURE AND PERMEABILITY OF THE SCALE OF SAISSETIA HEMISPHAERICA (TARG).

INTRODUCTION

The adult females of many families of Coccoidea (Homoptera), are sessile pests on a large number of tropical and sub-tropical plants, and need a more efficient protective covering than more active species.

The usual type of protective covering found is a thickened dorsal scale, secreted by the insect and impregnated with differing amounts of waxy material and waste products of metabolism, (Coccidae and Diaspididae), and in some families, for example the Lacciferidae, resinous materials obtained from the host plant. In other families, more active species secrete a powdery or filamentous wax covering (Pseudococcidae). The presence of the protective scale makes the economically important species difficult to control, as penetration of the scale by insecticides is limited.

In the present work, the structure and permeability of Saissetia hemisphaerica (Targ), a typical member of the family Coccidae, was studied.

Formation of the scale

Baranyovits (1953) describes the formation of the scale by Aonidiella aurantii (Mask) in the following manner. The active first instar nymph, or crawler, comes to rest, inserts its stylets into the plant (citrus) and begins to feed

intracellularly (Bodenheimer 1957). Glands scattered over the dorsal surface of the body secrete fine "silken" filaments and these are distributed over the whole insect body as a result of pivoting movements about the inserted stylets. Eventually the insect is covered with a tangle of loose silk. Berlese (1909) also described secretion of silk threads by immature scale insects.

A glutinous liquid secreted by the malipighian tubules is now distributed in the same way and, being absorbed on to the silk, forms a soft, apparently homogenous mass which subsequently toughens to form the scale. Because the alimentary system is blind, there being no connection between the mid and hind gut, provision is made for the products of metabolism to pass directly into the haemocoele and be incorporated with the "secretions" deposited in the scale. Wax filled haemocytes are known to occur in the haemolymph of some species of Coccids and Aphids (in Imms, revised edition 1957).

It is not known whether this method of laying down the basic structure of the scale is common to all scale insects; the presence of amino acids similar to those of silk suggests that the structure of the scale of S. hemisphaerica may be formed in this manner.

CHEMICAL CONTROL OF SCALE INSECTS

Scale insects are one of the most difficult groups of

insect pests to control. The possession of a dorsal scale — like structure, impregnated with wax or resin in many species, precludes the effective use of insecticidal materials for direct penetration to the insect surface. A second feature, is that fairly rapid development of resistance to some control measures is known to occur.

One of the most effective control measures used, is fumigation with hydrogen cyanide (HCN) gas, but in 1914–15 it was noticed that certain strains of the red citrus scale, Aonidiella aurantii (Mask), and the black scale, Saissetia oleae (Bern) were resistant to HCN (Quayle 1938). Dickson (1941) found that the difference in susceptibility was a sex–linked character, and Lindgren (1941) and Yust el al. (1943) demonstrated that resistance was not connected with the possession of the scale covering in the adult.

Ebeling (1939) found that the impermeability of the scale by sprays could be overcome by using spray solutions having a low advancing contact angle which penetrated beneath the edge of the scale, and through the mass of waxy threads secreted beneath Aonidiella.

Oils and oil emulsion sprays containing insecticides, must be used carefully to avoid phytotoxicity. DDT and methyl parathion successfully control a number of armoured scale insects (Diaspididae; Brown 1951). γ-BHC sprays are not very

effective against soft scales (Coccidae). Brown (1951) found
that the crawlers of _Saissetia_ _oleae_ (Bern) are able to
tolerate γ-BHC, but not DDT, the crawlers being unable to settle
on surfaces treated with this material.

HCN fumigation is still practised for control of
non-resistant strains of certain scale insects. Spray solu-
tions having rapid spreading powers are used to penetrate
beneath the edge of the scales; the combination of oils and
insecticides in sprays results in better control of scale
insects than that obtained by either material used alone.

THE STRUCTURE OF THE SCALE OF SAISSETIA HEMISPHAERICA.

Physical structure.

Sections of adult scale insects were prepared using the method of Sinha (1953). Insects were fixed in a mixture of a saturated solution of picric acid in 90 per cent alcohol (75 parts), formalin (25 parts) and concentrated nitric acid (5 parts) for 2 days to soften the scale material. The insects were then dehydrated in 90 per cent and absolute alcohol for 16 hours. The insects were placed in clove oil for 1 day, and then in clove oil saturated with celloidin. After infiltration of this solution the insects were placed in chloroform for 12 hours. When hard the block was trimmed and transferred to a mixture of paraffin (1 part) and chloroform (5 parts) for 12 hours, at 78°C. A single change of melted paraffin wax was sufficient to complete the production of the block.

Sections of the insects were cut at 10 μ; a part of a typical section is shown in Figure 17.

Smith (1944) described the structure of the scale of Saissetia nigra and stated that it consisted of cell units, or 'tesselation' units. The thickness of these units increased with age and in the mature insect ventral ridges expand laterally to form box-like structures each containing a space or cell. Several cells of this type are shown in the scale structure in Figure 17. From each cell a minute pore connects to a gland duct through which wax passes to the surface.
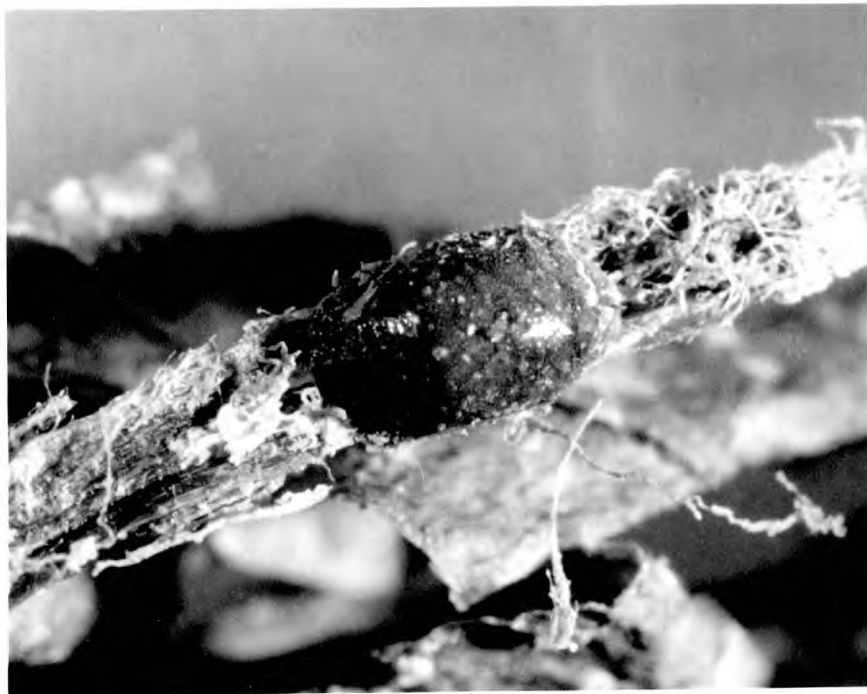
Figure 16. General appearance of dorsal
scale of Saissetia hemisphaerica. X c.17.
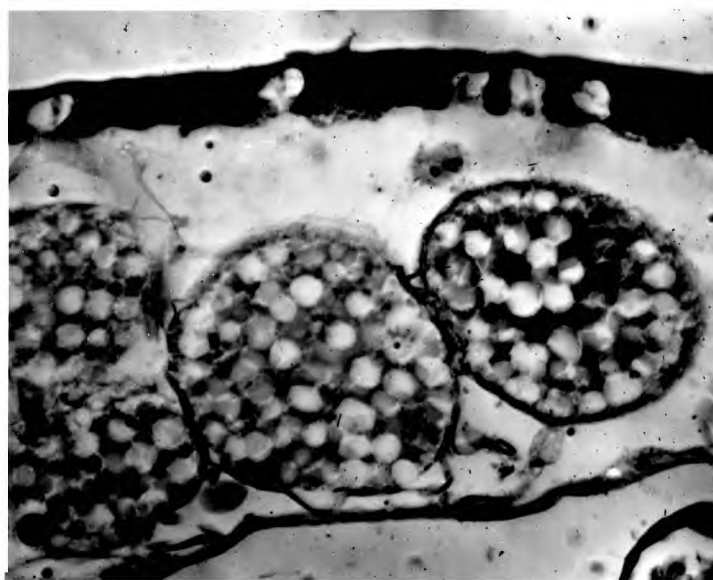
Figure 17. Vertical section through
S.hemisphaerica showing structure of
dorsal scale and maturing ovaries. X c.1250

In sections of S.hemisphaerica no pores were found, prob-ably due to the thickness of the sections. Sinha (1953) states that sections are best cut between 8μ and 12μ.

The possible method of formation of the scale has already been described.

The external appearance of the dorsal scale of S.hemis -phaerica is shown in Figure 16.

Chemical structure.

Attempts to identify the constituents of Coccid wax have been made by Chibnall et al. (1934a), Hackman (1951), Gilby and Alexander (1957) and Lower (1959). Chibnall et al. (1934a) stated that the wax produced by Tachardia lacca Kerr. is comp-osed of primary alcohols (C_{26-34}) in the alcohol soluble fract-ion and primary alcohols (C_{30-36}) and n-fatty acids (C_{30-34}) in the alcohol insoluble-benzene soluble fraction. Cochineal wax from Coccus cacti was found to consist of cocceryl alcohol (15-keto-n-tetratriacontan.1) and two acids, n-triacontanoic acid and 13-keto-n-dotriacontanoic acid, compounds closely related to those found by Blount et al. (1937) in the wax of Adelges strobi.

Gilby and Alexander (1957) investigated the effects of age, climatic conditions and host plant on the wax of Ceroplast-es destructor (Newstead) and concluded that, whatever the cond-itions, the wax was typical, consisting of paraffin-chain acids, alcohols and esters. Hackman (1951), working with the same

species, detected no paraffin hydrocarbons. The more recent work of Baker et al.(1960) and Gilby and Cox (1953) has thrown doubt on the presence of free alcohols in insect waxes.

Lower (1959) found that the scale of Austrotachardia acaciae (Mask). contained 7.8 per cent wax and 44.2 per cent of a lac type material by weight and smaller quantities of unidentified materials. In view of the complicated nature of the wax no attempts were made to identify the constituents in the time available; however, preliminary investigations into the possible presence of silk in the scale were made (Berlese, 1909; Baranyovits,1953).

Baranyovits (1953) found that none of the typical wax solvents attacked the scale of Aonidiella aurantii even after immersion for over a year, although the scales were dissolved by boiling N. sodium hydroxide. Scales of S.hemisphaerica were boiled in benzene under reflux to remove the surface wax; the bodies of the insects were removed by gentle boiling in sodium hydroxide solution until only the scale remained. These scales were hydroysed in 6N. hydrochloric acid for 10 hours.

Chromatograms were produced by exactly the same methods already descibed for the chromatograms of P.spumarius spittle. The amino acids(identified in Table 14) are very similar to those of spittle, except that cysteic acid, leucine and iso-leucine were found in addition, (Figure 19). It is shown later that apart from these three amino acids, the amino acid comp-
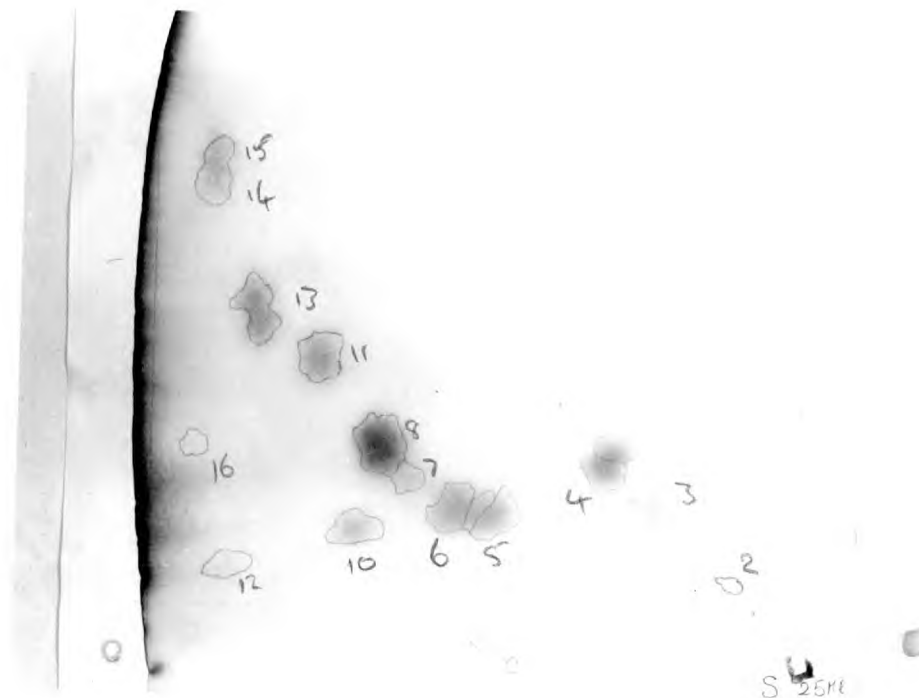
Figure 19. Chromatogram of amino
acids present in hydrolysed
scale of S.hemisphaerica.

See Table 14 for identification.

Direction 1. Butanol—Acetic acid.

Direction 2. Phenol.

osition of the silk cocoon of <u>Plodia</u> <u>interpunctella</u> is also very similar.

The pigment present in the scales of <u>S.hemisphaerica</u> was extracted in two fractions, one in boiling water and the other, possibly more closely bound to the structural material of the scale, in boiling sodium hydroxide. The two fractions were obtained after 6 and 15 hours boiling respectively.

Table 26a. and Figure 18 show that the absorption spectra of the two fractions is almost exactly similar. Lower (1959) found the absorption peak of the dye obtained from <u>Austrotachardia</u> <u>acaciae</u> to be at approximately 450m , while Gilby (1957) found that for hydrolysed wax of <u>Ceroplastes</u> <u>destructor</u> the absorption maximum was at 227.5m .

Extraction of pigment from Scale of _Saissetia hemisphaerica_ (7-Thary)

O – Water extract

● – Sodium hydroxide extract

Absorptiometer reading.

| Wavelength mμ | 410 | 430 | 470 | 490 | 520 | 550 | 575 | 600 | 680 |
| Ilford Filter No | 600 | 601 | 602 | 603 | 604 | 605 | 606 | 607 | 608 |

Figure 18.

140.
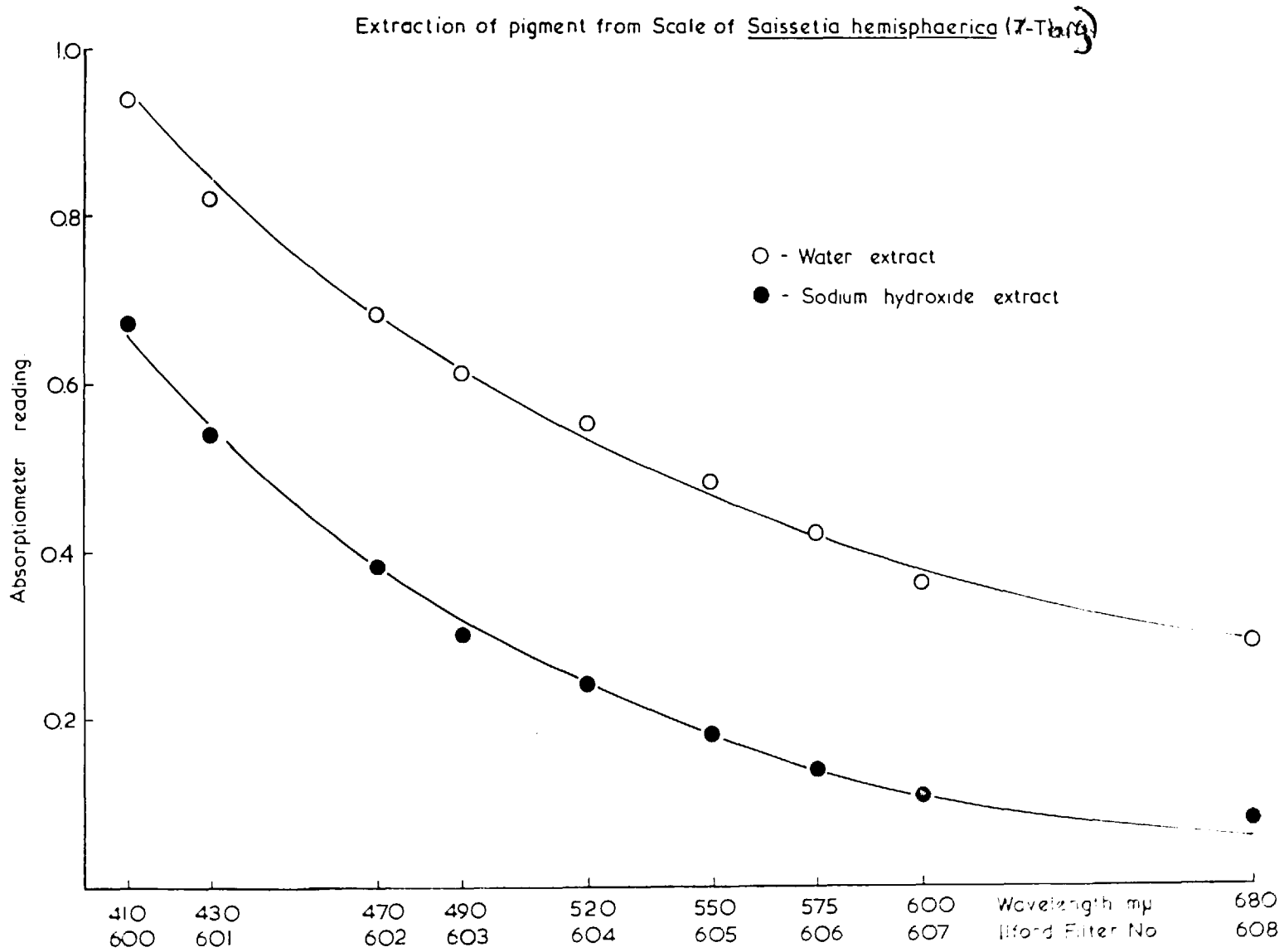
PERMEABILITY OF THE SCALE OF SAISSETIA HEMISPHAERICA (TARG)

The permeability of the scale of S.hemisphaerica was investigated by immersion in alcoholic dye solutions, aqueous solutions of cobalt chloride and solutions of cobalt naphthenate in a paraffin hydrocarbon. The latter two tests made use of precipitation of insoluble cobalt compounds after addition of 1-nitroso-2-naphthol and gaseous hydrogen sulphide respectively, to the original solutions. It was hoped that precipitation of these compounds would take place wherever the original solutions had penetrated the insect.

All insects were immersed on a piece of the vegetation to which they were attached, and were examined under a microscope to discover sites of accumulation of the dye or the precipitates.

Permeability to an alcoholic dye solution

Young adult insects, which had not started oviposition, were immersed in a one per cent solution of eosin in ethanol for 1, 6, 12 and 24 hour periods.

After washing in clean solvent, the insects were removed from the vegetation and examined for sites of dye accumulation. Some concentration of dye was observed where irregularities of the scale structure occurred, mainly around the edges of the scale and on the ventral surface of the body. Sections of these insects were cut, by the method of Sinha (1953)

described earlier. No dye was found to have penetrated the dorsal scale structure of the insect.

Permeability to an aqueous solution

Insects were immersed in a 5 per cent solution of cobalt chloride for the same periods of time as in the previous test. The addition of a few drops of a one per cent solution of 1-nitroso-2-naphthol caused the formation of a reddish-brown precipitate, the nature of which is not exactly known. The insects remained in this solution for 2 hours; Wigglesworth (1950) using a similar technique with oily solutions under reduced pressure to investigate tracheal penetration, stated that precipitation should take place as rapidly as possible, to prevent the fluid which had penetrated breaking up into short lengths.

No evidence of penetration of the scale structure or of the insect itself was found in sections of the insects. Accumulation of the precipitate was found around the edges of the scales, as with the alcoholic dye solution.

Permeability to a hydrocarbon solution

The technique described by Wigglesworth (1950) was used to investigate possible permeability of the scale to an oily solution under reduced pressure. Wigglesworth states that an oily solution is necessary and found that cobalt naphthenate in the solution gave good results; an intensely black

precipitate is formed after treatment of the oily solution
with hydrogen sulphide.

Young adult insects were placed in the side arm of a
glass tube containing 8 per cent cobalt naphthenate in
n-decane solution connected to a vacuum pump. The system
was evacuated to a pressure of 10mm. Hg and an inert gas,
nitrogen, was introduced restoring the pressure to atmospheric
level. This procedure was repeated twice. After the final
evacuation, the insects were tipped into the solution, and
after immersion for one minute, the pressure was restored
gently, but rapidly, by admission of air.

The insects were removed from the solution, washed twice
with clean decane and then hydrogen sulphide was bubbled
through decane saturated with hydrogen sulphide containing
the insects, for one hour. The reaction between hydrogen
sulphide and cobalt naphthanate produces a black precipitate
of cobalt sulphide.

Before the insects were prepared for section cutting,
microscopic examination of the insects showed a large amount
of precipitate around the edges of the scale and on the
ventral surface.

Sections of the insects were cut by the method described
earlier. The precipitate was not found in the cell units of
the scale structure and examination of a large number of

sections failed to reveal the presence of the precipitate in the wax pore ducts.

The failure of decane solution to penetrate into or through the scale via the wax pore ducts suggests that the pore ducts possibly contain material for secretion which blocked the entry of the solution.

Exposure to saturated γ-BHC vapour

Ovipositing adults of S. hemisphaerica, still attached to vegetation, were confined over solid γ-BHC crystals in a narrow glass tube, at $20°C$. The relative humidity within the exposure tubes was maintained at 70 %. The insects were placed on a tightly fitting perforated partition placed down the centre of the tube above the insecticide. The partition prevented the possibility of contact with the solid insecticide, as some insects, during fumigation, withdrew their stylets from the vegetation and moved short distances.

The insects were immobilized by the insecticide vapour within a few hours; total mortality was observed after 24 hours. The criterion of death used was inability to move any appendage, after removal from the vegetation, on stimulation with a needle.

In the time available, it was not possible to determine the exact age and stage of development of the insects used in this experiment. Most of the eggs laid by the adults

before exposure to the insecticide vapour hatched, but the young insects, or crawlers, which emerged, were killed within about 6 hours.

Discussion of results.

These experiments show that penetration of the solutions occurred peripherally at the edges of the scale and that alcoholic, aqueous and decane solutions all fail to penetrate the scale structure.

The preliminary experiments with δ-BHC vapour show that the vapour is absorbed by the insects despite the presence of the scale, but provides no evidence for or against the permeability of the scale structure to vapours. The simplest explanation is that γ-BHC vapour enters at the periphery of the scale.

THE STRUCTURE AND PERMEABILITY OF THE PUPAL COCOON OF PLODIA INTERPUNCTELLA (Hb.)

INTRODUCTION

Plodia interpunctella (Hb.), the Indian meal moth, is an important pest of stored flour and milled grain products in many parts of the world. The female moth lays its eggs in preferably the coarser grades of suitable stored products. Under temperate conditions, including southern Great Britain, the life cycle from egg to adult takes about four weeks to complete (Cotton 1943). Towards the end of the last larval instar, just before pupation, many Phycitidae migrate upwards away from the food medium in which they have been living, seeking a suitable site for pupation. Richards and Waloff (1946) describe how the random progress of Ephestia elutella Hb. larvae in the food medium alters to a powerful upwards urge causing an apparently uninfested stockpile to become a crawling mass of larvae. At this time Plodia and Ephestia larvae secrete a continuous silk thread which gives rise to the characteristic webbing effect associated with these pests, also further contaminating the stockpile. When the silk cocoon is eventually spun, it is of sufficiently open texture to reveal the outline of the pupating larva or pupa within. A similar structure is also described by Beeson (1910)

in his account of the Toon shoot borer, <u>Hypsipyla robusta</u>
Moore (Lepidoptera, Phycitidae).

THE CHEMICAL CONTROL OF PEST SPECIES OF PHYCITIDAE

The economically important species of Phycitidae are
controlled by fumigation, contact insecticides applied as
dusts or fogs, or more rarely by dessicating and abrasive
dusts (Brown 1951).

Examples of fumigants used in bulked stored grain are
carbon disulphide, ethylene dichloride, chloropicrin and methyl
bromide, the latter being outstanding for fumigation of
bagged material due to its great powers of penetration.

Chlorinated hydrocarbon insecticides protect stored
grain without impairing germination. DDT and γ-BHC have
both been used successfully, but the taint problem of γ-BHC
is an important factor. Synergised pyrethrum sprays kill the
adult moths (Owen and Waloff 1946) but fail to kill the
larvae already inside the infested food medium. Potter
(1935, 1938) investigated the effect of residual films of
insecticide applied as sprays for control of <u>Ephestia</u>
<u>elutella</u> Hb. and <u>Plodia interpunctella</u> (Hb.) and found that
a thin invisible film of pyrethrum in white oil deposited on
exposed surfaces killed larvae and moths on contact. During
the adult emergence period regular spraying was necessary to

maintain the residual film.

The use of finely ground powders as dessicating agents is limited because they reduce the flow properties of bulk grain (Pirie 1951), but a silica aerogel has been used in foodstuffs (Cotton and Frankenfeld 1949).

Most control methods used concentrate on the eradication of the larval and adult stages of these insects, as their mobility helps the control agent to achieve its purpose. The pupal stage, quiescent, partially concealed and protected by its cocoon is a less likely target for control measures.

THE STRUCTURE OF THE PUPAL SILK COCOON OF
PLODIA INTERPUNCTELLA (Hb)

Migrating larvae of P.interpunctella search out a crack or crevice in which to pupate. When settled the larva produces a small pad of silk upon which the main body of the cocoon is spun. The cocoon is very flimsy in structure and the outline of the pupa can often be easily made out. Beeson (1910) describes a similar sort of structure for the pupal cocoon of Hypsipyla robusta (Moore), a Phycitid occurring in India.

Silk is secreted by the labial glands in Lepidoptera in the form of fibroinogen which, on extrusion, denatures into an elastic protein fibroin (in Imms, revised edition,1957). Two concentric cores of fibroin are present in the silk of Bombyx mori L. coated with a gelatinous protein, sericin. Attempts to identify the production sites of the two proteins in the silk of Bombyx mori (Machida,1927) by differential staining techniques indicated that sericin is secreted throughout the whole length of the mid division of the labial gland, while the fibroinogen is produced in the posterior portion only.

After extraction in hot water of a large number of cocoons of P.interpunctella no residue indicating the presence of sericin was found. Whole cocoons were, therefore, hydrolysed by the technique described earlier and amino acid chromatograms were

prepared. The amino acids found are shown in Table 14 and Figure 20, and are seen to be very similar to those found in both hydrolysed spittle of P.spumarius and the scale of S.hemisphaerica.

Figures given for the water content of silk cocoons of Bombyx mori vary from 5.1 per cent (Abderhalden and Behrend, 1909) to 12.5 per cent (Kellner,1884). The water content of the silk cocoon of P.interpunctella of three different ages is given in Table 27; the highest water content was found in the cocoons of the youngest pupae indicating that there is a certain amount of water loss from the cocoon during development.
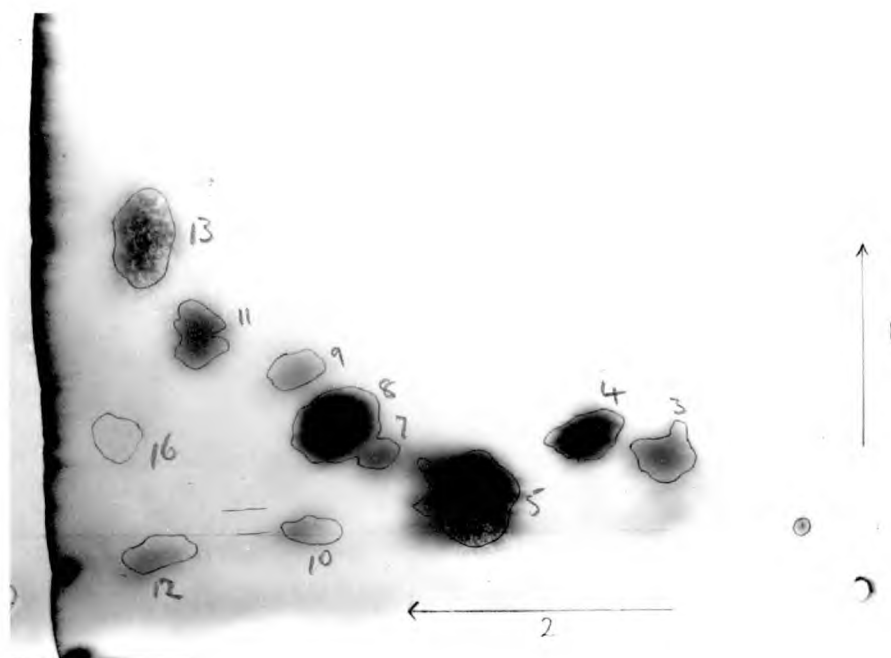
Figure 20. Chromatogram of amino
acids present in hydrolysed
silk of P.interpuntella.

See Table 14 for identification.

Direction 1. Butanol—Acetic acid.

Direction 2. Phenol.

THE PERMEABILITY OF THE PUPAL SILK COCOON OF
PLODIA INTERPUNCTELLA (Hb)

To investigate the permeability of the pupal cocoon the following experiments were carried out:

a) measured amounts of a 5 per cent solution of γ-BHC in acetone were applied to protected and unprotected pupae, at 25°C.

b) protected and unprotected pupae were exposed to saturated vapour of γ-BHC at a number of different temperatures.

Mortality of pupae was used as the criterion of permeability in these two experiments.

c) Both types of pupae were sprayed with solutions of dyes in the spray tower. Permeability was assessed by comparison of the intensities of the coloured solutions prepared from the recovered dye.

P. interpunctella was the only insect used in the present work which could easily be used in both protected and unprotected states, that is with and without the protective covering respectively. In the experiments where mortality was measured, to avoid possible differences in response due to physiological changes after handling, naturally unprotected pupae were used in preference to removing cocoons already formed.

TOPICAL APPLICATION

Experimental Procedure

Measured amounts of a 5 per cent solution of γ-BHC in acetone were applied to the thorax of the insects with an Agla micrometer syringe. The tip of the canula was bent downwards to minimise loss due to the volatile solution running back along it. After a preliminary test to find a suitable range of dosages the following were used — 2, 5, 8 and 10 containing respectively 0.1, 0.25, 0.4 and 0.5 mg. of insecticide.

A series of batches of pupae, both protected and unprotected, were taken on successive days after pupation, that is pupae one to nine days old. About 30 pupae of each age were treated with each dose of insecticide. Protected pupae were placed on a filter paper in a petri dish and treated with the insecticide solution. The solvent was allowed to evaporate and the pupae were placed on fresh filter paper in the dish. Unprotected pupae were treated in the same manner, except that for the higher dosage rates the solution was applied in successive small quantities and the solvent was allowed to evaporate before the next drop was applied. This procedure prevented the possible run-off of large drops from the smooth surface of the pupae (Figure 21). Topical application to the silk cocoon of protected pupae (Figure 22)
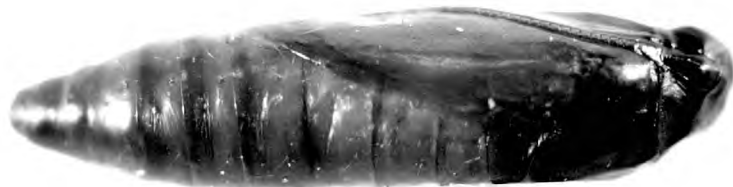
Figure 21. Pupa of Plodia interpunctella
without cocoon — 'unprotected'.   X c.16

Figure 22. Pupa of P. interpunctella with
cocoon — 'protected'.   X c.12

allowed the insecticide solution to spread over the cocoon and the surface of the pupa within.

Pupae used as controls were treated with the same amounts of acetone alone. Treatment and post-treatment conditions for the pupae were those used for the Plodia cultures, 25°C ± 0.5°C and 70 per cent ± 5 per cent relative humidity. Regular observations of the insects were made and emerging adult moths were removed to prevent disturbance of the remaining pupae.

In estimating mortality, adults emerging from pupae within ten days of pupation were recorded as live. Insects which completed their pupal development but failed to emerge successfully were recorded as dead.

Results and Discussion

The results are shown in Tables 28 and 29 and Graph 10.

The different stages in the life history of a holometabolous insect (egg, larva, pupa, adult) may differ considerably, both between and within stages, in their susceptibility to an insecticide under identical conditions. Du Cha nois (1947) experimented with house flies, Musca domestica L., and found that the second instar larva was more susceptible than the pupa, the most resistant stage, by a factor of about 190 measured in mg./litre at the L.D.50 value.

Graph 10.

Mortality of protected & unprotected pupae of <u>Plodia interpunctella</u> (Hb) after topical application of Y-BHC in acetone

O — Protected pupae
● — Unprotected pupae
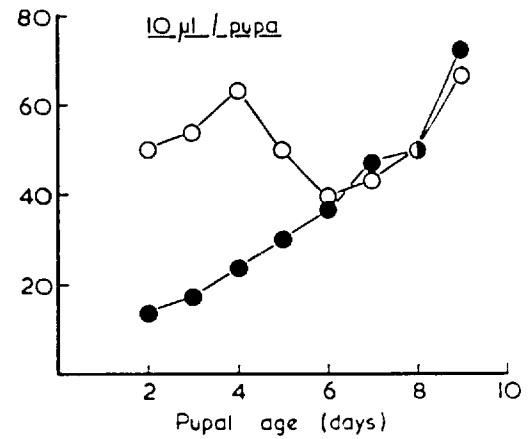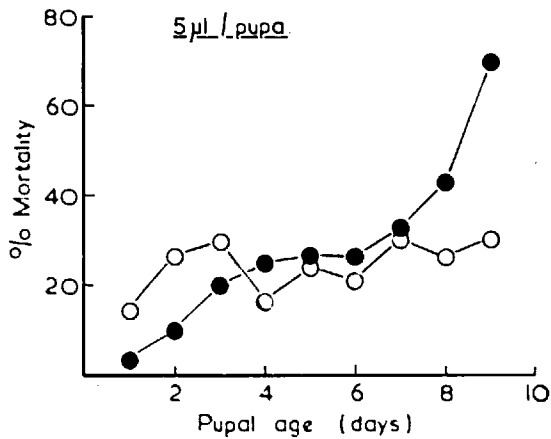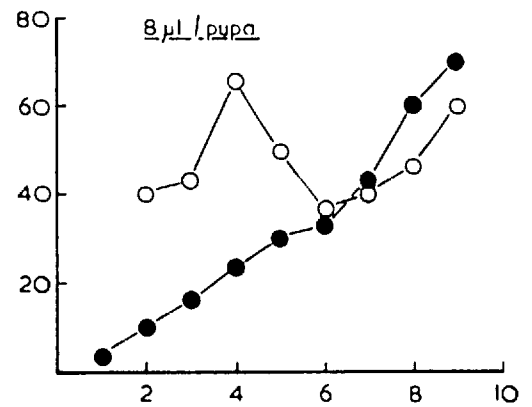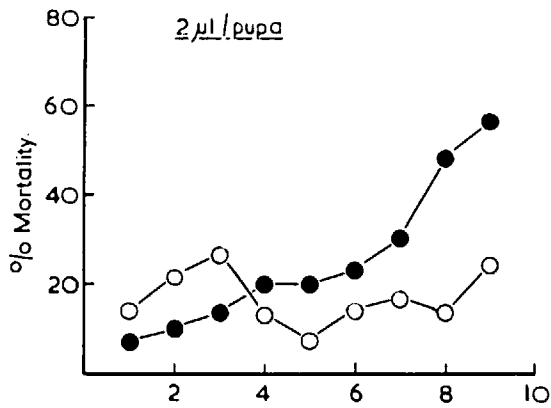
Mukerjea (1953) suggested that because of its changing metabolic rate, and often added protection, the pupa is usually one of the most resistant stages. He found that one day old pupae of Tenebrio molitor L. and Diataraxia oleracea L. and those about to emerge (8 and 20 days old respectively) were much less resistant to DDT and pyrethrins than pupae 4 and 10 days old respectively. As great physiological and morphological changes occur during pupal development this change in susceptibility is not unexpected.

In the present work Graph10 shows only small differences in susceptibility of protected and unprotected pupae. Mortality increased with age of unprotected pupae at all four application rates but for protected pupae age at time of application seems to have much less effect. When δ-BHC was applied to the younger protected pupae it was thought that the presence of the cocoon might help to maintain insecticide vapour around the pupa, thus leading possibly to greater susceptibility. This seems to have been the case for the two higher dosage rates.

Regression lines were obtained (Table 30) for each day's treatment of both types of pupa. The slopes were flat, and at days 2 to 4 the actual concentration of insecticide had little effect on response in unprotected pupae, but they appear to be much less susceptible than protected pupae of

these ages. At days 5 and 6 the LD.50 values suggest that the susceptibility of the protected pupae was greater, and on days 8 and 9 that the unprotected pupae were more susceptible. The LD.50 values on day 7 were almost equal; however, a t-test applied to the results for days 5 to 9 showed none of these differences to be significant at the 5 per cent probability level (Table 30).

To determine whether pupae, in the middle stages of development, were more resistant than either earlier or later stages, further t-tests were applied to the LD.50 values at 5 and 9 days for unprotected pupae and at days 1 and 4, and 4 and 9 for protected pupae. These tests showed no significant differences.

It appears from these results that for the range of dosages applied, the pupal cocoon has little or no protective value against topical application of insecticide at 25°C.

It is surprising that no significant differences were found for insecticide resistance between any developmental stages of pupae; further experiments involving large numbers of pupae are obviously necessary to clarify and expand these results.

EXPOSURE OF PUPAE TO SATURATED γ-BHC VAPOUR

The protective value of the pupal cocoon of P.inter-punctella was investigated by exposing pupae to saturated γ-BHC vapour. The experiments were designed to study the effect of the insecticide under the following conditions

A. State of pupae

Pupae were used in normally protected (P_1) and unprotected (U) states for the duration of the treatment and recovery periods. A further series of pupae (P_2) were used in the protected state for the treatment period and carefully removed from their cocoons for the recovery period, to investigate the possibility of prolonged fumigant action within the cocoon.

B. Age of pupae

Pupae of three ages, 8-14 hours (A_1), 90-96 hours (A_2), and 190-196 hours old (A_3) were exposed to determine whether insecticide resistance varied during pupal development.

C. Temperature

Pupae of the ages and states described above were exposed to the insecticide vapour at 15°C, 25°C and 30°C for the treatment and recovery periods; further pupae were exposed at 15°C and subsequently removed to 25°C surroundings for recovery.

The time taken for pupae to complete development varied with the treatment temperature and also with the age at

the start of exposure. Adults emerged from the youngest
pupae kept at 25°C throughout after 9 to 10 days, while
emergence from similar pupae kept at 15°C occurred after
20 to 27 days. The youngest pupae treated at 15°C and
allowed to recover at 25°C completed development in 12 to
16 days.

All pupae were reared and maintained at 25°C before
exposure.

Experimental procedure

The exposure chambers used in these experiments (Figure 9)
have been described in detail earlier. The atmosphere in the
chambers was allowed to saturate with ɣ-BHC vapour for 72
hours before the insects were introduced.

Seven exposure periods were used for the 8 to 14 hours
old pupae (A_1), 12, 24, 48, 96, 144, 192 and 216 hours, and
six for the 90 to 96 hours old pupae (A_2), 12, 24, 48, 72,
96 and 120 hours. The oldest pupae, 190 to 196 hours old
(A_3), could only be exposed for short periods (12 and 24
hours). There were five replicates of ten pupae for each
exposure period. Unprotected pupae were placed on the gauze
in the chambers and after exposure were removed to clean
petri dishes for recovery at the appropriate temperature.
Protected pupae were similarly treated, except that 20 pupae
were originally exposed, half of which were removed to petri

dishes after treatment, with their cocoons left intact (P_1).
The remaining 10 pupae were removed from their cocoons (P_2)
before being placed in petri dishes to recover.

One replicate of 10 pupae was used as a control for each
exposure period. Control mortalities were higher at $15^{\circ}C$
than at any other temperature, but were generally low.

Results and Discussion

The results are shown in Tables 31 to 37, and summarised
in Graph 11.

At first sight the lines in Graph 11 did not sufficiently
resemble a sigmoid curve to suggest a true regression rela-
tionship. Such a relationship would be expected in this
type of experiment. Probit calculations were done on these
lines, Table 38, and the low values obtained for χ^2 confirmed
that they are dosage—mortality regressions, the dosage being
a concentration—time product in which the concentration
remained constant.

Values for the LT50 are also given in Table 38. A large
number of comparisons between the LT50 values, using the
test (Finney, 1947) are possible; most of the LT50 values

162.

Graph 11.

A1.   **A2**   **A3**
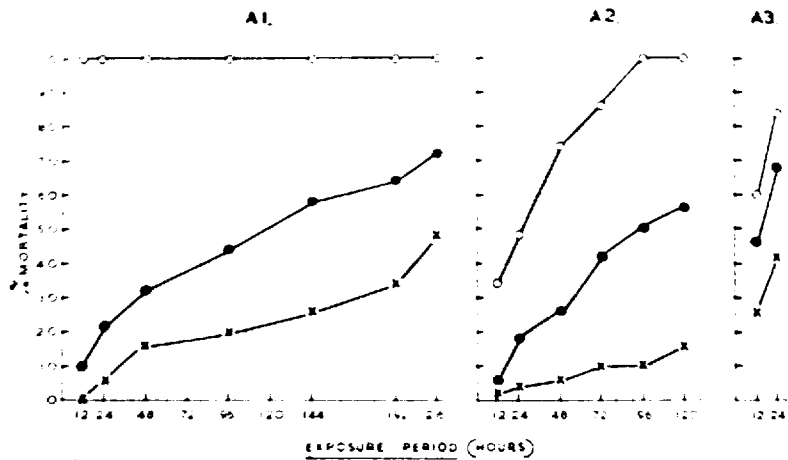
Y-BHC FUMIGATION OF UNPROTECTED PUPAE.
AGE (HRS). A1. 8-14.   TEMPERATURE. O-15° C.
A2. 90-96.   x-25° C.
A3.190-196.   ●-TREATED AT 15°C, RECOVERY AT 25°C.

A1.   **A2.**   **A3.**
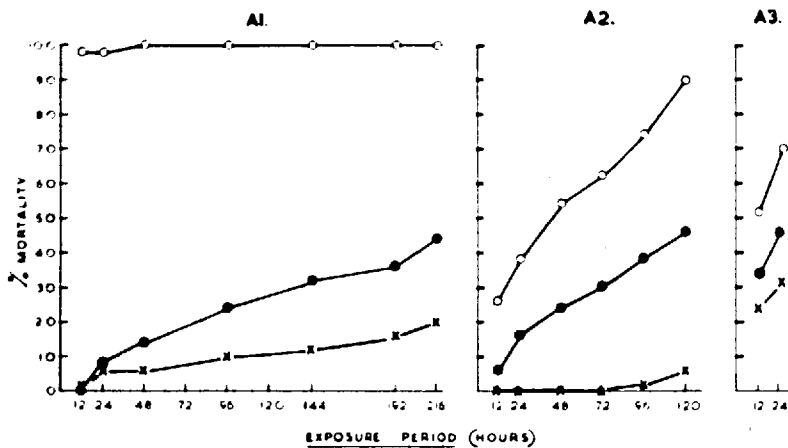
Y-BHC FUMIGATION OF PROTECTED PUPAE NOT REMOVED FROM COCOON AFTER TREATMENT.
AGE (HRS). A1. 8-14.   TEMPERATURE. O-15° C.
A2.90-96.   x-25° C.
A3.190-196.   ●-TREATED AT 15°C, RECOVERY AT 25° C.

A1.   **A2.**   **A3.**
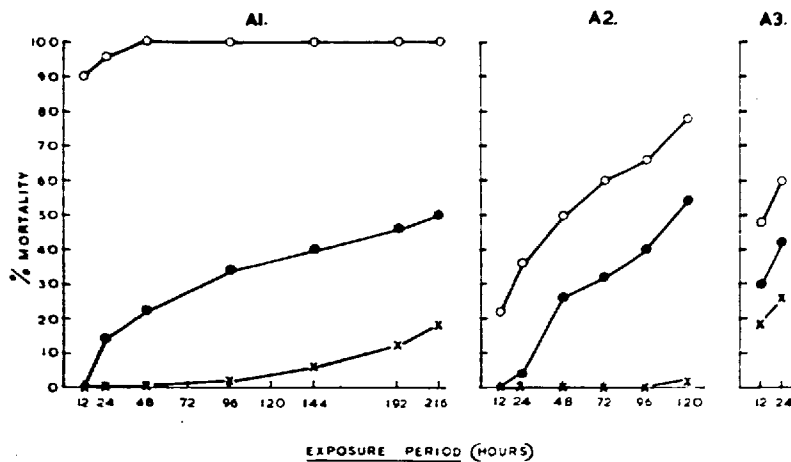
Y-BHC FUMIGATION OF PROTECTED PUPAE REMOVED FROM COCOON AFTER TREATMENT.
AGE (HRS). A1. 8-14.   TEMPERATURE. O-15° C.
A2.90-96.   x-25° C.
A3.190-196.   ●-TREATED AT 15°C, RECOVERY AT 25° C.

for different pupal states, and different ages are not
significantly different when compared at the same temperature
value.

The presence of the pupal cocoon at ages one and two
did not significantly reduce the susceptibility of the pupae,
except for pupae of age two (90-96 hours old) exposed at
$15^{\circ}C$ and for pupae of age one (8-14 hours old) exposed at
$15^{\circ}C$ and allowed to recover at $25^{\circ}C$. The results for
protected pupae (P_2), removed from their cocoons after
treatment, are similar to the results for protected pupae
(P_1), which remained in their cocoons.

In all cases exposure at the lowest temperature, $15^{\circ}C$,
was more effective than at $25^{\circ}C$, and as might be expected,
exposure at $15^{\circ}C$, followed by recovery at $25^{\circ}C$, gave inter-
mediate results. Exposure of the youngest pupae (A_1) at $15^{\circ}C$
produced a complete and rapid kill, irrespective of the state
of the pupa. A similar rapid kill was observed when pupae
of all three ages were exposed to γ-BHC vapour at $30^{\circ}C$.

As mentioned earlier, in the topical application experi-
ments, it was considered possible that pupae in the middle
stage of development might be less susceptible than pupae
at earlier or later stages of development. Exposure of
pupae at $15^{\circ}C$, generally resulted in lower mortality of
pupae of age two, but this is not always the case,

particularly when the cocoon was present throughout.

It appears from these results that the presence of the cocoon during treatment does not reduce the susceptibility of the pupae to γ-BHC vapour, and that in general there is no significant difference in susceptibility of pupae at any of the three stages of pupal development used.

The effect of temperature

The effects of the different temperature regimes employed in these experiments a re quite different.

At 15°C mortality was complete and rapid with the youngest pupae in all states but less marked for the two older groups of pupae. At 30°C the kill was rapid and complete regardless of the age or state of the pupae. Exposure at 25° resulted in fairly low mortality, highest for the unprotected pupae and similar with both types of protected pupae. Pupae in any state exposed at 15°C and removed to 25°C for recovery show intermediate mortalities in every case.

The toxicity of all fumigants is affected by temperature, as well as moisture, stage of development and physiological condition of organisms (Shepard 1937). Moore (1936) stated that mortality of insects may be greater at lower temperatures which favour adsorption of gas, rather than at high temperatures which favour chemical and physiological action, but which decrease the surface adsorption.

McIntosh (1954) and Munson et al. (1954) found that a negative coefficient of kill of Oryzaephilus surinamensis (L) was obtained with DFDT. [DFDT—(1, 1-bis (p-fluorophenyl 1)— 2,2,2 = trichloroethane)] In both cases the insects were reared at 30°C. McIntosh obtained his negative coefficient between 11°C and 30°C. Munson exposed insects treated at 30°C to 34°C for a short period to increase penetration and obtained his negative coefficient between 8°C and 34°C. Between 18°C and 34°C, however, DFDT was found to give a positive coefficient 7 hours after treatment. McIntosh (1954) stated that the temperature of greatest resistance of Oryzaephilus surinamensis may well be between 11°C and 30°C, and that there is no simple general physical rule connecting the toxicity of any one compound with its vapour pressure or temperature.

Munson (1953) found, during work on liposoluble insecticides, that cockroaches preconditioned at 23°C compared with those preconditioned at 34°C for two weeks were more resistant to the action of DDT when both were held at 23°C after treatment. It is thought that all insects possess lipoids which are subject to temperature influences, and as many of the more effective insecticides are liposoluble, negative coefficients of action with temperature and preconditioning effects should occur with many compounds (Munson et al.1954).

DDT has a physiological action on nerve membranes which are lipoidal in nature. Yamasaki and Ishii (1953a) found that the negative coefficient of action of DDT in the cockroach could not be explained by the penetrability of the cuticle by DDT, nor by the detoxification of the material, but found that the susceptibility of the nervous system was greater at lower temperatures. Yamasaki and Ishii (1953b) using oscillographic techniques proved that γ-BHC also acts on the nerves in the cockroach.

Hurst (1949), found that mature larvae of <u>Calliphora erytharocephala</u>, were extremely resistant to crystalline DDT at 20°C but after contact at 36°C for 2 hours, sufficient DDT was absorbed to cause paralysis when the temperature was reduced. Hurst concluded that DDT could be thrown out of internal lipids by chilling, and thus act on the peripheral nervous system.

The following possible explanations of the variation of the coefficient of action from negative to positive were put forward by Munson et al. (1954). Internal lipids effect a storage property which would be increased at higher temperatures removing the poison from the site of lethal action. Cuticular lipids can prevent or facilitate penetration of an insecticide depending on its liposolubility and the temperature.

Rates of absorption, detoxification, excretion, circulation of haemolymph and repair or replacement of damaged tissues are all possible factors in a complex of equilibria and reactions occurring simultaneously.

From the results obtained in these experiments, it appears that there is an optimum temperature of resistance of <u>Plodia</u> <u>interpunctella</u> pupae to γ-BHC vapour, which may be between 15°C and 30°C. Increasing the temperature from 15°C to 25°C after exposure, resulted in a decrease in mortality suggesting that γ-BHC exhibits a negative coefficient of kill below 25°C. Unfortunately, pupae exposed at 30°C, were not removed to 25°C for recovery as the kill at 30°C was rapid. It appears therefore, that γ-BHC possesses both a positive and negative coefficient of kill between 15°C and 30^{C}C, a possible temperature of greatest resistance occurring somewhere between these two values.

Further experiments using a different and greater range of temperatures would be of value in determining the exact method of action, and possibly the temperature of greatest resistance to γ-BHC and other insecticides.

The permeability of the cocoon to sprays.

To determine the permeability of the pupal cocoon of
P.interpunctella to sprays, protected and unprotected pupae
were sprayed with water and oil soluble dye solutions. The sol-
utions sprayed were 1 per cent methylene blue in water and 1
per cent Oil Red O in hexane and Odourless kerosene. No
results were obtained with the latter spray as it was not poss-
ible to remove the cocoon from protected pupae without trans-
ferring some of the dye.

5 replicates of 20 pupae of each type were sprayed at the
same time to avoid replication differences between sprays. 10 ml
of solution was sprayed; the spray details and results are
in Table 39. Unprotected pupae were washed with water or with
hexane; protected pupae were first carefully removed from their
cocoons before washing. The intensities of the dye solutions
prepared were compared against the appropriate solvent blank
in 4cm. cells in a Hilger 'Spekker' photoabsorptiometer.
Ilford filter No.606 was used for the water solution; filter
No. 604 was used for the hexane solution.

The results of these spraying experiments are given in
Table 39. The results show that the pupal cocoon is fairly
effective in preventing sprays from reaching the insect surface,
more so for the water spray than for the hexane spray, a fact
which is probably explained by the lower viscosity of hexane
and by its greater volatility. Droplet sizes ranged from $40-260\mu$.

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SUMMARY

1. The types of protective coverings secreted by insects are briefly discussed.

2. The structure and permeability of the protective coverings secreted by four selected insect species have been studied.

3. Permeability was estimated by direct measurement of dyes recovered on insects sprayed with dye solutions and indirectly by assessing mortality caused by an insecticide, γ-BHC, applied to protected and unprotected insects.

4. Apparatus designed for this work includes chambers used to study the penetration of vapour of γ-B.H.C and radio-active γ-BHC. A Spray Tower was designed to accommodate material ranging in size from insects in petri dishes to small trees. A description of the Tower and its performance is given.

5. Permeability of the wax-wool covering of <u>Cnaphalodes</u> <u>strobilobius</u> to Oil emulsion sprays and to vapour of γ-BHC has been investigated. The method of penetration of the covering by the insecticide vapour was determined by using the vapour phase of Carbon-14 labelled γ-BHC.

6. The chemical structure of the spittle secreted by nymphs of <u>Philaenus</u> <u>spumarius</u> was investigated by histochemical and acid hydrolysis techniques. The rate of dissolution of spittle masses by a number of groups of compounds and the possible mode of action of the compounds has been examined. The protective nature of the spittle was studied by subject-

ing spittle masses to sprays, dessication and fumigation.

7. Attempts made to penetrate the dorsal scale of <u>Saissetia</u> <u>hemisphaerica</u> by alcoholic, aqueous and oily solvents are reported. The results of acid hydrolysis to determine protein in the scale are also described.

8. The amino acid composition of the pupal silk cocoon of <u>Plodia</u> <u>interpunctella</u> has been investigated. Permeability of the cocoon was assessed by topical application of insecticide in acetone solution, by exposure of protected and unprotected pupae to saturated vapour of γ-BHC, and by spraying pupae of both types with dye solutions. Vapour penetration of the cocoon was investigated using pupae of three different ages under different temperature conditions.

9. A brief discussion of the value of the protective coverings is given.

GENERAL DISCUSSION

The intention of this work was to study the structure and permeability of the protective coverings secreted by four selected insect species. The coverings were chosen to represent, as far as possible, the major types of protective covering materials utilised by insects and because of this, the investigations carried out fell naturally into four sections. The study of the structure and permeability of each type of secretion needed different techniques and in this discussion the value of the protective coverings of the four insects is compared.

The physical structure and appearance of the four coverings is completely different. In one species only, P.spumarius does there appear to be complete coverage, but in this insect awareness of the external conditions suggests that sensory reception is highly organised. There may, in fact, be some connection with the outside of the secretion; this possibility would benefit from further investigation.

In P.interpunctella the outline of the pupa can be clearly seen through the structure of the cocoon. The cocoon appears to be highly permeable to insecticide applied topically or in the vapou phase; permeability was reduced only in the spraying experiments but here the nature of the solvent appeared to play a part.

The aphid, C.strobilobius and the Coccid, S.Hemisphaerica.

apparently well protected, were shown to be easily penetrated by certain treatments, the wax-wool covering of the aphid being inefficient protection against materials which wet the fibres of the covering and the vapour of the insecticide used; the dorsal scale of the Coccid was not penetrated in this work, but the insect was easily reached by materials which penetrated beneath the edge of the scale.

Protein material was found in three of the coverings and in Philaenus, where it constituted a very small fraction of the spittle covering, materials which attacked the protein, such as acids, were found to be highly effective in destroying the covering. The protein in the scale of Saissetia is probably present as a basis for further secretion; it is this other material which is believed to render the scale practically impermeable. The silk cocoon of Plodia pupae is probably produced to prevent mechanical damage as the silk fibres appear to possess no property other than forming a physical barrier; as such the pupal cocoon had little or no protective value in the present work.

The secretion of Cnaphalodes, which is mostly wax, could be penetrated by materials which lowered the absorbing powers of the wax. Thus the addition of a wetter to the spray solutions used allowed greater and more rapid penetration to occur. Similarly, the deposition of molecules of a lipophilic insect-

icide such as γ-BHC will occur until the wax becomes saturated at this point a dynamic equilibrium will be set up within the covering which then becomes freely permeable. The results of the penetration experiments using saturated vapour of Carbon-14 labelled γ-BHC indicated that this equilibrium point was reached after about 8 hours and that after that time deposition of the insecticide on the insect surface increased fairly rapidly, while the amount adsorbed on to the wax of the covering remained fairly constant.

The protective coverings secreted by these four insect species are probably used naturally for protection from adverse climatic conditions, except perhaps in Plodia, and from predatory and parasitic insects.and other animals. From the results of the investigations carried out it is certain that control measures,for these and probably other similarly protected insects, which make use of the inherent weaknesses of the protective coverings will be more efficient.

BIBLIOGRAPHY

Abderhalden E. and Behrend L. 1909. Z.physiol. Chem. 59. 236

Alexander P. and Block R.J. 1960. Analytical Methods of
                    Protein Chemistry. Vol.2. Pergammon Press.

Allen T.C.,Dicke R.J. and Brooks J.W. 1943. Soap.N.Y. 19 (4)
                    94-96, 121.

de Almeida A.A. 1962. D.I.C. Thesis. Imperial Coll..Lond. Univ.

App B.A. and Weaver C.R. 1953 J. econ. Ent.46 (1). 182

Armstrong G., Bradbury F.R. and Standen H. 1951.
                    Ann. appl. Biol. 38 (3). 555.

Baker G., Pepper J.H., Johnson L.H. and Hastings E. 1960.
                    J. Insect Physiol. 5. 47

Baranyovits F. 1953. Endeavour 12 (48). 202.

Bard J.H. 1961. M.Sc. Thesis. Imperial College, London Univ.

Barker G.E. 1948. Soap. N.Y. 24 (6). 46, 65.

Batelli A. 1891. Monitore zool. ital. 2 (7)

Beament J.W.L. 1955. J.Exptl. Biol. 32. 514.

Beeson C.F.C. 1910. Indian For. Rec. 7.

Benson A.A. 1952. J.Am. Chem. Soc. 72. 1710.

Berlese A. 1909. Gli Insetti. Milano.

Blochmann.F. 1889. Biol. Centralb. 9. 271.

Borner C. 1909. Biol. Centralb. 29. 118.

Block R.J., Durrum E.L. and Zweig G. 1955. Paper chromatog —
                    raphy and Paper Electrophoresis. Acad.Press. N.Y.

Blount B.K.,Chibnall A.C. and el Mangouri H.A. 1937
                    Biochem.J. 31 (2). 1375.

Bodenheimer F.S. 1951. Citrus Entomology. W.Junke, The Hague.

Bottimer L.J. 1945. Soap.N.Y. 21 (2). 151

Brown A.W.A. 1951. Insect control by chemicals.
                    Wiley and Sons Inc. N.Y.

Burdon E.R. 1908a. J. econ. Biol. 2. 1, 63.

     "   1908b.   ibid.   2. 119.

Campbell F.L. and Sullivan W.N. 1938. Soap. N.Y. 14 (6) 119,149

Cecil R. 1930. Ohio Journ. Science. 30 .120

Chibnall A.C.,Latner A.L.,Williams E.F. and Ayre C.A. 1934a.
Biochem J. 28. 313

Chibnall A.C.,Piper S.H.,Pollard A.,Williams E.F. and Sahai P.
1934b. Biochem.J. 28. 2189.

Chibnall A.C. and Piper S.H. 1934c. ibid. 2209.

Cholodkovsky N.A. 1911. Zool. Anz. 37. 172.

Cotton R.T. 1943. Insect pests of Stored Grain. Burgess, Minn.

Cotton R.T. and Frankenfeld J.C. 1949. Assoc. Operative
Millers Bull. 1660-1665.

Crooke M. 1952. For.Commn.Lond.,Forest Record No. 17.

David W.A.L. 1946. Ann. appl. Biol. 33 (2). 133

Day M.F. 1949. Aust.J.scient.Res.Ser.B.(Biol.Sc) 2 (4). 421

Dean R.W. 1947. J.econ.Ent. 40. 183

   "   1951. Comm. to Chem.—Biol.Coordination Center.

Dickson R.C. 1941. Higardia 13 (9). 515

Dorman S.C. and Hall W.E. 1953. J.econ.Ent. 46 (1). 151

Du Chanois F.R. 1947.   ibid. 40. 749

Ebeling W. 1939. Hilgardia 12 (11). 665.

   "   1951. Comm. to Chem.—Biol. Coordination Center.

Edwards W.H. 1896. Hemiptera (Homoptera). London.

Ewan H.G. 1961. Tech.Bull.U.S.Dep.Agric. 1250.

Fahey J.E., Wilson M.C. and Rusk H.W. 1960. J.econ.Ent. 53. 960

Fewkes D.W. 1960. Nature, Lond. 188. 167.

   "   1961a. J.econ.Ent. 54 (4). 771

   "   1961b. Personal communication.

Finney D.J. 1947. Probit Analysis. Camb. Univ. Press.

Fisher R.A. 1936. Statistical Methods for Research Workers.
Oliver and Boyd, Lond.

Flamant G. 1962. Phytoma 14 (134). 23

Gahan C.J. 1918. Proc.ent.Soc. Lond. 1918. 153.

Gast R. and Early J. 1956. Agric. Chem. 11 (4). 42,136,139.

Gilby A.R. 1957. Archs. Biochem. Biophys. 67 (2). 307

Gilby A.R. and Alexander A.E. 1957. Archs.Biochem.Biophys.
67 (2). 302

Gilby A.R. and Cox M.E. 1963. J. Insect Physiol. 9 (5). 671

Gosswald K.,Schulze E.F. and Kloft W. 1963. Symposium on the
use of Radio-isotopes and Radiation. Athens.April 1963.

Greenslade R.M. 1934. East malling Res.Sta.Ann.Rep. 1934. 185.

Gruner M. 1900. Zool. Anz. 23. 431

"   1901.   Inaugural Dissertation, Berlin.

Guilbeau-Braxton H. 1908. Am. Nat.T. 62.

Gurr E. 1958. Methods of Analytical Histology and Histochemistry
Leonard Hill Ltd., Lond.

Hackman R.H. 1951. Archs. Biochem. Biophys. 33 (1). 150

Haurowitz F. 1950. Chemistry and Biology of Proteins.
Acad. Press. N.Y.

Hewlett P.S. 1946. Ann. appl. Biol. 33 (3) 303.

Hoskins W.M. and Caldwell A.H.Jnr. 1947. Soap N.Y.23 (4). 143.

ten Houten J.G. and Kraak M. 1949. Ann. appl. Biol. 36 (3) 394

Howden G.F. and Marshall A.T. 1961. Nature, Lond. 189. 509

Hurst H. 1949.   ibid. 163. 286

Imms A.D. 1957. A general textbook of Entomoligy (revised
edition;Richards O.W. and Davies R.G.).Methuen.

Karrer P. 1950. Organic Chemistry. Elsevier Publ. Co. N.Y.

Kato K. 1958. Science Reports of Saitoma Univ.(Ser.B.) 3. 33

Kerr T.W. Jnr. 1953. J. econ. Ent. 46 (2). 353.

Kershaw J.C. 1914. Psyche. Camb. Mass. 21. 65

Kirkaldy G.W. 1906. Bull. exp.Sta. Hawaiian Sug.Pl. Ass.1. 269

Koehler C.S. and Gyrisco G.G. 1957. J. econ. Ent. 50 (3) 346

Latter O.H. 1897. Trans. ent. Soc. Lond. 1897. 113

Lees A.D. and Beament J.W.L. 1948. Quart.J.micr.Sci. 89. 291

Lewis C.T. 1963. Symposium. International Atomic Energy Agency.
Vienna, 1963.

Licent E. 1912. Cellule 28.

Lightly A.A. and Faulkner R. 1964. Quart. J. For. 57 (4) 293

Lindgren D.L. 1941. Hilgardia 13 (9). 491.

Lison L. 1953. Histochimie et Cytochimie animales. Villars, Paris

Locke M. 1961. J. Biophys. Biochem. Cytol. 10. 589

Lower H.F. 1959. Trans. roy. Soc. S. Aust. 82. 175

Machida J. 1927. J. Coll. Agric. imp. Univ., Tokyo 9 119

Marshall D.S. and Gyrisco G.G. 1951. J. econ. Ent. 44 (3) 289

May K.R. 1945. J. scient. Instrum. 22. 186

McGovran E.R. and Mayer E.L. 1943. Publs. Am. Ass. Advmt. Sci. 20

McIntosh A.H. 1954. Bull. ent. Res. 45. 137

Moore W. 1936. J. econ. Ent. 21 (1). 65

Mukerjea T.D. 1953. Bull. ent. Res. 44 (1). 121

Munson S.C. 1953. J. econ. Ent. 46. 657

Munson S.C., Padilla G.M. and Weissman M.L. 1954. J. econ. Ent. 47 (4

Nelson F.C. 1937. Jl. N.Y. ent. Soc. 45. 341

O'Kane W.C., Glover L.C. and Blickle R.L. 1941.

New Hamp. Agri. Exp. Sta. Tech. Bull. 76.

Osborn H. 1916. Maine Exp. Sta. Bull. 254. 265.

Owen R.W. and Waloff N. 1946. Ann. appl. Biol. 33. 387.

Parkin E.A. 1951. J. Sci. Fd. Agric. 2 (3). 136.

Parr H.C.M. and Busvine J.R. 1948. Ann. appl. Biol. 35 (3). 359

Partridge S.M. 1948. Biochem. J. 42. 238.

Pesson P. 1956. Boll. Lab. Zool. gen. agr. Portici. 33. 341

Petersen B.B. 1962. Dansk. Skovforen Tidsskr. 47. 118

Petty B.K. 1946. Sci. Bull. Dep. Agric. S. Afr. No. 267.

Pigman W. 1957. The Carbohydrates. Acad. Press. N.Y.

Pirie H. 1951. Chem. and Ind. Feb. 10. 1951. 100.

Porta A. 1901. Monitore zool. ital. 12. 57

Potter C. 1935. Ann. appl. Biol. 22 (4). 769.

    "   1938.   ibid.   25 (4). 836

    "   1941.   ibid.   28 (2). 142

    "   1952.   ibid.   39 (1). 1

Quayle H.J. 1938. Hilgardia 11 (5). 183

Rayner A.C. and Hurtig H. 1952. Suffield Exp. Sta. Tech. Paper 22.

Richards O.W. and Waloff N. 1946. Trans.R.ent.Soc.Lond. 97(2)253

Roan C.C. and Kearns C.W. 1948. Soap N.Y. 24 (5). 133,149

Rockstein M. 1964. The Physiology of Insecta. Acad. Press. N.Y.

Rogojanu V. 1934. Z. mikr.—anat. Forsch. 37.151

Rohwer S.A. 1949. Agric Chem. 4 (8). 35, 75.

Russell M.P. 1961. J. econ. Ent. 54 (4). 812.

Schneider—Orelli O. 1950. Mitt.schweiz.Anst.forstl.VersWes. 26.

Shepard H.H. 1937. Univ. Minnesota Agr.Exp.Sta.Tech.Bull. 120. 3

Simkover H.E. and Shenefelt R.D. 1952. J.econ.Ent. 45 (1). 11

Sinha R.N. 1953. Stain Tech. 28 (5). 249

Smith I. 1958. Chromatographic Techniques. Interscience, N.Y.

Smith R.H. 1944. Hilgardia. 16 (5). 225

Speyer E.R. 1919. Ann. appl. Biol. 6. 171

Steffan A.W. 1962. Z. angew. Ent. 50 (3). 328

Steven H.M. 1917. Proc. roy. Soc. Edinb. 37. 356

Stevens T.M. 1956. Ann. ent. Soc. Amer. 49. 617

Stoker R.I. 1948. Ann. appl. Biol. 35 (1). 110

Stultz H.T. 1939. Rep. ent. Soc. Ont. 70. 72

Subba Rao N.V. and Pollard A.E. 1951. J.Sci.Fd. Agric. 2 176

Sulc K. 1911. Arch. Naturgesch. 99. 147

Tattersfield F. and Morris H.M. 1924. Bull. ent. Res. 14 (3)223

Taylor F.S. 1957. Organic Chemistry. Heineman Ltd., Lond.

Tsuji H. 1958. Jap. J. appl. Ent. Zool. 2 (1). 17

    " 1959. ibid. 3 (1). 34

    " 1960. ibid. 4 (3). 173

Tzanakakis M.E. 1959. Hilgardia 29 (5). 205

Varty I.W. 1956. Bull. For. Comm., Lond. 26.

Vlitos A.J. and Merry C.A.F. 1962. Nature, Lond. 194. 211

Warth A.H. 1956. The Chemistry and Technology of Wax's.

                                                      Reinhold, N.Y.

Waters H.A. 1937. J. econ. Ent. 30. 179.

Way M.J. 1949. Ann. appl. Biol. 36 (1). 86

Weaver C.R. 1958. J. econ. Ent. 51 (5). 743

Webb J.E. 1947. Bull. ent. Res. <u>38</u> (2). 209

Weber H. 1930. Biologie der Hemipteren. Julius Springer,
Berlin.

Wigglesworth V.B. 1950. Quart. J. micr. Sci. <u>91</u> (2). 217

Williams G.C. 1964. Ann. appl. Biol. <u>53</u> (3). 459

Wilson H.A. and Dorsey C.R. 1954. Ann. ent. Soc. Amer. <u>50</u>.399

Yamasaki T. and Ishii T. 1953a. Oyo-Kontyu. <u>9</u>. 87.

              "   1953b. Botyu-Kagaku. <u>19</u>. 106

Yust H.R., Nelson H.D. and Busbey R.L. 1943. J. econ. Ent.
<u>36</u> (5). 744

Ziegler H. and Ziegler I. 1958. Z. vergl. Physiol. <u>40</u>. 549

TABLE 1   Mortality of migrating larvae of
                 P.interpunctella in petri dishes
     Ten replicates of ten larvae for each age.

Days after start of migration	No. larvae	No. pupae pro- duced	% mortality	No. adults pro- duced	% mortality	Total % mortality
1	100	73	27.0	67	8.2	33
2	100	88	12.0	85	3.2	15
3	100	82	18.0	66	19.5	34
4	100	74	26.0	57	23.0	43
5	100	38	62.0	28	26.3	72

TABLE 2

     Numbers of protected and unprotected pupae produced in
67 petri dishes. Ten larvae in each petri dish.

Type of pupa produced	Number of pupae	% pupae
Pupae with cocoon—"protected"	394	59
Pupae without cocoon—"unprotected"	216	32
Total mortality	60	9
Total	670	

TABLE 3

Results of observations of the number of larvae in petri dishes, for production of the optimum number of pupae with cocoons.

No. larvae per dish	Total Number of larvae used	Number of pupae produced				No. of larvae pupae dead.	
		"Protected"		"Unprotected"			
		Number	%	Number	%	Number	%
1	10	3	30.	7	70	0	
2	20	3	15	16	80	1	5
3	30	9	30	21	70	0	
4	40	8	20	32	80	0	
5	50	18	36	28	56	4	8
6	60	17	28.3	35	58.3	8	13.3
7	70	31	44.3	35	50	4	5.7
8	80	27	33.8	51	63.8	2	2.5
9	90	44	48.9	34	37.8	12	13.3
10	100	68	68	22	22	10	10
11	33	17	51.5	11	33.3	5	15.2
12	36	14	38.9	15	41.7	7	19.4
Totals	619	259		307		53	

TABLE 4.

Analysis of spray deposits of γ-BHC from acetone solution
(Conditions of test:— room temperature, $22^{\circ}C$ $2^{\circ}C$;   air
pressure of atomization, 76.0 cm.Hg;   volume of fluid sprayed,
10 mls;   time taken to spray, 19 secs;   time for spray
deposition, 3 mins;   lower shutter opened 5 secs. after
spraying finished;   deposits in mgs. on 3 in. by 1 in. glass
slides;   spray solution, 5%   BHC in acetone)

Position Trial	1 (periphery)	2 (periphery)	3 (periphery)	4 (periphery)	5 (centre)	Mean (mgs)
1	1.6	1.5	1.6	1.5	1.6	1.56
2	1.4	1.5	1.5	1.6	1.4	1.48
3	1.4	1.4	1.4	1.5	1.3	1.40
4	1.3	1.7	1.4	1.7	1.6	1.54
5	1.5	1.1	1.6	1.3	1.2	1.34
6	1.2	1.3	1.4	1.4	1.1	1.28
7	1.4	1.3	1.3	1.6	1.3	1.38
8	1.3	1.3	1.6	1.3	1.6	1.42
9	1.5	1.2	1.4	1.5	1.1	1.34
10	1.4	1.4	1.4	1.3	1.3	1.36
Means	1.40	1.37	1.46	1.47	1.35	1.41

Between positions variation $F = 1.40$ not significant at
$P = 0.05$.

Between trials variation $\underline{F = 2.58}$ Significant at P = 0.05

Standard deviation for single deposit $\underline{\pm\ 0.138}$

Standard error of mean of ten deposits at one position

$$\underline{\pm\ 0.044}$$

Standard error of mean of five deposits at one trial

$$\underline{\pm\ 0.062}$$

TABLE 5

Analysis of spray deposits of methylene blue dye
from aqueous solution.

(Conditions of test—: as Table 4 except, time taken to spray
22 secs; spray solution, 1% methylene blue in distilled
water).

Position Trial	1 (periphery)	2 (periphery)	3 (periphery)	4 (periphery)	5 (centre)	Mean (mgs)
1	0.38	0.39	0.39	0.38	0.38	0.384
2	0.43	0.43	0.42	0.43	0.42	0.426
3	0.42	0.42	0.41	0.39	0.41	0.410
4	0.44	0.43	0.42	0.42	0.40	0.422
5	0.39	0.37	0.39	0.40	0.41	0.392
6	0.39	0.39	0.40	0.40	0.41	0.398
7	0.41	0.43	0.41	0.40	0.40	0.410
8	0.43	0.42	0.41	0.42	0.38	0.412
9	0.38	0.39	0.40	0.39	0.40	0.392
10	0.40	0.41	0.39	0.41	0.39	0.400
Means	0.407	0.408	0.404	0.404	0.400	0.405

Between positions variation $F = 0.67$ not significant at
$P = 0.05$

Between trials variation $F = 6.39$ significant at $P = 0.05$

Standard deviation for single deposit ± 0.012

Standard error of mean of ten deposits at one position

± 0.0038

Standard error of mean of five deposits at one trial

± 0.0054

TABLE 6

Analysis of spray deposit of Risella oil (R.17)

(Conditions of test –: as Table 4 except, time taken to spray 115 secs; spray solution, Risella oil (R.17).

Position	1	2	3	4	5	mean
Trial	(peri- phery)	(peri- phery)	(peri- phery)	(peri- phery)	(centre)	(mgs)
1	12.8	12.8	13.4	12.8	12.2	12.80
2	14.1	13.9	14.3	13.6	13.7	13.92
3	13.6	13.8	12.7	13.1	13.2	13.28
4	12.7	13.4	14.2	12.8	12.4	13.10
5	13.1	12.9	13.6	13.3	12.7	13.12
6	12.9	13.2	13.1	12.9	13.2	13.06
Means	13.20	13.33	13.55	13.08	12.90	13.21

Between positions variation $F = 2.46$ not significant at $P = 0.05$

Between trials variation $F = 4.87$ significant at $P = 0.05$

Standard deviation for single deposit ± 0.384

Standard error of mean of six deposits at one position

± 0.121

Standard error of mean of five deposits at one trial

± 0.172

TABLE 7

Some Physical constants of oils used in
experimental work.

Physical constants	Odourless kerosene	Risella oil (R.17)
Mean molecular weight	200	280
Initial boiling point, ^{O}C	180	288
Flash point, ^{O}F	160	310
Viscosity (Redwood 1 at $70^{O}F$)	31	125
Specific gravity 60/60°F	0.790	0.870

TABLE 8   Mortality, after 24 and 72 hours, of C. strobilobius sprayed on Larch trees with a 6:3:0.2 v/v emulsion of odourless kerosene, water and Lissapol NX, containing different concentrations of γ-BHC. (Conditions of test: temperature, 22°C; air pressure of atomization, 76 cm. Hg; volume sprayed, 5 mls; time for deposition, 3 mins;

% concentration of γ-BHC (mgs.)	NO. insects	Mortality after 24 hours		Mortality after 72 hours	
		No. dead	%Mortality	No.dead	% Mortality.
0.1	150	32	21.3	43	28.7
1.0	150	67	44.7	71	47.3
10.0	150	81	54.0	100	66.7
100.0	150	119	79.3	129	86.0
1000.0	150	145	96.7	142	94.7
Control Average		15	10.0	17	11.3

P = 0.05.

24 hours.   LD.50 = 5.5   0.002 mgs/litre.   Y = 3.87 + 0.65x
χ^2 = 8.45

72 hours.   LD.50 = 2.6   0.001 mgs/litre.   Y = 4.13 + 0.61x
χ^2 = 0.23

t test, (P = 0.05) confirmed that difference between 1 and 3 day treatments was not significant (t = 2.34)

TABLE 9 Mortality, after 24 and 72 hours, of C.strobilobius sprayed on Larch trees, with a 6:3:2v/v emulsion of odourless kerosène, water and Lissapol NX, containing different concentrations of γ-BHC.

(Conditions of test: as Table 8 except, time to spray, 33 secs.)

% Concentration of γ-BHC (mgs)	No. insects	Mortality after 24 hours		Mortality after 72 hours	
		No. dead	%mortality	No.dead	%Mortality
0.1	150	18	12.0	20	13.3
1.0	150	40	26.7	43	28.7
10	150	66	44.0	76	50.7
100	150	93	62.0	101	67.3
1000	150	130	86.7	139	92.7
Control		23	15.3	19	12.7

P = 0.05.

24 hours. LD.50 = 4.90 ± 0.011mgs/100ml. Y = 3.04 + 0.73x
$\chi^2 = 4.69$

72 hours. LD.50 = 2.19 ± 0.005 mgs/100ml. Y = 3.24 + 0.75x
χ^2 4.88.

t test, (P = 0.05), confirmed that difference between 1 and 3 day treatments was significant (t = 2.67)

Differences between emulsion sprays

t test, (P = 0.05), confirmed that differences between 1 day and 3 day treatments with the two emulsions were significant.

1 day treatment : t = 7.04
3 day treatment : t = 6.74

TABLE 10

Mortality of C. strobilobius exposed to
saturated vapour of ɣ-BHC.

(Conditions of test—: temperature, $25^{\circ}C \pm 2^{\circ}C$; relative
humidity of chamber, 75 per cent.)

| Exposure period (hrs) | Number of insects exposed | Mortality of insects | | Control mortality |
		No. dead	% mortality	
6	25	0	0	0
12	25	1	4	0
18	25	7	28	1
24	25	10	40	0
30	25	14	56	2
36	25	18	72	0
42	25	20	80	0
48	25	23	92	1

Equation of regression line —: $Y = 4.25x - 1.06$

LT.50.value = 26.73 ± 0.027 (hours) $\chi^2 = 3.46$

The fiducial limits to the LT.50 value are 23.7 and 30.9
hours.   (t = 1.96 at 5 per cent level of probability)

TABLE 11

Exposure of C. strobilobius to vapour of C-14 labelled γ-BHC

(Conditions of test-: temperature of exposure, $25^\circ C \pm 0.3^\circ C$; relative humidity, 75 per cent; exposure period, 30 mins; chambers equilibrated for 12 hours before use; 10 insects exposed)

C-14 standard. Counts (cps) before sample counts	Background counts of tubes 5 mls. scintillator liquid (cps)			Time (secs) for 400 counts of tubes scintillator liquid samples (10 insects)			C-14 standard. Counts (cps) after sample counts.
	A	B	C	wax-wool ex-tract A	Insect surface extract B	Insect internal extract C	
421.4	2.0	1.2	1.6	29.0	30.6	226.8	405.9
423.7	2.6	1.9	1.4	27.7	27.2	224.4	416.1
425.6	1.6	2.0	1.7	29.6	30.8	218.7	415.6
410.4	2.7	1.8	2.4	27.7	28.9	222.1	413.4
422.8	1.9	2.2	2.0	27.8	30.9	221.0	413.6
Means 420.8	2.2	1.8	1.8	28.4	29.7	222.2	412.9

cps/insect	1.41	1.35	1.80
corrected cps/insect (sample cps - background cps)	1.19	1.17	0.00

Counting efficiency of C-14 standard (516 cps at 100 per cent efficiency)

Average cps. for ten readings, 416.9; 80.8 per cent efficiency.

TABLE 12

Summary of results of exposure of C.strobilobius to vapour of C-14 labelled γ-BHC

(Conditions of tests —∵ as Table 11 except, exposure periods ranging from 30 mins. to 48 hours)

Exposure periods (hrs)	Hexane extracts. Gms. x 10⁻⁹ γ-BHC			C-14 standard Average cps	per cent counting efficiency for C-14 standard
	Wax—wool extract	Insect surface extract	Insect internal extract		
0.5	1.34	1.32	0.00	416.9	80.8
1	6.10	3.38	0.24	402.8	78.1
2	9.01	6.24	0.42	389.0	75.4
4	12.64	8.77	0.70	409.9	79.5
6	14.18	10.49	1.54	408.7	79.2
8	16.10	16.03	2.07	413.5	80.2
12	18.46	26.93	2.70	422.2	81.8
18	20.60	31.20	3.27	412.5	80.0
24	22.16	33.12	3.93	414.4	80.3
36	22.77	36.91	5.53	422.0	81.8
48	23.76	41.50	6.81	421.6	81.7

TABLE 13   Host plants of the two most common species
of Cercopidae found.

Neophilaenus lineatus L.	Philaenus spumarius (F)	
Graminae Agrostis tenuis	Compositae	Achillea millefolium
Alopecurus pratensis		Artemisia vulgaris
Arrhenatherum elatius		Centaurea nigra
Deschampsia caespitosa		Cirsium arvense
Festuca rubra		Cirsium vulgare
Holcus lanatus		Helianthus annus
Holcus mollis	Campanulaceae	Campanula persificola
Lolium perennae	Caryophyllaceae	Dianthus carthusianorum
Phleum pratense	Cruciferae	Alliaria officinalis
Poa annua		Cytisus sp
Poa pratensis	Hypericaceae	Hypericum perforatum
	Labiatae	Salvia pratensis
	Leguminosae	Trifolium pratense
	Onagraceae	Chamaenerion angustifolium
	Plantaginaceae	Plantago lanceolata
	Polygonaceae	Rumex scutatus
	Rosaceae	Filipendula ulmaria
	Rubiaceae	Gallium cruciata
		Gallium mollugo
	Scrophulariaceae	Veronica sp.
	Solanaceae	Lycium sp.
	Umbelliferae	Anthriscus sylvestris

<u>TABLE 14</u>   Amino acids found in acid hydol-
<u>Figures 15,19,20.</u>   ysates of the protective coverings
of <u>P.spumarius</u>, <u>S.hemisphaerica</u>
and <u>P.interpunctella.</u>

Amino acid	R_f value Phenol run	<u>P. spum- arius.</u>	<u>S. hemis- phaerica.</u>	<u>P.inter- punctella.</u>
1. Cystine	20.	x		
2. Cysteic acid	7		x	
3. Aspartic acid	17	x	x	x
4. Glutanic acid	26	x	x	x
5. Serine	35	x	x	x
6. Glycine	42	x	x	x
7. Threonine	48	x	x	x
8. Alanine	58	x	x	x
9. Tyrosine	60	x	*	x
10. Glutamine	57	x	x	x
11. Valine	78	x	x	x
12. Arginine	86	x	x	x
13. Phenyl- alanine	84	x	x	x
14. Leucine	85		x	
15. Iso − leucine	85		x	
16. Proline	90	x	x	x

x— indicates presence of amino acid.

TABLE 15
Graph 3

Dissolution of P.spumarius spittle in solutions of Mineral Acids. Times in minutes;

Acid	Hydrochloric	Nitric	Sulphuric	o-Phosphoric
Acid Normality	HCl	HNO_3	H_2SO_4	H_3PO_4
0.001N	7.0 6.0 7.5 5.5 8.5	11.0 10.0 9.5 11.5 10.5	13.5 12.0 13.5 11.5 12.5	18.5 21.0 17.5 21.0 17.0
0.01N	2.5 5.0 4.5 3.0 4.5	6.0 5.5 7.5 6.5 5.0	9.0 11.5 10.5 11.0 10.0	7.5 6.0 8.0 7.5 6.0
0.1N	2.0 3.5 3.5 2.5 3.5	5.0 6.0 5.0 5.5 4.5	9.5 8.0 9.0 8.0 8.0	4.0 2.0 2.5 1.5 2.5
1.0N	15.0 12.0 14.0 12.5 12.5	8.0 8.5 7.5 6.5 7.0	2.5 4.0 3.0 4.5 3.5	4.5 3.5 3.5 3.0 2.5
10.0N	17.5 17.5 17.5 16.5 18.0	8.0 8.5 7.0 9.0 7.5	8.0 8.0 6.5 9.5 8.0	8.0 4.5 6.0 7.0 5.5
Average (mins)				
0.001N	7.0	10.5	12.6	19.0
0.01N	3.9	6.1	10.4	7.0
0.1N	3.0	5.2	8.5	2.5
1.0N	13.2	7.5	3.5	3.4
10.0N	17.4	8.0	8.0	6.2

TABLE 16
Graph 4

Dissolution of P.spumarius spittle in solutions of Aliphatic Monobasic Acids. Times in minutes.

Acid	Formic	Acetic	Propionic
Acid Normality	H.COOH	CH$_3$.COOH	C$_2$H$_5$.COOH
0.001N		12.0,13.0,12.5, 11.5,11.5	11.5,12.0,11.0 11.5,11.0
0.01N	12.5,13.5,12.5, 12.0,13.5	9.0,10.0,9.0, 9.5,9.5	8.5,9.0,8.5,7.5, 9.5
0.05N	10.5,9.0,10.0, 11.0,11.0	8.5,7.0,7.5, 7.5,8.0	6.5,6.5,6.0,6.5, 6.5
0.1N	9.0,9.5,9.0,8.5, 9.0	6.5,6.5,7.0, 6.0,6.5	5.5,5.0,5.0,4.5, 5.5
1.0N	6.0,5.5,5.5,6.0 5.5	3.5,4.0,4.5, 4.5,3.5	3.5,4.0.3.0,4.5, 3.0
Average (mins)			
0.001N		12.1	11.4
0.01N	12.8	9.4	8.7
0.05N	10.3	7.7	6.2
0.1N	9.0	6.5	5.1
1.0N	5.7	4.0	3.6

TABLE 17

Graph 5   Dissolution of P.spumarius spittle in solutions of Aliphatic Dibasic Acids.   Times in Seconds.

Acid	Oxalic	Malonic	Succinic
Acid Normality	$(COOH)_2$	$(COOH)_2CH_2$	$(COOH.CH_2)_2$
0.1N	125,120,125,145, 135	230,250,235,260, 250	350,345,360,370, 375
0.2N	100,100,95,115, 120	105,130,115,120, 125	180,190,185,210 195
0.5N	85,95,85,110,105	105,110,100,95, 100	130,120,115,125, 130
1.0N	185,200,180,210, 215	160,150,185,155, 180	145,165,155,140 145
Average (secs)			
0.1N	130	251	360
0.2N	106	119	192
0.5N	96	102	124
1.0N	198	166	150

TABLE 18
Graph 6

Dissolution of P.spumarius spittle in solutions of Chloro—acetic Acids. Times in minutes.

Acid	Monochloro-acetic	Dichloro-acetic	Trichloro-acetic	Acetic
Acid Normality	$CH_2Cl.COOH$	$CH.Cl_2.COOH$	$C.Cl_3.COOH$	CH_3COOH
0.001N	9.0,10.0, 10.5,12.0 11.5	9.0,10.0, 8.0,10.5, 10.0	5.5,6.0,7.0 7.5,8.5	12.0,13.0, 12.5,11.5, 11.5
0.01N	7.0,8.0,8.0, 8.5,8.5	6.0,5.5,8.0 6.0,7.0	3.0,3.5,2.5 4.0,4.0	9.0,10.0, 9.0,9.5,9.5
0.05N			0.5,1.0,2.5 1.5,2.0	8.5,7.0,7.5 7.5,8.0
0.1N	3.5,5.5,4.5, 6.5,6.5	3.5,4.0,3.5 5.0,6.0	3.0,3.0,4.5 4.0,3.0	6.5,6.5,7.0 6.0,6.5
1.0N	3.5,4.0,3.5, 5.5,5.5	7.5,6.5,5.0 7.0,6.5	8.0,9.0,8.5 10.0,9.5	3.5,4.0,4.5 4.5,3.5

Average (mins)				
0.001N	10.6	9.5	7.0	12.1
0.01N	8.0	6.5	3.4	9.4
0.05N			1.5	7.7
0.1N	5.3	4.4	3.5 R.	6.5
1.0N	4.4	6.5	9.0 R.	4.0

TABLE 19   Dissolution of P.spumarius spittle in solutions
Graph 7   of Aliphatic Monohydroxy Alcohols.   Times in seconds.

Alcohol	Methanol	Ethanol	n—Propanol	tert—Butanol
% concentration	CH_3OH	C_2H_5OH	$n-C_3H_7OH$	$tert-C_4H_9OH$
15			330,320,325, 315,315	
20		$>$400	185,165,190, 170,180	
30	$>$400	370,350,350 375,350	105,75,80, 100,90	
40	410,405,380, 385,395	215,205,205 200,215	25,20,30,30, 25	
50	170,150,155, 170,165	95,105,85 105,80	20,10,15,30,10,5,20,15,10 25	
60	110,115,110, 95,105	55,40,60,45 50	10,15,10,20,10,5,10,10,5 15	
70	55,40,50,50, 55	20,20,30,35 20	10,5,10,15, 15	$<$10 R.
80	35,30,15,35, 30	10,10,15,20 15	$<$10 R.	$<$5 R.
90	$<$15 R.	$<$10 R.	$<$5 R.	$<$5 R.

Average (secs)				
15			321	
20		$>$400	178	
30	$>$400	359	90	
40	395	208	26	
50	162	94	20	12
60	107	50	14	8
70	50	25	11	$<$10 R.
80	29	14	$<$10 R.	$<$5 R.
90	$<$15 R.	$<$10 R.	$<$5 R.	$<$5 R.

TABLE   20   Dissolution of P.spumarius spittle in solutions
               of Acetone.   Times in seconds.

Graph   8

% concentration	Acetone $(CH_3)_2CO$	Average (secs)
40	320,285,295,305,295	300
50	95,120,120,105,120	112
60	65,50,70,55,60	60
70	30,35,40,20,15	28
80	10,5,10,15,10	10
90	< 5 R	< 5 R
100	< 5 R	< 5 R

TABLE 21   Dissolution of P.spumarius spittle in solutions
Graph   9   of Inorganic Hydroxides.   Times in seconds.

Hydroxide	Potassium	Sodium
Hydroxide Normality	KOH	NaOH
0.001N	280,285,270,265,275	225,240,235,230,230
0.01N	205,190,180,185,190	160,150,155,170,165
0.1N	100,100,105,105,110	80,90,80,90,85
1.0N	40,35,45,50,40	25,20,20,30,25
10.0N	10,10,5,15,10	
Average (secs)		
0.001N	275	233
0.01N	190	160
0.1N	104	85
1.0N	42	24
10.0N	10	

TABLE 22

Physical constants of Acids used for dissolution
of P.Spumarius spittle.

Graphs 3 to 6.
Tables 15 to 18.

Acids	Formula	Molecular Weight	Optimum observed normality for dissolution.	Molarity at optimum normality	pKa value
Mineral acids					
Hydrochloric	HCl	36.5	0.1	0.1	−7
Nitric	HNO_3	63	0.1	0.1	−1.64
Sulphuric	H_2SO_4	98	1.0	0.5	−3, +2
o-Phosphoric	H_3PO_4	98	0.1	0.03	2.1,7.2, 11.9
Aliphatic Mono-basic acids					
Formic	H.COOH	46			3.75
Acetic	CH_3COOH	60			4.76
Propionic	C_2H_5COOH	74			4.87
Aliphatic Dibasic acids					
Oxalic	$(COOH)_2$	90	0.5	0.25	1.27,4.27
Malonic	$(COOH)_2CH_2$	104	0.5	0.25	2.86,5.70
Succinic	$(COOH.CH_2)_2$	118	0.5	0.25	4.21,5.64
Chloro-acetic acids					
Monochloro-acetic	$CH_2Cl.COOH$	94.5			2.87
Dichloro-acetic	$CHCl_2COOH$	129	0.1	0.1	1.25
Trichloro-acetic	CCl_3COOH	163.5	0.05	0.05	0.66

All pKa values measured at $25^{\circ}C$ except
at $18^{\circ}C$
at $20^{\circ}C$

TABLE 23

Mortality of P.spumarius nymphs after spraying
spittle masses with δ-BHC in n-decane emulsion

(Conditions of test— room temperature, 21°C;
air pressure of atomization, 76.0 cm.Hg; volume
of fluid sprayed, 10 mls; average time to spray
32 secs; time for spray deposition, 3 mins.)

% δ-BHC in emulsion (mgs.)	Number of nymphs sprayed.	Instar 3		Instar 4	
		No. dead	% mortality	No. dead	% mortality
0.01	25	1	4	0	0
0.1	25	7	28	4	16
1.0	25	13	52	9	36
10	25	20	80	18	72
100	25	25	100	25	100

L.D. 50, Instar 3. 0.0071 ± 0.0026 (Y = 3.36 + 0.89x)

L.D. 50, Instar 4. 0.0178 ± 0.0061 (Y = 2.68 + 1.03x)

t test confirmed that difference between treatments of the
two instars was not significant (t = 1.83)

TABLE   24

Mortality of P.spumarius nymphs after spraying spittle masses with Sulphuric Acid solutions followed by ∖-BHC in n-decane emulsion.

(Conditions of test —: as Table 23 , except average time to spray acid solutions, 24 secs.)

No. nymphs	Normality of Acid Spray	% ∖-BHC in emulsion (mgs.)	Instar 3		Instar 4		Control mortality (Nos.)	
			No. dead	% mortality	No. dead	% mortality	Instar 3	Instar 4
25	0.1N	10	18	72	17	68	0	0
25		50	24	96	22	88	0	0
25		100	25	100	25	100	0	0
25	0.5N	10	17	68	17	68	1	0
25		50	23	92	20	80	0	0
25		100	25	100	25	100	0	0
25	1.0N	10	21	84	18	72	0	0
25		50	24	96	23	92	1	1
25		100	25	100	25	100	2	0

TABLE 25   Survival times of P.spumarius nymphs exposed
to saturated ϒ-BHC vapour.

(Conditions of test: Room temperature,18°C;
Relative humidity, 79 per cent).

25 nymphs of each instar exposed.

Nymphal instar	Average time to death in hours.
1	4.5 ± 0.5
2	6.0 ± 1.0
3	5.5 ± 0.5
4	9.0 ± 1.0
5	10.5 ± 1.5

Control mortality one   (instar 3)

TABLE 26   Survival times of P.spumarius nymphs subjected
to dehydration over Potassium hydroxide at
room temperature, (18°C).

Instar	Number of nymphs	Time to death (mins) after leaving spittle
1	25	110 ± 20
2	25	275 ± 35
3	25	210 ± 20
4	25	430 ± 30
5	25	490 ± 20

Control mortality nil.

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TABLE 26a.

Figure 18   Extraction of pigment from Scale of
S. hemisphaerica by water and Sodium
hydroxide.

Ilford Filter Number	Wavelength m μ.	Absorptiometer Reading	
		Water	Sodium hydroxide
600	410	.941	.670
601	430	.818	.535
602	470	.675	.381
603	490	.607	.298
604	520	.553	.244
605	550	.481	.184
606	575	.422	.143
607	600	.362	.107
608	680	.293	.076

TABLE 27.
  Water content of silk cocoons, pereent by weight,
  of P. interpunctella of different ages.

Pupal age (days)	original weight of 25 cocoons (mgs)	Weight after drying (mgs)	% water content
1	7.3	6.5	11.0
1	6.7	6.0	10.4
1	7.1	6.4	9.9
4	7.4	6.8	8.1
4	7.0	6.4	8.6
4	6.4	5.9	7.8
9	6.7	6.3	6.0
9	7.3	6.8	6.9
9	7.7	7.2	6.5

TABLE 228

Graph 10

Mortality of unprotected pupae of P. interpunc-
tella of different ages, after topical appli-
cation of a 5 per cent solution of γ-BHC in
acetone.

(Conditions of test: temperature, 25°C;
relative humidity in chambers, 70 per cent).

Dose applied	2		5		8		10	
Pupal age (days)	No. pupae	No. dead	No. pupae	No. dead	No. pupae	No. dead	No. pupae	No. dead
1	30	2	30	1	30	1		
2	30	3	30	3	30	3	30	4
3	30	4	30	6	30	5	30	5
4	30	6	28	7	30	7	30	7
5	30	6	30	8	30	9	30	9(1)
6	30	7	30	8	30	10	30	11
7	30	9	30	10	30	13	30	14(2)
8	29	14	30	13	30	18	30	15
9	30	17	30	21	30	21(1)	29	21(1)

Control mortalities in brackets.

See Table 30 for Probit analysis.

TABLE 29 Mortality of protected pupae of P.interpunctella
Graph   of different ages, after topical application of
            a 5 per cent solution of γ-BHC in acetone.
            (Conditions of test: as Table 28).

Dose applied	2		5		8		10	
Pupal age (days)	No. pupae	No. dead	No. pupae	No. dead	No. pupae	No. dead	No. pupae	No. dead
1	29	4	28	4				
2	28	6	30	8	30	12	28	14
3	30	8	30	9(1)	30	13	30	16
4	30	4(1)	30	5	29	19(1)	30	19
5	28	2	29	7	28	14	30	15
6	29	4	29	6	30	11	28	11
7	30	5	30	9	30	12	30	13(1)
8	30	4	30	8	30	14(2)	30	15
9	29	7	30	9	30	18	30	20

Control mortalities in brackets.

See Table 30 for Probit analysis.

TABLE 30

Probit analysis of results in Tables 28
and 29 . Mortality of protected and
unprotected pupae of P.interpunctella,
after topical application of BHC in
acetone, at 25°C.

Pupal age (days) unprotec-ted	χ^2	Regression equation Y =	LD.50 mls.5% γ-BHC in ace-tone	$\pm$ standard error	LD.50 mgs. γ-BHC	t dF=4 P=0.05
2	0.16	3.64 + 0.17x				0.285
3	0.36	3.89 + 0.19x				0.348
4	0.11	4.14 + 0.16x				0.327
5	0.03	4.01 + 0.48x	0.115	0.352	5.74	0.817
6	0.15	4.07 + 0.55x	0.049	0.093	2.45	0.538
7	0.31	4.19 + 0.66x	0.017	0.015	0.85	0.209
8	1.47	4.84 + 0.23x	0.005	0.006	0.24	0.561
9	0.17	5.00 + 0.61x	0.001	0.001	0.05	1.440

Protected

2	0.89	3.77 + 1.10x	0.013	0.006	0.65	
3	1.09	3.97 + 0.98x	0.011	0.005	0.56	
4	6.59	2.69 + 2.64x	0.007	0.001	0.37	
5	0.68	2.83 + 2.25x	0.009	0.002	0.47	
6	0.50	3.47 + 1.25x	0.017	0.008	0.85	
7	0.02	3.65 + 1.19x	0.014	0.006	0.69	
8	0.37	3.16 + 1.81x	0.010	0.002	0.51	
9	2.77	3.64 + 1.69x	0.007	0.001	0.33	

TABLE 31

The effect of γ-BHC vapour on unprotected pupae of
P. interpunctella exposed 8–14 hours after pupation,
at different temperatures.

Numbers of pupae killed.

Ten pupae used for each replicate at each exposure
period.

Temperature	Replicate	Exposure period (hours)						
		12	24	48	96	144	192	216
25°C Treatment and Post Treatment	1	0	1	2	1	3	3	4
	2	0	0	1	3	1	4	6
	3	0	1	1	2	3	3	5
	4	0	1	2	2	2	3	5
	5	0	0	2	2	4	4	4
	Totals	0	3	8	10(1)	13	17	24
15°C Treatment and Post Treatment	1	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10
	4	10	10	10	10	10	10	10
	5	10	10	10	10	10	10	10
	Totals	50	50	50	50(1)	50(1)	50(2)	50(3)
15°C Treatment 25°C Post Treatment	1	1	2	3	4	5	6	7
	2	0	3	2	5	4	7	8
	3	2	1	4	3	7	7	6
	4	1	2	3	4	6	5	7
	5	1	3	4	6	7	7	8
	Totals	5	11	16	22	29(1)	32	36(2)

Control mortalities in brackets

(Ten pupae used for each exposure period at the
different temperatures).

TABLE 32

The effect of γ-BHC vapour on protected pupae (P_1) (remaining in their cocoons after treatment) of $P.$ interpunctella, exposed 8–14 hours after pupation, at different temperatures.

Numbers of pupae killed.

Ten pupae used for each replicate at each exposure period

Temperature	Replicate	Exposure Period (hours)						
		12	24	48	96	144	192	216
25°C Treatment and Post Treatment	1	0	1	0	1	2	1	2
	2	0	1	1	.0	1	2	2
	3	0	0	1	2	1	2	2
	4	1	0	0	1	1	1	2
	5	0	1	1	1	1	2	2
	Totals	1	3	3	5	6	8	10
15°C Treatment and Post Treatment	1	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10
	3	9	10	10	10	10	10	10
	4	10	9	10	10	10	10	10
	5	10	10	10	10	10	10	10
	Totals	49	49	50	50(1)	50	50(1)	50(1)
15°C Treatment 25°C Post Treatment	1	0	1	2	2	3	4	4
	2	0	1	1	3	3	3	4
	3	0	1	2	3	4	4	5
	4	0	1	1	2	3	3	5
	5	0	0	1	2	3	4	4
	Totals	0	4	7	12	16	18(1)	22(1)

Control mortalities in brackets.

(Ten pupae used for each exposure period at the different temperatures).

TABLE 33

The effect of γ BHC vapour on protected pupae (P_2)
(pupae removed from cocoons after treatment) of
P. interpunctella, exposed 8—14 hours after pupation,
at different temperatures

Numbers of pupae killed.

Ten pupae used for each replicate at each exposure
period.

Temperature oC	Replicate	Exposure period (hours)						
		12	24	48	96	144	192	216
25°C Treatment and Post Treatment	1	0	0	0	1	1	2	2
	2	0	0	0	0	0	0	1
	3	0	0	0	0	1	1	2
	4	0	0	0	0	1	3	2
	5	0	0	0	0	0	0	2
	Totals	0	0	0	1	3	6	9
15°C Treatment and Post Treatment	1	9	10	10	10	10	10	10
	2	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10
	4	8	9	10	10	10	10	10
	5	8	9	10	10	10	10	10
	Totals	45	48	50	50(1)	50(1)	50	50(1)
15°C Treatment 25°C Post Treatment	1	0	1	2	3	4	5	5
	2	0	2	2	3	3	4	5
	3	0	1	3	4	5	5	5
	4	0	1	2	3	5	5	6
	5	0	2	2	4	3	4	4
	Totals	0	7	11	17	20	23(1)	25(1)

Control mortalities in brackets

(Ten pupae used for each exposure period at the
different temperatures)

TABLE 34

The effect of γ-BHC Vapour on unprotected pupae of P interpunctella exposed 90—96 hours after pupation, at different temperatures.

Numbers of pupae killed.

Ten pupae used for each replicate at each exposure period

Temp- erature °C	Replicate	Exposure period (hours)					
		12	24	48	72	96	120
.25°C Treatment and Post treatment	1	0	0	1	1	0	1
	2	0	0	0	1	1	2
	3	0	0	1	0	2	1
	4	1	1	1	2	1	2
	5	0	1	0	1	1	2
	Totals	1	2	3	5	5	8
15°C Treatment and Post treatment	1	4	4	8	8	10	10
	2	3	4	6	9	10	10
	3	3	5	8	9	10	10
	4	4	6	7	8	10	10
	5	3	5	8	9	10	10
	Totals	17	24	37	43	50(1)	50(2)
15°C Treatment 25°C Post Treatment	1	0	1	3	5	5	4
	2	1	3	2	4	6	6
	3	1	1	3	3	5	5
	4	0	2	2	4	5	6
	5	1	2	3	5	4	7
	Totals	3	9	13(1)	21	25	28(1)

Control mortalities in brackets.

(Ten pupae used for each exposure period at the different temperatures).

TABLE 35

The effect of BHC vapour on protected pupae(P_1)
(remaining in their cocoons after treatment)
of P. interpunctella exposed 90—96 hours after
pupation, at different temperatures.

Numbers of pupae killed

Ten pupae used for each replicate at each
exposure period.

Temp- erature ^{o}C	Replicate	Exposure period (hours)					
		12	24	48	72	96	120
25°C Treatment and Post Treatment	1	0	0	0	0	0	0
	2	0	0	0	0	1	0
	3	0	0	0	0	0	1
	4	0	0	0	0	0	0
	5	0	0	0	0	0	2
	Totals	0	0	0	0(1)	1	3
15°C Treatment and Post treatment	1	2	5	6	5	6	9
	2	2	3	4	5	8	9
	3	2	4	6	6	7	8
	4	3	3	6	7	7	9
	5	4	4	5	8	9	10
	Totals	13	19	27(1)	31	37	45(1)
15°C Treatment 25°C Post Treatment	1	0	2	1	3	4	4
	2	1	2	2	3	5	5
	3	1	1	3	4	3	5
	4	1	1	2	3	3	4
	5	0	2	4	2	4	5
	Totals	3	8	12	15	19	23

Control mortalies in brackets

(Ten pupae used for each exposure period at the different
temperatures)

TABLE 36

The effect of γ —BHC vapour on protected
pupae (P_2) (pupae removed from cocoons after
treatment) of <u>P. interpunctella</u>, exposed
90—96 hours after pupation, at different
temperatures.
Numbers of pupae killed
Ten pupae used for each replicate at
each exposure period.

Temperature °C	Replicate	Exposure period (hours)					
		12	24	48	72	96	120
25°C Treatment and Post treatment	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	1
	5	0	0	0	0	0	0
	Totals	0	0	0	0	0	1
15°C Treatment and Post treatment	1	3	4	5	7	6	8
	2	2	3	6	6	6	8
	3	1	3	4	4	7	8
	4	3	5	3	7	8	7
	5	2	3	7	6	6	8
	Totals	11	18	25	30(1)	33	39 (2)
15°C Treatment 25°C Post treatment	1	0	0	3	5	5	7
	2	0	1	2	3	4	6
	3	0	0	3	2	3	5
	4	0	1	3	3	4	5
	5	0	0	2	3	4	4
	Totals	0	2	13	16	20	27 (1)

Control mortalities in brackets

(Ten pupae used for each exposure period at the
different temperatures)

TABLE 37

The effect of γ-BHC vapour on pupae of
P. interpunctella of all types, exposed
190-196 hours after pupation, at different
temperatures.

Numbers of pupae killed.

Ten pupae used for each replicate at each
exposure period.

Temperature °C	Replicates	Exposure periods (hours)					
		Unprotected pupae		Protected pupae (P$_1$) remaining in cocoon after treatment		Protected pupae (P$_2$) removed from cocoon after treatment	
		12	24	12	24	12	24
25°C Treatment and Post treatment	1	3	3	2	4	1	2
	2	2	5	3	3	3	4
	3	4	6	2	2	3	2
	4	2	3	3	5	0	1
	5	2	4	4	2	2	4
Totals		13	21	12	16	9	13
15°C Treatment and Post treatment	1	6	7	4	7	5	4
	2	8	9	7	5	3	7
	3	4	8	4	6	3	5
	4	7	10	5	9	6	8
	5	5	8	6	8	7	6
Totals		30	42(2)	26	35(1)	24	30(1)
15°C Treatment 25°C Post treatment	1	3	6	2	4	3	2
	2	5	6	3	4	3	4
	3	4	8	4	6	4	6
	4	4	6	5	5	3	4
	5	7	8	3	4	2	5
Totals		23	34	17	23	15	21

Control mortalities in brackets

(Ten pupae used for each exposure period at the
different temperatures).

TABLE 38

Probit analysis of results in Tables 31 to 36
Mortality of pupae protected throughout (P_1),
pupae protected during exposure only (P_2) and
unprotected pupae (U), exposed to saturated
vapour of BHC at different ages and at different
temperatures.

Coding of pupae	χ^2	Regression equation $Y =$	LT.50 (hours)	$\pm$ standard error	Probability level P
Unprotected pupae					
U					
A_1. 25	4.36	$1.25 + 1.51x$	302.0	62.51	$0.7 > P > 0.5$
A_1. 15 25	1.09	$1.89 + 1.52x$	112.2	13.42	$0.9 > P$
A_2. 25	0.42	$1.82 + 1.01x$	1380.0	1615.57	$0.9 > P$
A_2. 15	9.03	$1.39 + 2.64x$	23.4	2.32	$0.1 > P > 0.05$
A_2. 15–25	0.80	$1.27 + 1.85x$	102.3	13.88	$0.9 > P$
Protected pupae P_1					
A_1. 25	0.96	$2.12 + 0.83x$	2951.0	3142.52	$0.9 > P$
A_1. 15–25	2.72	$0.93 + 1.64x$	302.0	85.29	$0.9 > P > 0.7$
A_2. 15	4.91	$2.38 + 1.67x$	37.2	4.27	$0.3 > P > 0.1$
A_2. 15–25	0.52	$2.04 + 1.35x$	154.9	36.70	$0.9 > P$
Protected pupae P_2					
A_1. 25	0.18	$-3.89 + 3.41x$	407.4	118.06	$0.9 > P$
A_1. 15–25	4.39	$1.54 + 1.48x$	218.8	37.74	$0.7 > P > 0.5$
A_2. 15	0.85	$2.48 + 1.50x$	47.9	5.83	$0.9 > P$
A_2. 15–25	3.60	$-0.24 + 2.56x$	112.2	12.13	$0.5 > P > 0.3$

TABLE 39. Permeability of the pupal cocoon of
P.interpunctella to aqueous and oil
sprays containg dyes.

(Conditions of test — temperature, $22^{\circ}C$; spray
solutions, 1 per cent methylene blue in water,
1 per cent Oil Red O in hexane; spray pressure,
76 cm.Hg; time to spray, water 22 secs., hexane
18 secs.; volume sprayed, 10 ml.; spray depos-
ition, 3 mins..)

5 replicates of 25 protected and unprotected pupae
sprayed with each solution.

Replicate	Weight of Dye recovered (mg. x 10^{-2})			
	Water spray		Hexane spray	
	U	P	U	P
1.	5.8	0.9	5.2	2.0
2.	6.1	1.2	5.0	1.7
3.	5.5	1.0	5.6	2.1
4.	5.6	0.7	5.5	2.3
5.	5.9	0.9	5.1	1.9
Means	5.78	0.94	5.28	2.0