THE REACTIONS OF CERTAIN INSECT SPECIES TO TREATMENT WITH TOXIC SUBSTANCES DISPERSED IN MOVING AIR

A Thesis submitted by

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ABSTRACT

Fumigation apparatus capable of passing fumigant/air mixtures at controlled velocities over test insects is described. The apparatus was employed to test the effects of wind speed on the toxicities of three fumigants - hydrogen cyanide, nicotine and DDVP to three insect species.

The toxicities of nicotine and DDVP were found to be enhanced as the gas velocity was increased, while the toxicity of hydrogen cyanide was barely affected. The enhanced toxic effect was expressed as a reduction in the exposure time required for the insects to accumulate a median lethal dose. The continued enhancement of the toxic effect with increasing velocity declined slowly until a steady state was reached, at a velocity characteristic for each insect species. The toxicity of nicotine was the most affected by gas velocity and this fumigant was used to investigate the factors affecting its absorption at various wind speeds. Insects assayed for nicotine after fumigation showed fumigant residues which followed a similar pattern, with respect to wind speed, as did the toxicity figures.

Nicotine was found to accumulate in the epicuticular waxes which act as a fumigant reservoir; the residues in the waxes varied with velocity, whereas residues within the insect

remained unaffected. Absorption was shown to increase with velocity up to a limit when the fumigant available at the surface in the gas phase becomes equal to, or greater than, that lost from the surface by diffusion into the substrate. This situation was also found to occur with beeswax and paraffin wax.

The solubilities of nicotine and DDVP in various epicuticular waxes were measured and an attempt made to relate the solubility figures to some characteristics of the waxes.

C O N T E N T S

I N T R O D U C T I O N

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INTRODUCTION

1. GENERAL

The use of toxic compounds in the vapour phase for the control of insect pests is well established. Hydrogen cyanide was used to control citrus scale in 1886 and since this time many compounds have been, and continue to be, screened with a view to their use as fumigants.

Fumigants operate in the gaseous phase and it follows that their use, though flexible, is confined to enclosed spaces such as buildings, aeroplanes, specially constructed chambers, or temporarily erected tents of impermeable fabric. Similarly, a fumigant is able to penetrate packed commodies, a property denied to other insecticides. Soil too may be fumigated, a fabric covering being advisable to retain fumigants with high vapour pressures.

The toxic effect is due to a proportion of the total fumigant accumulated by an insect during fumigation. This quantity increases with both time of exposure and fumigant concentration, and it has been found by experiment that the toxic dose is proportional to the product of these parameters. The effectiveness of a fumigant is then expressed in terms of a concentration time product or CT_p. It follows that low concentrations involve impractically long exposure times with the added possibility that a number of the more inaccessible insects may take up insufficient poison, or they may also be able to eliminate the gas at a greater rate than that at which it is taken up. High con-

centrations often manifest undesirable side effects and may be unnecessary, and, therefore, uneconomic. Tainting and unacceptable residues in food stuffs become evident and the viability of plant material is reduced. In general, the biological effect caused by any one CT product for a fumigant is similar over a wide range of concentrations and times. Discrepancies occur, however, where extremely long times or high concentrations are involved.

There is frequently considerable latitude in the practice of fumigation, between the CT product necessary to ensure control of the pest, and that at which objectionable side effects occur. However, occasion does arise when these two critical figures approximate; at such times even distribution of the fumigant is essential in order that deep seated infestations may be adequately dosed while the upper CT_p limit is not exceeded in the more accessible regions. For this reason and because considerable layering of the fumigant is experienced when large spaces are fumigated, some mechanical means of stirring the air fumigant mixture by means of hand-operated punkahs (Page and Lubatti, 1933) or by motor driven fans (Monro, Buckland and King, 1955) is necessary. Both methods have been assessed by Call (1952). A number of other methods for achieving uniform distribution of fumigant have also been tested (see Page and Lubatti, 1963, for review).

While stirring is normally to be advocated whenever practicable, it is inadvisable for leaky or porous structures

(Page and Lubatti, 1949; Blackith, 1953) or for fumigations under plastic sheeting, unless rigorous attention is paid to the prevention of leaks. Stirring introduces small pressure differences and where any porosity exists in the enclosing medium there will be a loss of fumigant and air at high pressure points and dilution with incoming air in the low pressure regions.

Attendant to the even distribution in the free space brought about by stirring, is the enhanced penetration of packed commodities which occurs (Page and Lubatti, 1940; Turtle, 1941; Page and Lubatti and Russel, 1949; Lubatti and Bunday, 1958). This 'turtle' effect as it is known is not always present with slow or moderate stirring (Page and Lubatti, 1940), and, as even rigorous stirring is insufficient to produce the pressure differences necessary for bulk transference through the treated commodity, these authors attribute the effect to some unspecified surface phenomenon.

Physically, sorption and desorption (discussed later, Section 4) are similar, one being the converse of the other. Desorption or, more commonly, evaporation of water from a cylinder, wet at the bottom and open at the top, has been shown on theoretical grounds to be proportional to the square root of the velocity across the top of the tube (Jeffreys, 1918). This has been substantiated experimentally by Ramsay, (1935a). If, however, the approaching air was moist and a drying agent is substituted for the wet surface within the cylinder, the

resultant water movement will be reversed, and a situation is arrived at analogous to the sorption of fumigant on the drying agent and the sorption of fumigant in the interstices of packed commodities.

The main barrier to the penetration of boxed dried fruits lies in the wrapping and the box itself (Page and Lubatti, 1937). It would seem reasonable to suppose that if this first stage in the penetration could be accelerated then the overall penetration will be much enhanced. Aerodynamic considerations, such as that proposed by Jeffreys (1918) suggests that this might well occur at small interleaved gaps in the wrapping. This effect would also continue to be apparent even when the scale of the system is reduced considerably beyond that of an insect which presents a similar obstacle to fumigant entry in that it is surrounded by a hard relatively impermeable 'box' penetrated infrequently by the pores of the insects' respiratory system.

The biological effects of bulk movement of fumigant air mixtures past insects have escaped attention, and since preliminary studies showed that enhanced mortalities were obtained when nicotine as a vapour is blown over test insects, it is with an investigation of this and associated phenomena that this thesis is primarily concerned.

2. THE UPTAKE OF FUMIGANTS BY INSECTS

It is generally held that gaseous insecticides take effect from entry through the respiratory system, and only when in liquid or solid form act as contact poisons entering the insect through the cuticle. There is, however, considerable evidence to indicate that cuticular uptake and penetration are important contributory factors in the entry of both high and low vapour pressure fumigants. The term 'fumigant' is used here in the widest sense, embracing any toxic chemical dispersed as vapour in the gas phase.

Entry by way of the respiratory system is known to occur. Hazelhoff (1928) notes that in atmospheres containing excess carbon dioxide, the spiracles remain open and that this aids the penetration of toxic gasses. Similarly, the presence of carbon dioxide during fumigation with chloropicrin and ethylene oxide increased the efficacy of the treatment (Cotton, 1930, 1932). The toxicity of methyl bromide has also been shown to be directly related with the rate of respiration (Bond, 1956).

The spiracles when open aid fumigant transfer. The rate of diffusion through the small pores is more in proportion with their perimeters than with their areas (Brown and Escombe, 1900). The tracheae penetrate throughout the insect and diffusion in the gas phase occurs some 10^6 times as fast as in the most permeable of tissues (Krogh, 1919, 1941). Thus, if the distance a toxic molecule must travel in the tissue is a modest ten-thousandth of the gaseous pathway from spiracle to tracheole,

the time taken in the tissues is at a minimum one hundred times as long as that spent in the tracheae (Buck, 1962). The above consideration concerns diffusion of oxygen and carbon dioxide but is also applicable to fumigant molecules. Fumigant diffusion is more likely to be modified by sorption on the tracheal wall with the probable reduction in the rate of transfer in the gas phase. Rates of uptake and distribution by the haemolymph are not likely to exceed gaseous diffusion which occurs more readily for small molecules, whereas permeation velocities bear no simple relationship to molecular size or mass; a more important factor is that of the solubility of the diffusing substance in the medium (Barrer, 1951). Another is the extent to which the structure swells - the more open the structure becomes, the less would be the expected resistance to permeability. A third factor of probable importance in biological systems, where the phases are not homogeneous, is one of surface diffusion, and this may conceivably proceed at a rate closer to that of diffusion through a gas than through liquid or solid media.

It would seem on balance, therefore, that entry by way of the respiratory system is the more likely. However, penetration of the cuticle has been shown to occur to an extent which varies with fumigant. The uptake of hydrogen cyanide is reduced sixty-six per cent. by sealing the spiracles (Bond, 1961 - b); a considerable proportion is left which must enter by another route, and by way of the cuticle would

seem the only other plausible pathway. This fumigant causes rapid paralysis but continues to be absorbed linearly from a constant concentration for at least ninety-six hours, when eight times the lethal dose has accumulated (Bond, 1961,a). Entry is thought to be mainly through the spiracles, as in the paralysed condition the spiracle lips are held apart.

The larval cadelle will slowly accumulate a toxic quantity of methyl bromide even when access to the tracheae is denied (Monro, 1965).

The toxicity of DDVP vapour to flying locusts is found to be greater by a factor of ten than the maximum expected value derived from a knowledge of the tracheal ventilation rate and the median lethal dose by injection. It is concluded that most of the DDVP must be picked up by absorption (MacCuaig and Watts, 1963).

The tracheal ventilation rate of the confused flour beetle does not materially affect uptake of aldrin vapour. Total uptake and the fluxes of aldrin across the cuticle being similar for both dead and live insects (Lewis, 1965).

It would appear from these reports that the cuticle plays an important role, where cuticle penetration occurs, in supplementing uptake by the tracheal system, and may well be the dominant factor concerned in the entry of others, to which the cuticle offers small resistance. The cuticle and its structure must now be assessed as an aid or barrier to the transfer of molecules into the insect system.

3. PERMEABILITY OF INSECT CUTICLE

The insect cuticle is fundamental to an insect's existence; it acts both as skeleton and protective covering, preventing the insect from physical and chemical injury and from excessive water loss.

Early studies on the modes of entry of insect poisons concerned those insecticides in common use at the time and were thought to be respiratory, such as nicotine (McIndoo, 1916), or stomach poisons, such as arsenic.

Nicotine was later shown to be able to enter through the integument (Richardson, et al, 1934; Glover and Richardson, 1936), and that the nicotine molecule is taken up more readily from solution than is the lipophobe nicotine ion (Richardson, 1945).

The cement and wax layers which restrict the egress of water also oppose the entry of insecticides (Wigglesworth, 1945) and some nonelectrolytes (Treherne, 1957). Injury or abrasion to the wax layer accelerates the onset of toxic symptoms.

The structure of the cuticle in relation to its permeability to water and insecticides has been reviewed (Wigglesworth, 1948, 1957) where a schematic conception of the cuticle is presented and has been reviewed more recently by Ebeling (1964) and Locke (1964).

The cuticle is part lipoid, part aqueous. Beneath the peripheral lipid layer, the cuticle is mainly aqueous, con-

sisting of a fibrous matrix of long chain molecules of polyacetylglucosamine, arranged in a series of lamellae. Traversing the cuticle from the lipid layer to the cellular epidermis are very many pore canals whose function it is, together with the less numerous ducts from the dermal glands, to transport the wax to the surface after ecdysis and injury (Dennel, 1958). The diameter of the pore canals varies between species but is of the order 0.1 - 1 μ. In some species the canals remain open, that is their contents remain fluid, long after moulting (Locke, 1961) and in others the contents solidify (Wigglesworth, 1948). In others, some of the contents solidify and may become sclerotized, but an annular space remains around the solidified filament (Way, 1948).

The most likely path taken by substances, especially lipophilic ones entering the insect through the cuticle is by way of the pore canals (Wigglesworth, 1942) and support for this hypothesis is found in the relative rates of penetration of DDT through the compound eye of Protophormia where no pore canals are to be found, and through other sites on the insect where pore canals are numerous (Lewis, 1954). This view appears to be generally accepted (Ebling, 1964) though other routes must exist where pore canals are absent (Brown, 1951).

There is much evidence which suggests, however, that the epicuticular waxes, though forming a very thin layer over the cuticle, present an obstacle for substances entering the

insect. This layer in itself may be separated into three distinct regions (Beament, 1945). The tightly packed monolayer at the interface with the polyphenol layer is known to be the main barrier to water with the outer cement layer also taking part (Beament, 1961).

Disruption of the waxes by solvents allow for their more rapid uptake (Lennox, 1940; O'Kane, et al, 1940; Morozov, 1935; Umbach, 1934). Entry through the larval cuticle of Calliphora larvae is greatly accelerated in the presence of kerosene (Hurst, 1940; 1943). The affect being attributed to the increased permeability of the outer liped layer in the presence of apolar substances. Insects whose epicuticles stain most readily with Sudan III are more susceptible to Pyrethrum (Klinger, 1936). A similar trend has been found for the larval instars of the sugarbeet web worm where a progressive diminution of the fat content of the cuticle is associated with a decrease in their susceptibility to Pyrethrum sprays (Pepper and Hastings, 1943).

The isomers of benzene hexachloride have been shown to penetrate grain weevils in proportion to their solubilities in hydrocarbon solvents, (Armstrong, et al, 1951). Pyrethrum enters the cuticle of Rhodnius progressively more quickly when dissolved in the more volatile paraffins; here the thickness of the endocuticle is important in slowing down the rate of entry (Wigglesworth, 1942). This has been held to suggest that the smaller the penetrating molecule, the more rapidly

it will diffuse through the cuticle, a hypothesis supported by Hurst (1943) who finds that the lower members of a homologous series are more effective in entry than the heavier members. The greater assistance in penetration afforded by lighter solvents has also been demonstrated (Webb and Green, 1946) and more recently (Hadaway and Barlow, 1958). Closer scrutiny by Lewis (1963) discloses that while absorption of dieldrin increases with decrease in carrier viscosity, the absorption of the carriers themselves is independent of viscosity. It is suggested that one likely solvent effect is on the organization of the epicuticular wax.

Some oils, especially in the presence of oleic acid, will pass right through the cuticle and appear in the cells of the epidermis and in the dermal glands. It is not possible to see them in the cuticle in transit so they must pass through in droplets or particles beyond the resolving power of the microscope (Wigglesworth, 1942). It would seem most reasonable to assume that substances passing through the cuticle do so by diffusion and are dispersed as individual molecules through the system. A correlation has been suggested between the hardness and blackness of insect cuticle and its permeability (see Wigglesworth, 1948).

Both mealworms and cockroaches develop toxic symptoms more rapidly when minute droplets of pure nicotine are applied to unsclerotized areas of cuticle (O'Kane, et al, 1933), and oil has been observed to penetrate more rapidly through the thin

articulating membranes of bristles than the normal cuticle (Wilcoxon and Hartzell, 1933; Klinger, 1936; Wigglesworth, 1942). Most of the conclusions are drawn from observations of toxicity which are not to be relied upon for comparisons between species unless another means of comparison of tolerance is available - by injection or ingestion for example. Insects may also react differently when similar but separated areas of cuticle are treated; for example, the toxic symptoms arising from DDT poisoning vary with the amount of nervous tissue beneath the sites of application (Lewis, 1954).

It is evident that many compounds, some of them toxic to insects, are able to penetrate the cuticle, and once in the epidermis diffuse into the haemolymph to be distributed to all parts of the insect. To enter most easily, a substance must be, to some extent, both wax and water soluble in order to permeate epicuticular waxes and then be taken up by the endocuticle, and finally the haemolymph (Webb and Green, 1946). Since lipid material extends through the cuticle to the epidermis in the pore canals, the affinity for water may be less important than that for fats. The epidermis is cellular and the lipoproteins of the cell membranes are possible routes for hydrophobe substances to the basement membranes. Internally, the basement membrane is irrigated by the haemolymph, an efficient transport system even for hydrophobes.

4. SORPTION

From the preceding sections it has been seen that some penetration of the cuticle by fumigants has been established and that many solid insecticides, once in contact with the outer surface, will penetrate the cuticle unaided. The initation of penetration of a fumigant will depend on intimate contact between the fumigant molecules and the insect cuticle.

Solids are composed of particles which exert forces of mutual attraction on one another and which, at the surface of the solid, extend beyond the boundary. Neighbouring molecules of the adjacent phase are attracted by interacting fields of force, causing them to be held in numbers sufficient to satisfy the surface forces available. In this type of reaction, the substrate (here the solid) is termed the sorbent and the attracted phase the sorbate. Where the attraction is purely physical it is termed adsorption and when this is followed by a transfer of electrons amounting to a chemical reaction, the whole process is known as chemisorption. It is these cohesive forces which cause the familar phenomena of adhesion between solids and the wetting of solid surfaces by liquids.

The forces involved in adsorption are of many types; description of these may be found in the literature on surface chemistry - see, for example, Gregg (1961) or the comprehensive review by Honig (1954).

The total attractiveness of two molecular species is found by summation of the attractive forces. At equilibrium the

attractive forces are balanced by a repulsive force arising from the interpenetration of the two electron clouds.

The magnitude of the fields of force surrounding a molecule has been estimated for a number of simple examples (Honig, 1954). Reference to Fig. 1 reproduced from Gregg (1961) shows that the potential energy of an isolated atom increases once it has approached within about 7Å of the surface of a co-valent crystal.

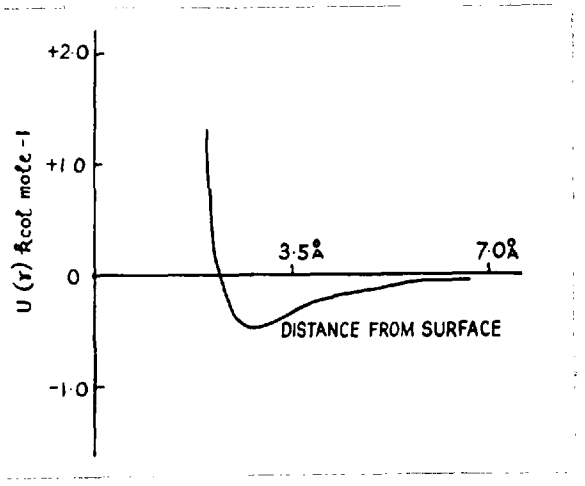

Fig. 1 The potential energy between an isolated atom and an ideal covalent crystal as a function of the distance of the atom from the surface (Gregg, 1961).

The additional attractive force experienced by highly polar molecules has been shown to be only slightly greater than the dispersive forces even when these are at their maximum (de Boer, 1956). It may be assumed, therefore, that the total

forces which exist, even in extreme instances, are not effective at distances much greater than 20Å above a solid continuum and while surface irregularities may modify the two main types of attractive force, the effect is only small.

The distance over which mutual attraction becomes operative does not necessarily increase in proportion with the size of molecular species; even if this were so, the field of attraction cannot be expected to extend beyond the order of 100Å.

When a fumigant is stirred, the distances over which fumigant molecules must travel by diffusion alone are considerably reduced. This will be discussed in a later section (6). However, these distances which are of millimeter order are vastly in excess of the maximum possible distance of attraction of a sorbent for a sorbate, and it may be concluded that even rapid stirring will have little effect on the phenomena of sorption, though as will be discussed later (section 6) the process as a whole may be enhanced where other factors determine the rate at which absorption proceeds.

Currently accepted theories of gas adsorption visualize the adsorbed layer as being monomolecular, or one molecule thick at low pressures which become multimolecular as the saturated vapour pressure of the sorbate is approached. Much will depend on the nature of the sorbent surface and the molecular species involved. In general, at relative pressures below 0.25, where a pressure of 1 represents saturation for any

particular gas, the adsorbed molecules form a monolayer (Gregg, 1961).

This is the condition found when there is only one molecular species in the gas phase and the monolayer consists solely of these molecules. When, however, more than one vapour is present, the monolayer will consist of a mixture of molecules. The relative numbers of each per unit of surface depends on the relative concentrations of the component vapours, their affinities for the sorbent, respective vapour pressures, and the overall temperature and pressure. Thus, when a fumigant is introduced into an air surface system, fumigant molecules will gradually displace some of the various component molecules of air, oxygen, nitrogen, carbon dioxide, water, etc. previously present on the surface. Equilibration in so complex a system occurs slowly, in contrast with the process in the simple case of a uniform plane surface exposed to a single gaseous molecular species. Now if the sorbent is at all porous or if the sorbate (in this instance a fumigant) is at all soluble in the sorbent, there will be a drift by diffusion into the substrate. This movement produces a deficiency of fumigant in the monolayer which is made up from the gas phase by adsorption of free molecules which come within range of the now available attractive forces. This monolayer must not be pictured as a more or less static layer; molecules are continually moving between the sorbed and gaseous state interchanging with other molecules. A dynamic equilibrium is

attained which, if disturbed by solution, is restored by further sorption. The converse may also occur when equilibrium is upset by a preponderance of fumigant in the sorbent.

One further point which must be borne in mind when considering the epicuticle and adsorption is that the true surface area of an insect is much greater than the apparent projected area by a factor which may be as large as ten and which depends on the insect species concerned (Lockey, 1960).

5. THE EFFECTS OF AIR MOVEMENT ON THE UPTAKE OF AEROSOLS AND FUMIGANTS.

Investigation of air movement in relation to the uptake of toxic chemicals by insects has been confined mainly to studies of spray droplets and flying insects. The factors affecting the uptake of particulate dispersions in moving air are discussed by Townsend (1948); Gregory (1951); Ranz and Wong (1952); Jarman (1959,a,b) and Hadaway and Barlow (1965). The most important are the particle's weight and size, air speed and the size and shape of the object on which the particles impact.

For resting adult mosquitoes, David and Bracey (1946) find a linear relationship between mortality and the speed with which an insecticidal mist is passed over them. Pickup from mists with particle diameters less than 10 μ has also been shown to be largely due to the flight movements themselves The vast majority of these particles are accumulated by the

wings when the insects are flying, but the distribution is at random over the whole insect when impaction is due to droplet movement alone.

Flying locusts, in particular, have been shown to pick up considerable quantities of spray on their wings, and this quantity may make an important contribution to the fatal dose (MacCuaig, 1962,b).

La Mer and Hockberg (1949) sprayed mosquito adults in a wind tunnel and found the median lethal dose to increase rapidly with decreasing drop size. A parallel increase in the toxicity of an insecticidal spray to locusts has been found (Wootten and Sawyer, 1954). Kennedy and others (1948) have also shown smaller drops to be more effective than large ones, while Lycopodium spores (32 μ diameter) are deposited on cylinders in greater numbers with increasing velocity (Gregory, 1951).

Maximum uptake by locusts from a spray has been shown to occur with particles of 60 μ (MacCuaig, 1962,a), a figure close to that of 50 μ predicted on theoretical grounds (Townsend, 1948).

The evidence for the effects of air movement on sorption and desorption is conflicting in that some systems are affected while others are not.

The sorption of hydrogen cyanide by wheat is unaffected by the rate of flow (Lubatti, 1944) nor is flow important in the sorption of chloropicrin by wheat (Northover, 1965).

Contrary to this is the accumulation of inorganic bromide by wheat during fumigation with methyl bromide. Higher levels of residual bromine are to be found after fumigation with continuous stirring than in a still atmosphere (Heuser, 1959).

Ramsay (1935,a,b,) has shown that evaporation from a wetted surface increases with the square root of the velocity of approaching air and that loss of water from the tracheal system of the cockroach is considerably affected by wind velocity when the spiracles are open, particularly when the animal is positioned transversely to the air stream.

Allamand and Burrage (1928) find that rate of aeration does not affect the loss of carbon tetrachloride from charcoal in terms of the quantity removed per litre; in terms of time, however, this does represent an increase in the rate of loss. In general, gas absorption in packed absorption towers varies as the 0.8 power of the gas velocity (Sherwood, 1937).

These few examples indicate that in some instances air movement is important in governing the rate of absorption or the reverse process desorption. The dynamic aspects of these processes will now be discussed.

6. FLUID DYNAMIC THEORY AS IT AFFECTS FUMIGANT UPTAKE

(a) SORPTION

Consider an infinite fumigation system at rest with the concentration of fumigant P and in this, an absorbent at whose surface the concentration is P'. As absorption occurs, adsorption sites will become vacant at the surface, the balance

being restored by sorption of air fumigant from the gas phase, which in turn lowers the concentration P' above the surface. Diffusion will now occur through the gas phase in proportion to the concentration gradient P-P'/d, where d is the distance between the points where the concentrations are P and P' respectively. As absorption continues, d becomes very large with a corresponding decrease in the rate of diffusion, and where this is the governing factor, it follows that the rate of absorption will be reduced similarly. The effect of air movement over the sorbent is to limit d.

Above a plane surface parallel to a steady air flow, there exists a velocity gradient which extends from the surface where the air velocity is zero to a certain distance above the surface where the velocity is that of the approaching air. This boundary or viscous layer is caused by the viscosity of the air. The molecules close to the surface are held back and these in turn affect the molecules a little further away. The thickness of the viscous layer is zero at the leading edge of the surface and increases asymptotically with distance from the leading edge in the direction of the stream. The thickness d of the boundary layer at a distance x from the leading edge is given by:

$$\frac{d}{x} = \frac{4.64}{2\sqrt{ux/v}},$$

where u is the free stream velocity and v the kinematic viscosity of air at the temperature of the system (For the derivation of this equation, which is due to von Karman (1921) see, for example, Eckert and

Drake (1959) or Schlichting (1955).

As the velocity of the air stream is increased, a state is reached where the increasing shear stresses in the air cause turbulence to set in. Boundary layer thicknesses under these conditions differ only slightly from those in laminar flow. However, convection in the turbulent zone will be supplementary to diffusion and becomes a more important factor in the transfer of a fumigant from the air mass to the sorbent. Even in turbulent boundary layers a laminar sublayer exists at the surface. The thicknesses of these boundary layers d and d' respectively are related by expressions of the same form as that given above. They are for the turbulent boundary layer:

$$\frac{d}{x} = \frac{0.376}{\sqrt[5]{ux/v}},$$

and for the laminar sublayer

$$\frac{d'}{d} = \frac{194}{0.\sqrt[7]{ux/v}},$$

(Eckert and Drake, 1959).

There is a point in a fully established boundary layer in laminar flow where it is possible for an increase in the fumigant/air velocity to have little effect in enhancing absorption. In laminar flow no convection occurs and at a certain distance from the leading edge where the boundary layer is increasing in thickness slowly with distance downstream, a steady state will be attained when the fumigant lost to the surface from the boundary layer is replaced by diffusion

through the free stream. An increase in the gas velocity will reduce the boundary thickness and close to the leading edge enhanced absorption is likely to be found. Further downstream, however, diffusion will remain the rate determining process. It is extremely unlikely that such conditions will be experienced by most biological material. This is especially true of an insect where the surfaces are short and not truly planar. This will lead to some degree of mixing in the free stream and turbulence in the boundary layer, except perhaps for very low gas velocities.

It may be seen from the equations given above that the thicknesses of the boundary layers remain in inverse proportion to a power of the free stream velocity, thus as the velocity u increases, the boundary thickness d decreases. It is also evident that when this thickness is considered with respect to the rate of diffusion for unit area, given by $P-P'/d$, an increase in u causes an increase in $P-P'/d$. Thus, for a freely sorbing substrate, an increase in sorption is to be expected with increased air speed. Both heat and mass transfer are facilitated more in turbulent than in laminar flow. In turbulent flow the outer edge of the boundary layer is maintained at the concentration in the bulk gas by convection, whereas under conditions of laminar flow, the slower diffusion process is more important, except perhaps in the early stages during development of the boundary layer. For practical engineering purposes the solution of mass transfer

problems in turbulent flow differs little from laminar flow; the latter, however, are simpler to calculate and serve as good models for study of the basic phenomena (Erkert and Drake, 1959).

6. (b) POSSIBLE EFFECTS ON DIFFUSION INTO THE TRACHEAL SYSTEM.

Reference has been made above (2) to the pore diffusion effect where small pores in a surface are found to pass gasses and vapours by diffusion in greater quantity than would be expected from consideration of their areas, and the co-efficient of diffusion of the respective gas. A spiracle level with a plane surface will be presented with similar concentrations as the neighbouring regions of epicuticular wax; where the rate of absorption by the wax is greater than the rate of diffusion to the surface, fumigant can only pass into the spiracle at a rate of the same order as that for an equivalent area of wax. But when the rate of arrival at the surface may exceed that at which it is taken up by the epicuticular waxes, a spiracle will be able to pass fumigant at an increased rate by a pore diffusion effect. This effect is unlikely to be so marked as that found in an isolated pore open at both sides, since spiracles commonly occur in cuticular depressions and the space behind is encroached upon by the tracheal walls. Mechanical ventilation of the trachae will, to a certain extent, aid transfer through the spiracles. Many insect species ventilate the respiratory system by rhythmic contractions of the

abdomen, and many, when in flight, are ventilated unidirectionally by co-ordinated movement of the spiracular valves (Buck, 1962; Miller, 1964).

Ramsey (1935,b) has also shown a considerable increase in water loss from cockroaches placed across an air stream compared with those orientated axially. Similarly, this orientation would increase fumigant uptake. Once fumigant molecules have entered the tracheal system, there is little to prevent their removal from the gas phase. The surface area presented by the tracheae is considerable and the cuticle, where it exists, is extremely thin (Locke, 1957; 1964). Another factor, probably of only slight importance, is that respiration rates are known to increase with the onset of poisoning (Keister and Buck, 1964).

An enhanced effect may also occur similar to that propounded by Jeffreys (1918) in which it is deduced that the rate of water loss from a cylinder, wet at the bottom and open at the top, is inversely proportional to the square root of the velocity of air passing across the open top. In this deduction no account is taken of possible turbulent effects within the cylinder, but as turbulence would not be expected in insect tracheae, except perhaps in close proximity to the opening, a similar relationship may govern fumigant transfer here.

To summarize, it is evident that fumigant transfer through the spiracles occurs and is likely to be enhanced by

air movement over the insect, though the quantities involved remain unpredictable. The proportion of fumigant entering in this way may be only a small contribution to the toxic dose when the cuticle as a whole offers little resistance to fumigant penetration.

PART I

INSECTS, MATERIALS, APPARATUS AND METHODS

CONTENTS

I.

1. INSECTS

Insect species were chosen for ease of breeding in large numbers, for contrasting surface character and to provide some size variation within the scope of the fumigation apparatus. With the exception of Periplaneta adults, cultures of each species were maintained in the insectary at the Field Station.

Supella supellectilium (Serville) Dictyoptera, Blattidae.

Second instar nymphs were used for toxicity studies for fumigant uptake, and for samples of the epicuticular wax.

Periplaneta americana (L) Dictyoptera, Blattidae.

Adults, kindly supplied by Mr. Haskins of the Pest Infestation Laboratory, Slough, were used for fumigation of their forewings and for samples of their epicuticular waxes.

Schistocera gregaria Forsk, Orthoptera, Acrididae.

First instar hoppers were used for knockdown and paralysis studies.

Oryzaephilus mercator (Fauvel), Coleoptera, Cucujidae.

Adults were used for toxicity tests, fumigant uptake, and for epicuticular wax samples.

Tenebrio molitor (L) Coleoptera, Tenebrionidae.

Adults were used for fumigation of their elytra and for samples of epicuticular wax.

Coccinella septempunctata (L) Coleoptera, Coccinellidae.

Adults were captured in the field and used for knockdown and paralysis tests.

Musca domestica (L) Diptera, Muscidae.

Adults were used for toxicity tests, for measurements of fumigant uptake and for samples of wings and epicuticular wax.

I.

2. MATERIALS

FUMIGANTS

Hydrogen Cyanide

Hydrogen cyanide is a colourless liquid, vapour pressure 610 mm Hg at 20°C which boils at 26°C to a colourless gas with an almond like smell. The commercial fumigant used was supplied by I.C.I. Ltd. as liquid in steel containers.

Nicotine

Nicotine was obtained as the pure compound from Hopkin & Williams Ltd. It is a colourless, odourless liquid when pure. On exposure to air it darkens and becomes more viscous giving off a strong unpleasant smell. B.pt.247°C vapour pressure 0.04 mm Hg at 20°C.

DDVP or Dichlorvos

A 98% pure sample was obtained from CIBA Ltd. and was used for standard solutions for gas liquid chromatography (GLC) analysis. Resinous pellets containing 10% DDVP were used to generate toxic atmospheres during toxicity tests. These are manufactured under the trade name 'Vapona' by Shell Chemical Co. Ltd.

REAGENTS

Chloroform

Reagent grade chloroform was used for extraction procedures. No material interfering with the nicotine peak was found when the solvent was concentrated 250:1; in the extraction procedures concentration rarely exceeded 50:1. The chloroform used for extraction of epicuticular waxes was first redistilled through carefully cleaned apparatus.

Sodium sulphate

Dehydration of chloroform following insect residue extractions was carried out with reagent grade anhydrous sodium sulphate. No interfering chromatogram peaks were found.

Magnesium oxide

Clean up of insect and wax extracts was carried out on columns of reagent grade magnesium oxide 5 cm long and 1 cm diameter. The interfering material present in this chemical (Fig. 2) was removed by allowing a quantity of chloroform, at least three times the oxide volume, to pass through before use.

The standard solutions of hydrochloric acid and potassium hydroxide used for extraction of nicotine from waxes were made up from Analar grade chemicals.

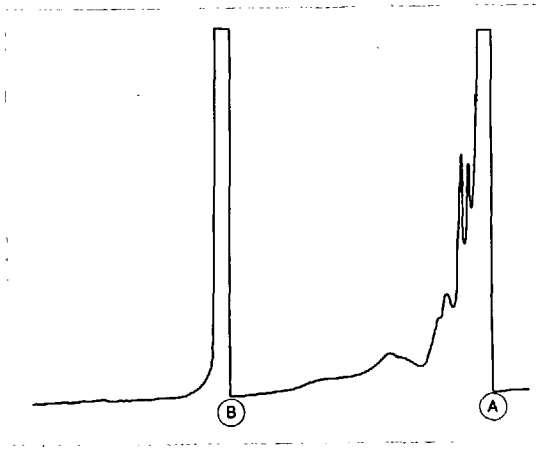

Fig. 2 Gas chromatograms of chloroform eluted from MgO column.

A. Sub-sample of concentrate from first 10 ml eluted, showing interfering material.

B. Sub-sample of concentrate from 20-30 ml fraction, showing clean solvent.

I - 3. APPARATUS

(a) FUMIGATION WIND TUNNELS

Three wind tunnels were constructed. An apparatus was required capable of passing fumigant/air mixtures over test insects at a required range of velocities up to 400 cm/sec and preferably at constant concentration. Either of two principal systems may be employed to fulfil these conditions. One is open ended, in which clean air is drawn through a chamber or chambers to control temperature, humidity and fumigant concentration, passed through the experimental chamber and finally released to the atmosphere. The other method is to use a closed circuit where the fumigant and air

are recirculated. A small reconditioning chamber is required to replace fumigant lost by absorption.

An open ended wind tunnel, in which the experimental conditions could be controlled with precision at slow rates of flow was first tested and found to be unreliable in maintaining nicotine concentrations at flows much above twelve litres per minute (115 cm/sec). Accordingly, the fumigant wind tunnels were designed on a closed circuit pattern, fundamentally more suited to higher gas flows.

WIND TUNNEL I

The apparatus is illustrated diagrammatically in Fig.3

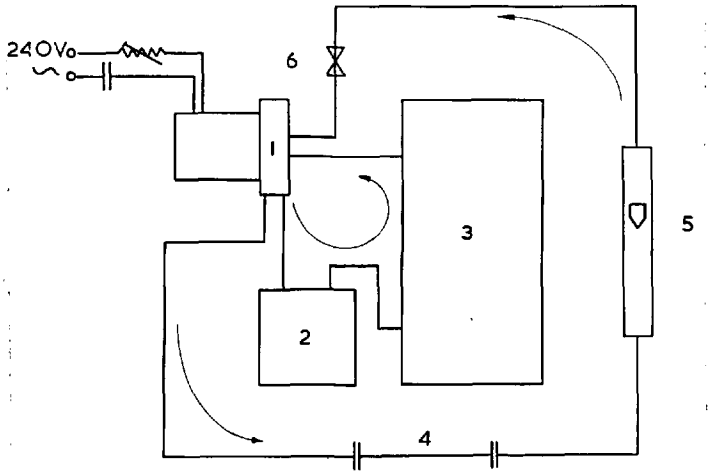

Fig. 3 Fumigant wind tunnel I.

The air/fumigant mixture is circulated by a centrifugal fan (1) through either of two separate loops, one containing a saturator (2) and a reservoir (3) and the other the experimental insect exposure tube (4) and a flowmeter (5) of the

rotameter type. Mixing of the two streams takes place in the impeller chamber of the pump. The saturator is a small glass vessel of about 500 ml capacity in which about 1 ml of pure nicotine was placed at the start of a series of fumigations. The reservoir consisted of a steel container with an approximate capacity of 20 litres, in which were a number of aluminium vanes to aid efficient mixing and to provide a larger surface area for nicotine absorption. The outlet returns to the inlet manifold of the pump.

The second loop carried the air/fumigant mixture through two gentle curves to the insect exposure chamber (Fig. 4)

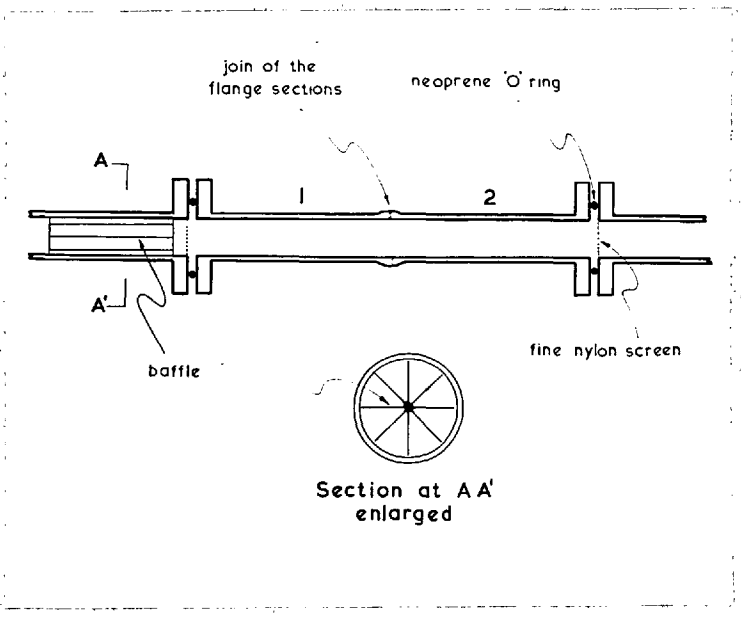

Fig. 4 Insect exposure chamber

This consisted of two standard Quickfit flat-flange joints (1 and 2) fused end to end with the flanges facing outwards. Two similar joints were fixed in the circuit, spaced sufficiently far apart to take the exposure chamber. Across

the flanges of these joints pieces of fine nylon mesh were stretched, partly to prevent insects crawling or being blown into the rest of the apparatus, and partly as an aid to the even distribution of air entering the exposure chamber. As an additional aid to steady air flow, a baffle was fitted within the joint at the motor end of the exposure chamber. This consisted of 2" x $\frac{5}{8}$" coverslips cut longitudinally and cemented together to form a baffle, star-shaped in cross section, and a close fit within the first joint (Fig. 4). Neoprene 'O' rings cemented over the nylon formed a gas-tight seal.

Two interchangeable flowmeters were used, one a metric 7A Rotameter covered the range 1-10 litres per minute, the other, a No. 12A, a range of 5-50 litres per minute. The flow in the experimental loop was controlled roughly by means of a rheostat governing the motor speed, and more delicately with the valve (6). The various constituent parts of the apparatus were linked together with $\frac{1}{2}$" OD composition lead piping, using soldered joints where practicable or butt joints, sleeved with polythene tube, sealed and held in place with 'Bondcrete' cement.

WIND TUNNEL II

The apparatus described above was considered unsuitable for hydrogen cyanide fumigation. Accordingly an all glass apparatus was constructed embodying a reservoir of 100 l flask which possessed two outlets diametrically opposed (Fig. 5)

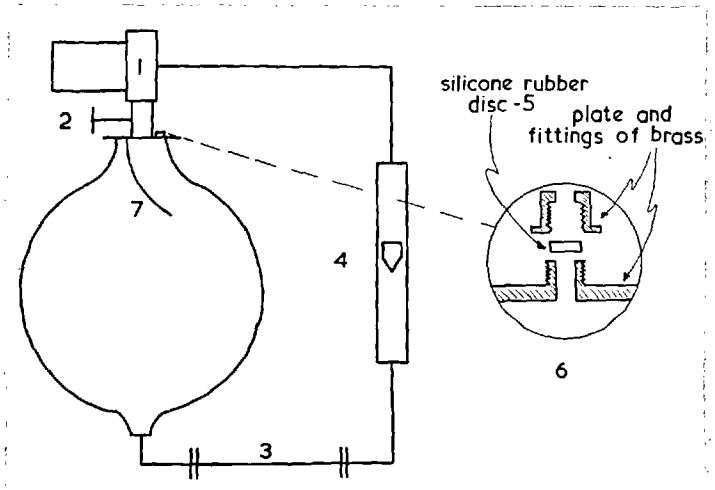

Fig. 5 Fumigant wind tunnel II

The same motor (1), valve (2), exposure chamber (3) and flowmeters (4) were used as before, and these were interconnected by Standard B 24 glass sockets and glass right-angle bends with B 24 cones at either end. Compression copper pipe fittings, in which the metal rings were replaced by neoprene 'O' rings were used to join longer lengths of glass tubing and to allow some flexibility to the flow circuit. The apparatus was dosed by injection of liquid hydrogen cyanide through a rubber septum (5) mounted in the brass flange fitting (6) closing the neck of the reservoir. A baffle (7) was fitted within the reservoir to mix the fumigant.

WIND TUNNEL III

A third wind tunnel, designed for use within a desiccator is illustrated in Fig. 6.

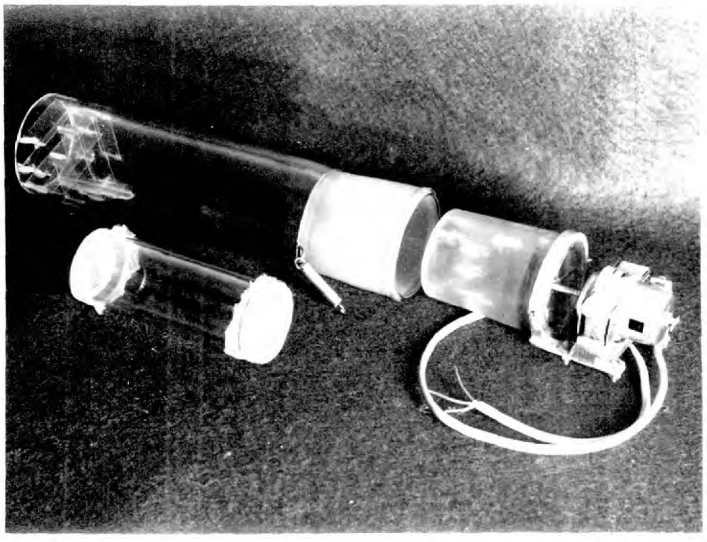

Fig. 6 Fumigant wind tunnel III
and insect cage.

Fumigant velocity is controlled by the motor speed, which is, in turn controlled by a rheostat. The velocity in the tunnel, measured with a small deflecting vane anemometer was plotted on graph paper against the motor speed measured with a stroboscope. Thus a predetermined velocity could be established by suitable adjustment to the motor speed. When the stroboscope was not available for this purpose the motor speed was assessed electronically. (See Appendix). The maximum wind speed is restricted to about 150 cm/sec by the limitations of the motor. Baffles constructed of glass coverslips, cut to size, were cemented into place. A cage to contain live insects, also illustrated in Fig. 6, was cut from 1" OD thin-walled glass tube. End caps were of nylon mesh held in place with rubber bands. Fumigations employing this wind tunnel

were carried out in a large sweet jar in a horizontal position. DDVP vapour was generated from 'Vapona' pellets.

The wind tunnel was set up with the insects to be fumigated inside and positioned within the fumigation chamber through the neck of the jar. Electrical connection was made by a co-axial socket located in a hole in the lid and made gas tight with cement. A silicone rubber septum, similar to that in the second wind tunnel, allowed gas samples to be taken with a glass syringe.

WINDSPEED AND VELOCITY PROFILES

The windspeed within the exposure chamber of tunnels I and II is calculated from the volume of air passing in unit time, indicated by the flowmeter, and the cross-sectional area of the chamber. This area was 1.77 sq. cm. Thus the two flowmeters covered velocities between 10 and 470 cm/sec. A 5 cm/sec velocity, used on a number of occasions, was found by extrapolation at the lower end of the flowmeter.

Zero windspeed was obtained by almost closing the fine control valve. With the pump running the gas flow through the chamber was sufficient to replace fumigant lost by absorption. Uptake of nicotine by beeswax under these conditions agreed with that absorbed in a still nicotine atmosphere within a desiccator.

The nature of the flow in the chamber, whether laminar or turbulent, affects not only the absorption coefficient of a freely absorbing medium, but also the velocity profile

across the tube. A much wider range of velocities occur in laminar flow (Fig. 7a) than in turbulent flow (Fig. 7b).

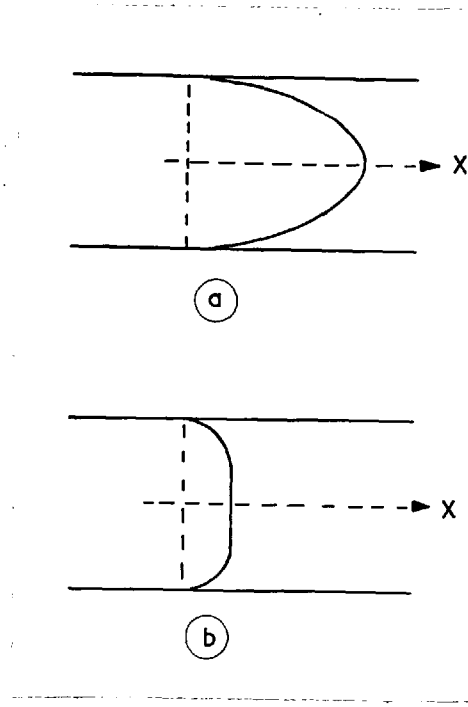

Fig. 7 Velocity profiles across tubes

(a) in laminar flow x axis - velocity;
(b) in turbulent flow x axis - mean velocity.

Turbulence may be expected in a pipe of circular cross section when the value $K = \frac{\rho v d}{\eta}$ exceeds a certain value. For ordinary smooth-walled tubes K can be taken as 2000 (International Critical Tables).

Thus, the velocity at which the flow in this particular exposure chamber may be turbulent is about 200 cm/sec for air. Observation of the movement of a fine glass fibre suspended in

the air stream showed that turbulence appeared in an empty exposure chamber between 43 and 53 cm/sec and that the fan speed and the valve setting had no effect on these values.

3. (b) MODIFICATIONS TO A DESICCATOR FOR FUMIGATIONS WITH PRESSURE FLUCTUATIONS.

One series of nicotine fumigations was carried out with superimposed pressure fluctuations. A 4½ l desiccator with a central hole of about 1¼" diameter in the lid was used as the fumigation chamber, the hole being closed with a removable polythene bung. A petri dish in the desiccator well contained a filter paper soaked in nicotine, which proved sufficient to maintain a steady toxic concentration during a series of fumigations.

An electric clock-motor, fitted with a paddle, was mounted in the desiccator to provide gentle stirring, electrical connections were made by two wires led through a hole in the bung. A two-way tap was also mounted in the bung. One branch led to a filter pump, and when the tap was turned to this position, the pressure within the desiccator was reduced. The other branch led to 3 bubblers filled with nicotine and thence to the atmosphere; when turned to this position normal pressure was restored with fumigant laden air.

The pressure in the desiccator was measured with a mercury manometer connected by a polythene tube through the bung. Gas samples were withdrawn by syringe through a piece of silicone rubber inserted in a fourth hole in the bung.

The insect cages were of copper gauze about 3" long and 1" diameter. During a fumigation the cages rested on a coarse gauze shelf (2 mesh to the inch) above the well. The cages were inserted and withdrawn with a hooked wire, through the central hole.

3. (c) GAS-LIQUID CHROMATOGRAPHY.

As a number of fumigant insecticides were to be used, their assay by the technique of gas-liquid chromatography (GLC) with a hydrogen flame detector was assessed, found suitable and adopted. An apparatus was constructed around a detector made of brass and similar to that described by Onkiehong (1960). The jet and pick up electrode were of platinum. Gas flows were monitored by rotameters and controlled by fine needle valves. The columns used were packed in borosilicate glass tubing and were enclosed in a steam jacket. Silicone rubber 'O' rings were used to seal the columns in brass fittings (for details see Appendix). The conductivity of the hydrogen flame was measured by a cathode follower impedance convertor circuit similar to that described by Smith (1960) - see Appendix for circuit diagram. The 6 SN7 valve was replaced by an E 80 CC, the output being fed into a 1 mV potentiometric recorder. A series of shunts appropriate to the input impedance of the recorder were arranged to give a stepped output with two-fold increments. At the most sensitive setting, baseline noise was about 3% of full scale deflection (FSD).

3. (d) SOLVENT CONCENTRATION.

Many of the fumigant solutions extracted from insects and waxes were too dilute to be assayed directly by GLC and had to be concentrated. The Kuderna-Danish technique was used for this concentration, in which the solvent is removed through a fractionating column, thus preventing the escape of higher boiling material. The apparatus was constructed of three parts. The lower portion consisted of a 10 ml graduated test tube, above which there was a pear-shaped vessel, 60 ml in capacity supporting a vigreux column of 4 to 5". B 14 cones and sockets connected each part. In use, a clean pin was dropped inside the apparatus to prevent bumping, and the lower two inches of the graduated tube immersed in a water bath. When most of the solvent had boiled away and about 0.5 ml remained, the apparatus was removed from the water bath and allowed to cool. The inside walls were then washed down with one or two mls of solvent delivered from a teat pipette. Surprisingly, this operation was found to be almost superfluous for reproducable results, but was included as a precaution. The bulb and vigreux could now be replaced by a B 14 stopper until the solvent was subsampled and assayed by GLC.

I.

4. METHODS - ANALYTICAL PROCEDURES

(a) Hydrogen Cyanide

Determinations of hydrogen cyanide were carried out by a slight modification of the micromethod (Lubatti, 1935) in which the gas is absorbed in aqueous sodium bicarbonate and titrated with iodine. Gas samples were withdrawn through the rubber septum used for dosing the apparatus, with an all glass 20 ml syringe, fitted with a long needle (2" x 26 gauge). The gas sample taken depended on the concentration and was varied from 5 ml for high concentrations, above 10 mg/l, to 15 ml for low concentrations. The gas sample was slowly bubbled through 1 ml 0.3 per cent sodium bicarbonate, contained in a micro test-tube. Little loss of cyanide could be detected by smell, and as the results agreed well, it may be assumed that substantially all the cyanide was absorbed in this way.

The above procedure was carried out before and after a fumigation to assess the gas concentrations. During a fumigation, or a series of fumigations, 0.5 ml gas samples were assayed with the hydrogen flame detector. Considerable tailing of the hydrogen cyanide peak was experienced at room temperatures, the most suitable column and temperature for the purpose were found to be a column of 10% polyethyleneglycol succinate 48" long and one sixteenth inches diameter run at 100°C. In this way, the cyanide was held back sufficiently for the

recorder to follow the detector response, and enabled samples to be analysed every five minutes if required. Peak areas are difficult to determine under these conditions owing to the narrow cyanide peak. Peak heights gave estimates agreeing to within 5%.

(b) NICOTINE

Preliminary quantative estimations of nicotine were made by an extension of the colorimetric method described by Rintakul and Hennen (1950) and Blackith (1953). Attempts to increase the sensitivity further by reducing the volumes of acid and alkali used and by extraction of the colour complex with smaller volumes of chloroform were satisfactory only for gas samples taken from the wind tunnel, using evacuated vessels of 200 ml capacity. Assays by this method for the small quantities of nicotine residues in insects were inconsistent.

The majority of the nicotine assays were carried out with the GLC apparatus described above. Polyglycol columns have been found most suitable for the assay of tobacco alkaloids, including nicotine (Quin, 1959). The column used here was 80-100 mesh alkali washed 'celite 545'(washed with sodium bicarbonate) supporting 10% w/w stationary phase of 4 parts polyethylene glycol 400 and one part dinonyl phthalate, steam jacketed at 100°C.

With an injection port pressure (see Fig. in Appendix) of 10 pounds per square inch (psi), the nitrogen flow rate

was about 30 ml/min; hydrogen flow was maintained at 80 ml/min and air at 800 ml/min. Calibration graphs were prepared by injection of samples of a standard solution of pure nicotine in chloroform or n-hexane. The graphs were linear at the more sensitive settings of the instrument. The product of peak height and width at half height were used for peak mensuration. This assessment was found to be more precise than a measure of peak height alone.

Gas concentrations were determined by taking 15 ml of air/fumigant mixture in an all-glass syringe and injecting into the inlet port of the gas-liquid chromatography apparatus. The nicotine peaks are compared with those from a standard solution made up in chloroform or hexane. Concentrations determined in this way were lower than those determined by colorimetric analysis of vacuum samples. There are two possible reasons for this. The first, and probably minor one, is that a gas sample taken in the syringe is increased to the pressure within the injection port as soon as the rubber septum is pierced; this could cause some condensation of nicotine from the sample which may or may not have reached equilibrium by the time the sample has been expelled from the syringe. The chromatogram peak will therefore give a lower value for the nicotine present in the sample than the true one, since some of the fumigant will remain on the walls of the syringe. The second reason is that the colorimetric method for nicotine assay has been shown by Blackith (1953) not to be

specific for nicotine but also to react to similar compounds of lower molecular weight. Thus, gas samples assayed colorimetrically will give a value higher than the correct one when impurities are present.

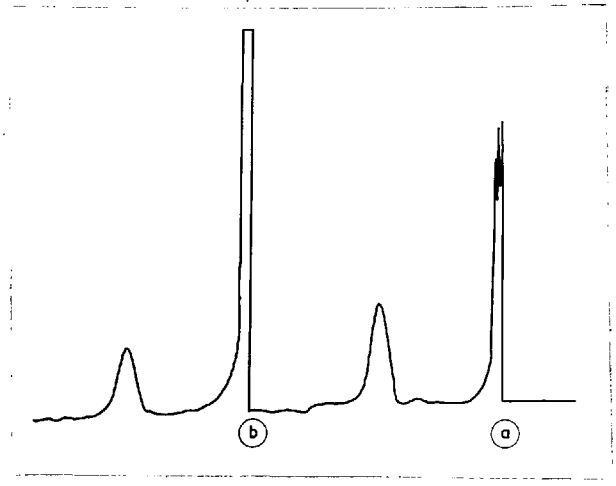

Fig. 8 Gas chromatograms of nicotine air samples.
(a) fresh nicotine, concentration - 18 µg/l.
(b) after 3 hours, concentration - 12 µg/l.

Fig. 8a shows a chromatogram of a gas sample taken from the wind tunnel while the nicotine in the saturator was still fresh. There are at least three impurities, barely separated on this column, which are eluted almost immediately. After a short interval of 2-3 hours, this triple peak may be seen to be greater, and the nicotine peak to have diminished (Fig. 8b).

From this value the concentration of nicotine fell slowly and reached a value of 5 µg/l after a period of 3-4 weeks; below this figure the nicotine peak cannot be measured accurately.

Since analysis by GLC showed the quantities of the impurities present to vary, it was felt that a more reliable estimate of concentration would be obtained by this method than by colorimetry. Loss in the syringe is probably small, the concentration of nicotine present in the gas samples being considerably lower than the calculated saturation value. If, after injection of a gas sample, the syringe is allowed to stand with the plunger withdrawn to the 15 ml mark for 10-15 minutes, allowing sufficient time for the residual nicotine to evaporate, the nicotine peak following injection of this sample cannot be distinguished from the baseline noise. It may be concluded that the concentrations determined by GLC are only likely to differ from the true value when saturation is approached. The concentrations found in the wind tunnel were well below the calculated saturation figure.

Extraction of Nicotine from Fumigated Insects

Three possible methods were tested. The first, developed from the colorimetric analysis method, involved extraction of nicotine as the hydrochloride from an insect homogenate in dilute hydrochloric acid (.04N). The filtrate was made up to 15 ml, added to a separating funnel, and made alkaline by the addition of a small quantity of strong, aqueous caustic potash (see Fig. 9).

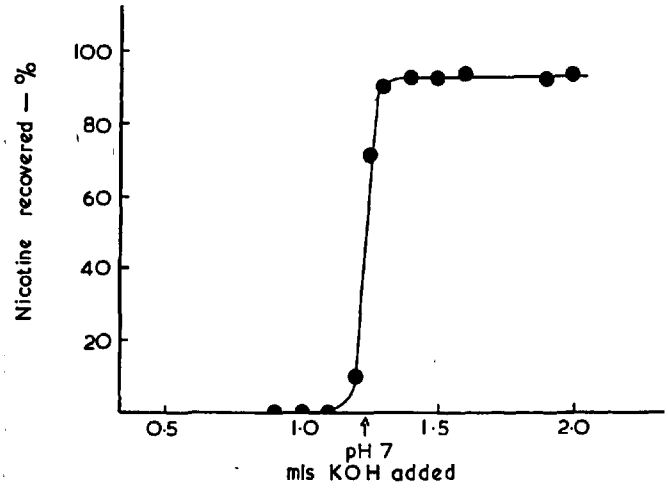

Fig. 9 Recovery of nicotine from aqueous solutions of different pH (500 µg nicotine in 15 ml .04N HC l).

The freed nicotine was extracted by three successive washings of 20,10 and 10 mls chloroform, which, after dehydration with anhydrous sodium sulphate was concentrated to a known volume of about 2 mls. A concentrate subsemple of 10 µl was subsequently assayed by GLC. This method is not entirely suited to all insect material as there is a tendency to form a stable emulsion in the separating funnel, owing, possibly to the action of soaps formed by hydrolysis and esterification of some insect lipids. The second method was to extract the homogenate with dimethyl sulphoxide, a solvent with small affinity for fats. This extract proved difficult to chromatogram satisfactorily owing to its decomposition above 100°C. and the nicotine present could not be easily

separated by column chromatography with another solvent. A straight-forward extraction of the insect homogenate with chloroform was best, the dehydrated solution being run through 5 cms. of magnesium oxide before concentration. Small amounts of co-extracted material led to a baseline drift of the subsequent chromatograms which was small enough to be acceptable.

Extractions from beeswax and paraffin wax were made by shaking with dilute hydrochloric acid, warmed above the melting point of the wax. Following cooling and filtration, the acid solution was made alkaline, and the same procedure followed as above. Chloroform was found to be the most suitable solvent for extracting nicotine from an alkaline aqueous medium (see Fig. 10).

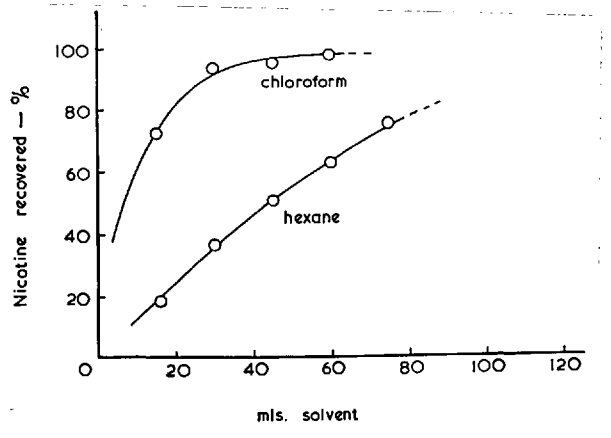

Fig 10 Recovery of nicotine from aqueous alkaline solution by chloroform and hexane.

(c) DDVP

DDVP was successfully chromatogrammed on a 24" x 1/16" column of 100-120 mesh acid-washed 'celite 545' supporting 10% polyethylene glycol succinate. (Retention time about ten minutes at 100°C and at an inlet pressure of 10 psi). The concentrations, however, at which this fumigant is biologically active are low, less than 1 µg per litre, and samples of 20 ml were barely distinguishable from baseline noise at the most sensitive setting of the instrument. Larger gas samples could not be taken for two reasons: firstly, there was a tendency to blow the flame out, and secondly, the sample could not be introduced quickly enough to provide a sharp peak. Long time intervals of injection tend to produce flattened rather than sharp peaks, and these cannot be satisfactorily characterized with simple linear measurements.

PART II

TOXICITY AND ABSORPTION OF FUMIGANT IN RELATION TO FUMIGANT/AIR VELOCITY

CONTENTS

II

1. INTRODUCTION

Fumigants enter insects by either or both of two routes - through the integument and through the tracheal system. The relative importance of each route varies with the fumigant and insect concerned. In both routes the maintenance of the bulk phase concentration in proximity to the surface of the insect must affect the rate of entry, and, in the tracheal route, pressure differences and behaviour of the spiracles might be expected also to exert an influence. Theoretical considerations predict an influence of air movement on both the factors affecting rate of entry and that the magnitude of this influence will depend on the insect-fumigant system studied.

An investigation into the relative importance of these two entry routes and the influence of gas velocity in facilitating entry by these routes was carried out. Hydrogen cyanide penetrates mainly through the respiratory system and was used to investigate the effect of fumigant movement on tracheal absorption. Nicotine and DDVP are known to be able to penetrate the integuement and were used to investigate the effects of fumigant movement on cuticular absorption. Nicotine showed the greatest increase in efficacy when streamed over test insects and was used in subsequent experiments to investigate the manner in which this effect was produced.

The effects of different treatments or methods of application of a toxin may be simply assessed by a comparison of insect responses to each treatment. However, a more precise estimate is obtainable by a comparison of the dosage response relationship for each treatment. Additional information concerning the rates of action and availability of the toxin may be extracted from this method of analysis.

As already noted, dosage rates in fumigation technique are assessed in terms of concentration time products. Thus the dosage may be altered by changing either or both of these variables. Within certain limiting values of concentration and time, the toxic effect is proportional to the concentration time product. Beyond these limits the concentration time product required to produce a definite toxic effect increases. In the limit, when either of the parameters is small enough, the required concentration time product is infinitely large. In these investigations, the concentrations have been held as steady as possible and time taken as the variable parameter.

It follows from theoretical considerations of gas absorption that an increase in fumigant/air velocity is likely to bring about increased absorption and therefore higher mortalities. There are, however, a number of other possible factors which cannot be ignored when considering gas absorption by insects. It may be that insects in reacting to higher flow rates absorb more fumigant by increased respiration or tracheal ventilation; or, perhaps, by attempting evasive

action in the higher wind speeds, larger areas of more sensitive cuticle may be exposed. Desiccation may also be an important supplement to the toxic action of the fumigant. Many of the experiments described in this section were designed to assess the validity of these theories. In the latter half of the section, the absorption of fumigant by insects is investigated by chemical assay.

II

2. THE TOXICITY OF FUMIGANTS

The method of treatment was similar for all insects and fumigants, and consisted of serial fumigations in time for each of a number of gas velocities. Fifty or more adult Oryzaephilus could be exposed at any one time. Both Musca adults and Supella nymphs are larger, and fifty was the maximum number that could be accommodated simultaneously. This limitation was emphasized at gas velocities above 300 cm/sec, when the insects would tend to accumulate at the down wind end of the chamber and form a plug which considerably restricted the flow. The test insects were placed in the exposure chamber, without anaesthetic whenever possible; when this procedure was impracticable (as it was with Supella), small quantities of carbon dioxide were used and the insects allowed to recover fully before being fumigated.

Generally, five fumigations were carried out for each gas velocity used, the time limits being determined approximately beforehand by fumigating batches of ten insects over a wide

range of time intervals. A twenty-four hour recovery period was allowed, the insects being confined in 3 x 1 inch specimen tubes, capped with muslin, and held at the temperature of the fumigation (20° ± 2°C). The mortality was recorded at the end of this time.

Fumigant/air velocity effects are reflected by changes in the median lethal exposure times (MLT) obtained at various flow rates. The dosage mortality data for each velocity have been analysed with a logarithmic probability transformation (Finney, 1947); an example is shown in Fig. 11, and the MLT's plotted with their respective velocities (see Figs. 12, 13 and 14). The 95% confidence limits are also shown.

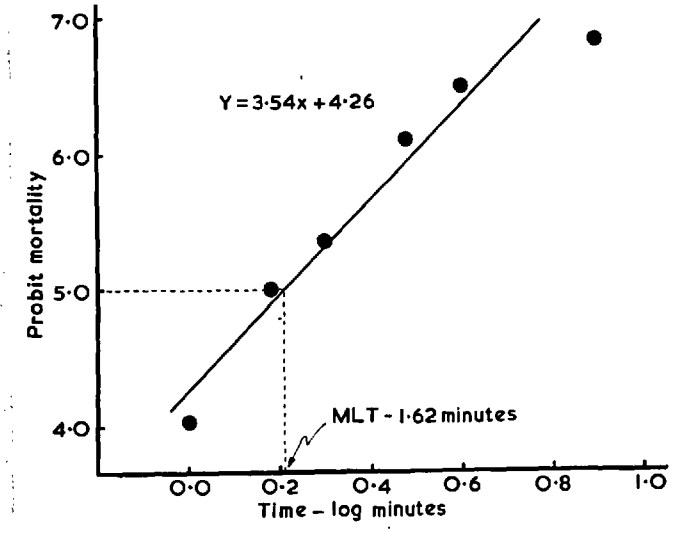

Fig. 11 The toxicity data, in probits, plotted with log exposure times, for fumigations of Supella nymphs with hydrogen cyanide at 95 cm/sec. The calculated regression line is shown.

Hydrogen cyanide fumigations were carried out in the all-glass wind tunnel I, nicotine in wind tunnel II and DDVP in wind tunnel III (Part I).

It is evident from these curves that while increase in velocity has little effect on the toxicity of hydrogen cyanide it greatly enhances the toxicity of nicotine and DDVP. Furthermore, toxicity does not increase linearly with velocity but falls off exponentially at a flow which is characteristic to the insect species.

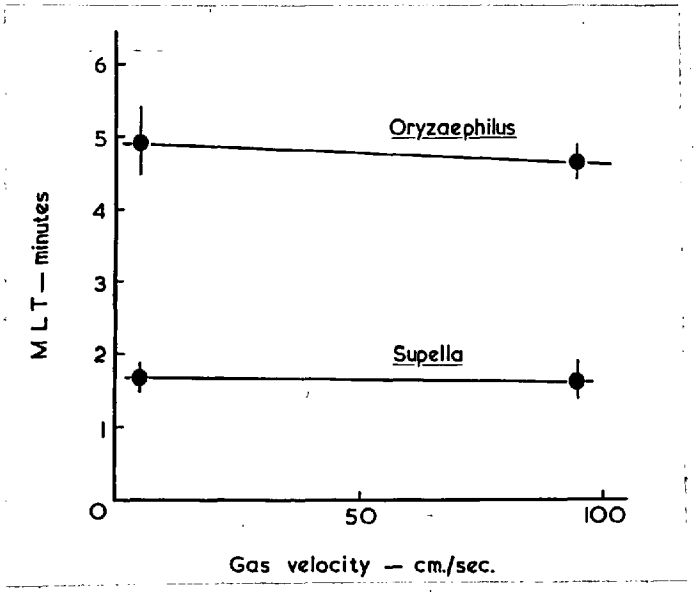

Fig. 12 Effect of fumigant velocity on the toxicity of hydrogen cyanide (Data in Appendix - Table A 1).

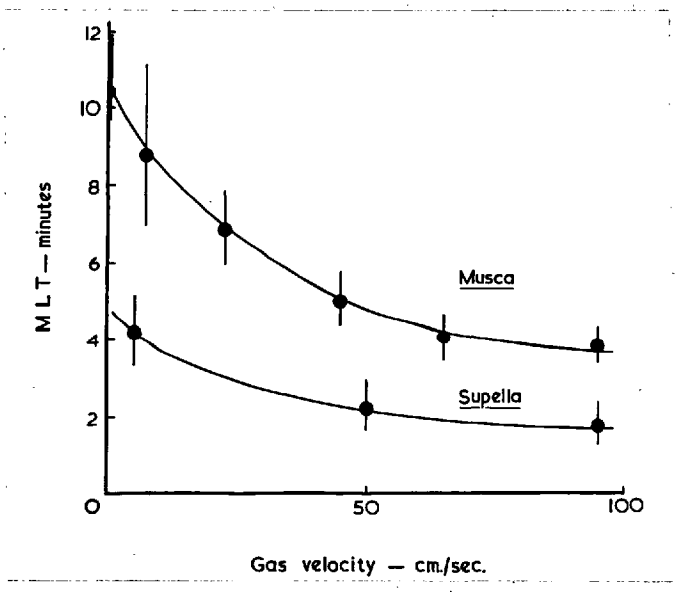

Fig. 13 Effect of fumigant velocity on the toxicity of nicotine (Data in Appendix - Table A 2)

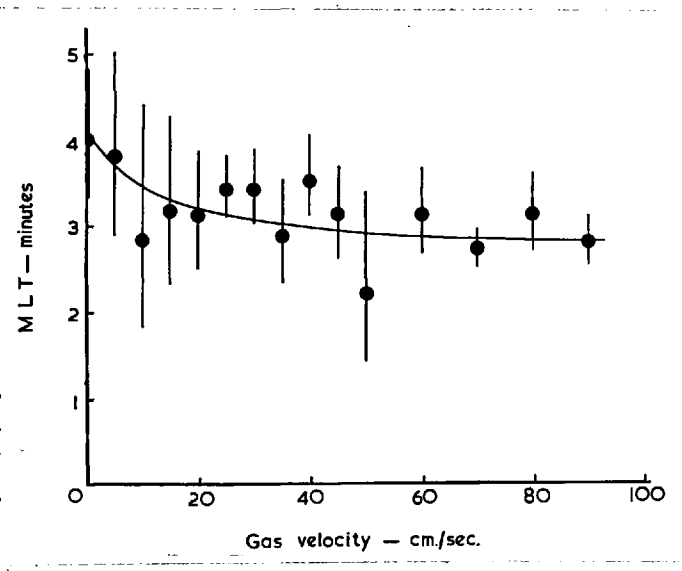

Fig. 13a Effect of fumigant velocity on the toxicity of nicotine (Oryzaephilus) (Data in Appendix - Table A 2)

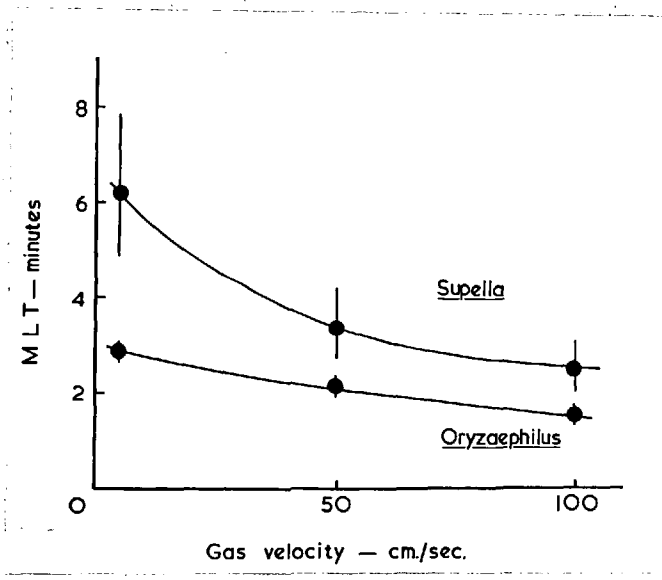

Fig. 14 Effect of fumigant velocity on the toxicity of DDVP (Data in Appendix - Table A 3)

II

3. INSECT BEHAVIOUR DURING FUMIGATION

A series of investigations into the behaviour of the insects during fumigation was conducted. A study was made of the time taken to paralyse the insects at different gas velocities, their behaviour during this time, and of the proportion of the total exposure time during which it was possible for tracheal ventilation to occur through open spiracles.

Fumigated insects exhibit toxic symptoms almost immediately after the commencement of a fumigation, and, irrespective of gas velocity, the insects become agitated, in-

co-ordinated and finally paralysed. The degree of agitation varies among individuals and appears in no way related to velocity. At the concentrations used, the time taken for both nicotine and hydrogen cyanide to paralyse the insects is short, between one and two minutes, and for the remainder of the fumigation the majority are to be observed upside down with legs flexed. Insects are taken to be paralysed when mobility and involuntary movement have both ceased; small tremors were discounted. Paralysis times of insects fumigated individually or in pairs were recorded, twenty-five of the three species used previously being treated at each of two widely separated velocities, namely 5 and 90 cm/sec. A similar treatment was given to twenty-five Schistocerca nymphs and twenty-five Coccinella adults. The mean values are reproduced in Table I.

TABLE I

	$\bar{t}_{(25)}$ - minutes	
	5 cm/sec	90 cm/sec
Supella	1.54	1.54
Musca	1.74	1.53
Oryzaephilus	1.80	1.71
Schistocerca	1.61	1.64
Coccinella	1.58	1.51

(Complete data is reproduced in Table A 4 in the Appendix).

The variation between individuals is considerable; the means, however, vary little with flow rate. Application of the t-test showed there to be no significante between the paralysis times for the species tested at the two velocities used, except for Musca where the difference was just significant at the 5% level. In view of the small sample and the considerable variation, this result may be spurious. There were differences of only small significance between species, and it may be concluded that in general gas velocity has little effect on the time taken to paralyse these insects.

Tracheal ventilation

The extent of tracheal ventilation was assessed, first by observation of the thoracic spiracles of adult houseflies during fumigation, and secondly, by a series of fumigations in which the pressure fluctuations were superimposed in an attempt to pump more fumigant into the insect by way of the tracheae. These thoracic spiracles are more readily visible than the very much smaller abdominal spiracles, and, in addition, are closer to the central ganglion of the nervous system. Since nicotine acts primarily on the nervous system and the spiracles probably account for a large proportion of respiratory exchange, especially during flight, it would seem reasonable to suppose they will be the most important in fumigant uptake by the respiratory system. The insects were viewed with a binocular microscope through a glass cover slip which was

cemented over a hole blown in the side of the exposure tube. Each insect was lightly anaesthetised and cemented to a short length ($1\frac{1}{2}$"-2") of soft iron wire, the point of attachment being the dorsum of the thorax between the wing bases. The hind legs were removed and when the insect had fully recovered it was placed in the exposure tube and positioned beneath the window by means of a magnet outside the tube. The optimum viewing positions are shown in the Appendix.

The apparatus was then run at a pre-determined flow rate with nicotine present and a record made of the time that the spiracles were open, while the insects were exposed for the MLT appropriate to the velocity.

Six insects were inspected for the activity of the meso-thoracic spiracle and six for the meta-thoracic spiracle at each of the flow rates tested. A summation of the times for which each spiracle was open is reproduced graphically in Fig. 15 as a percentage of the median lethal time plotted with respect to velocity. It can be seen that as the velocity is increased a tendency exists for the spiracles to be open for shorter proportions of the median lethal time. Thus, in experiments at different velocities the tracheal system could only take up a median lethal dose if the reduction in opening time at higher flows is offset by improved tracheal ventilation caused by the air movement.

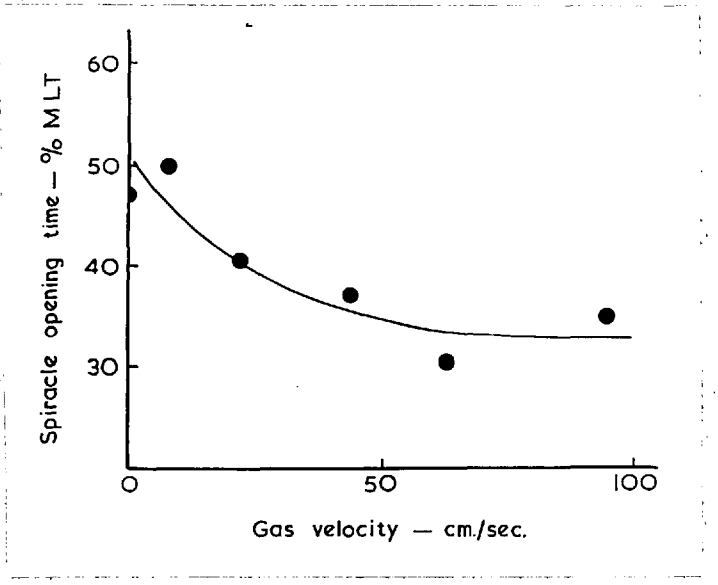

Fig. 15 The percentage of the MLT during which the thoracic spiracles were open. The MLT at any gas velocity is obtained from the curve for Musca shown in Fig. 13. (Data in Appendix - Table A5)

II

4. FUMIGATION WITH SUPERIMPOSED PRESSURE FLUCTUATION

In an attempt to enhance tracheal uptake of nicotine and to determine its importance in the absorption of a lethal dose, a series of fumigations were carried out in the modified desiccator described in Part I, in which, by superimposing pressure fluctuations, nicotine vapour might be 'pumped' into the respiratory system.

The effects of low pressures on insects is well documented; (Boyle, 1670) was the first to record that insects could be killed by subjecting them to reduced pressures for some time. His theory that death was due to lack of oxygen

was later shown to be unlikely and that mortality was more likely caused by desiccation (Back and Cotton, 1925; Lutz, 1929; Fisk and Shepard, 1938). Livingstone and Reed (1940) substantiated by El Nahal (1953) directly correlated mortality at reduced pressures with water loss. Bhambhani (1956) showed that at very low pressures (2-3 mm Hg) water loss was greatly reduced and that there was no mortality. Low pressures have been found to enhance the toxic effect of fumigants; most research has shown that at pressures exceeding 200 mm Hg, or thereabouts, mortalities closely approach those found at atmospheric pressures (Monro, 1961). The effect of reduced pressures in enhancing the mortality during fumigation is thought to be caused by the more rapid diffusion of the fumigant brought about by a reduction in the number of air molecules which normally impede the diffusion process.

In the present experiment low pressures of this order were avoided and two series of control fumigations were carried out; one at atmospheric pressure and one at the lowest pressure of the fluctuation cycle, namely 500 mm Hg. In the third series, the pressure was altered from atmospheric, 755 mm Hg, to 500 mm Hg and back to 755 mm Hg three times per minute. The test insect was the housefly Musca domestica.

For a statistical analysis of the dosage response relationships, CTp's have had to be used for the dosage parameter, since with pressure fluctuation, the concentration

was found to increase during the course of a fumigation. No significant differences are to be observed in the mortalities; the data may be fitted with three parallel lines which are not significantly separated. The X^2 analysis is shown in Table II.

TABLE II - ANALYSIS OF X^2

Source	X^2	Degrees of freedom
Position	0.22	2
Parallelism	1.74	2
Linearity	10.96	12

(Summarized from Table A6)

It appears from these results that the tracheal system is much less important than the cuticle in absorbing lethal doses of nicotine vapour.

II

5. AIRING OFF AFTER FUMIGATION

After fumigation with high vapour pressure fumigants, methyl bromide for example, a considerable proportion of the absorbed fumigant may be desorbed from the fumigated commodity. It has also been shown recently (Perry, et al, 1964) that between 40-50% of aldrin and 23-38% of dieldrin applied topically to houseflies may be lost through volatilization. If low vapour pressure fumigants are lost from insects after fumigation, then airing at increased wind speed must aid removal in the same way that it aids absorption.

To test this hypothesis, batches of 50 houseflies were fumigated for different times arranged to provide a number of mortalities after the 24-hour recovery period. Directly after

fumigation, each group was divided in two and each sub-group of 25 was weighed. One sub-group from each group was allowed to recover in 3 x 1 inch specimen tubes as before; the other sub-group was confined in a tube with copper gauze across either end and exposed to clean air at a wind speed of about 100 cm/sec. This treatment was continued for six hours after the end of fumigation, and the insects allowed to recover in still air. The mortality in both groups was recorded 24 hours after fumigation, at which time the insects were also re-weighed (Appendix table A 7). Regression analysis shows that the mortalities in the two groups are not significantly different. χ^2 for parallelism with one degree of freedom is 0.087, and the difference between the MLT's is less than the standard error. There was a trend, however, for lower mortalities to occur among the aired insects. The weight lost, a mean of 20% in the aired and 17% in the unaired insects, must arise mainly from loss of water, very little excrement being deposited in the recovery tubes.

It is evident that little, if any, nicotine is lost during forced airing, though there is more water lost during this treatment than during recovery in still air. Since mortality is slightly reduced, however, the toxic action of the fumigant would appear to be more important than the desiccating action.

II

6. ABSORPTION OF FUMIGANT

Absorption of the median lethal dose

It has been established that insect behaviour is not affected by fumigant movement and probably has little influence on any enhanced toxicity. If mortality is due to the toxic action of the fumigant alone, then insects fumigated at time and flow conditions described by the toxicity/velocity curves above (Figs. 12-14) would be expected to have acquired similar doses, since these curves describe CTp conditions at which there is a 50% mortality. To verify the levels of poison present at the end of a median lethal fumigation, batches of 50 houseflies were fumigated with nicotine at a number of velocities and times in accord with the toxicity-flow curve (Fig. 13). After exposure, each batch was weighed and the nicotine present assessed by the colorimetric method described above (Part I). The results are reproduced here in terms of parts per million of nicotine absorbed (Fig. 16)

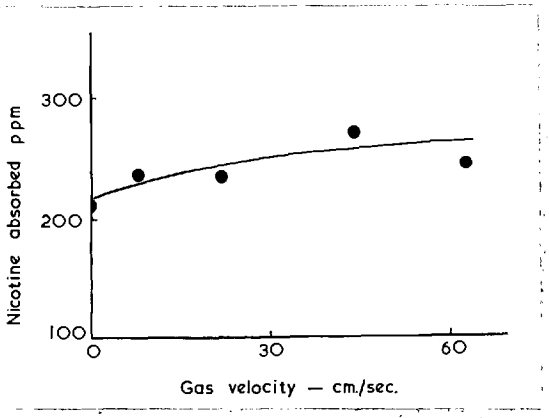

Fig. 16 Absorption of nicotine by Musca exposed at a number of gas velocities and for the appropriate MLT. (Data in Appendix - Table A 8).

Absorption at Different Fumigant Velocities

In order to explore further the effects of velocity on the uptake of nicotine, insect fumigations at differing velocities were carried out. The durations of exposure were similar for any one insect species and were sufficient to provide a measurable nicotine residue at the end of fumigation.

Oryzaephilus mercator

Batches of fifty adults were fumigated at the flow rates used in the previous experiment for periods of 10, 15 and 25 minutes. At the end of each fumigation the beetles were weighed and the nicotine assayed by the colorimetric method. Nicotine concentrations during fumigation were taken by vacuum sample and assayed colorimetrically. The results are expressed graphically in Fig. 17.

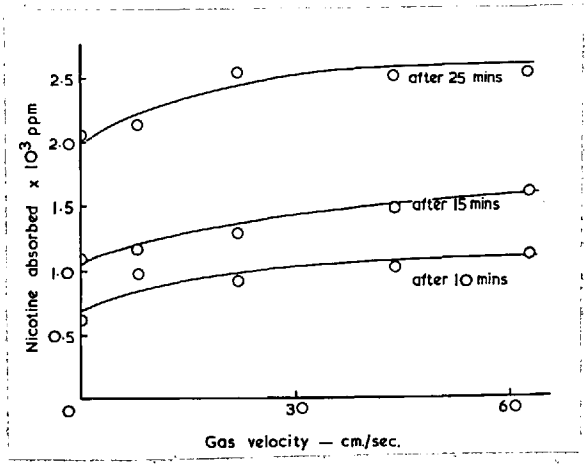

Fig. 17 Absorption of nicotine by *Oryzaephilus* at a number of gas velocities.
(Data in Appendix - Table A 9)

Musca domestica

Batches of about fifteen houseflies were fumigated with nicotine for ten minutes at varies wind speeds, after which they were counted, weighed and assayed for nicotine by GLC. Nicotine concentrations were assessed before, during and after each fumigation, again by GLC, and a mean of these three values taken for the concentration prevailing during that fumigation. The concentration ranged from 9.9 to 15.6 µg/l and the mean uptake for each fumigation has been corrected to a concentration of 10 µg/l by multiplying by a factor $\frac{10}{x}$ where x is the concentration of the particular fumigation. This correction factor is probably a fair approximation, since the relation of nicotine sorption to concentration is likely to be linear at low concentration. Absorption with flow rate is expressed graphically in Fig. 18.

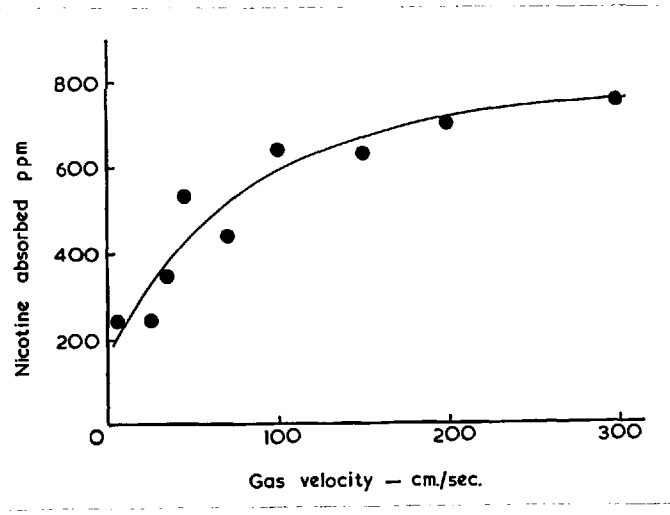

Fig. 18 Absorption of nicotine by Musca at a number of gas velocities. (Data in Appendix - Table A 10)

Supella supellectilium

Fumigations were similar to those described above. 30 second-instar nymphs were used and were fumigated for 10 minutes before counting, weighing and determination of nicotine absorbed. (Fig. 19)

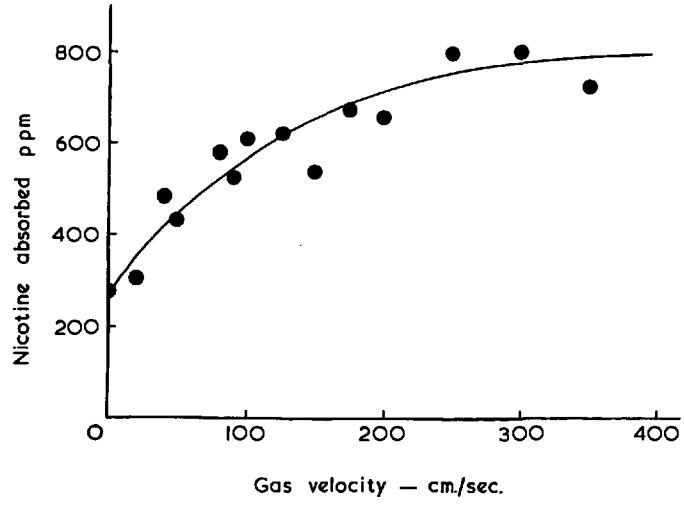

Fig. 19 Absorption of nicotine by Supella at a number of gas velocities. (Data in Appendix - Table A 11)

II

7. ABSORPTION BY DEAD AND LIVE INSECTS

It was shown in the last experiment that uptake of fumigant is proportional to the toxicity, and that insect behaviour and paralysis are unaffected by gas velocity. Uptake of nicotine is, therefore, a passive physical process in that it is uninfluenced by the insects' activity, and thus may well be similar in both dead and live houseflies.

Houseflies were treated with chloroform vapour and allowed to

air for about 30 minutes in order to ensure that the insects were dead and that little chloroform remained. These flies were fumigated in triplicate for 10 minutes in wind tunnel I at each of three velocities, 5, 40 and 95 cm/sec. Each replicate consisted of 15 dead and 15 live houseflies, separated in the exposure chamber by a piece of copper gauze. The order in which the insects were fumigated was alternated - that is for one fumigation dead insects were placed in the upstream end of the chamber and for the following fumigation the positions were reversed. At the end of each fumigation the insects were assayed for nicotine as before; the results shown here (Fig. 20) indicate that there is no difference in absorption between dead and live houseflies.

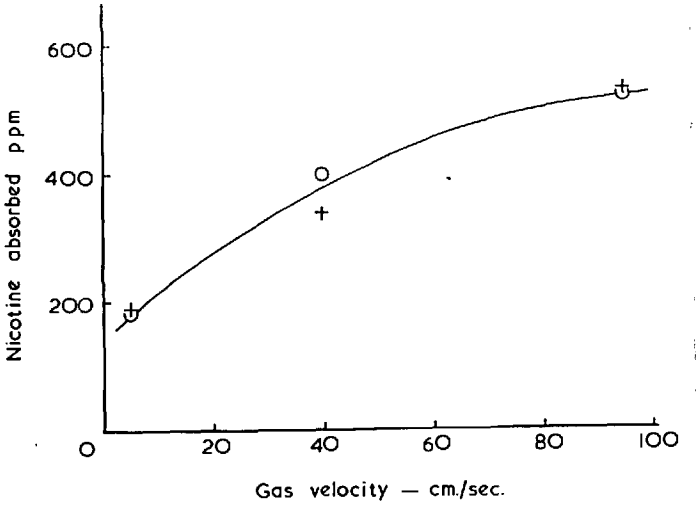

Fig. 20 Absorption of nicotine by Musca at a number of gas velocities (Data in Appendix - Table A12)
o dead insects
† live insects

II

8. NICOTINE ABSORPTION BY HOUSEFLIES WITH NO EPICUTICULAR WAXES.

Absorption of nicotine by houseflies is now established as a passive physical process occurring probably at the surface of the cuticle and supplemented to a small extent by tracheal uptake. If the surface of the cuticle is important then a change in surface composition may alter the character of absorption. The epicuticular waxes forming the outermost part of the insect cuticle may be readily removed with solvents. Insects are, of course, killed during washing with solvent, but this is not material to the experiment since absorption by dead and live insects has been shown to be the same. Thus, any differences in absorption shown by insects, in which the waxes are removed, will demonstrate the effect of the epicuticular wax on fumigant uptake and penetration. Insects were de-waxed by three washings in hot chloroform because hot solvent has been shown to be more effective than cold in exposing the underlying polyphenol layer of the cuticle (Wigglesworth, 1945).

Houseflies in this waxless condition were compared with normal flies which were previously killed with chloroform vapour by exposing treated and untreated houseflies in batches of twenty to three velocities of nicotine for a period of six minutes, after which fumigant determinations were carried out on each group. The results are shown in Fig. 21 where it is seen that a marked difference occurs, both in the total fumigant absorbed, and in the rate at which absorption increases with

the velocity. It must be concluded from this observation that the epicuticular waxes are an aid to fumigant absorption and are not so efficient in restricting the entry of nicotine as the underlying layers of the cuticle.

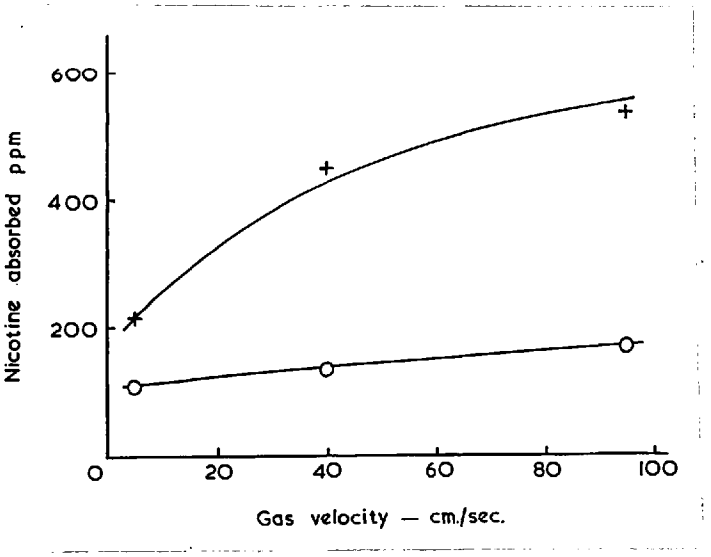

Fig. 21 Absorption of nicotine by Musca at a number of gas velocities (Data in Appendix - Table A 13)

+ normal insects
o insects in which the epicuticular wax has been removed.

II

9. THE DISTRIBUTION OF ABSORBED NICOTINE

The marked difference in uptake which occurs between normal houseflies and those in which the epicuticular wax has been removed suggests that the wax layer is a more effective sorbent for nicotine which then passes into the insects, or that it acts as a reservoir since absorption by the wax is greater than the underlying cuticle. To determine where the

fumigant accumulates, houseflies were fumigated at a number of gas velocities as before. At the end of the fumigation, the insects were weighed and the epicuticular waxes removed with 3 x 10 ml washings of warm chloroform. Nicotine determinations were carried out on the wax extract and on the remaining flies. The results are shown in Fig. 22, corrected to concentrations of 10 µg/l. The quantities of nicotine present in the waxes are expressed in terms of µg per fly; the total quantity of nicotine is expressed in µg/g of fly.

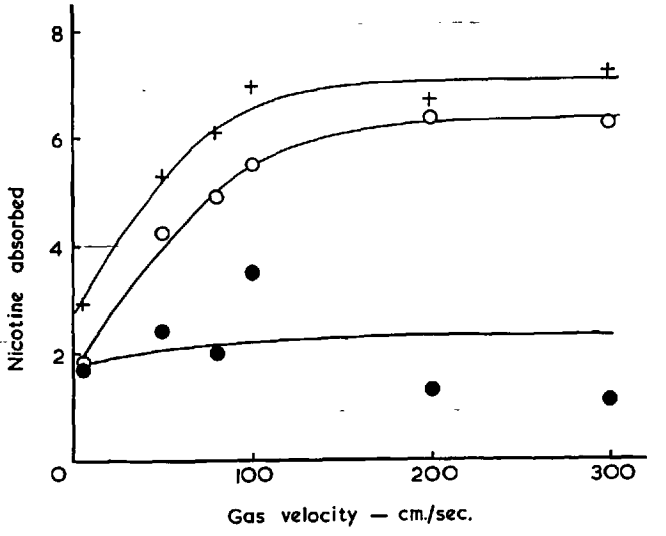

Fig. 22 Absorption of nicotine by Musca at a number of gas velocities. (Data in Appendix - Table A 14)

o epicuticular waxes ordinate µg/fly
● remainder of insect ordinate µg/fly
+ total ordinate $x10^2$ ppm

It is evident that a considerable proportion of the accumulated nicotine was present in the epicuticular waxes at the end of exposure, and that this quantity increased with flow rate until a velocity was reached at which the absorption rate became a maximum. Nicotine residues within the insect were more variable and appear to be independent of velocity.

II

10. THE EFFECT OF REPLACING THE EPICUTICULAR WAXES WITH BEESWAX

An experiment was carried out with Oryzaephilus in which uptake by normal dead insects was compared with that by dead insects that had had their epicuticular waxes removed with solvent and were re-coated with a thin layer of beeswax.

The aim of this exercise was twofold. The toxicity/velocity curve (Fig. 13a) and the absorption/velocity curves (Fig. 17) for this beetle both tend to an asymptotic value at a considerably lower velocity than that found for either of the other two species tested. This might perhaps be due to the small size of this beetle, which, when lying on the floor of the exposure chamber, might be more affected by the boundary layer relating to the chamber rather than to the insects themselves. Alternatively, the rate of absorption may be governed by the character of the epicuticular waxes. Thus, if the epicuticular surface were to be replaced with a material of different absorption properties then the absorption/velocity curve might take one of two forms. Either it would be similar

to that already recorded for this insect (when it may be assumed that size is the governing criterion) or dissimilar when it would appear that the character of the surface was more important.

Beeswax is later shown to possess absorption properties differing from the epicuticular wax of Oryzaephilus adults and was used as an alternative epibuticular surface.

The epicuticular waxes were stripped from the insects in a manner similar to that employed for the housefly, i.e. by three quick washings with hot chloroform. The insects were allowed to dry and then placed on a filter paper in a 5 cm buchner funnel, above a buchner flask attached to a filter pump. With the pump running, a warm solution (30-40°C) of about 10% beeswax, in 60/80 petroleum ether, was poured into the funnel. When dry, the beetles were removed from the funnel and allowed to air off completely on a warm oven. Fifty at a time were taken and exposed in glass tubes with nylon mesh ends in the small wind tunnel III. In tandem with these, in a second cage, dead untreated insects were fumigated. After a 20 minute exposure, each batch was assayed for nicotine by the method already described. The results are expressed graphically in Fig. 23 where it may be seen that the order of uptake is similar for both groups, but there appears to be a significant difference in the velocity at which the rate of absorption nears the asymtotic value.

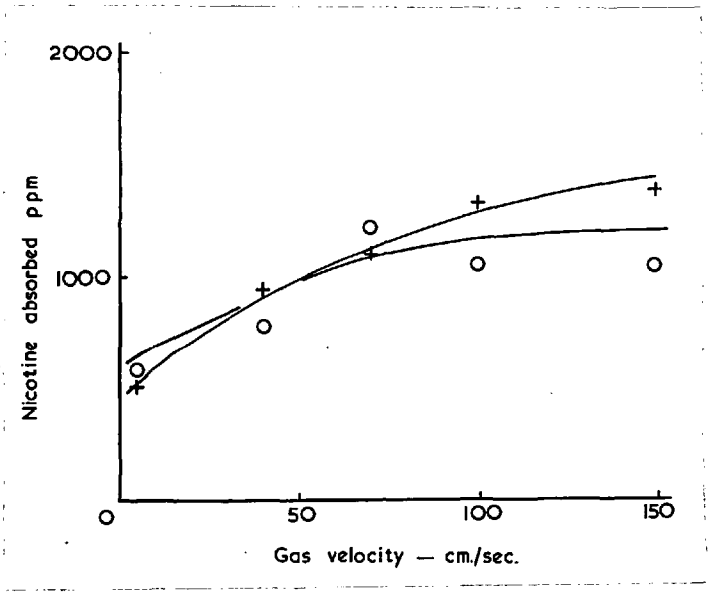

Fig. 23 Absorption of nicotine by Oryzaephilus at a number of gas velocities (Data in Appendix - Table A 14)
o normal insects
+ beeswax - coated insects

From these results it may be concluded that it is more the character of the epicuticle and not the size of the insect which is the governing factor in preventing continued increase of absorption with velocity.

II

11. DISCUSSION

Toxicity data derived from dosage/response analyses are only readily comparable when the regressions are parallel. Extreme instances with a lack of parallelism are observed in the mortalities occurring after fumigation with chloropicrin and ethylene oxide, where LC_{50} values indicate equivalent toxicities, and LC_{100} values establish ethylene oxide as con-

siderably the more effective fumigant (Negherbon, 1959). This situation frequently occurs when comparisons are made between toxins. For comparison between treatments of the same toxin, however, variations in slope may be attributed to differences, afforded by the treatment, in the availability of the toxin at the sight of action. The precision in the estimation of a dosage in a regression analysis decreases on either side of the median lethal dose; thus, where complete mortality is not required, as it is in the economic practice of fumigation, comparison at the median lethal dosage level is more suitable. In these experiments the regression coefficients for each series are of similar order and thus comparison of MLT's is valid.

It is evident from the earlier experiments, described in this section, that the toxicity of certain fumigants is enhanced by streaming them over the test insects. Both nicotine and DDVP are shown to possess this property. Comparison of median lethal exposure times at constant concentration and at different fumigant/air velocities demonstrates that the toxicit of these fumigants is enhanced by low wind speeds and that a velocity characteristic of each species is reached, above which there is little increment in toxic effect. This situation is reflected in the quantities of nicotine absorbed by insects with increasing wind speed. The quantity of absorbed toxin is sensibly similar at median lethal exposures over a wide velocity range, and the rate of absorption increases with velocity, conforming to the shape of the toxicity wind speed relationship. A similarity between the curves is to be expected if it is assumed, not unreasonably, that mortality is

related to fumigant absorbed.

It is feasible that mortality, resulting from fumigant action, might be enhanced by water loss. Ramsay (1935b) demonstrated that evaporation of water from the cockroach increased as the velocity of the surrounding air was increased. A five-fold increase in velocity, from 4-20 m/sec, raises the loss from the surface by a third of its former value, and the loss from the tracheae by a factor of seven. In view of this report and of the conditions imposed in the present wind tunnel, some increase in evaporation would be expected, though Ramsay's velocities were considerably the greater. Weighings of insects before and after fumigation, and after the 24 hour recovery period, showed total losses not much greater than 20% by weight and less than those which in the absence of fumigant cause death. (30% for the cockroach - Gunn, 1935). Thus it seems likely that water loss is unimportant, and it may be assumed that mortality is directly related to the amount of fumigant absorbed. This theory is supported by the results of the experiment in which insects were 'aired' after fumigation. There was a slightly higher mortality in those insects which lost least weight, also indicating that the toxic effect of the fumigant is more important than water loss.

At the end of a fumigation, the greater proportion of the absorbed nicotine is to be found in the epicuticular waxes. The residue present in the rest of the insect may have entered by way of the tracheae or by penetration through the cuticle.

Observation of spiracular activity suggests that uptake by the tracheae would fall as the velocity is increased since their opening time is shorter at higher flows. It is unlikely, however, that uptake by the respiratory system remains constant as the velocity is changed, but more likely that it increases in a manner similar to that of water loss (Ramsay, 1935b). Summation of the two processes would be expected to give the constant uptake found experimentally in the internal tissues.

The brief paralysis times exhibited by a wide range of insect species during exposure to both nicotine and hydrogen cyanide indicates that this condition is probably caused by fumigant entering through the spiracles, penetration of the cuticle being a slow process. Hydrogen cyanide is known to enter mainly through the tracheae, and since an increase in gas velocity does not alter the toxicity of this fumigant at the concentrations used and the times taken to paralyse the insects by both this fumigant and nicotine are similar at high and low velocities, it may be concluded that movement of these fumigants past the insects has a negligible effect on tracheal uptake.

DDVP is slower acting than either nicotine or hydrogen cyanide, though it is faster than many insecticides - benzene hexachloride and DDT for example, and that it should cause paralysis some 20 minutes after exposure is a reflection more of the mode of action of this particular compound than of its

failure to penetrate the tracheal system at all readily. As with nicotine, the toxic dose of DDVP probably accumulates in the epicuticular waxes of the cuticle, and becomes effective over a period of time by reason of its slow release into the haemolymph.

A theoretical estimation of the importance of the respiratory system for fumigant uptake in flow conditions is made possible by calculation of the order of tracheal aeration that could occur and hence the maximum quantity of fumigant that may enter in this way.

Consider a generalized insect 5 mm in diameter, orientated transversely to an air stream of velocity u cm/sec (inset Fig. 24). Let there be spiracles with a diameter of 200 μ situated diametrically opposite one another in the horicontal plane and let them be interconnected with a trachea of the same diameter.

At the upstream spiracle there will be an increase in pressure caused by the kinetic energy of the impinging air stream which, derived from the Bernoulli equation, is given by:

$$p = \rho u^2/2,$$

where ρ is the air density.

At the downstream spiracle a reduction in pressure occurs which, under optimum conditions, is approximately equal and opposite to the freestream impact pressure given above (Erkert and Drake, 1959). Thus the pressure drop across the length of

the trachea is given by:

$$p = \rho u^2.$$

The air flow through the trachea will be laminar because the diameter is small, and the volume passing is given by the Poisseuille equation,

$$V = \frac{\pi r^4 p}{8 l \eta},$$

where V is the volume passed in unit time, r is the radius of the tube, p is the pressure drop across length l and η is the viscosity of the air at the prevailing temperature.

Substituting for p in this expression, the volume passed

$$V = \frac{\pi r^4 \rho u^2}{8 l \eta}.$$

If the generalized insect is given 10 of these tracheae and is fumigated for 10 minutes in a nicotine concentration of 10 µg/l, the amount of nicotine taken up, assuming all passing through to be absorbed, is shown for different wind speeds in Fig. 24.

The quantity added to this by diffusion is minimal, convection being a more efficient process in mass transfer. The approximate amount diffusing into these 20 spiracles for the same fumigation conditions given above is shown by the dotted line in Fig. 24.

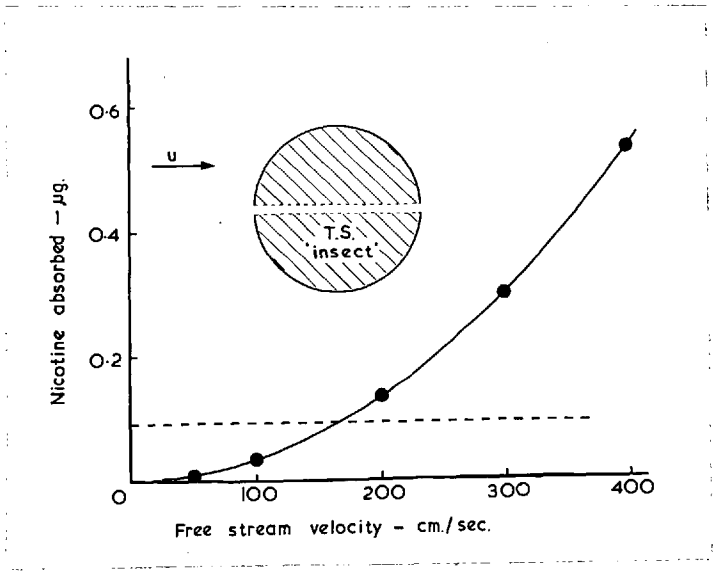

Fig. 24 Theoretical effect of freestream velocity on tracheal absorption. Nicotine absorbed by 10 tracheae during a 10 minute exposure at a concentration of 10 µg/l.
——— by convection
- - - - by diffusion.

The figure was arrived at by application of the diffusion equation

$$m = KA \frac{(p - p')\, t}{d},$$

where m is the mass diffusing in time t between two planes of area A and concentrations p and p', separated by a distance d. K is the diffusion coefficient for the gas through the medium concerned, in this case nicotine and air, and has been calculated as 0.05 cm^2/sec. from the Gilliland equation (see Perry, 1941, p 1168).

Again optimum conditions have been assumed. The concentration of 5 µg/l is assumed to be maintained at the spiracle face and is reduced, by absorption, to zero at a distance of 200 µ into the trachea.

It is emphasized that the figures shown in Fig. 24 are greater than would be found in practice. The tracheae seldom cross the insect directly; their diameter is reduced internally and the spiracle opening is typically less than the tracheal diameter as well as being fringed with hairs. It is also unlikely that fumigant absorption will be so rapid that the diffusion distance is reduced to 200 µ as used for the calculation. All these factors will retard the forced ventilation of the system and lead to a total uptake less than that shown. Even so, the amount of nicotine absorbed in this way is a small proportion of the quantity 2 µg/fly, found by experiment in the internal tissues after a similar fumigation.

These results are not necessarily in contradiction of those of Ramsay (1935,b). The highest wind speed used in this study was the same as the lowest used by the above author, and it may be seen from Fig. 24 that it is at this wind speed (400 cm/sec) that tracheal ventilation begins to increase more rapidly as the freestream velocity is increased. Cockroaches, also, have larger tracheae, and as the volume of air passed by the tracheae is proportional to the fourth power of the radius, considerably more forced ventilation will occur in this insect than in the smaller insects studied here.

The experimental results support the theory, and it may be concluded that, for nicotine at least, air movement at the velocities used has little effect in increasing tracheal ventilation and that most of the nicotine found within the insect has arrived there by penetration of the cuticle, after first being sorbed by the epicuticular waxes.

The reactions of insects to air movement during a fumigation is similar at all velocities; thus, exposure of more sensitive areas of the integument is not important in increasing absorption with flow. Absorption by dead insects is similar to that by live insects, and from this, and evidence discussed so far, it may be concluded that the enhanced toxicity arising from streaming nicotine and DDVP over the insects is caused by increased absorption at the cuticle surface.

PART III

ABSORPTION BY INERT SYSTEMS

III

1. INTRODUCTION

In the previous section both toxicity and absorption of fumigant were shown to increase with gas velocity and that this increase did not continue with velocity but asymptotically approached a limiting value. It therefore seemed appropriate to study fumigant uptake by simple systems in which a number of the variables present in biological material could be reduced. Absorption of fumigant, where this is affected by gas velocity, was shown to occur mainly in the epicuticular waxes; thus attention was turned to the absorption characteristics of a number of waxes, including those of the epicuticle. It was impracticable to use the epicuticular waxes themselves for much of this investigation, however, and beeswax was chosen as the alternative. Beeswax is similar to epicuticular wax, its main constituents being esters of myricyl alcohol - chain length 30 carbon atoms (Gilmour, 1961). The variation of uptake of nicotine by beeswax rods with changes in the fumigant velocity, the surface area of the wax and the macrosculpture on the surface, was investigated.

The absorption rate from still air and the solubilities of nicotine and DDVP in a number of epicuticular waxes are described.

The availability of the fumigants with increasing gas velocity to liquid systems, having fewer absorption barriers, was also studied.

III

2. EFFECTS OF FUMIGANT VELOCITY ON ABSORPTION BY BEESWAX AND PARAFFIN WAX

Rods of beeswax and paraffin wax (m. pt. 58-62°C) were exposed to nicotine vapour at a number of velocities, and after exposure assayed for nicotine. The rods were 40 mm long by 4 mm diameter, having a surface area of about 530 mm^2. This size is convenient to handle and is large enough to provide measurable uptake even at low flow rates. The wax rods were prepared by drawing molten wax at about 70°C into glass tubes, 4 mm internal diameter, which were first wetted internally with glycerol. The glycerol prevented the wax from sticking to the glass. When cool, the wax was easily withdrawn from the tube and after washing in distilled water and drying, was ready to be cut into appropriate lengths. The hollow ends formed by contraction during cooling were removed and returned to the molten wax. The rods were held in position in the centre of the exposure chamber of wind tunnel I with a modified paper clip. The inner end of the wire forming a standard paper clip (28 mm x 7 mm) was lifted above the plane of the clip so that it lay parallel to its old position and the remaining three longitudinal members, but about 4 mm above them and in the median line of the clip when viewed from above. The wax rods were held to this limb of the clip by gently warming the wire which could then be pushed into the end of the rod to a depth of 1-2 mm. This formed a firm joint which could be easily broken when the wax

was required for analysis (see Fig. 27). Paraffin and beeswax rods were fumigated for 20 minutes in concentrations of 5 µg/l. Fumigant concentrations and absorbed nicotine were assessed by the methods described in Part I.

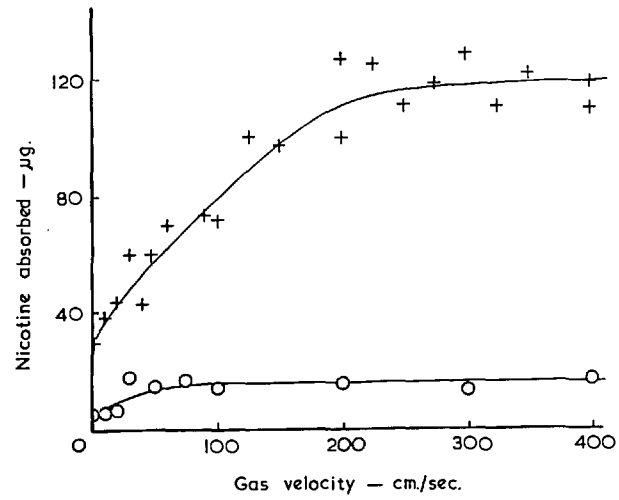

Fig. 25 Absorption of nicotine by beeswax and paraffin wax rods with change in gas velocity.
(Data in Appendix - Table A 16)

+ Beeswax
o Paraffin wax.

The results are shown in Fig. 25, from which it is evident that the initial absorption rates and the limiting velocities, above which there is no further increase in absorption rate, both differ considerably. This restriction on further absorption is not due to saturation of the whole wax rod, since smaller wax rods, with correspondingly smaller surface area, continued to absorb linearly with time at a gas

velocity of 300 cm/sec. for longer periods (see Fig. 26).

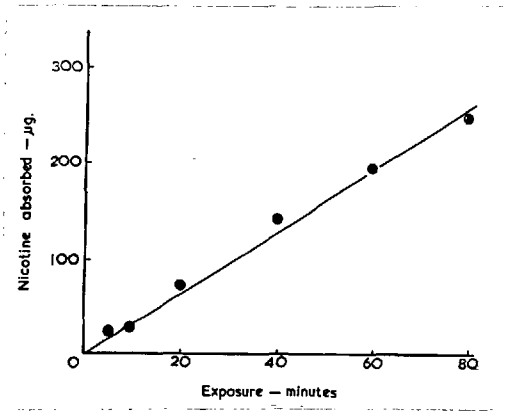

Fig. 26 Absorption of nicotine with time at a gas velocity of 300 cm/sec. (Nicotine 5 µg/l; beeswax rods 24 x 4 mm).

Ebling (1961) has shown the rate of movement of an oil soluble red dye through beeswax to be of the order of 125 µ per 24 hours. If nicotine were to diffuse through beeswax at a comparable rate, then its absorption by these rods would be expected to continue for 16 days, though the absorption time curve would probably begin to depart from its linear course after about half this time. The same argument applies to paraffin wax, and it would seem more plausible that the absorption rate, where this becomes steady with increasing velocity, is an indication of the maximum possible rate of uptake by the wax which is governed by the rate of diffusion of nicotine into the interior of the wax. The term 'saturation velocity' is here used to describe the velocity at which the maximum rate of absorption is approached.

III

3. EFFECT OF SURFACE SCULPTURE

An experiment was designed to investigate the possible effect of turbulence on the transfer of nicotine from the gas phase to the wax. Annular or longitudinal grooves were cut in the surface of otherwise plain wax rods so that, though different forms were exposed to the air stream, the total surface area was the same for both rods. Any difference in uptake between rods with these two groove patterns might indicate the importance of turbulence in aiding absorption. At low velocities, where the flow in the exposure chamber is known to be laminar, turbulence will set in as the fumigant passes over the annular grooves, whereas the longitudinal grooves should maintain steady flow conditions. At higher rates, when the flow becomes turbulent, there will be little difference in the flow patterns and absorption by both sculpture patterns should be similar.

The grooves were cut with the wax rod held in a lathe chuck. 15 annular grooves were cut, using the lathe to turn the wax, and 15 longitudinal grooves cut by operating the tool feed mechanism with the rod stationary and turning the rod through 24° between grooves. The cutting tool consisted of a short length of stainless steel wire, cut square at the end. A form of microsculpture was achieved by making many shallow holes with a fine pin over the entire surface (Fig. 27).

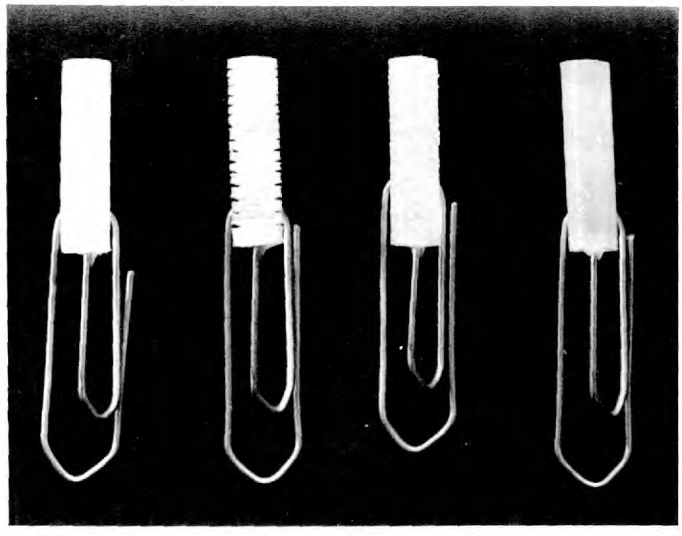

Fig. 27 Sculptured and plain beeswax rods showing method of support.

The rods were fumigated for 20 minutes at the velocities indicated, after which they were assayed for nicotine. The results are shown in Fig. 28. There is considerable variation in the quantities absorbed, possibly due to the difficulty of cutting clean grooves, with the result that the surface areas found in practice vary from the calculated value. No great difference is apparent in the rate of absorption by the two types of macrosculpture.

The results of microsculpturing are shown in Fig. 29. Apart from increasing the surface area and thus the total fumigant absorbed, this form of sculpture did not appear to bring about any difference in uptake between laminar and turbulent flow, nor did it appear to affect the saturation velocity.

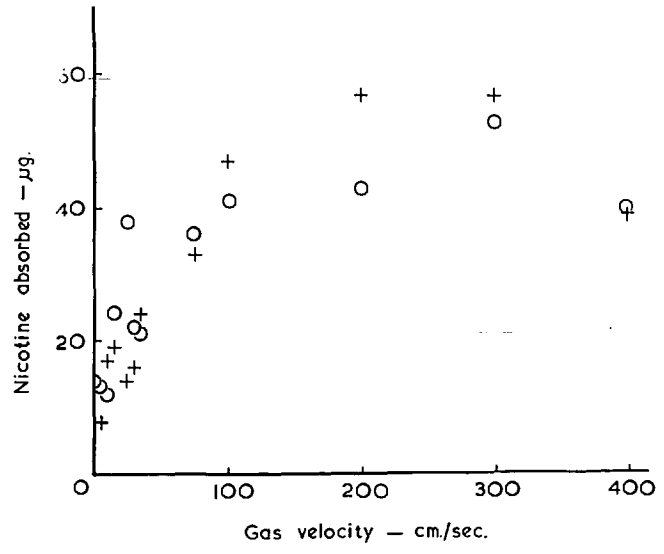

Fig. 28 Absorption of nicotine by grooved beeswax rods.
(Data in Appendix - Table A 17)

ϕ Longitudinal grooves
o Annular grooves.

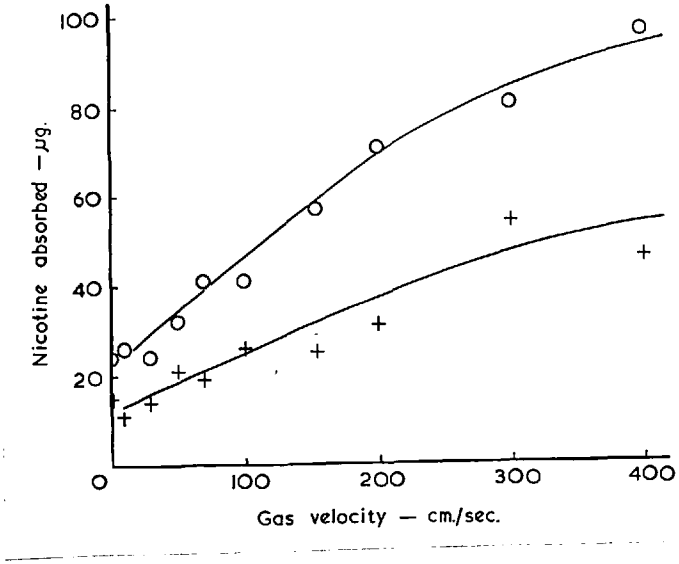

Fig. 29 Absorption of nicotine by plain and microsculptured beeswax rods.
(Data in Appendix - Table A 18)

ϕ Plain surface
o Microsculptured surface.

III

4. ABSORPTION BY WAX RODS OF DIFFERENT LENGTHS

To show the effect of altering the length of the wax rod and therefore the surface area available for absorption, a number of rods of various lengths were exposed to nicotine vapour for 20 minutes at different gas velocities, after which they were assayed for nicotine (Fig. 30).

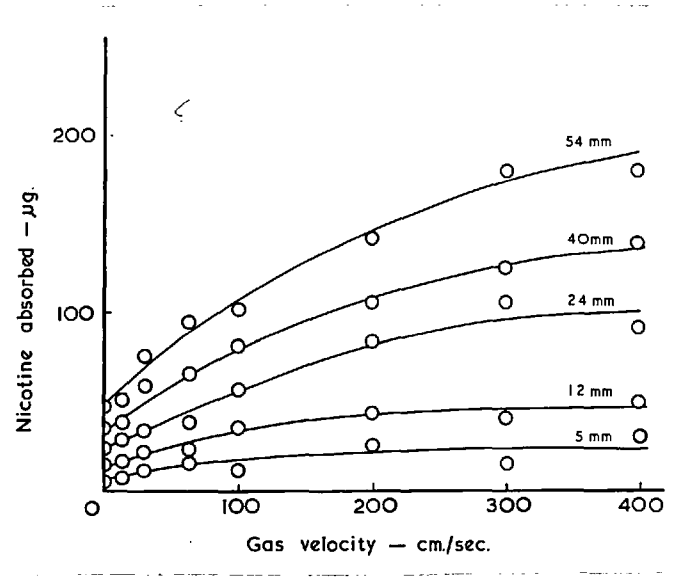

Fig. 30 Absorption of nicotine by beeswax rods of different length with change in gas velocity.
(Data in Appendix - Table A19)

From this experiment further evidence may be derived that the term saturation velocity is in agreement with the definition that it is the velocity at which the rate of absorption reaches an upper limiting value. It will be seen that if the maximum values for the quantities of nicotine absorbed by each rod are extracted from the curves of Fig.30

and are converted to the quantities absorbed per unit area in unit time, the figures arrived at are 1.4, 1.4, 1.3, 1.3, and 1.3 $\mu g/cm^2/min$, which compare well with 1.2 $\mu g/cm^2/min$ from the first experiment in this section. Since the experiment covers a wide range of wax areas, 0.88 cm^2 to 7.04 cm^2, it would appear that this rate is indeed the fastest rate at which nicotine can be absorbed by beeswax from the gas phase.

The results from this experiment also illustrate the dependence of absorption on fluid dynamic conditions and the importance of the boundary layer. From the inspection of the curves shown in Fig. 30, it may be seen that saturation velocity is lower for the shorter rods. If the ends of the rods are neglected (in practice the front end is probably absorbing at the maximum rate at quite low velocities) and the side of the cylinder is pictured as a plane surface lying parallel with the air stream, a boundary layer of a thickness determined by the velocity is established along the length of the surface. Now let the maximum rate of absorption occur over length l when the boundary thickness is equal to or less than the distance d in Fig. 31. As the wind velocity is increased, so the boundary layer decreases in thickness and the distance l is increased. Therefore, at low wind speeds only a small portion at the upstream end of a rod is absorbing at its maximum rate, and thus for shorter rods the saturation velocity is lower than for longer rods.

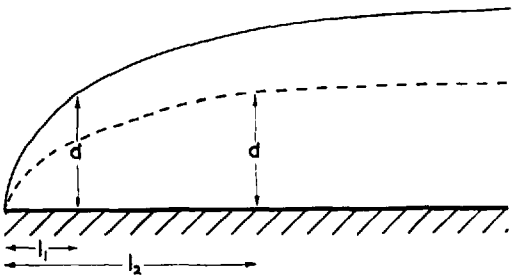

Fig. 31 Typical boundary layer profile formed above a plain surface lying parallel to a gas flow

——————— Freestream velocity u_1

- - - - - - - - - - Freestream velocity $u_2 > u_1$

(Vertical scale exaggerated tenfold)

In practice the total absorbed by a rod includes the quantity absorbed by the end faces. It was mentioned above that the front face is probably absorbing at a relatively low gas velocity. The downstream face is in a turbulent wake formed from air that has already lost much of its fumigant in passing down the length of the rod, and will thus approach the maximum rate of absorption slowly.

III

5. EFFECT OF AIRING AFTER ABSORPTION OF NICOTINE

It was recorded in Part II that slightly lower mortalities were found among houseflies that had been exposed to a current of clean air after fumigation than among the insects allowed to recover in a muslin capped specimen tube. With this in mind it was proposed to find out if nicotine was lost

from beeswax by airing after fumigation.

Since, in separate fumigations, some variation occurs in quantities of nicotine absorbed, comparison between rods fumigated individually is undesirable. Thus, for this experiment rods 40 mm long x 4 mm diameter were fumigated and after fumigation they were halved; one half was assayed for nicotine immediately and the other half aired in a 400 cm/sec air stream for a certain time before assay. The wax rods were exposed to fumigant at 350 cm/sec for the following reasons. Above the saturation velocity for beeswax nicotine is absorbed at a steady rate over all the exposed surface and total absorption by the two halves is, therefore, found to agree experimentally within 10% at 350 cm/sec. The result of airing one of the pieces is shown in Fig. 32 where it may be seen that a considerable quantity of nicotine is lost, the residue being perhaps held irreversibly by the fatty acid in the wax.

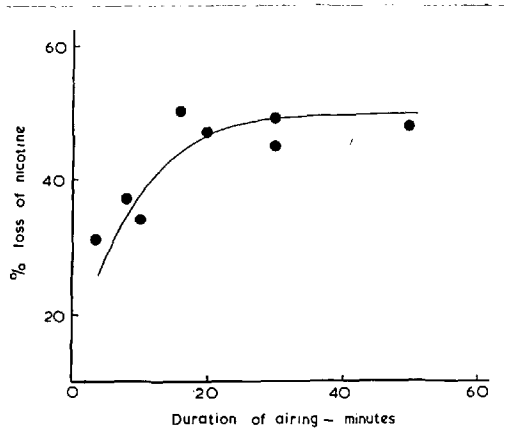

Fig. 32 Loss of absorbed nicotine during airing.
(Data in Appendix - Table A 20)

6. ABSORPTION BY DIFFERENT INSECT CUTICLES WITH SIMILAR SURFACE AREA

It has been shown that nicotine accumulates in the epicuticular waxes and that the saturation velocity varies with each species investigated. It follows from the different saturation velocities recorded that the cockroach is able to absorb the fumigant faster than the housefly which in turn is able to absorb faster than Oryzaephilus. This difference in absorption rate would appear to be a reflection of the absorbing power of the epicuticular waxes. The wax on the epicuticle of the roach takes the form of a mobile grease, while that of Oryzaephilus is a much harder and higher melting point wax. Generally, stored product insects and many others living in dry surroundings have hard waxes. The housefly wax is likely to be intermediate between these extremes, and it seems reasonable to suppose that the solubility of nicotine in the mobile wax of the roach would be greater than in the harder wax of the housefly, and even greater than in the hardest wax of Oryzaephilus. Indeed, it has been shown that DDT crystals dissolve more readily in the softer epicuticular waxes and that insects with a soft wax are more susceptible to this toxin than those with harder waxes, (Pradhan, et al, 1952).

Since the rate of diffusion of a substance through a solid is generally proportional to the solubility of the substance in that solid (Barrer, 1951), it follows that the

rate of diffusion of nicotine into the epicuticular waxes is greater for the cockroach, intermediate for the housefly and least for Oryzaephilus. This is precisely the arrangement shown by the saturation velocities. As gas velocity increases, more nicotine is made available at the surface and this continues to be removed by the roach at velocities greater than that found for either of the other two insects. Thus, the rate of removal from the surface by the roach wax and therefore the rate of diffusion through this wax appears to be greater than it is for the housefly, which in turn is greater than in Oryzaephilus.

It seemed expedient at this juncture to measure the basic rate of uptake for each insect cuticle. Before this could be done, however, a knowledge of the respective cuticular areas was desirable. It is fortunate that accurate determinations of epicuticular surface areas have been made for parts of insects similar to those used here. The true surface areas of Periplaneta forewings, Musca wings and Tenebrio elytra have been shown to be larger than their apparent projected areas by factors of 8.2, 2.3, and 6.7 respectively (Lockey, 1960). Accordingly, sufficient wing material was accumulated for the determination of absorbed nicotine after fumigation. In order that the whole surface should be available for nicotine absorption, the insect material was threaded on fine stainless steel pins (inset Fig. 33). The increase in the surface area

where the material is pierced by the pin is small in comparison with the total surface even for housefly wings. Fumigant penetration to the internal surfaces of the wings through the pin lesion is likely to be a slow process, if it occurs at all, and may be ignored for short exposure times.

Absorption of nicotine from still air per square centimetre of true surface is shown in Fig. 33, the true surface being calculated from the projected areas, measured prior to fumigation, increased by the factors given above.

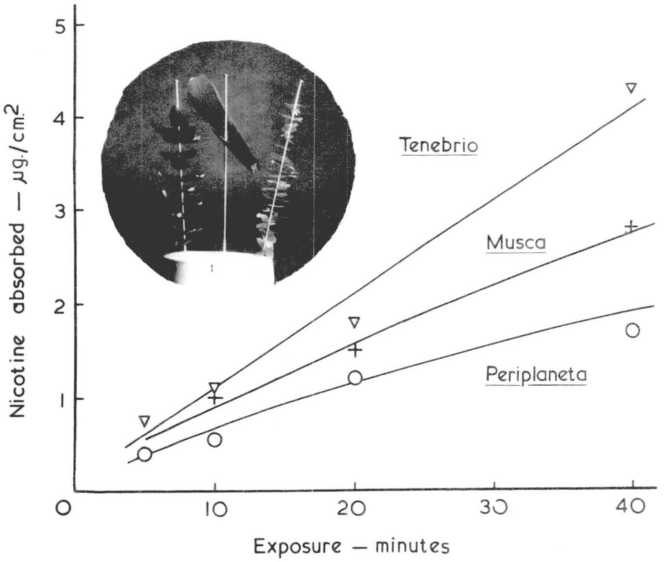

Fig. 33 Absorption of nicotine by insect cuticles. (Inset: method of support) (Data in Appendix - Table A 21).

o Periplaneta forewings
+ Musca wings
v Tenebrio elytra.

The rates of absorption found were the reverse of those expected. In unit area, most nicotine is absorbed by Tenebrio and least by Periplaneta cuticle. This may be caused either by Tenebrio wax absorbing nicotine at a rate higher than that of the other samples or possibly by the underlying Tenebrio cuticle taking up nicotine from the wax layer at the greater rate. In the following experiment the solubility of nicotine in these and other waxes was determined and showed that the former reason was more likely.

III

7. ABSORPTION AND DESORPTION OF NICOTINE AND DDVP BY WAXES

It has been suggested that the first stage in the pickup of solid insecticides is by simple solution in the epicuticular wax (Armstrong, et al, 1952); (McIntosh, 1957). This is generally accepted. However, there is little information available on the capacity of epicuticular waxes for dissolving either fumigant or solid insecticides. Bond (1959) showed the epicuticular waxes of Calandra to contain 1.1 µg/g of insect of hydrogen cyanide following a 4-hour fumigation at 8 mg/l (at a rough estimate the amount of hydrogen cyanide absorbed is not more than 0.01% w/w in the wax). The capacity of epicuticular waxes to hold fumigants in solution is relevant to any discussion concerning fumigant absorption, and on this account the solubility of nicotine and DDVP in a number of waxes was

determined. The method used was as follows.

The epicuticular waxes were extracted from the dead intact insects, contained within a filter funnel, by three quick washings with hot chloroform. The filter paper was previously cleaned, also with hot chloroform. The filtrate was concentrated and evaporated to dryness in a small tube on a hot water bath. When dry and cool a polythene cap was fitted and the wax stored until required.

A fine glass capillary tube was drawn and cut into 4 cm lengths. One end of each of these lengths was sealed in a flame and the last 5-6 mm bent over to form a hook, after which the remaining end was also sealed. The tubes were hung by their hooks from a wire frame and were carefully washed with hot chloroform and hot water and dried in a dust free area; subsequent handling was carried out with a pair of forceps to avoid transfer of unwanted material to the glass. After weighing, each tube was carefully coated with a thin layer of an epicuticular wax by smearing a small quantity of wax on the lower 3 cm and gently warming over an alcohol flame until the wax had formed an even film over the glass. When cool, the tubes were weighed again to determine, by difference, the amount of wax on each. All weighings were on a Cahn microbalance reading to 0.5 µg. Shellac was applied to two tubes by dipping them in a solution of shellac in chloroform and allowing the chloroform to evaporate. By repeating this process an appropriate thickness of shellac

was built up. Glass capillary tube, rather than thin rod, was used to reduce the weight of wax support to a minimum, thus enhancing the sensitivity of the balance.

Fumigant solubility was measured by placing the waxed tubes in a glass jar at 20°C with a nicotine atmosphere of about 5 µg/l. They were removed individually for weighing at increasing intervals of time until little further increase in weight occurred, at which time the fumigant atmosphere was removed and the fumigant allowed to desorb in dust free air. Weights were again recorded at increasing intervals of time until a constant weight was attained. A similar procedure was followed for exposure to DDVP, the fumigant being generated by 'Vapona' pellets. Plain glass tubes showed no increase or decrease in weight. The quantities of nicotine and DDVP absorbed and the amount remaining in equilibrium with pure air are shown in Figs. 34, 35, 36 and 37; the fumigant sorbed is expressed as a percentage of the original weight of wax and is plotted with respect to time. Complete saturation with nicotine was not reached, the experiment was terminated at this point, however, as the Tenebrio wax had taken up so much nicotine that a fluid drop had formed at the bottom of the tube which threatened to drop off when handled.

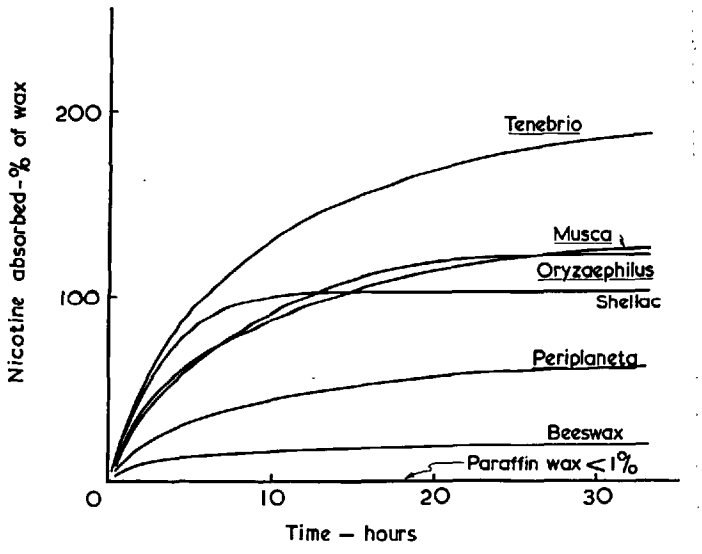

Fig. 34 Absorption of nicotine by waxes. (Data in Appendix - Table A 22)

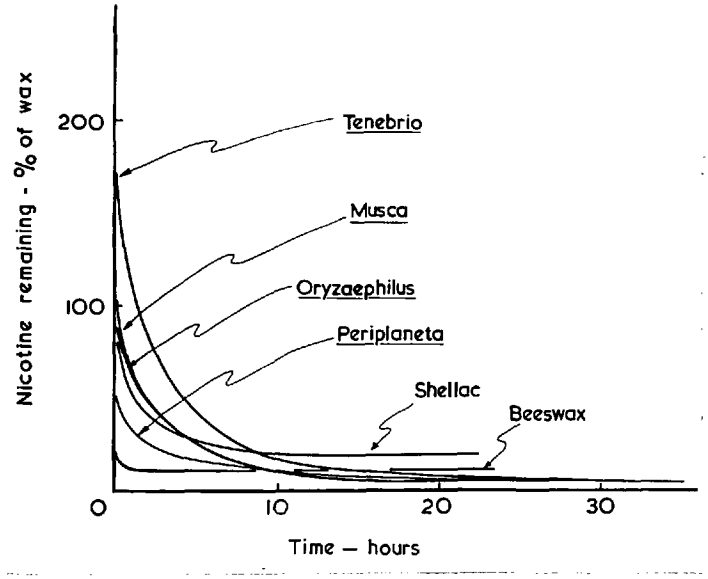

Fig. 35 Desorption of nicotine from waxes. (Data in Appendix - Table A 22)

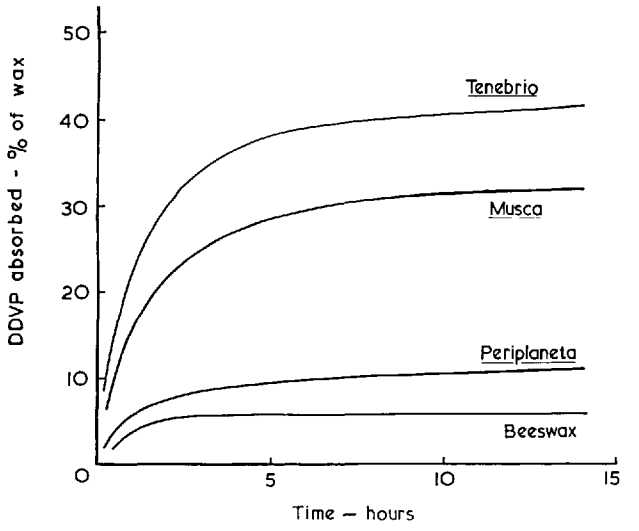

Fig. 36 Absorption of DDVP by waxes. (Data in Appendix - Table A 23)

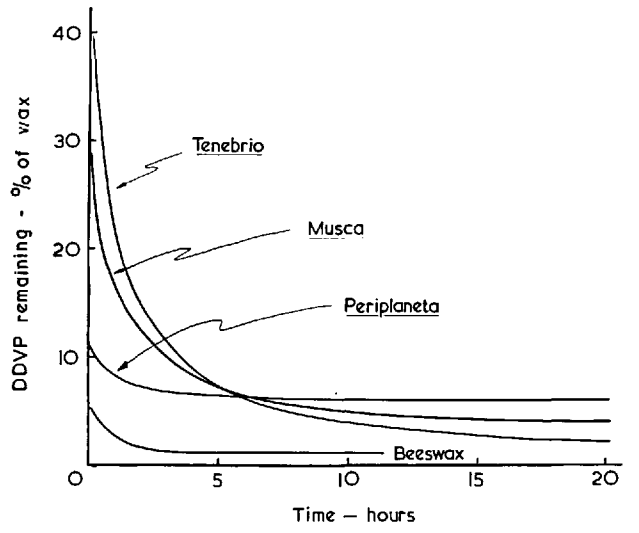

Fig. 37 Desorption of DDVP from waxes. (Data in Appendix - Table A 23)

The results of these experiments are in agreement with the previous experiment and show that the harder epicuticular waxes are capable of absorbing more nicotine than softer waxes.

The same order of solubility was also found for DDVP, though with this fumigant the total quantities taken up are appreciably less than for nicotine.

There appears to be little difference in the relative proportions of nicotine absorbed by the extracted epicuticular waxes in this experiment, and those found in the previous experiment where the waxes remained in situ. Calculations of the thicknesses of the extracted waxes as they occur on the glass tubes indicate that they were about 10 times the naturally occurring thicknesses. Equilibrium will be established more slowly in a thick layer of wax which may account for the differences in the initial rates of absorption exemplified by the shellac film about 0.1 micron thick, and the Oryzaephilus wax film about 1 micron thick.

III

8. SOME PHYSICAL PROPERTIES OF THE EPICUTICULAR WAXES

Epicuticular waxes are known to be complex mixtures of long chain fatty acids, alcohols, paraffins and esters in various proportions, and have been recently reviewed by Locke (1964) and Hackman (1964). Composition has been fully investigated in the American roach (Gilby and Cox, 1963) and

the mormon cricket (Baker, et al, 1960). In this section a number of the physical properties that might affect the solubility of fumigants in the waxes is described. An important one is the softness of the wax (Pradhan, et al, 1952), and another, more important possibly for nicotine which is a basic compound, the acidity of the wax. These two properties were determined for the epicuticular waxes used in these experiments by modifications of the basic methods described by Fryer and Weston,(1918). The wax was obtained by washing the insects in warm chloroform as before.

Capillary tubes were prepared, each containing a small sample of wax. The ends were sealed and each capillary in turn was fixed beside a thermometer bulb and lowered with the thermometer into a water bath. The wax was observed with the aid of a low power binocular microscope while the bath was slowly warmed with a microburner. A stirrer maintained an even temperature and aided heat transfer to the thermometer and capillary.

Waxes, being complex mixtures of hydrocarbons, do not possess precise melting points as found for pure chemical compounds, but a melting range, during which, as the temperature is raised, they undergo a number of transitional states. The more varied the components, the more amorphous is the wax and the wider the melting range. The temperature of two states may be determined readily; these are the temperature of incipient fusion when the wax softens, begins to clear and

usually forms a meniscus when confined in a capillary tube, and the temperature of complete fusion at which the wax becomes a completely clear liquid. Between these temperatures both opaque solid and clear liquid coexist, the solid portion being the higher melting point components.

With the exception of Periplaneta wax, which immediately after extraction was a pale yellow grease, the waxes from the other insects all had similar melting ranges. Incipient fusion occurred at 34°, 37° and 38° respectively for Musca, Oryzaephilus and Tenebrio, while complete fusion occurred only above 100°C in all cases. Periplaneta wax, after standing for a few weeks, gave similar figures for the melting range, 34° incipient fusion and complete fusion above 100°C. Musca and Oryzaephilus waxes were opaque and yellow, clearing at about 110°C to clear yellow liquids. Tenebrio wax was opaque and white, clearing to a colourless liquid at about 110°C.

The acid value of a wax is a measure of the free fatty acids present in the wax and is obtained simply by titration of a solution of the wax in a suitable solvent, with aqueous caustic potash or soda; the acid value is defined as the number of mg of caustic potash required to neutralise the acid present in 1 gm of wax.

Only small quantities of wax were available and the titration using phenolphthalein as an indicator was carried out in a small glass specimen tube standing on a white tile.

0.1 N caustic soda was added with a 10 µl Hamilton syringe to a weighed quantity of epicuticular wax, dissolved in 2:1 alcoholic benzene. The respective acid values were Periplaneta 40, Musca 80, Oryzaephilus 43, Tenebrio 160, beeswax 12, and shellac 47. These values may not be very precise as, owing to the small amount of wax available, the blank titration formed a considerable proportion of the total reading for waxes low in free acids. However, the accepted values for beeswax and chinese insect wax (similar to shellac) are of the same order, namely, 17-21 and 63 respectively, and it may be taken that the values determined in this experiment are not grossly exaggerated.

The solubilities of the fumigants in these waxes are more closely correlated with the acid value than with the melting point. Why this should be is not certain; it may be that the fatty acids of the waxes are the only components which take part in the solution, though this would seem unlikely as alcohols and esters are not generally poor solvents. The correlation may be purely fortuitous.

III

9. THE ABSORPTION OF FUMIGANTS BY LIQUIDS

It has been assumed previously that fumigant is made increasingly available as the wind speed in the exposure chamber is raised, and it has been argued from this that the exponential behaviour of the absorption and toxicity curves are caused by slow fumigant penetration in the absorbing

median. This would seem to be a reasonable assumption, especially as fumigant concentrations are maintained at the higher flows. However, in order to show that this hypothesis is correct, flow fumigations were carried out using liquids as the absorbing media. Provided the volume of liquid is large compared to the quantities of fumigant absorbed, this medium should present a negligible barrier to absorption compared with the solids used previously.

Fumigations over a number of velocities up to 400 cm/sec were carried out in a nicotine concentration of about 10 µg/l. A small glass cup containing 0.2 ml dilute sulphuric acid was placed within the exposure tube. Nicotine vapour was absorbed by the acid, which, after a 20 minute exposure was washed into a separating funnel and assayed for nicotine as described in Part I.

Hydrogen cyanide fumigations were carried out in a similar manner to those for nicotine. The gas was absorbed in 0.2 ml of 0.7% aqueous sodium bicarbonate in the same cup that was used for nicotine absorption. Exposures were for 3 minutes at a concentration of 1.7 mg/l, after which the bicarbonate was washed into a small glass vessel and titrated with iodine, as described in Part I.

DDVP was absorbed in n-decane exposed to the fumigant in depressions (15/64" deep x 15/64" diam.) drilled in a piece of perspex measuring 1" x 2" x ¼". After each fumigation the remaining decane was assayed for DDVP by direct injection of

a known amount into the GLC apparatus (see Part I). Approximately 10% of the decane evaporated during the fumigation (the exact amount depended on the velocity) and as the final volume could not be estimated accurately enough for the total DDVP absorbed to be calculated, the DDVP present is expressed as parts per million of the decane sampled.

The velocities for nicotine and hydrogen cyanide absorption have been corrected by a factor (x 1.59, or 177/111) which is the ratio of the cross sectional area of the tunnel to the area remaining after inclusion of the glass vessel, and gives a more correct value for the velocity over the liquid surface. The results are shown in Figs. 38, 39 and 40, from which it is evident that as the gas velocity is raised fumigant is made increasingly available to systems capable of absorbing them.

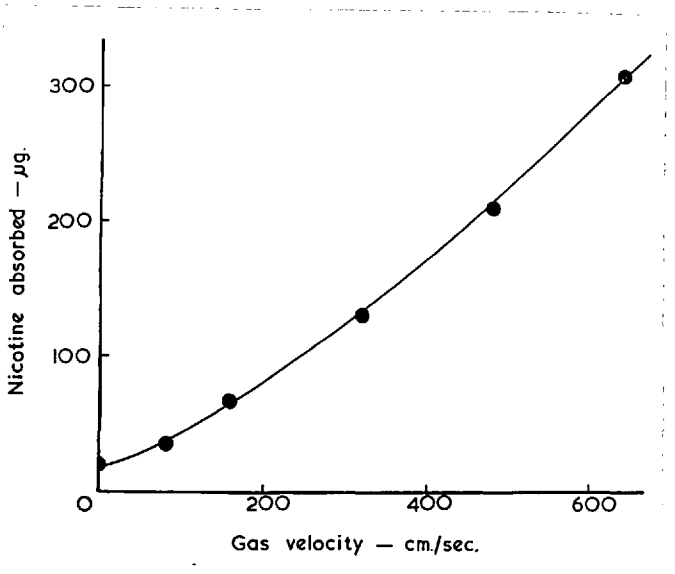

Fig. 38 Absorption of nicotine by dilute sulphuric acid with change in gas velocity.
(Data in Appendix - Table A 24).

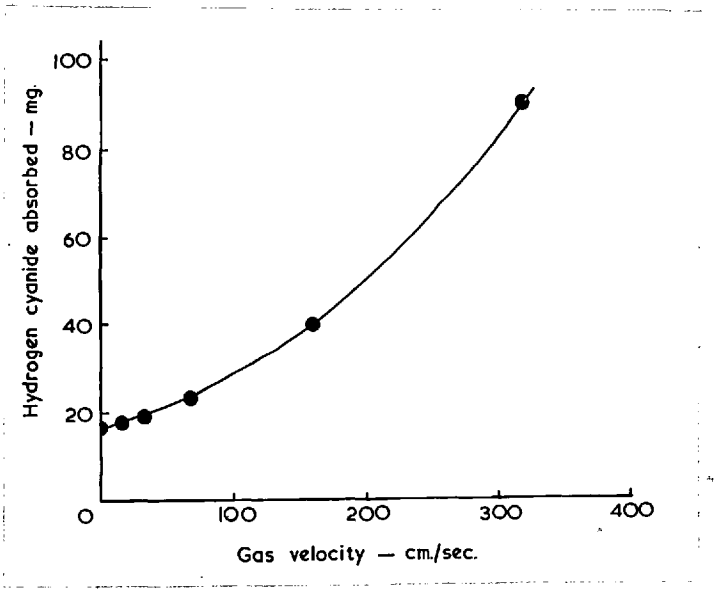

Fig. 39 Absorption of hydrogen cyanide by dilute aqueous sodium bicarbonate with change in gas velocity. (Data in Appendix - Table A 25).

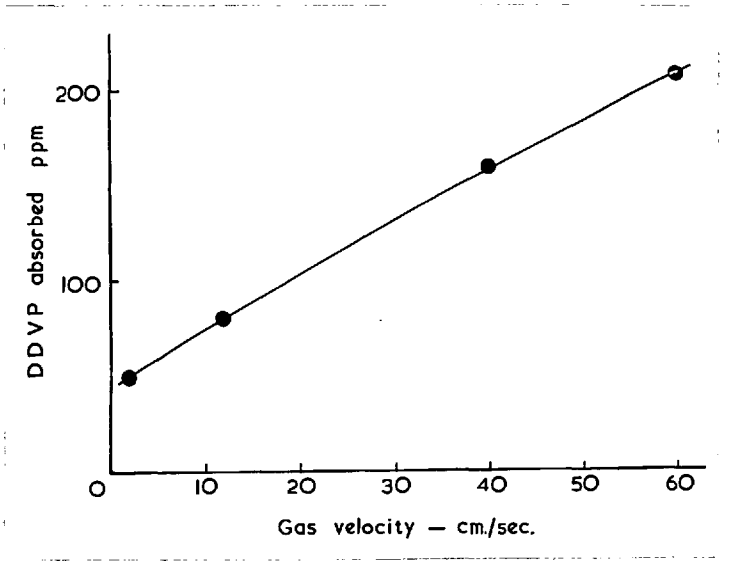

Fig. 40 Absorption of DDVP by n-decane with change in gas velocity. (Data in Appendix - Table A 26)

The curves for nicotine and hydrogen cyanide absorption show a small increase in the rate of absorption as the gas velocity increases, contrary to the exponential decrease expected from a reduction in boundary layer thickness. This type of absorption in which the curves are convex to the velocity axis, is most probably caused by the amount of stirring, induced by the air movement, in the liquid sorbents. Movement in the liquid was seen to increase at higher flows, though a measure of the degree of mixing could not be made. It is evident that minor barriers to absorption also occur in liquids.

III

10. DISCUSSION

To avoid confusion one criterion that has been withheld from discussion of saturation velocity so far is that of the Reynolds number. For a solid moving through air a dimensionless relationship exists between the wind speed, a linear dimension of the solid and the air viscosity, which is known as the Reynolds number and is equal to $\frac{ux}{v}$.

It was shown in the introduction to this thesis that the boundary layers were denoted by equations in which this relationship played a prominent role. Now it can be shown that for solids of varying size, but similar shape and orientation, the boundary layer conditions at any given Reynolds number are similar. Thus, for a small object, one

Reynolds number is attained at a higher velocity than for a larger object. Similarly, if the saturation velocity is reached at a Reynolds number descriptive of the appropriate boundary condition, then for small objects the windspeed at which this is attained is higher than it is for larger objects. Some separation in the saturation velocities was found to occur in practice, but the following arguments show that in these experiments this separation is more likely to be caused by differences in absorptive properties than by the respective sizes of each insect.

The saturation velocity for Oryzaephilus was found to be much lower than that for the other insects tested. This order of velocities can only be caused by the upper limit to the rate of fumigant uptake by this insect being lower than that in the other species, since if the limit to the rate of absorption were to be the same in all cases, the saturation velocity would increase as the size was reduced. An indication that size was not the limiting factor is provided by the experiment in which the epicuticular waxes of Oryzaephilus were replaced with beeswax. The normal change in the rate of absorption with increasing velocity was altered by the beeswax coating, whereas if size had been the governing factor in restricting the saturation velocity the beeswax coating would not affect the shape of the absorption curve though the quantity of fumigant absorbed might have been altered.

Another indication that size is not the prime factor limiting the rate of absorption at the surface is provided by

the experiment in which beeswax and paraffin wax rods were fumigated. The absorption rates of the two waxes in rods of identical shape are widely separated both in the quantity absorbed and in the velocity at which the limit is attained. Again, since size is constant, the absorbing property of each wax is the major factor in determining the limit to the rate of absorption.

The saturation velocities for the housefly and cockroach were also shown to be different. The cockroach nymphs were of a comparable size to the houseflies and any difference in saturation velocity is probably caused by their respective maximum absorption rates. The difference in microstructure is probably not important in view of the results reported in this section on the effects on sorption of a sculptured surface. Also, a hairy surface would be expected to induce a thicker boundary layer, the hairs being supplementary to the viscous property of the air in reducing the velocity near the surface, and given similar rates of absorption the free stream velocity at which saturation occurs must be higher for a surface of this kind than for a smooth surface. The reverse was found experimentally; the saturation velocity for the rough surface (housefly) was lower than the saturation velocity for the smoother surface (cockroach).

Beeswax rods showed a reduction in saturation velocity as length was reduced, but this is not strictly comparable with a true size reduction. Only one dimension was altered

in the experiment whereas the Reynolds number concept requires that all three dimensions should change proportionately. It is concluded, therefore, that in all these experiments the absorptive capacities of the exposed surfaces, rather than effects of size, were the major factors in determining saturation velocity in each case.

The relationship between epicuticular waxes derived from insects and their capacities to absorb the fumigants, nicotine and DDVP, is of interest. Contrary to previously recorded data (Pradhan, et al, 1952) both nicotine and DDVP were found to be more soluble in hard than in soft wax though the data from the above authors may be misleading in this context. It is reported that the solution of DDT crystals in the soft epicuticular wax of the Lepidopterous larva Euproctis lunata was much more rapid than in the hard wax of Trogoderma granaria, a phenomenon that may equally well be caused by a corresponding difference in the availability of the crystals which were found to sink more quickly into soft than into hard wax. Here, too, absorption was from a solid phase which is likely to differ appreciably from fumigant absorption. The rate of absorption of nicotine per unit area was very much greater by the epicuticular wax in which it was more soluble, than it was for the less active waxes, which is in agreement with the general rule relating rates of diffusion

and solubility. That waxes with similar melting points should show such diverse properties is, perhaps, not surprising when it is remembered that the relative proportions of the component compounds vary considerably - see, for example, the differences between the waxes of two insects more closely related than those used in this study - the mormon cricket (Baker, et al, 1960) and the American roach (Gilby and Cox, 1963). A summary of the proportions of the major constituents is given below: (Table III, Summarized from Hackman, 1964):

Chemical Group	Proportion present per cent wax	
	Cricket	Cockroach
Hydrocarbons	48-58	76.7
Free Acids	15-18	7.2
Aldehydes	- -	8.9
Esters	9-11	5.0

It has been demonstrated that the saturation velocity with nicotine for the stored product beetle Oryzaephilus is less than that for either the housefly or the cockroach, indictating that the fumigant is more soluble in the softer wax of the cockroach than in the housefly or the beetle, a situation later shown to be untrue. The reason for this is revealed when the epicuticular waxes are studied in greater detail.

The epicuticular wax layer is divided into three distinguishable parts; on the inside is a layer in which the wax molecules are tightly packed and orientated by the underlying polyphenol layer. At the surface a 'cement' layer exists

which is considerably harder than the remaining wax and is also very thin, at most a few molecules deep. Between these layers lies the bulk of epicuticular wax in which the molecules are less well ordered than at the boundaries. (Wigglesworth, 1945 and 1948; Beament, 1955). It is the cement layer that is the concern of the present discussion. Beament (1955) suggests that shellac is mainly cement from the lac insect while Locke (1964) likens the cement layer to varnish. The thickness of this outer layer is known to vary considerably, being thickest in the exposed cuticles of many beetles and thinnest in the mobile layer of the cockroach, as first demonstrated by Ramsay (1935 b). It is intuitive that these outer layers are likely to present a greater resistance to the penetration of fumigant molecules than the remaining wax, and that the thicker, harder cement of a beetle will be traversed with less facility by the fumigant than the outer layer of opposite extreme found in the cockroach.

In the argument that follows it is assumed that Tenebrio and Oryzaephilus have cement layers of a similar order of thickness and that the epicuticular waxes of Periplaneta and Supella are also similar to one another, a supposition which is not unreasonable.

Consider the absorption process by two contrasting epicuticular waxes, one a hard wax with a thick cement layer and the other a soft wax with a thin cement layer at the

surface.

When the rate of absorption is low, uptake will be governed primarily by diffusion influenced by the partial pressure difference between the fumigant in the air mass and at the cuticle surface. The partial pressure difference will be greater in the wax through which the fumigant is able to pass more readily, i.e., the harder wax, and uptake will be proportionately greater.

As the fumigant/air mixture is moved at increasing speed over the surface, the fumigant becomes more freely available and waxes continue to absorb in the order given above untilaa stage is reached at which the fumigant transfer through one of the cement layers is at a maximum. It is most likely that the cement layer on the hard wax will be the first to be effected in this way and at this point an increase in the availability of the fumigant will have little further effect on the rate of absorption by that cement layer and the underlying epicuticular wax. The softer wax with a smaller barrier at the surface will continue to absorb fumigant more quickly until the limiting rate is attained. Under these circumstances, this type of insect would be expected to possess the lower saturation velocity, a phenomenon which was found to occur in practice, and it follows from this argument and from the experimental results that the housefly possesses a cement layer that is intermediate in permeability and structure between that of the beetle and the cockroach.

The cement layers were also shown to modify the rate of absorption to a considerable degree in still air conditions. Comparison of the rates of absorption of epicuticular waxes in situ with their absorption rates when extracted and coated on glass shows that absorption by the extracted samples is more rapid. In the extracted waxes, the components of the cement layer are incorporated in the bulk of the wax by the extraction process, and thus there is no relatively impermeable barrier at the surface. After 40 minutes exposure, absorption by the waxes of Periplaneta, Musca and Tenebrio, in situ is 1.7, 2.8, and 4.3 $\mu g/cm^2$ respectively, and for the extracted waxes the figures are approximately: 14, 62 and 148 $\mu g/cm^2$ respectively. It is evident from these figures that the rate of absorption in the natural state is considerably less than that found for extracted material, and that the relative rates of absorption for each species of natural to extracted waxes (Periplaneta 1:8, Musca 1:22 and Tenebrio 1:34) indicates that the cement layers restrict nicotine uptake in proportion to their probable thicknesses.

The difference in thickness of the wax layers could cause this inequality but it is argued that absorption by natural waxes had not departed seriously from linearity with time and therefore saturation and an accompanying slowing down of the rate of absorption was not approached. Calculation of the maximum amount of nicotine that could theoretically be absorbed by the epicuticular waxes in situ also shows that the

waxes in this experiment have not absorbed more than 30% of the maximum possible. (The maximum amount of nicotine expected in situ was calculated from the total quantity that the waxes are known to be able to absorb - Fig. 34 - and the amount of wax present on each cuticle sample derived from Lockey, 1960).

A little nicotine appears to be lost from flies forcibly aired after fumigation. It also seems likely that a proportion is lost in still air, though no experiments were devised to show this, for the following reasons. Desorption of nicotine from epicuticular waxes derived from insects was shown to be a considerably more rapid process than the initial absorption (Figs. 34 and 35). This experiment was carried out in still air. The median lethal dose accumulated during an MLT exposure of houseflies was shown in an early experiment to be increased slightly at the higher velocities. It is conceivable that this is because more nicotine is lost from these insects during the initial stages of the subsequent recovery period. At low velocities the duration of exposure is longer than at high velocities, and it follows that the quantities of nicotine absorbed in this time will be distributed in greater depth in the cuticle and will be less readily desorbed than the more concentrated doses nearer the surface; in order to achieve the same toxic effect, i.e., a 50% mortality, more fumigant must be accumulated during shorter exposures than during longer ones. This reasoning is

supported by the results of the experiment in which insects were aired after fumigation. Though this experiment was not in itself conclusive, a trend was apparent which is in agreement with the other results discussed above.

At this stage it is possible to introduce a tentative explanation for there being no increase in the toxicity of hydrogen cyanide when this fumigant is passed over insects at increasing velocities.

If a graph is plotted of fumigant concentration against the time required to produce a 50% mortality, a relationship such as that shown in Fig. 41 is found.

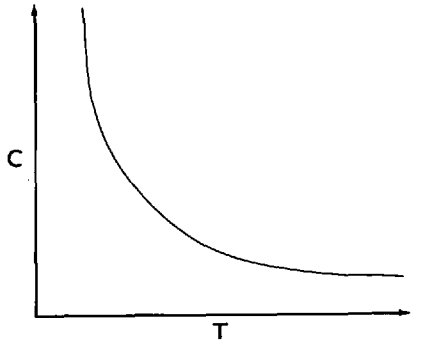

Fig. 41 Typical concentration vs time relation for CT_{50} of hydrogen cyanide.

At a certain low concentration, the insect is able to survive unharmed. Small increases in concentration, above this level, bring about large reductions in the median lethal exposure times which decrease as the concentration is increased. Eventually, a concentration is reached where further increase

has little effect in reducing the MLT. Absorption by an insect which will absorb fumigant readily, but to which an upper limit is fixed with regard to rate of uptake, satisfies the above conditions. When the fumigant concentration is sufficiently high to cause the rate of uptake to reach this limit, increasing the concentration further has no effect. The effect of passing the fumigant/air mixture over the insect is similar to that of increasing the concentration, in that more fumigant is made available at the surface. In the experiments described in Part II, the concentration used was of the order of that used in practical fumigation procedure, and for many insect species this is close to the point at which further increase in concentration has no effect on toxicity. Thus, it follows that air movement will not be effective in increasing the fumigant's toxicity at these concentrations.

At this concentration the insect must be absorbing fumigant at the maximum possible rate, which is the same as that occurring at the saturation velocities with other fumigants used here, and this rate is determined by the insect system and not by the gas phase. Since hydrogen cyanide enters mainly by way of the respiratory system, absorption by this route must be at a maximum. Hence, any forced ventilation of the respiratory system, brought about by increasing the gas velocity, will not appreciably increase the quantities absorbed.

If the fumigant concentration is reduced to a level at

which small alteration causes considerable differences in the LT_{50}, it is conceivable that the fumigant's toxicity will be enhanced by movement of the bulk phase over the insect. This was found to occur in one unreplicated experiment, in which two consecutive 4 hour fumigations were carried out, one in slowly moving air and the other at a gas velocity of 100 cm/sec. The concentration of hydrogen cyanide was about 0.4 mg/l at the start of the first fumigation and had fallen to less than 0.2 mg/l at the end of the second. The resulting mortalities in 100 Oryzaephilus adults in each fumigation were 38% and 66% respectively. Unfortunately, it was not possible to maintain steady concentrations in the apparatus concerned for the long time intervals required, and no further quantitative estimations of the flow effect were made, owing to this difficulty of standardizing the conditions.

CONCLUSIONS

One cannot draw many general conclusions from this study in view of the small number of fumigants used, of which only one, namely, nicotine was studied in any detail. However, it is apparent that absorption of the low vapour pressure fumigants is governed by gas absorption laws as they relate to bulk movement of the gas phase. The reason for the movement being ineffective in increasing the toxicity of hydrogen cyanide is most probably that the concentrations found in practice are already sufficient for the insect to absorb the gas at the maximum rate. Thus, though absorption may be linear with time, any increase in the availability of the fumigant to the insect, caused either by an increase in concentration or by movement of the bulk gas phase, will not induce any further increase in the rate of absorption.

The rather intangible concept of the maximum rate of absorption is well illustrated in this study. There is a limit to the amount of fumigant that an insect can take up in unit time and this has been shown to occur at a definite gas velocity that has been termed a saturation velocity. Further study with a more detailed and critical approach might separate the effects of Reynolds number from the absorptive capacity of the surface in determining the saturation velocity. The wind tunnels used in this investigation fell short of the optimum from an aerodynamic standpoint. The

saturation velocity is not a fixed parameter for any one wax or insect; it indicates when the upper limit to the rate of absorption has been attained and this is as dependent on the concentration as on the boundary layer.

Further evidence is given for the restriction by the cement layer on the penetration of insecticides, and it is shown how a thick hard layer is more restrictive than a thinner one though the wax beneath the thicker layer is more receptive. The cuticle underlying the surface wax was shown to be an even greater barrier to the penetration of nicotine.

The technique of determining saturation velocities for a fixed set of conditions may prove to be a useful tool in assessing the relative importance of the constituent parts of the cuticle in regulating the entry of insecticides, and is suggested as a topic for further study. Estimations of the rate of penetration of substances applied to surfaces as liquids or solids are likely to be erroneous. Liquids may disrupt the lattice arrangement of the underlying components, a disruption being known to occur with epicuticular waxes: and solids, by virtue of their rigidity, present small and uncertain surfaces of contact to absorbing media.

By streaming the substance, as a vapour, over test surfaces and measuring the absorption which occurs with increasing gas velocity, an upper limit to the rate of absorp-

tion may be found, and by subjecting each constituent layer of the cuticle to this treatment, a composite picture may be obtained of the importance of each component in the conduction of insecticidal and other substances through the cuticle. It should be borne in mind in attempting a series of experiments of this nature that the upper limit to the rate of absorption found is that of the system as a whole. For example, a monomolecular layer which may be freely penetrated by any particular substance would appear falsely impermeable if situated over a less readily conducting substrate.

This technique is, of course, only applicable to substances which are able to exist in sufficient quantities in the vapour phase. The exact concentrations and velocities required depend on the rate of absorption, which in turn is affected by the affinity of the sorbent for the sorbate, and can be determined only by experiment.

Absorption of nicotine by the tracheal system appears to be a relatively unimportant mechanism of penetration, and it seems reasonable to suppose that this is true in general for fumigants capable of penetrating the integument in more than minimal quantities.

Conclusions from experiments reported here, which demonstrate little further absorption to occur when the tracheae are forcibly ventilated, apply only to two rather special cases. Nicotine was shown to enter mainly through the cuticle, and a large increase in tracheal absorption would be

required to add significantly to the total amount absorbed. Hydrogen cyanide was used at concentrations which were high enough to cause absorption to have closely approached the upper limiting rate, and for this reason an increase in availability could not appreciably affect the quantities absorbed. There were indications that at lower concentrations the toxicity of hydrogen cyanide was enhanced by increased gas velocity, and this would provide a fruitful field for further work.

One interesting observation, which may easily be extended to other insecticides that possess a measurable vapour pressure, is the relative solubilities found for DDVP and nicotine in the epicuticular waxes. Both these fumigants were more soluble in hard than in soft wax. The reasons for this are not clear; it may be that the more crystaline structure of the harder wax provides more or larger intermolecular spaces to be filled with fumigant molecules. Or it may be that the structure of the hard wax is able to swell as the fumigant is absorbed, thus making room for more solute in the hard than in the soft wax. Either possibility is open to further investigation.

A practical application stemming from this investigation may arise in the increasing use of compounds of low vapour pressure as spatial fumigants. The concentration of a substance of this nature in the vapour phase is inherently low and thus the rate of absorption of the substance by insects

will be below the maximum possible. Many of the insecticidal compounds with low though workable vapour pressures are more lipophilic than those with higher vapour pressures, and partly because of this property they are able to penetrate the insect integument more rapidly. With the introduction of stirring, insects exposed to air movement are able to absorb fumigant at a greater rate from the moving air. Insects buried in crevices in buildings or goods would not be greatly affected by stirring, but if the fumigant were to include an irritant, insects would be stimulated to move and in their wandering might emerge to where moving air would bring about a more rapid and more certain accumulation of a toxic dose than before. The advantages in persuading flying insects to take wing during fumigation with low vapour pressure fumigants are obvious. Flight would not necessarily have to be swift; inspection of the toxicity velocity curves for nicotine and DDVP shows that the greatest increase in effect occurs at the lower gas velocities.

The stirring of high vapour pressure fumigants to enhance toxicity, as distinct from penetration of goods, has been limited probably by their slow uptake by insects and at normally applied concentrations, increased absorption brought about by air movement is only slight.

Two problems exist, however, when fumigants of low vapour pressure are stirred. The first is that considerable losses are likely by sorption on to all available surfaces necessi-

tating the inclusion of a fumigant generator in the apparatus, and the second is that many of these fumigants are toxicalogically very active, and care would have to be taken in any scheme of this kind to guard against the accumulation of toxic residues.

SUMMARY

Three fumigant wind tunnels are described in which fumigant/air mixtures may be passed over test insects at controlled velocities. The effects of altering wind speed during fumigation with hydrogen cyanide, nicotine and DDVP were determined by assessing the relative toxicities of the fumigants to three insects species Supella supellectilium, Musca domestica and Oryzaephilus mercator at different flows.

The toxicity of hydrogen cyanide at normally applied concentrations was unaffected by the gas velocity during the fumigation. The toxicities of both nicotine and DDVP to three species increased proportionally with wind speed at the lower velocities, and approached asymptotically to an upper limit at a velocity characteristic for each insect; increasing the flow above this velocity had little further effect in increasing the toxicity.

Observations of the spiracles during the accumulation of a median lethal dose showed that the proportion of the time during which they were open and tracheal ventilation could take place was reduced as the velocity was increased. In theory, similar quantities might be taken up by the tracheae at different gas velocities since air movement would be expected to enhance fumigant transfer into the tracheae, thus offsetting the reduced time available for tracheal absorption.

The observed increase in toxicity at higher flows could not be attributed to behavioural factors and was shown to be caused by the increased absorption of fumigants. The gas velocity at which no further increase in fumigant toxicity occurred coincided with the limit to the increasing rate of fumigant absorption and was termed saturation velocity.

Nicotine was found to be absorbed more slowly by cuticle from which the epicuticular waxes had been removed than by normal cuticle. It was shown that the waxes acted as a reservoir in which a large proportion of the accumulated nicotine exerted a toxic effect after the fumigation period.

A saturation velocity was shown to exist for isolated waxes when these were fumigated at a number of wind speeds, and to occur when the rate of absorption by the substrate reaches an upper limit imposed by the rate of diffusion of the fumigant into the substrate. No similar saturation velocity was found when liquids were exposed to moving fumigant.

Both nicotine and DDVP were found to be more soluble in hard than in soft epicuticular waxes. Cement layers were found to be of some importance in restricting the rate of absorption, though the underlying cuticle was shown to provide an even greater barrier as nicotine accumulated in the epicuticular waxes during fumigation.

The acid values of the epicuticular waxes from the insects used were shown to increase with wax hardness, though

the melting ranges were not found to differ markedly. The solubility of both nicotine and DDVP was greater in those waxes with the higher acid numbers.

Absorption of fumigants by liquid systems was shown to continue to increase with gas velocity and not to approach a limiting value as it does for insects and isolated waxes.

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APPENDIX

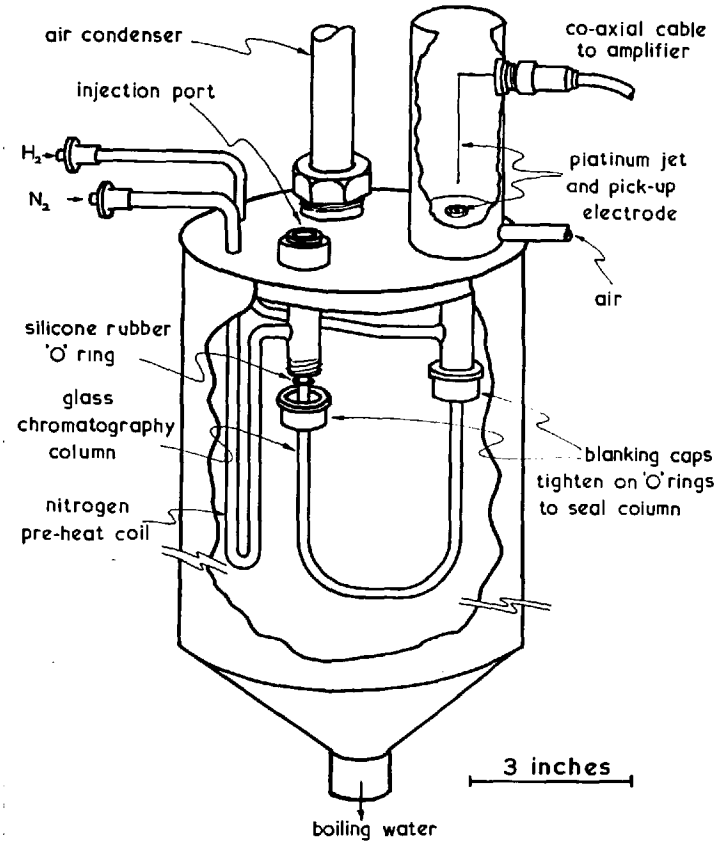

Fig. 42 Cut away drawing of part of GLC apparatus showing lay out and method of securing columns.

All parts of brass or copper unless otherwise stated.

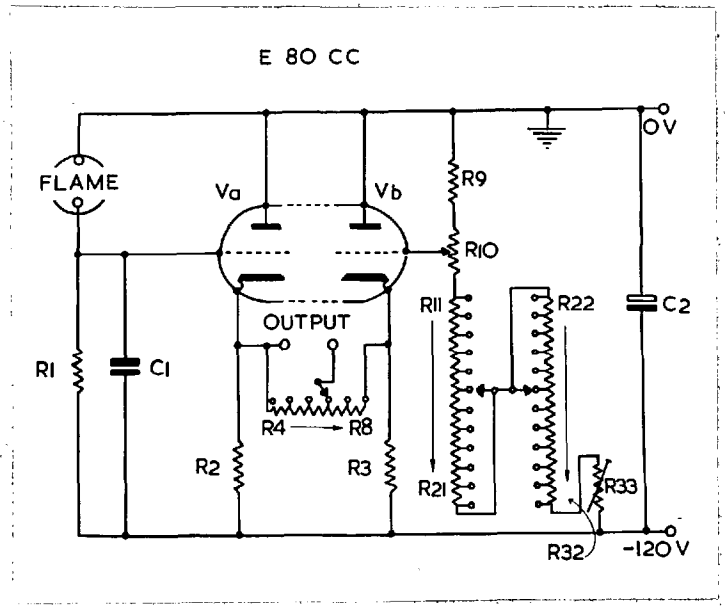

Fig. 43 Circuit diagram of Cathode-follower impedance converter. (Modified from Smith 1960)

Components.

Resistors ½W		Capacitors	
1	1 KMohm	1	.005 mfd paper
2,3	51 Kohm	2	1.0 mfd electrolytic
4,5	24 ohm		
6	51 ohm		
7	100 ohm		
8	200 ohm		
9	150 Kohm	Valve	
10	50 ohm w/w pot.	Va,Vb,	E80CC
11-21	20 ohm		
22-32	200 ohm		
33	100 Kohm preset		

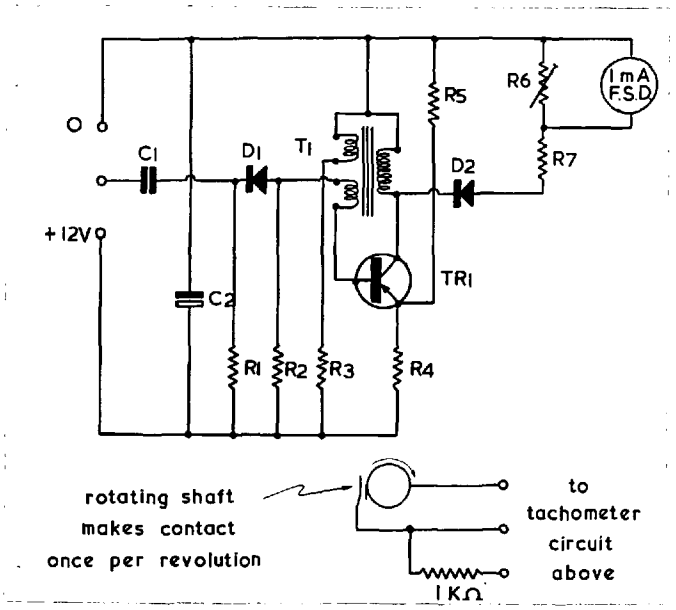

Fig. 44 Tachometer circuit for measuring motor speed in wind tunnel III.

COMPONENTS

Resistors ½W

1,2	270	ohm
3	125	ohm 5W
4	56	ohm
5	1	Kohm
6	250	ohm w/w pot
7	1.9	Kohm

Capacitors

1	0.001	mfd. ceramic
2	100	mfd. electrolytic

Pulse transformer
Deakin Phillips Electronics Ltd.,
Staines, Middlesex.

Semi-conductors

D1	OA 81
D2	OA 5
TR1	OC 71

IN ALL TOXICITY DATA: N = number of insects used
R = mortality 24 hours after fumigation.

Table A1
Toxicity data supporting Fig. 12

HYDROGEN CYANIDE FUMIGATIONS

Concentration 5 mg/l.

Supella supellectilium

Time minutes	Gas velocity cm/sec. 5		95	
	N	R	N	R
1.0	30	4	30	5
1.5	30	10	30	15
2.0	30	23	30	19
3.0	30	26	30	26
4.0	30	29	30	28
8.0	30	30	30	29
	χ^2 2.89		χ^2 2.98	
	b = 5.02 ± 0.69		b = 3.54 ± 0.52	
	m = 0.22 ± 0.03		m = 0.21 ± 0.03	

Oryzaephilus mercator

Time minutes	Gas velocity cm/sec. 5		95	
	N	R	N	R
2.0	100	6	100	3
3.0	100	7	100	11
4.0	100	20	100	27
6.0	100	73	100	78
8.0	100	92	100	95
	χ^2 18.75		χ^2 5.62	
	b = 5.77 ± 0.42		b = 6.38 ± 0.46	
	m = 0.69 ± 0.01		m = 0.67 ± 0.01	

Table A2
Toxicity data supporting Fig. 13

NICOTINE FUMIGATIONS

Concentration 5 µg/l

Supella supellectilium

Time minutes	Gas velocity cm/sec. 5 N	5 R	50 N	50 R	95 N	95 R
1	30	3	28	6	30	11
2	30	4	20	9	30	15
4	30	13	22	16	30	22
8	30	24	16	14	30	28
16	30	29	24	24	30	30
	χ^2 2.95		χ^2 1.18		χ^2 2.48	
	b = 2.72 ± 0.36		b = 2.43 ± 0.42		b = 2.14 ± 0.36	
	m = 0.62 ± 0.05		m = 0.34 ± 0.06		m = 0.24 ± 0.06	

Musca domestica

Time minutes	Gas velocity cm/sec. 0 N	0 R	7 N	7 R	23 N	23 R	45 N	45 R	65 N	65 R	95 N	95 R
3.0		-	50	0	50	2	50	8	50	12	50	18
5.0		-	50	2	50	9	50	29	50	38	50	33
7.0		-	50	18	50	29	50	36	50	39	50	41
8.5	50	6		-		-		-		-		-
9.0	50	7	50	24	50	36	50	40	50	46	50	49
9.5	50	12		-		-		-		-		-
10.0	50	22		-		-		-		-		-

ANALYSIS

cm/sec	χ^2	b	m
0	1.45	15.32 ± 4.08	1.02 ± 0.02
7	0.30	6.29 ± 3.79	0.92 ± 0.05
23	2.18	5.26 ± 0.81	0.84 ± 0.03
45	0.21	3.82 ± 1.80	0.70 ± 0.03
65	4.03	3.93 ± 0.67	0.60 ± 0.03
95	2.87	4.15 ± 0.61	0.58 ± 0.03

Table A2 contd.
Toxicity data supporting Fig. 13a.

NICOTINE FUMIGATIONS

Concentration 5 µg/l

Oryzaephilus mercator

Time mins.	Gas velocity cm/sec. 0		5		10		15		20		25		30		35	
	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R
3.17	-		-		-		-		28	14	28	12	29	9	24	13
3.98	23	11	29	13	25	17	25	18	24	17	24	14	25	19	25	22
5.02	24	18	25	20	25	22	35	30	28	22	35	33	33	30	31	28
6.32	27	22	28	21	27	23	31	28	26	23	24	23	23	21	25	24
7.93	23	21	27	22	23	23	27	27	28	27	25	25	22	21	25	25
10.00	25	25	28	26	28	27	26	26	-		-		-		-	

	40		45		50		60		70		80		90	
1.60	-		-		-		-		-		25	1	25	1
2.00	-		-		-		28	5	25	2	26	1	29	8
2.52	21	8	21	7	25	12	27	10	25	11	24	4	25	9
3.17	24	7	24	8	32	29	26	8	25	18	27	19	29	22
3.98	25	12	29	26	24	19	26	18	28	27	25	17	24	16
5.02	27	23	27	24	24	23	25	24	26	24	-		-	
6.32	27	24	24	23	24	23	-		-		-		-	

ANALYSIS

Gas velocity

cm/sec	χ^2	b	m
0	1.83	5.21 ± 1.21	0.60 ± 0.04
5	3.69	3.35 ± 0.89	0.58 ± 0.06
10	3.93	3.75 ± 1.20	0.45 ± 0.09
15	1.99	5.25 ± 1.56	0.50 ± 0.07
20	0.35	4.15 ± 0.98	0.49 ± 0.05
25	2.80	7.61 ± 1.44	0.54 ± 0.02
30	5.48	6.09 ± 1.16	0.54 ± 0.03
35	2.16	5.85 ± 1.45	0.46 ± 0.05
40	6.13	4.67 ± 0.93	0.55 ± 0.03
45	7.84	6.41 ± 1.14	0.49 ± 0.02
50	7.88	4.35 ± 1.15	0.34 ± 0.06
60	7.73	5.53 ± 0.94	0.50 ± 0.02
70	5.58	7.81 ± 1.16	0.44 ± 0.02
80	7.43	7.09 ± 1.16	0.50 ± 0.02
90	6.68	5.21 ± 0.94	0.45 ± 0.02

Table A3
Toxicity data supporting Fig. 14.

DDVP FUMIGATIONS

Concentration<1 µg/l

Supella supellectilium

Time minutes	Gas velocity cm/sec. 5		50		100	
	N	R	N	R	N	R
1.0	25	1	25	2	25	3
2.0	25	1	25	8	25	11
4.0	25	6	25	13	25	15
6.0	25	12	25	18	25	23
8.0	25	17	25	22	25	24

ANALYSIS

Gas velocity cm/sec	χ^2	b	m
5	2.40	2.91 ± 0.57	0.79 ± 0.05
50	1.00	2.61 ± 0.45	0.53 ± 0.05
100	3.14	3.01 ± 0.46	0.40 ± 0.46

Oryzaephilus mercator

Time minutes	Gas velocity cm/sec. 5		50		100	
	N	R	N	R	N	R
1.0	75	1	75	1	75	16
2.0	75	12	75	43	75	46
3.0	75	29	75	47	75	71
4.0	75	71	75	72	75	74
6.0	75	75	75	75	75	75

ANALYSIS

Gas velocity cm/sec	χ^2	b	m
5	22.32	7.17 ± 0.68	0.45 ± 0.01
50	19.46	5.40 ± 0.49	0.33 ± 0.02
100	5.07	4.87 ± 0.45	0.19 ± 0.02

Table A4.

Time in minutes taken to paralyse a number of insect species exposed to nicotine at two gas velocities and a concentration of about 10 µg/l.

Coccinella septempunctata (adult).		Schistocerca gregaria (hoppers).	
5 cm/sec	90 cm/sec	5 cm/sec	90 cm/sec
1.48	1.25	1.23	1.70
1.33	1.30	1.78	1.82
1.45	1.47	1.85	1.05
1.17	1.28	1.23	1.90
1.72	1.62	1.82	1.45
1.87	1.48	1.40	1.03
1.37	1.22	1.27	1.50
1.78	1.50	2.03	1.37
1.37	1.72	1.73	1.95
1.42	1.72	1.95	1.88
1.32	1.73	1.23	1.87
1.55	1.85	1.47	1.72
1.62	1.42	1.48	2.22
1.45	1.35	1.52	1.17
2.03	1.52	2.00	2.03
1.60	1.95	1.92	1.43
1.46	1.33	1.98	1.72
1.92	1.40	2.02	1.52
1.40	1.17	2.17	1.10
2.87	1.97	1.63	2.22
1.43	1.38	1.08	1.13
1.53	1.20	1.77	1.95
1.27	1.70	1.12	1.60
1.65	2.08	1.15	1.72
1.33	1.22	1.42	1.97
$\bar{x}$ 1.58	1.51	1.61	1.64
t = 0.717		t = 0.318	

Table A4 contd.

Supella supellectilium		Musca domestica		Oryzaephilus mercator	
5 cm/sec	90 cm/sec	5 cm/sec	90 cm/sec	5 cm/sec	90 cm/sec
1.47	1.12	1.42	1.17	1.80	1.58
1.55	1.28	2.00	1.42	1.83	1.78
1.97	1.55	2.25	1.50	2.25	2.45
1.35	1.62	1.50	2.17	1.77	1.48
1.98	1.32	1.58	1.17	2.07	1.82
1.48	2.15	1.66	1.25	2.07	1.92
1.62	1.32	1.66	2.03	1.58	1.40
1.45	1.58	1.92	1.08	1.66	1.43
1.66	1.27	2.17	1.33	1.88	2.00
1.18	1.62	2.25	1.92	1.48	1.48
1.63	1.25	1.33	1.25	1.73	1.50
1.75	1.68	1.83	1.60	2.00	1.52
1.57	1.47	2.17	2.00	1.52	1.38
2.15	1.38	1.42	1.00	1.66	1.73
1.27	1.78	1.73	1.42	2.03	1.90
1.40	1.05	1.80	1.75	1.52	1.45
1.22	2.13	1.08	1.28	1.60	1.60
1.55	1.37	1.25	1.43	1.92	1.65
1.82	1.70	1.83	1.66	1.25	1.50
1.27	1.35	1.48	1.17	1.73	1.58
1.57	1.67	1.50	1.58	1.77	2.25
1.75	1.98	2.25	1.83	1.52	2.17
1.68	1.33	2.20	1.75	1.83	1.66
1.22	1.53	1.45	1.58	2.33	2.00
1.05	2.10	1.75	2.00	2.25	1.58
$\bar{x}$ 1.54	1.54	1.74	1.53	1.80	1.71
t = 0.005		t = 2.15		t = 1.14	

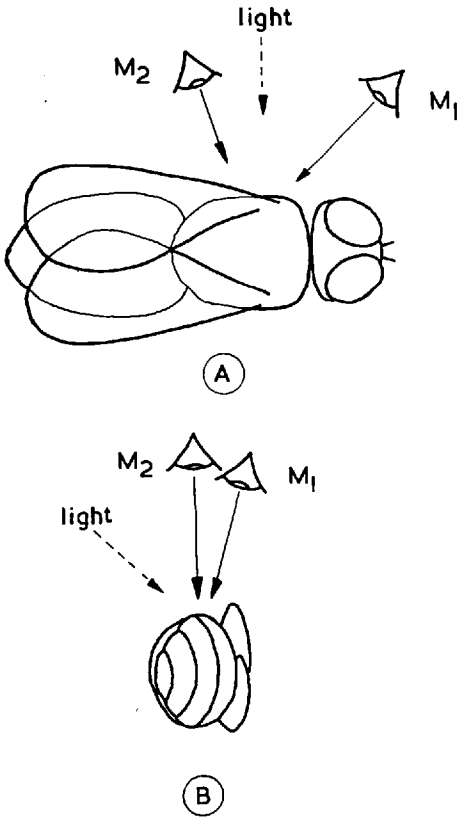

Fig. 45 Optimum positions for observation of the thoracic spiracles of Musca.

A plan view
B end view
M_1 meso-thoracic spiracle
M_2 meta-thoracic spiracle.

Table A5
Spiracle activity during fumigation with nicotine.

Gas velocity	Time spent open mins. - secs.					
cm/sec	0	8	22	44	63	95
	5-40	4-20	3-05	1-40	1-10	1-05
Meso-	4-30	4-10	2-50	1-55	1-25	1-15
	4-15	4-45	2-45	2-05	1-30	1-15
thorax	4-50	4-20	2-20	2-05	1-15	1-25
	5-20	4-00	3-10	1-50	1-30	1-20
	5-30	4-30	2-50	2-00	1-15	1-20
	4-10	4-25	2-25	2-10	1-35	1-15
Meta-	4-50	4-40	3-10	1-45	1-20	1-20
	4-40	4-25	2-40	1-50	1-15	1-15
thorax	5-30	4-10	2-30	1-50	1-10	1-10
	5-05	4-15	2-35	2-00	1-10	1-30
	5-30	4-30	2-45	2-05	1-25	1-15
mean-minutes	4.98	4.38	2.75	1.93	1.33	1.28
MLT (from Fig. 13)	10.4	8.6	6.8	5.2	4.2	3.7
% MLT open	47	50	40	37	30	35

Table A 6

Toxicity data from nicotine fumigation of Musca domestica with pressure fluctuations - CTp's expressed in µg.min/l.

1. 755 mm Hg.			2. 500 mm Hg.			3. 3 cycles/min 500/755/500		
CTp	N	R	CTp	N	R	CTp	N	R
32	30	1	36	30	2	56	30	4
48	30	3	54	30	1	84	30	5
64	30	4	72	30	3	112	30	7
96	30	11	108	30	11	168	30	19
128	30	12	144	30	17	224	30	26
192	30	20	216	30	23	336	30	27
0	30	0	0	30	0	0	30	0

ANALYSIS

For parallel lines: $b = 3.39 \pm 0.28$ $m_1 = 2.12 \pm 0.03$
$m_2 = 2.14 \pm 0.03$
$m_3 = 2.14 \pm 0.03$

for position	$\chi^2_{(2)}$	0.22
for parallelism	$\chi^2_{(2)}$	1.74
for linearity	$\chi^2_{(12)}$	10.96

Table A7
Toxicity and weight loss data of Musca domestica adults aired after fumigation.

The insects were exposed to a nicotine concentration of about 1 µg/l in still air within a desiccator.

Time of exposure minutes	Mortality among aired insects N	R	Mortality among allowed to recover normally N	R
6	25	8	25	12
12	25	12	25	8
24	25	11	25	15
48	25	18	25	22
96	25	25	25	25
	χ^2 8.54		χ^2 10.01	
	b = 1.58 ± 0.32		b = 1.72 ± 0.33	
	m = 1.19 ± 0.14		m = 1.07 ± 0.21	

For parallelism χ^2 = 0.087

Table A8 (Fig. 16).

Absorption of nicotine by Musca domestica adults exposed at a number of gas velocities for the appropriate MLT.

Velocity cm/sec	Time minutes	Wt. of 50 flies - mg	Nicotine recovered µg	ppm.
0	10.4	520	109	210
8	8.6	515	121	235
22	6.8	520	121	233
44	5.2	535	145	271
63	4.2	515	125	243

Table A 9.

Absorption of nicotine by Oryzaephilus mercator at a number of gas velocities.

Nicotine concentration: 120 µg/l by colorimetric assay. This value is high by a factor of about 10x, (see p.48-49).

Fumigant velocity cm/sec.	Absorption - ppm. 10 minutes	15 minutes	25 minutes
0	610	1090	2040
8	986	1160	2120
22	906	1290	2540
44	1010	1470	2510
63	1110	1510	2540

These figures may be high for the same reason as the gas concentration estimate. They are relative, however, and serve to show the approach to an asymptotic value and that absorption of this fumigant has departed only little from linearity with time after 25 minutes.

Table A 10. (Fig. 18).

Absorption of nicotine by Musca domestica at a number of gas velocities.

The quantities of nicotine absorbed have been corrected for concentrations of 10 µg/l.

Gas Velocity cm/sec.	Number of Insects	Wt. of insects mg.	Conc. µg/l.	Nicotine absorbed µg.	ppm	corrected ppm
0	15	190	15.6	42.1	222	142
5	16	205	11.7	58.8	287	245
15	15	200	12.3	60.5	303	246
25	15	185	12.8	82.0	443	346
35	17	200	9.9	105	525	530
70	15	170	14.4	107	630	438
100	15	180	13.3	153	850	640
150	14	165	14.5	151	915	630
200	12	148	11.5	119	804	700
300	12	140	12.0	127	908	750

Table A 11. (Fig. 19).

Absorption of nicotine by Supella supellectilium at a number of gas velocities.
(20 minute exposure, concentration 10 µg/l).

Gas velocity cm/sec.	Nicotine absorbed µg/g.
0	276
20	302
40	485
50	432
80	580
90	526
100	610
125	621
150	540
175	675
200	660
250	860
300	805
350	728

Table A 12. (Fig. 20).

Absorption of nicotine by dead and live houseflies.
(10 minute exposure, 10 µg/l).

Gas Velocity cm/sec.	Live insects (+) Wt. 15 insects mg	nicotine residue µg	mean ppm	Dead insects (o) Wt. 15 insects mg	nicotine residue µg	mean ppm
	170	28		150	28	
5	160	32	190	180	32	180
	155	32		170	30	
	150	60		160	82	
40	175	61	340	155	56	400
	160	45		160	53	
	175	87		150	79	
95	160	85	530	165	86	520
	165	90		175	86	

Table A 13. (Fig. 21)

Absorption of nicotine by Musca domestica with and without epicuticular wax.
(6 minute exposure, 12 µg/l).

Gas Velocity	Normal insects (+)			Insects with epicuticular wax removed (o)		
	Wt. 20 insects	nicotine residue		Wt. 20 insects	nicotine residue	
cm/sec.	mg	µg	ppm	mg	µg	ppm
5	265	57	215	260	29	110
40	275	124	450	265	36	135
95	260	136	535	260	44	170

Table A 14. (Fig. 22)

Location of absorbed nicotine
(10 µg/l).

Gas velocity	Insects		Nicotine absorbed waxes o	rest •	total +
cm/sec.	No.	Wt. mg	µg/fly	µg/fly	ppm
5	20	235	1.8	1.7	291
50	16	200	4.2	2.4	527
80	20	225	4.9	2.0	611
100	23	300	5.5	3.5	694
200	20	225	6.4	1.3	688
300	19	195	6.3	1.1	725

Table A 15 (Fig. 23).

Absorption of nicotine by normal and beeswax coated Oryzaephilus mercator (5 µg/l).

Gas velocity cm/sec.	beeswax coated ♦ Wt. of insects	nicotine residue µg	ppm	normal o Wt. of insects	nicotine residue µg	ppm
5	18	9	500	19	11	580
40	18	17	940	18	14	780
70	19	21	1100	18	22	1220
100	18	24	1330	18	19	1050
150	18	25	1390	18	19	1050

Table A 16 (Fig. 25).

Absorption of nicotine by beeswax and paraffin wax rods with change in gas velocity. (5 µg/l).

Gas velocity cm/sec	Nicotine absorbed beeswax µg	paraffin wax µg
0	30	5
10	38	6
20	44	7
30	60	18
40	40	-
50	60	15
60	70	-
75	-	17
90	74	-
100	72	14
125	100	-
150	97	-
200	100,127	-
225	129	-
250	111	-
275	119	-
300	129	13
325	111	-
350	122	-
400	110,119	17

Table A 17 (Fig. 28).

Absorption of nicotine by grooved beeswax rods (20 minutes, 10 µg/l).

Gas velocity cm/sec.	Nicotine absorbed - µg Circular grooves (o)	Annular grooves (+)
0	14	15
5	13	8
10	12	17
15	24	19
25	38	14
30	22	16
35	21	24
75	36	33
100	41	47
200	45	57
300	53	57
400	40	39

Table A 18 (Fig. 29).

Absorption of nicotine by plain and microsculptured beeswax rods.
(20 minutes, 10 µg/l).

Gas velocity cm/sec.	Nicotine absorbed - µg. microsculptured (o)	plain (+)
0	24	15
10	26	11
30	24	14
50	32	21
70	41	19
100	41	26
150	57	25
200	71	31
300	81	54
400	97	46

Table A 19 (Fig. 30)

Absorption of nicotine by beeswax rods of different length with change in gas velocity.
(20 minute exposure, 5 µg/l).

Gas velocity	Nicotine absorbed - µg.				
	5 mm	12 mm	24 mm	40 mm	54 mm
cm/sec	88 mm^2	176 mm^2	327 mm^2	528 mm^2	703 mm^2
0	5	14	23	36	47
10	8	16	29	38	51
30	12	22	34	59	76
60	16	23	39	66	94
100	11	38	57	81	101
200	26	43	83	106	141
300	15	41	106	125	180
400	31	50	91	139	180

Table A 20 (Fig. 32)

Loss of absorbed nicotine during airing.

Time of airing minutes	Nicotine lost %
4	31
8	37
10	34
16	50
20	47
30	45,49
50	48

Table A 21 (Fig. 33)

Absorption of nicotine by insect cuticle.
(5 µg/l).

		Projected area cm^2	True area cm^2	Nicotine absorbed µg 5 min	10 min	20 min	40 min
Periplaneta	(1 forewing)	5.20	42.7	17.1	23.9	51.3	72.6
Musca	(75 wings)	15.7	36.1	-	36.2	54.3	101
Tenebrio	(12 elytra)	4.91	32.9	25.0	36.2	59.2	141

Lockey, (1960) - see p. 101

Absorption per unit area - $\mu g/cm^2$

	5 min.	10 min.	20 min.	40 min.
Periplaneta	0.40	0.56	1.2	1.7
Musca	-	1.0	1.5	2.8
Tenebrio	0.76	1.1	1.8	4.3

Table A 22 (Figs. 34 and 35).

Absorption and desorption of nicotine with time by various waxes.

Material	Original wax present - µg.
Periplaneta	68
Musca	137
Tenebrio	278
Oryzaephilus	286
Beeswax	73
Paraffin wax	100
Shellac	30

Absorption

Material	Time hours	% nicotine absorbed 1	2	4	5	7	14	24	33
Periplaneta		13	12	31	-	37	50	59	63
Musca		26	39	58	-	74	100	120	125
Tenebrio		28	49	79	-	109	149	178	188
Oryzaephilus		21	34	-	65	-	103	119	120
Beeswax		7	10	13	-	16	17	19	20
Paraffin wax		1	-	1	-	1	1	1	1
Shellac		-	-	-	83	-	101	103	104

Desorption

Material	Time hours	% nicotine remaining 1	5	12	14	22	24	28	39
Periplaneta		34	19	-	7	7	-	6	6
Musca		66	26	-	8	6	-	5	6
Tenebrio		114	42	-	13	9	-	7	7
Oryzaephilus		48	20	6	-	-	5	-	5
Beeswax		14	12	-	-	12	-	-	12
Paraffin wax		0	-	-	-	-	-	-	-
Shellac		53	27	20	-	20	-	-	-

Table A 23 (Figs. 36 and 37).

Absorption and desorption of DDVP with time by various waxes.

Absorption

Material	Time hours	Wt. original wax - µg	% nicotine absorbed				
		0	1	2	4	8	14
Periplaneta		163	6	7	9	10	11
Musca		124	15	22	27	31	32
Tenebrio		135	22	30	37	40	42
Beeswax		86	3	7	6	5	6

Desorption

Material	Time hours	% nicotine remaining				
		1	2	5	10	20
Periplaneta		18	7	7	6	6
Musca		16	12	7	5	4
Tenebrio		22	15	7	4	2
Beeswax		2	2	1	1	1

Table A 24 (Fig. 38).

Absorption of nicotine by dilute sulphuric acid at a number of gas velocities.

Gas concentration 10 µg/l; 0.2 ml of 0.04N H_2SO_4.

Gas velocity - cm/sec. Indicated	Corrected	Nicotine absorbed µg.
0	0	20
50	80	36
100	160	68
200	320	130
300	481	210
400	641	309

Table A 25 (Fig. 39).

Absorption of hydrogen cyanide by dilute aqueous sodium bicarbonate at a number of gas velocities.

(gas concentration 1.7 mg/l; 0.2 ml 0.7% $NaHCO_3$ exposed for 3 minutes).

Gas velocity cm/sec Indicated	corrected	Hydrogen cyanide absorbed - µg
0	0	16.5
10	16	17.5
20	32	19.0
40	68	23.0
100	160	39.5
200	320	90.0

Table A 26 (Fig. 40).

Absorption of DDVP by n-decane at a number of gas velocities.

(gas concentration <1 µg/l exposed for 10 minutes).

Gas velocity cm/sec	DDVP absorbed ppm
2	50
12	80
40	160
60	208