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FINELY DIVIDED POWDERS**

WALTER EBELING

**RELATION OF LIPID ADSORPTIVITY
OF POWDERS
TO THEIR SUITABILITY AS
INSECTICIDE DILUENTS**

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**PHYSICO-CHEMICAL MECHANISMS FOR THE REMOVAL
OF INSECT WAX BY MEANS OF FINELY
DIVIDED POWDERS¹****WALTER EBELING²****INTRODUCTION**

ABOUT 95 per cent of the insect cuticle consists of a relatively thick inner part called the procuticle, composed of chitin, protein, and other compounds, and containing considerable quantities of water. Above the procuticle is the nonchitinous epicuticle, approximately 1 μ in thickness, which consists of an inner hydrophylic protein zone, called the cuticulin, a middle zone composed of lipid (usually a solid wax), and an outer zone of shellac-like material, the so-called "tectocuticle" or "cement" (Richards, 1951).³ Beament (1959) found that with some insects, wax in a volatile solvent migrates through the cement to form an outer layer of wax. It is the lipid of the epicuticle that protects insects from abnormally rapid loss of water.

A number of investigators have shown that when insects or mites crawl over films or layers of finely divided particles, particularly when the latter are deposited as dry dusts, they are susceptible to desiccation. This desiccation results from the removal by these particles of some of the very thin lipid layer of the epicuticle (Zacher and Künicke, 1931; Hockenyos, 1933; Germar, 1936; Zacher, 1937*a*, 1937*b*; Chiu, 1939; Alexander *et al.*, 1944; Kalmus, 1944; Parkin, 1944; Wigglesworth, 1944, 1945, 1947*a*, 1947*b*, 1957, 1958; Beament, 1945, 1958; Hunt, 1947; Cotton and Frankenfeld, 1949; David and Gardiner, 1950; Bartlett, 1951; Helvey, 1952; Glynne Jones, 1955; Holdgate and Seal, 1956; Nair, 1957; Ebeling and Wagner, 1959; Wagner and Ebeling, 1959; Tarshis, 1959, 1960, 1961; and Micks, 1960).

Some investigators have attributed the desiccating action of finely divided powders primarily or entirely to their ability to remove the protective lipid layer by abrasion (Wigglesworth, 1945, 1947*a*, 1958; Beament, 1945; Hunt, 1947; and David and Gardiner, 1950). Beament (1959) later reiterated his belief that stationary adsorptive powders applied to the "primary" wax layer do not increase its permeability to water, but that when applied

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³ See "Literature Cited" for citations, referred to in the text by author and date.

to the wax above the "cement," in a wax-cement-wax system, they increase water loss to a rate that is higher than that of the same cuticle with only a primary wax layer. Alexander *et al.* (1944) found that abrasive powers were more effective against the adults of some species of beetles and sorptive powders were more effective against the adults of others, as well as the larvae of all species tested. Other investigators, particularly in later years, have recognized that adsorption can be the dominant factor in the removal of lipid from most insect species, provided the powders used possess the required physical characteristics for a high degree of sorptiveness for wax. The majority of these investigators (Hurst, 1948; Helvey, 1952; Glynne Jones, 1955; Nair, 1957; and Ebeling and Wagner, 1959) demonstrated that the ability of finely divided powders to remove the epicuticular wax of the insects they investigated increased with increasing sorptiveness. All the highly effective powders have been nonabrasive.

There has been considerable controversy as to whether powders can cause lethal dehydration of insects entirely, or even in part, by adsorption of the solid wax. The adsorption of the "mobile grease" of the cockroach has been conceded (Wigglesworth, 1945; Beament, 1945). This controversy is of more than academic interest. Obviously, if the protective wax can be removed only by abrasion, the use of inert powders or dusts for the control of insects through desiccation must be confined to those species whose habitats and habits make them susceptible to the action of abrasives, primarily the granary weevils. In fact, powders whose action is entirely abrasive have been used successfully, in limited commercial practice, only against granary weevils, which abrade their bodies as they crawl among the kernels of grain. Even for such insects, however, the nonabrasive, but highly sorptive, silica aerogels have proved to be superior (Cotton and Frankenfeld, 1949). On the other hand, if the epicuticular wax can be removed by adsorption, many species, with a wide variety of habitats and habits, will be susceptible to the lethal effect of the sorptive powders. The insect need only accumulate the powder on the underside of its body as it crawls over the dusted surface (Ebeling and Wagner, 1959).

If insects are immobilized by death or otherwise before a sorptive powder is applied, the initiation of water loss is delayed for several hours, but then proceeds at a rate far in excess of that of undusted insects (Ebeling and Wagner, 1959).

It was the primary purpose of this investigation to demonstrate the adsorption of insect wax by means of finely divided powders, visually and gravimetrically, and to propose a physicochemical mechanism by which this action takes place. For ease of manipulation and for purposes of standardization, the experiments were made primarily with beeswax as a representative insect lipid. However, the removal of dyed lipid from the insect epicuticle by means of sorptive powders was photographically recorded.

An additional aim in this investigation was to correlate certain physical characteristics of finely divided powders with their ability to adsorb insect wax and with their insecticidal effectiveness.

MATERIALS

One of the premises in this investigation was that information obtained regarding the adsorption of beeswax would be applicable in a general way to the wax layers of many insect species. The chemistry of beeswax has been investigated in great detail, particularly by Warth (1956). It has the general composition of all insect waxes investigated to date, primarily comprising esters, wax acids, and hydrocarbons. However, its free alcohol content is very low (1 to 1.25 per cent), in contrast to the high quantities claimed for various insect waxes by previous investigators. Warth believes that although alcohols are initially produced in large quantity, they are soon oxidized by free oxygen to the corresponding acid molecules.

In one analysis of beeswax, esters comprised 71 per cent of the total and had a melting point of 64.5° C. Among the simple esters, the most abundant was myricyl palmitate, $C_{15}H_{31}CO.O.C_{26}H_{53}$. Free wax acids, mainly saturated, made up 13.5 to 14.5 per cent of the total wax. Hydrocarbons, mainly the saturated hentriacontane, $C_{31}H_{64}$, with a melting point of 68.4° to 69.0° C., constituted 10.5 to 13.5 per cent (Warth, 1956).

Since the glands that secrete the beeswax of commerce are merely specialized epidermal glands homologous with those that secrete the wax covering the epicuticle of the bee, it is reasonable to presume that their product is practically the same. Glynne Jones (1955) found that the melting point of the wax in the epicuticle of the bee is practically the same as that of extracted beeswax (62° – 65° C.).

The specific gravity of beeswax is 0.95 to 0.96. When distilling off 2.1 per cent of a beeswax sample in a ball-tube distillation apparatus at 210° to 230° C. and 0.08 mm. Hg, the writer found that, upon cooling, the material solidified, indicating no liquid fractions.

For the purposes of the present investigation, it was considered desirable to work with films of beeswax on glass or plastic vials. Films of different thicknesses, ranging from 0.32 to 65 μ , were obtained on the outer surfaces of stoppered vials by dipping them into solutions of beeswax in carbon tetrachloride in concentrations ranging from 1.5 to 50 per cent, or by dipping them into melted beeswax, usually the yellow beeswax of commerce. However, some work was done with a white wax, filtered and refined without the use of acid and with a minimum of heat. This wax is free of pollen, propolis, and other contaminants found in the yellow beeswax of commerce.

In one experiment, U.S.P. household paraffin was compared with beeswax in regard to loss of weight of the wax films by adsorption and by exposure to high temperatures. Paraffin is a mixture of solid hydrocarbons obtained from petroleum and has the general formula C_nH_{2n+2} , with the number of carbon atoms ranging from 22 to 30. The Parowax used in the present experiments has a melting point averaging about 51.5° C. and contains fractions ranging in melting point from 37° to 64° C.

The finely divided powders used in the experiments are described below. Some of the more pertinent physical characteristics are also listed in table 1.

(1) **Silica aerogel SG-68.** Silica gels are well-known dehydrating agents and adsorbents. They are amorphous, nonabrasive powders, formed as a

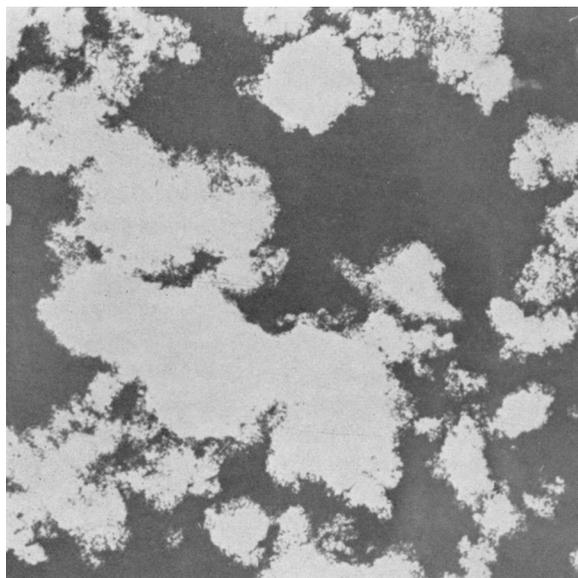


Fig. 1. Electron micrographs of silica aerogel powder SG-67, showing lacy structure evident at edges of the agglomerates, which comprise many minute particles approximately 0.01 to 0.05 μ in diameter. $\times 12,600$.

result of the primary chemical reaction of sodium silicate and sulfuric acid. A firm hydrogel is formed which is washed free of reaction salts. A granular, dehydrated silica gel results that can be reduced to very small particle size. Because of their noncrystalline structure, high purity (99.5 per cent SiO_2), and chemical inertness, silica gels are not injurious to human beings. They do not cause silicosis, but respirators are recommended for those who work for extended periods with the powder, to prevent irritation resulting when an appreciable amount of the material is inhaled.

The silica gels of lowest bulk density, and consequently of greatest porosity, are called aerogels. SG-68 is an extremely light, fluffy silica aerogel, with 82 per cent of the aggregates ranging from 0.1 to 3.2 μ in diameter, but electron micrographs show that these comprise myriads of tiny ultimate particles only 0.01 to 0.05 μ in diameter (fig. 1, showing SG-67). SG-68 has an average pore diameter of 115 \AA and a surface area (specific surface) of 300 square meters per gram, and will adsorb 300 per cent of its weight of linseed oil. It has a pH of 7.0. The pH of silica gels and aerogels is based on a 5 per cent slurry in distilled water.

(2) **Silica aerogel SG-67.** This product is the same as SG-68, except for 4.7 per cent of ammonium fluosilicate present in less than a continuous monolayer. It has a density of 4.5 pounds per cubic foot, as packed, and a pH of 2.6.

The fluoride is added to the silica hydrogel in the process of manufacture and was originally added to decrease the caking characteristics of the gel and to produce an improved silica flatting agent. However, the fluoride greatly improves the silica aerogel as an insecticide by causing the particles

to have a positive charge, thus enhancing their depositing ability and adherence when applied as a dust. In addition, silica aerogel appears to have the ability to disrupt the outermost layer of the epicuticle, which Richards (1957) has found to be the major cuticular barrier to ions. The penetration of the ion barrier, plus the adsorption of lipid, appears to allow the inorganic water-soluble toxicant to contact the water-bearing portion of the cuticle and to act as a contact insecticide (Ebeling and Wagner, 1959).

(3) **Silica gel AL-1.** This material differs from the two silica aerogels described above in having about three times higher density, a greater particle size, a much smaller pore size, and greater specific surface. It has a greater ability to adsorb water. Its pH in water slurry is 2.8.

(4) **Silikil.** This is a silica gel with a relatively low degree of wax adsorbency, low specific surface (36 meters²/g.), relatively high bulk density (approximately 3.5 times as high as SG-67), and with 85 per cent of the particles ranging from 1 to 5 μ . It has a pH of 4.25 in 5 per cent aqueous slurry.

(5) **Activated carbon.** This black powder is made from lignite. Ninety per cent passes through a 325-mesh screen. It has the largest pores of all powders used in this investigation and also a large specific surface (545 meters²/g.). The pH of the water extract is 9 to 11.

(6) **Olancha Clay.** One of four fuller's-earth-type clays, out of 162 finely divided powders tested, that were found to have outstanding ability to kill drywood termites by desiccation (Wagner and Ebeling, 1959). It is a montmorillonite clay with 58.6 per cent SiO₂ and 12.8 per cent Al₂O₃. It has a Scott Volumeter density of 16 to 18 pounds per cubic foot, with 91 per cent passing through a 325-mesh screen, a linseed-oil adsorption of 120 per cent, and a pH (in 1-5 slurry) of 6.75.

(7) **Attaclay.** This is another fuller's-earth-type clay with considerable ability to kill insects by desiccation. It is an attapulgite clay with 67.0 per cent SiO₂, 12.5 per cent Al₂O₃, and 11 per cent MgO. It has a Scott Volumeter density of 17.8 pounds per cubic foot, 85 per cent passing through a 325-mesh screen, a linseed-oil adsorption of 119 per cent, and a pH (in water slurry) of 6.9 to 7.1.

(8) **Insecticide Grade Pyrophyllite.** This material contains 75.0 per cent SiO₂ and 19.5 per cent Al₂O₃; a bulk density (fluffed) of 30 pounds per cubic foot; and 93.5 per cent passes through a 325-mesh screen. Pyrophyllite particles have no internal porosity or surface, and this powder can adsorb only 50 per cent of its weight of linseed oil. Its pH in water slurry is 6 to 7.

(9) **Barytes.** This material is used as a filler for rubber. It is 86.18 per cent BaSO₄. The powder is heavy, white, and 99.7 per cent passes through a 325-mesh screen.

(10) **Molecular Sieves.** These synthetic zeolites are alkali metal aluminum silicates, quite similar to many natural clays and feldspars. With most hydrated materials of this type, when the water of hydration is driven off, the crystal collapses or becomes rearranged. With Molecular Sieves, however, the structure of the crystal remains unchanged, which results in a network of empty pores and cavities that comprise about half the total volume of the crystals. Molecular Sieves have a pH in water slurry of approximately 10.

(11) **Sand.** Approximately equal parts of quartz and feldspar. The sand used was angular in shape and was ground to pass through a 200-mesh screen.

(12) **Carborundum.** Carborundum (silicon carbide—SiC) is second only to diamond dust in abrasiveness. Because of the limitations in the manufacture of screens, all numbers below 240 "grit" are nominal rather than absolute designations of mesh size. The particle sizes, for the bulk of the powder, are given for grits 400 and 600 in table 1.

Observation of Adsorption from Beeswax Layers with the Aid of a Microscope

According to Azaroff and Buerger (1958), most waxes are crystalline. In the present investigation, it was shown by X-ray crystallography, according to the powder method, that beeswax contains both crystalline and amorphous material. Although beeswax is a solid substance, the same physicochemical laws that pertain to the adsorption of ordinary liquids would be involved in the adsorption of the amorphous constituents. (Whenever adsorption of wax is referred to in the present paper, amorphous wax is implied.)

Most of the physical and organic chemists who were consulted regarding the apparent removal of wax from the epicuticle of insects by adsorption, as indicated by staining techniques (Ebeling and Wagner, 1959), were skeptical of the validity of this conclusion because of the relatively rapid rate of removal of the wax reported in the experiments in question. The numbers of minutes for 50 per cent kill of insects by placing them on a film of one of the silica gels, for example, were as follows: ants, 10; *Drosophila melanogaster*, 19; German cockroaches, 92; drywood termites, 152; and houseflies, 185 (Ebeling and Wagner, 1959). Therefore, it was considered desirable to obtain visual evidence of the rate of adsorption of insect wax and its migration over solid surfaces.

The ability of certain sorptive powders to adsorb solid wax was easily verified by direct observation. A silica aerogel (SG-67) was allowed to settle out from a cloud of dust formed in a 1,000-ml. beaker onto two microscope slides, one covered with a twenty-four-hour-old film of white beeswax about 65 μ in depth. When examined with a stereoscopic microscope at $\times 96$ magnification, the aggregates of silica aerogel on these slides were at first a translucent gray when resting on glass or wax. They retained this appearance when resting on glass. On beeswax, the smaller aggregates of the powder became white within an hour at 22° C., indicating wax adsorption. Within two hours, the wax had diffused over the entire surface of some aggregates, rising 10 to 20 μ up into the wax layer on which they were resting. Over a period of a few days, nearly all silica aerogel aggregates had gradually turned white, beginning at their bases and extending upward as far as 30 to 40 μ . Figure 2 contains photomicrographs of layers of silica aerogel on glass and on white beeswax three days after it was applied. The color of the waxed silica was similar to that of the freshly broken surface of a cake of beeswax. It contrasted so sharply with the color of the unimpregnated silica that the exact point to which the wax had migrated was dis-

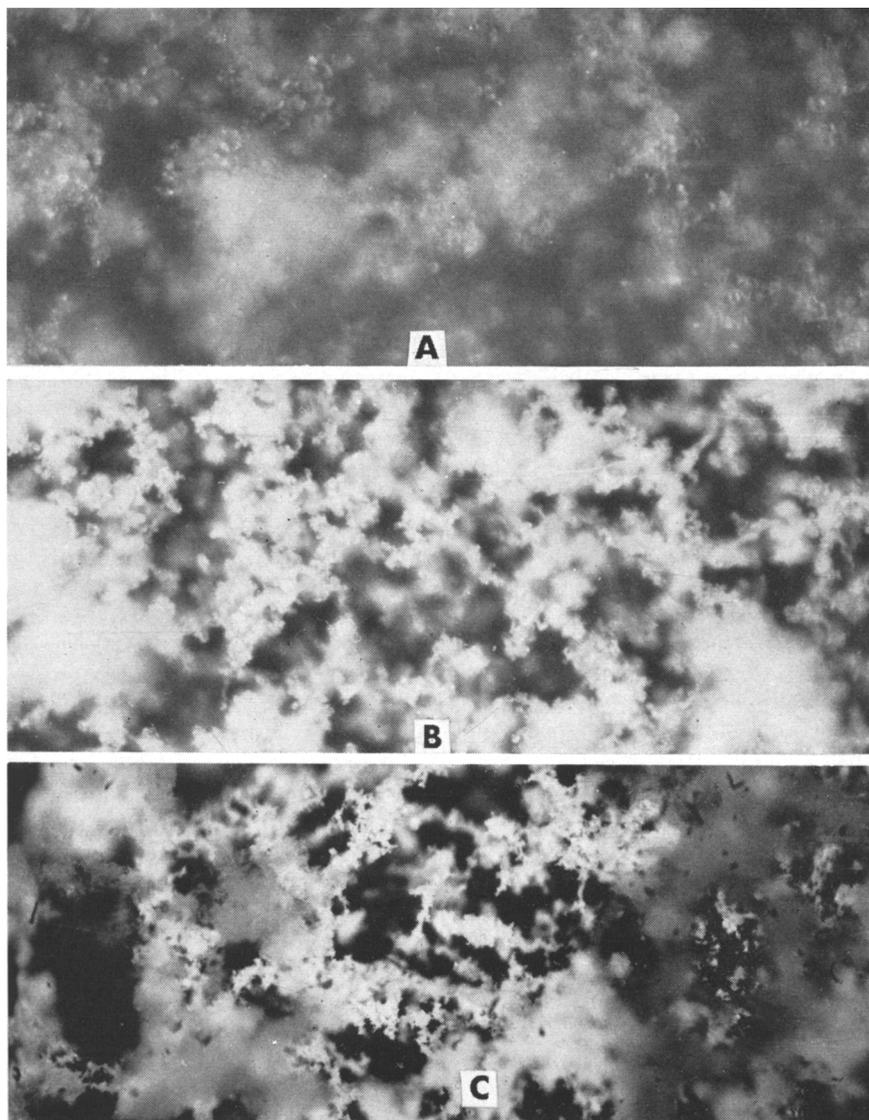


Fig. 2. Adsorption of insect wax by silica aerogel SG-67. *A*, Silica powder resting on glass, showing, out of focus, the gray, fluffy masses and, in focus, the gray, translucent, granular formations. *B*, Silica powder after twenty-four hours on beeswax, showing, out of focus, the typical, wax-impregnated, white, fluffy masses and, in focus, the white, opaque, granular formations, also typical of wax-impregnated silica. *C*, Silica powder after twenty-four hours on abdominal tergite of an American cockroach, showing, in focus, the bottom layer of lipid-impregnated, white, opaque, granular formation, and above this and out of focus, the gray masses of unimpregnated silica not reached by the available cockroach lipid.

In *A* and *B*, the focus is slightly above the substrate, and the out-of-focus masses of silica are farther from the substrate (nearer the camera); in *C*, the focus is on the layer of silica aggregates immediately adjoining the substrate.

tinctly defined. The portion of the silica that is completely in focus has a translucent, gray, granular appearance and a lacy design. This changes to an opaque white after it has been covered with a film of beeswax. This change in appearance can be readily seen in the figure.

After the silica aerogel resting on wax had turned white, it could be washed off with water, filtered, and the wax could be extracted with hot chloroform. Evaporation of the solvent on a glass slide left a wax deposit on which water drops had a 90° contact angle. Similar treatment of freshly dusted waxed slides did not result in a perceptible wax residue on slides on which the solvent was evaporated.

In order to facilitate observations, white beeswax was melted and dyed by the addition of 2.5 per cent by weight of Oil-Soluble Red. Microscope slides were dipped into the melted wax, in order to form a film on the glass, and then they were set aside for twenty-four hours. Powders were then deposited on the dyed wax films as before, by allowing them to settle out from clouds of dust formed in 1,000-ml. beakers placed upside down over the slides.

At 23° C., many of the smallest aggregates of the silica aerogels SG-67 and SG-68 were already completely saturated with the wax in fifteen minutes, as indicated by their red color. Subsequent diffusion of the wax over the silica aerogel particles proceeded approximately as described above with reference to the undyed wax. The wax did not saturate as large aggregates of Olancha Clay or Attaclay as of the silica aerogels. The powders that are least effective in killing insects by desiccation, such as pyrophyllite, talc, sulfur, and walnut-shell flour, did not adsorb wax, at least not to the extent of becoming visibly colored.

None of the powders changed color when placed on a layer of the powdered dye alone.

At a temperature of 23° C., silica aerogel adsorbed wax more rapidly at 15 and 42 per cent relative humidity than at 70 per cent. However, at 70 per cent relative humidity, all the smaller aggregates were saturated with wax in two hours. At 100 per cent relative humidity, the silica particles became visibly wet. Nevertheless, within a two-day period, many of the aggregates took on various shades of pink, ranging to red on some of the smaller aggregates. All the aggregates eventually became red. This discoloration cannot be attributed to the independent effect of the dye, for this powder is not soluble in water.

Dr. J. J. Bikerman has suggested to the writer that the apparent movement of the dyed wax over wet silica may be explained by the "Marangoni effect," which pertains to the movements in the surface of water caused by local variations in surface tension (Bikerman, 1958). In the present instance, the film of water on the silica particles might dissolve some wax molecules (presumably primarily the acids). The surface tension of the water would be reduced at the point of contamination. The tendency in such a circumstance is for the contaminating substance to be dispersed over the entire surface area of the water; in this case, the film covering the silica particles.

It is of interest to note that Ebeling and Wagner (1959) found that in seven hours, drywood termites lost 36.7 per cent of their body weight from

loss of water when placed on silica aerogel SG-67 at 100 per cent relative humidity, compared with 2 per cent loss when not in contact with the powder. This appears to be explainable only on the basis of the removal of cuticular lipids, even in a water-saturated atmosphere. It also indicates that, when the lipid protective covering on their epicuticle is removed, insects can lose water at an abnormal rate, even in a water-saturated atmosphere. Tarshis (1961) made similar observations with regard to cockroaches, fleas, and cheese mites.

Observation of Adsorption from Insect Cuticles

If, instead of dipping glass slides into melted wax, they are dipped into a hot solution of 3 per cent beeswax in carbon tetrachloride, films can be produced of about the same thickness as the insect lipid layer, 0.4μ or less. Only the smallest particles of a sorptive dust layer on such a thin wax film will then become completely covered with wax. These can be most easily seen if the wax is dyed. The larger aggregates adsorb some wax at their interfaces with the substrate, but often the quantity is not sufficient to be visible from above. As might be expected, the minute quantity of wax that is present in the epicuticle of most insects is also sufficient to cover only the smallest sorptive particles.

The wax of the insect epicuticle can be dyed with acetone solutions of Oil-Soluble Red, but the acetone, when evaporating, tends to bring to the surface additional quantities of lipid from below. To avoid this, the wax layers can be dyed by allowing a fine powder of Oil-Soluble Red dye to settle, from a confined cloud, onto the bodies of the insects. The dye is dissolved by the wax and spreads from each particle or aggregate until in about twenty-four hours the wax layer is quite uniformly dyed. The excess dye powder can then be blown and washed off.

A layer of SG-67 was allowed to settle on the bodies of *Eleodes* beetles, termite nymphs, fleas, and cockroaches that had been exposed to dye powder at 27° C. for twenty-four hours. With the exception of the newly killed cockroaches, which had a liberal supply of readily adsorbed lipid, the amount of wax adsorbed by the powder was as small as that adsorbed from the thin beeswax films. Only the smallest dust particles were uniformly colored by the dyed insect lipid. The adsorption was recorded by means of color transparencies.

Cockroaches had such an abundant supply of lipid that there was no need to dye it to facilitate observations on adsorption. Enough wax was adsorbed to be clearly evident as an opaque, white layer beneath the translucent, gray masses of unimpregnated lipid (fig. 2, *C*). On *Eleodes* beetles, large quantities of wax were adsorbed from the undersides of the elytra, but not from the upper sides, except along the suture between the elytra. On *Eleodes*, as well as other insect genera, the wax was always adsorbed in by far the greatest quantities along sutures and at the anterior and posterior margins of abdominal sclerites or at the junctures of body regions. In other areas, there was only enough wax to cover the smallest particles or aggregates of powder.

Migration of Wax Molecules Over Solid Surfaces

Adsorption, as here used, pertains only to the wax taken up at the interfaces of the silica particles and the wax substrate. The photomicrographs in figure 2 indicated that the wax was able to permeate the entire mass of a silica aerogel agglomerate. Therefore, besides being adsorbed at the interface, the wax molecules must have diffused upward in some manner over the silica surfaces, for distances of up to 30 or 40 μ .

The adsorption of wax and the movement of wax, or its constituents, over solid surfaces have been not only observed, but also systematically studied in previous investigations. These investigations showed that the mobility of molecules in waxes at room temperature is greater than might be concluded *a priori*.

In a mixture of paraffin wax fractions with a melting point of 69° C. and stearic acid (m.p. 68.2° C.), the surface molecules would normally be regarded as completely fixed, at least within relatively narrow limits. However, Rideal and Tadayon (1954a) demonstrated that when a drop of water was placed on such a surface, its contact angle became progressively lower, changing within a few hours from original values of over 100° to values ranging to approximately 60°, depending on the percentage of stearic acid, which ranged from 0.6 to 100 per cent. This indicated to the experimenters that the stearic acid molecules turned over so as to present a greater number of carboxyl groups to the water phase.⁴

The rate of "overturning" of the stearic acid molecules varied according to the metal substrate on which the monolayers occurred, for the metals had different degrees of "attraction" for stearic acid.

From wax layers on a given metal, stearic acid monolayers could be transferred to another metal to which the molecules were more strongly attracted. The order of attraction of a number of metals was platinum > copper = nickel > tin = aluminum > silver = mica.

It was shown by Rideal and Tadayon (1954b) that stearic acid molecules can be not only "overturned" and anchored to new surfaces, but that they can also migrate along the new surface for a considerable distance. They deposited a C₁₄-labeled monolayer of stearic acid over a 1.5-cm. area of mica substrate and determined its rate of diffusion beyond this area, using a direct counting technique. The surface diffusion at 35° C. was found to be 5.3×10^{-8} cm.²/sec. Surface diffusion of stearic acid over the first monolayer

⁴Dr. J. J. Bikerman has expressed to the writer the belief that the contact angles in the above experiment decreased because of diffusion of wax into water and its spread over the water surface (Marangoni effect), not because of an overturning of molecules. However, the mobility of molecules within a solid wax is demonstrated by a number of other phenomena, such as "bloom," a powder deposit of wax appearing on the surface of beeswax at ordinary room temperature (Bisson, Vansell, and Dye, 1940). Wax bloom may also be extended from the outer zone of shellac-like material of the insect epicuticle (Wigglesworth, 1957; Richards, 1958).

The rate of lateral movement of Oil-Soluble Red dye from a particle of this powder placed on beeswax is also of interest. The powder dissolves in the wax and spreads from the initial point of contact. The writer has measured the rate of progress of this dye, at 26.7° C., at about 125 μ per day, a distance that is about 500 times the thickness of the average lipid layer in the insect epicuticle.

of this compound deposited on a substrate was more rapid than that of the stearic acid comprising the monolayer itself. This may explain the deep color of the individual strands of silica aerogel aggregates resting on dyed wax in the present investigation, indicating deposits of much more than a monolayer of wax.

Professor W. F. Seyer, of the Department of Engineering, University of California, Los Angeles, informs us that if a piece of paraffin wax is placed on a clean metal, a layer will migrate over the metal at room temperature in sufficient quantity to lubricate it.

The phenomenon of migration of solids over solid surfaces is further illustrated by the migration of benzophenone over surfaces of mica, glass, silver, and polished quartz (Volmer, 1932), and the rapid penetration of insecticide, from large crystals, into dried mud blocks (Hadaway and Barlow, 1951).

It may be stated in general that molecules, atoms, and ions, present on the surface of a solid and not belonging to its lattice, can move along the surface (Bikerman, 1958), and the molecules comprising solid matter are no exception. In the case of waxes and crystals, the only factors that might be a source of surprise, to those not familiar with the phenomenon, are the speed and the extent of the process.

Physical Characteristics Affecting the Ability of a Powder to Remove Wax and to Kill Insects by Desiccation

In table 1 are listed, for sixteen powders, those physical characteristics that *a priori* might be assumed to affect the ability of these powders to remove wax from the insect epicuticle and thereby cause death of the insect by desiccation. The powders were selected to present a wide range of such characteristics as particle size, pore size, specific surface, and abrasiveness. An attempt was made to correlate these characteristics with (1) the ability of the powders to remove beeswax from plastic vials, and (2) the ability to knock down or kill four species of insects.

With regard to table 1, the data on pore diameters, specific surfaces, and to a large extent the particle sizes, were obtained from the manufacturers of the powders or from industrial testing laboratories, although some of the particle sizes were determined by the writer.

In order to determine the relative abrasiveness of the powders, pieces of vinyl plastic, of the type used for portfolio covers, were placed in a flat-bottomed glass bowl and covered with powder. The powder was then rubbed onto the plastic sheets in a uniform manner by means of a No. 8 rubber stopper. This was continued for one minute on each side of each sheet. Light from a microscope lamp was passed through the abraded area of each plastic sheet and recorded for intensity, five inches from the sheet, by means of an exposure meter with cover closed. The deviation of the exposure meter reading from that recorded from an unabraded sheet of the vinyl plastic indicated the number of foot-candles of light not reaching the exposure meter because of the increased opacity of the plastic sheet caused by the abrasion. This number was termed the "abrasive index."

TABLE 1

RELATION OF PARTICLE SIZE, PORE SIZE, SPECIFIC SURFACE, AND ABRASIVENESS OF SIXTEEN POWDERS TO BEESWAX ADSORPTION AND KNOCKDOWN OF FOUR SPECIES OF INSECTS*

Powder	Average particle size	Average pore diameter (angstroms)	Average specific surface (meters ² /g.)	Abrasive index†	Beeswax adsorption (mg./60 cm. ²)		Decreased opacity of wax films (foot-candles)	Period for 100 per cent. knockdown or mortality (hours)			
					Motionless 24 hours	In motion one hour		<i>Drosophila</i> (knock-down)	German cockroach (knock-down)	Drywood termite (mortality)	<i>Tribolium</i> beetle (mortality)
Silica aerogel SG-68.....	82% 0.1 - 3.2 μ	115	300	0	11.0 ± 1.1	6.1 ± 0.7	11.6 ± 1.6	1.9	0.97	3.1	22
Silica aerogel SG-67.....	82% 0.1 - 4.8 μ	115	247	0	12.5 ± 1.2	5.2 ± 0.4	14.4 ± 1.9	1.6	0.65	3.1	17
Silica gel AL-1.....	Average 10.0 μ	22	700	0	20.1 ± 1.0	8.3 ± 0.8	18.4 ± 1.9	1.5	0.03	1.7	29
Silikil.....	85% 1-5 μ	122	36	0	3.2 ± 0.36	3.2 ± 0.21	6.8 ± 1.0	3.0	5.4	9.6	162
Activated carbon.....	90% 325-mesh	335‡	545	1	15.9 ± 1.3	7.8 ± 0.7§	1.0	0.93	1.7	24
Olancha Clay.....	91% 325-mesh	22	130	2	12.2 ± 1.0	7.1 ± 0.8	12.2 ± 0.8	1.6	1.25	3.2	25
Attaclay.....	85% 325-mesh	28	120	2	9.2 ± 0.4	6.7 ± 0.5	10.6 ± 0.7	2.1	1.70	3.8	72
Insecticide Grade Pyrophyllite.....	93.5% 325-mesh	0	0.45	7	1.0 ± 0.1	0.7 ± 0.1	5.2 ± 0.5	8.0	26.0	25.0	264
Barytes.....	99.7% 325-mesh	0	1.0	1	0.0¶	0.0¶	0.0**	6.8	22.6	26.0	216
Molecular Sieves 5A.....	Average 2.7 μ	5	700-800	22	3.8 ± 0.3††	6.6 ± 1.0	3.2	3.5	12.4	168
Molecular Sieves 13X.....	Average 2.7 μ	13	700-800	17	6.5 ± 0.7††	10.8 ± 1.6	2.4	1.2	9.3	144
Sand.....	100% 200-mesh	0	?	20	0.0¶	0.0¶	2.0 ± 0.8	14.7	72.0	78.0	264
Carborundum 100.....	90% 100-mesh	0	?	22	0.0¶	0.0¶	0.3 ± 0.3	33.0	114.0	628.0	312
Carborundum 240.....	95% 200-mesh	0	?	22	0.0¶	0.0¶	0.8 ± 0.7	24.0	96.0	161.0	252
Carborundum 400.....	79% 5-19 μ	0	?	20	0.0¶	0.0¶	1.8 ± 1.0	10.8	43.0	45.0	224
Carborundum 600.....	85% 1-10 μ	0	?	15	0.8 ± 0.07	0.3 ± 0.03	2.4 ± 1.3	5.2	21.3	23.0	193

* Temperatures during the biological tests ranged from 20° to 29° C.; relative humidities, from 25 to 52 per cent. The temperature at the beginning of the experiment was 24° C.; relative humidity, 43 per cent. Temperatures during the beeswax adsorption tests were constant at 26.7° C.

† Decrease in foot-candles of light passing through vinyl plastic sheets abraded with the powder for one minute.

‡ Fifty per cent of the volume of the powder was made up of pores greater than 320-350 angstroms in diameter.

§ A minute film of this black powder could not be removed by washing and tended to decrease transparency.

|| Data for the montmorillonite clays in general. No specific determination has been made for Olancha Clay.

** Some of the particles became embedded in the wax, resulting in a net gain in weight of the wax film. Since these powders are practically nonporous, the particles that became embedded in the wax resulted in a slight net gain in weight.

†† The incorporation of Barytes into the wax film was so conspicuous as to be easily visible with the unaided eye and resulted in an increased opacity of 4.0 ± 0.7 foot-candles.

‡‡ Molecular Sieves reach high temperatures when agitated, resulting in the softening of the wax.

To determine the relative abilities of the powders to adsorb beeswax, plastic vials 2.5 cm. wide and 7.5 cm. long were dipped into melted white beeswax to form films of wax about 65μ in thickness and with an area of about 60 sq. cm. The vials, with lids, were placed in eight-ounce jars and covered with powder. Some were left standing for twenty-four hours, and others were turned end over end at the rate of 108 revolutions per minute, with a mechanical device, so that the powder would be in constant motion against the wax. This agitation was continued for one hour. At the end of each of these twenty-four-hour or one-hour periods, the vials were swabbed with cotton under running water. They were then air-dried and weighed, in order to determine the loss of weight caused by the adsorption of wax. Five vials were used for each of the sixteen powders tested. Five waxed vials, serving as checks, were not placed in powder, but were washed and weighed like the others. Weight lost by these vials was subtracted from that which was lost by the vials that had contact with powder.

With each decrease in the thickness of a wax layer, there should be a corresponding decrease in the opacity of the film. To test this assumption, glass microscope slides were coated on both sides with $0.4\text{-}\mu$ films of white beeswax. These were placed in various powders for six twenty-four-hour periods, after each of which the powders were removed, as previously described for the waxed vials. Five slides were used for each powder. The slides were then placed before a source of electric light, and the amount of light passing through the wax was determined by means of an exposure meter at a constant distance from the slides and in a fixed position.

The Action of Various Powders Against Insects

The pertinent physical characteristics of the powders having been determined, the next procedure was to determine their action against four species of insects. The test insects used were adult *Drosophila pseudoobscura*, adult males of the German cockroach (*Blattella germanica*), fully developed nymphs of the drywood termite (*Kaloterms minor*), and adults of the confused flour beetle (*Tribolium confusum*).

One cubic centimeter of the powder was placed in a four-ounce jelly jar with a bottom area of 45 sq. cm., and the insects were placed in the powder in the following numbers: *Drosophila*, approximately fifty (by aspiration from a mixing jar); cockroaches, five; termites, ten; and *Tribolium*, ten. All tests were made in triplicate. After the insects were placed in the jars, the latter were shaken so as to cover each insect completely with a film of powder. An excessive quantity of powder was used, in order to eliminate variables that could be attributed to inadequate or uneven deposit of powder such as might result if minimal quantities were used. It should be borne in mind, however, that if the quantity of powder per unit area is decreased beyond a certain point, the differences in the insecticidal effectiveness of powders of different bulk densities are greatly accentuated. The heavier powders may not provide a layer of sufficient bulk to enable the insects to develop a cloud of dust about their bodies or otherwise pick up a lethal quantity. For example, when SG-67 and Olancha Clay were used at the excessive quantity of 0.42 cc. per 6.5 sq. cm., the latter, which is 5.3 times greater in bulk

density, required 3.1 times longer to result in a 100 per cent knockdown of cockroaches by desiccation. However, when both powders were used in a thin film at only 1.5 mg. per 6.5 sq. cm., Olancha Clay required 12.6 times longer than SG-67 to give a 100 per cent knockdown.

In table 1, the only properties of the powders that are generally correlated with insecticidal effectiveness are their specific surfaces, their pore sizes, and their wax sorptivities. Although the specific surface of a powder is generally correlated with insecticidal effectiveness, this is not necessarily the case, for a powder may have a large specific surface but have pores that are too small to admit the large wax molecules. This is clearly the case with the Molecular Sieves. The two types used in these experiments, 5A and 13X, have uniform pores of 5 Å and 13 Å, respectively, in diameter. As the name implies, Molecular Sieves are designed to allow molecules of a certain size to pass through and to "sieve" out larger ones. For example, type 5A can adsorb propane and higher *n*-paraffins up to at least C₁₄, but not any of the *iso*-paraffins. Judging from the period required for perfectly dry Molecular Sieves 13X to knock down cockroaches, it is about equal to Olancha Clay in its ability to adsorb the epicuticular lipid of this insect. According to Beaumont (1955), the liquid solvent that gives cockroach lipid its mobile character may be about equal in quantity to the solid wax and probably consists of paraffins and alcohols of comparatively short chain lengths of the order of C₈-C₁₂. Such molecules could undoubtedly be adsorbed by the Molecular Sieves in sufficient quantity to result in a substantial increase in the intramolecular spacing of the remaining hard-wax molecules and a consequent passage of water through the epicuticle. However, Molecular Sieves 13X is inferior to Olancha Clay in its ability to adsorb the much harder beeswax, which contains few molecules below C₃₀. It is also inferior to Olancha Clay in its ability to kill insect species other than the cockroach, which possess wax of much higher average molecular dimensions.

On the other hand, a powder may have pores of adequately large diameters but may nevertheless have a small specific surface. An example of such a powder in table 1 is Silikil, which has a low wax sorptivity and relatively low insecticidal effectiveness, despite having large pores.

As shown in table 1, the silica gel AL-1 has a spectacular insecticidal effectiveness when it is dry. Cockroaches are knocked down in two minutes. However, a serious drawback in the use of both AL-1 and Molecular Sieves is that they have an especially high affinity for water. Therefore, they deteriorate rapidly, in their ability to adsorb insect wax and desiccate insects, when the dry powder is taken from a sealed can and exposed to air for a few hours, for they adsorb too much atmospheric moisture. For example, the manufacturers state that silica gel AL-1 adsorbs 24 per cent of its weight in water at 40 per cent relative humidity, 40 per cent at 80, but only 42 per cent at 100. In tests made by the writer, among desiccated powders allowed to remain at 60 per cent relative humidity for two weeks, AL-1 adsorbed 27.4 per cent of its weight in water and Molecular Sieves 13X adsorbed 29.3 per cent.

Insects are killed by dehydration when they have lost approximately half their water content, amounting to about a third of their body weight (Ebel-

ing and Wagner, 1959). Knockdown can occur long before this point is reached, however, depending on how rapidly the dehydration takes place. The rapid knockdown of cockroaches by the silica gel AL-1 is a measure of the rapid rate of water loss caused by this powder. Apparently, the insects were put into a state of shock about a half hour before they had suffered a sufficient loss of water to result in death. Normally, the dehydration caused by sorptive powders is not sufficiently rapid to cause a distinct knockdown. The activity of the insect declines gradually until death occurs. This is in contrast to the action of toxicants, which usually cause a rapid knockdown, but the period required to kill the insect is usually greater than that which is required when the same species is treated with the best sorptive powders (Ebeling and Wagner, 1959; Tarshis, 1959).

With the exception of AL-1 and Molecular Sieves, the powders shown in table 1 do not decrease to any important degree in their insecticidal effectiveness, regardless of the period during which they are exposed to air at ordinary humidities. The silica aerogel SG-67 adsorbs no water at 40 per cent relative humidity, 5 per cent of its weight at 80, and 80 per cent at 100. Thus, it retains excellent ability to adsorb wax up to nearly 100 per cent relative humidity. Then, in a water-saturated atmosphere, apparently the fluoride in this powder becomes highly effective against insects and mites (Ebeling and Wagner, 1959; Micks, 1960; and Tarshis, 1960, 1961). The same powder, without the fluoride, acts very slowly in a water-saturated atmosphere.

It appears (table 1) that a pore diameter of about 20 Å is the lower limit for effective wax adsorption, provided the powder has not had a chance to adsorb much atmospheric moisture.

Table 1 shows that all powders that were highly abrasive (Molecular Sieves, pyrophyllite, sand, and carborundum) were poor wax adsorbents and were also ineffective against the insects. There is nothing about abrasiveness *per se* that would decrease the ability of a powder to remove insect wax, but it happens that the abrasive powders are the most apt to be nonsorptive.

Barytes, also an insecticidally ineffective powder, is practically nonabrasive, but it is also nonsorptive and therefore relatively ineffective for killing insects by desiccation.

It will be noted that the *Tribolium* beetle, believed by some investigators to require an abrasive powder because of its protective tectocuticle, was relatively resistant to the action of sorptive powders. However, it required an average of only 13.9 per cent as long to obtain a 100 per cent kill of this beetle in sorptive powders as in the nonsorptive ones. With the exception of Barytes and Silikil, the nonsorptive powders were highly abrasive. On the average, *Tribolium* beetles treated with the abrasive powders died only 21.5 per cent more rapidly than those left in untreated containers.

Table 1 also shows that carborundum increased in insecticidal effectiveness with diminishing particle size. This confirms the results of David and Gardiner (1950), who ascribed the greater effectiveness of the finer powder to its greater ability to abrade the cuticle. When comparing 700-mesh carborundum (very fine) with 150-mesh (coarse), these investigators found that only the finer dust caused abrasion of the beetle *Rhizopertha*. They used the am-

moniacal silver nitrate staining technique of Wigglesworth (1945) for revealing the abraded areas. Except for the tips of the appendages, abrasion was noted only where two parts of the body moved against each other, such as the mouth parts, leg joints and sides of the abdomen, and under the margins of the elytra.

David and Gardiner (1950) followed Wigglesworth (1944, 1945, 1947a) and others in attributing lethal action to the abrasion of the cuticle and consequent water loss. Abrasion can probably account for a low order of insecticidal action in some instances. However, the specific surface of any powder greatly increases with decreasing particle size, even though the powder be nonporous. A slight ability to adsorb wax was demonstrated with carborundum 600 (table 1). Also, it should be borne in mind that the ability of carborundum to adhere to an insect may increase as much as 31-fold with decreasing particle size from the "240" to the "600" grade (David and Gardiner, 1950). These factors must be taken into account when speculating on the mode of insecticidal action of the finer grades of carborundum and other nonporous powders.

Barytes increased the weight of wax films to the extent of 2.4 ± 0.34 mg./60 cm². This heavy (but nonsorptive and virtually nonabrasive) powder has a greater tendency than the other powders to incorporate itself into the wax film, even when not in motion over the wax surface. Nevertheless, when compared with the untreated checks, Barytes can be seen to have a certain feeble insecticidal effectiveness, comparable to that of pyrophyllite and the finer grades of carborundum. Besides Barytes, the other heavy and nonsorptive powders (pyrophyllite, Molecular Sieves, sand, and carborundum) had some tendency to incorporate themselves into the wax film. For some of these (sand, carborundum 100 and 400), this resulted in a slight net gain in weight. No doubt all of the above powders adsorbed some wax, and in the case of pyrophyllite and carborundum 600, as well as the porous Molecular Sieves, this resulted in a net loss in weight of wax. The sorptive ability of a powder appears to be more satisfactorily indicated by the resulting decrease in opacity of the wax film. As shown in table 1, all powders except Barytes increased the amount of light passing through a beeswax film.

The increase in the opacity of the glass slides caused by the double layer (total of 0.8μ) of beeswax averaged about 48 foot-candles. This maximum opacity was decreased by the six successive adsorptions in the various powders to the extent shown in the table (averages for the five slides). When these figures are compared with the quantities of beeswax adsorbed, also shown in table 1, they indicate that the ability of powders to decrease the opacity of wax films is approximately proportional to their ability to remove wax, as indicated by gravimetric analysis. This, in turn, is determined primarily by the adsorbency of the powders.

The above experiment confirms the findings of Helvey (1952) in tests with the larvae of the Mexican bean beetle, *Epilachna varivestis*. His electron micrographs showed that the powders with little or no insecticidal value had particles containing sharp edges and points, and that the most effective powders were also the least abrasive.

Alexander *et al.* (1944) found that inert finely divided powders increased

in their ability to kill insects as they decreased in particle size down to the optimum size of about 1 μ . Nevertheless, in the present experiments, the highly abrasive Molecular Sieves, with an average particle diameter of 2.7 μ , and 600-mesh carborundum, with 85 per cent from 1 to 10 μ , were inferior in insecticidal effectiveness to sorptive 325-mesh powders such as activated carbon, Olancha Clay, and Attaclay, which have an average particle-size range of from 15 to 18 μ .

As stated previously, some sorptive powders have the ability to adsorb wax at 100 per cent relative humidity, and cause the insects to lose water. Silica aerogels appear to be particularly effective in this connection. In one experi-

TABLE 2
WEIGHT LOSS AND MORTALITY OF GERMAN
COCKROACHES LEFT FOR TWENTY-FOUR
HOURS IN VARIOUS POWDERS AT 100
PER CENT RELATIVE HUMIDITY*

Powder	Weight loss (per cent)	Mortality (per cent)
SG-68.....	41.7	100
Olancha Clay.....	24.8	46.7
Activated carbon.....	25.5	53.3
AL-1.....	9.4	0.0
Molecular Sieves 13X.....	7.0	0.0
Carborundum 600.....	2.8	0.0
None.....	1.3	0.0

* The powders were left for eighteen hours at 100 per cent relative humidity before the insects were added. The temperature was 24.5° C. while they were in the powder.

ment, six powders were placed in four-ounce jelly jars and left for twenty-four hours at 100 per cent relative humidity. The humid chambers were glass desiccators with an inside diameter of 20.3 cm. and containing 720 ml. of distilled water. Then, for each powder, five male German cockroaches were placed in each of three jars; the jars were briefly shaken to insure a complete film of powder on each insect, and left in the humid chamber. Table 2 shows the per cent loss of weight and per cent mortality of the cockroaches after they had been left in the powder for twenty-four hours. Powder adhering to their bodies was removed with a camel's-hair brush before they were weighed. Experiments with freshly dusted cockroaches showed that the brief period required for cleaning and weighing the insects in the ambient atmosphere was not sufficiently long to account for any significant weight loss.

The above experiment might be subject to the criticism that the removal of the cover at the time the cockroaches were placed in the desiccator temporarily decreased the relative humidity in this chamber below 100 per cent. Therefore, an experiment was made in which it was not necessary to remove the cover, for a hole was bored in the rubber stopper at its apex. This hole was normally taped over, but a forceps could be extended through it so as to effect certain manipulations without removing the cover.

Silica aerogel SG-68 was placed in a jelly jar of 7.5 cm. diameter at the usual rate of 1 cc. per 45 sq. cm. This jar also contained an upright vial in which were ten male German cockroaches, confined by means of a gauze cover

held in place with a rubber band. Another vial with ten cockroaches was placed in the desiccator, but outside the jelly jar. After the SG-68 and the cockroaches had remained in the jar for twenty-four hours, the cockroaches were liberated by means of the forceps and crawled about in the powder. Twenty-four hours later, these cockroaches, as well as the check insects, were removed from the desiccator, cleaned, and weighed. The insects in the SG-68 had lost 42.5 per cent of their weight, and the check insects, 3.3 per cent. In an adjoining desiccator in which the same experiment was made except that the lid was removed to place the insects into the desiccator, the cockroaches in SG-68 lost 42.3 per cent of their weight, and the check insects, 3.4 per cent. In both cases, the insects left in the powder were all dead. This indicated that the momentary removal of the lid of the desiccator resulted in no significant difference in the rate of desiccation of the insects. During this experiment, temperatures ranged from 23.3° to 26.7° C.

Insects can lose water at 100 per cent relative humidity only when they are left in continuous contact with the powder. Apparently, water is released from the insect cuticle, even in a water-saturated atmosphere, if the protective wax layer is removed. However, this water must be continuously sponged away by the powder in order that more water may be released from the cuticle. It was shown by Ebeling and Wagner (1959) that when drywood termites were shaken in a sorptive powder, so as to remove their lipid, and the powder was then removed, they did not die in a water-saturated atmosphere. However, they all died within one day if exposed to normal conditions of relative humidity. If left in the water-saturated environment for four days, then placed in the ambient atmosphere, they all survived, having had time to restore the wax removed by the powder.

The ability of certain powders to cause insects to lose water at 100 per cent relative humidity is of great academic interest, but of little or no practical value. However, the addition of a monomolecular layer (4.7 per cent) of ammonium fluosilicate to SG-68 results in the product referred to in this investigation as SG-67. This powder kills cockroaches and fleas about as rapidly at 100 per cent relative humidity as it does under a wide range of humidities, down to as low as 20 per cent. It is believed that the silica continues to remove wax, even in a water-saturated atmosphere. This would allow the fluoride to contact the water-bearing protein layers below the protective lipid and become toxic to the insect, even though normally it cannot be used as a contact insecticide.

Rate of Wax Adsorption with One Exposure

The inner walls of four glass adsorptive tubes were coated with white beeswax by means of a hot 50 per cent solution of the wax in carbon tetrachloride, containing 1 per cent Oil-Soluble Red dye. Twenty-four hours later, the tubes were placed in a Klett-Summerson photoelectric colorimeter, and readings were made. Then silica aerogel SG-67 was poured into the test tubes and emptied by tapping. This left a film of powder on the wax surface. Colorimeter readings were then made in one, four, eight, and forty-four hours. The increases in the readings resulting from the silica aerogel alone, immediately after application, were subtracted from subsequent readings. The results are shown in figure 3.

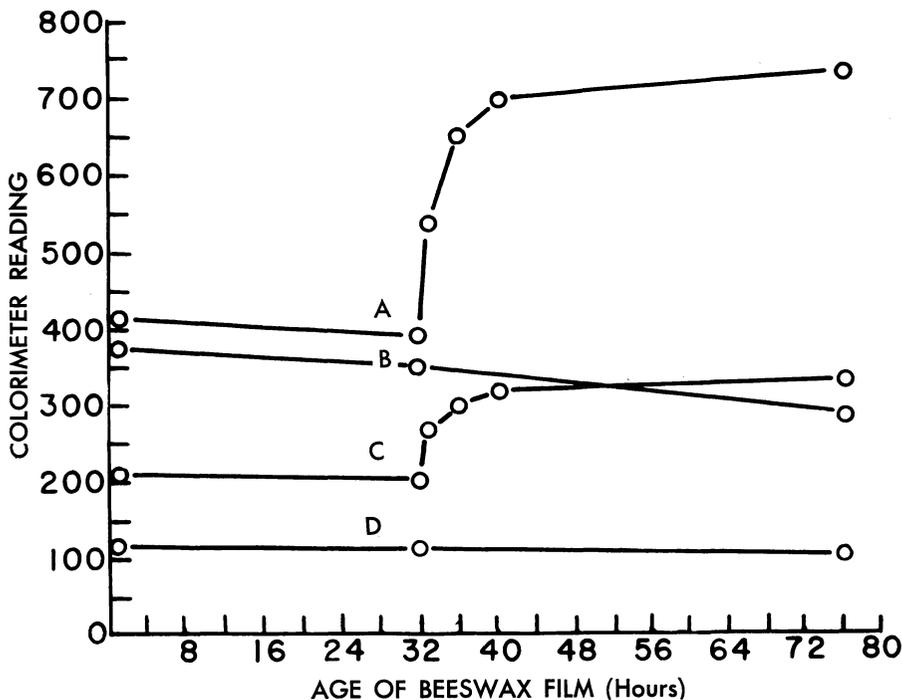


Fig. 3. Net gain in optical density of dyed beeswax-silica aerogel layers after contact of films *A* and *C* with silica aerogel SG-67 for thirty-two hours, indicating rate of adsorption of wax with a single exposure to the sorptive powder. The optical density reading resulting from the powder itself was subtracted from the total.

Within one hour, there occurred a sharp increase in the opacity of the wax-silica aerogel layers. This was believed to be caused by the adsorption of the dyed wax by the silica particles, resulting in a scattering of light with a consequent increase in the optical density readings on the colorimeter. Before adsorption, the dyed wax had existed in translucent films with a minimum of opacity.

Within forty-four hours, the colorimeter readings appeared to have reached approximately their maximum values, indicating that about all the wax that could be adsorbed with a single layer of powder had already been removed. However, 46 per cent of the maximum adsorption had occurred in one hour, 76 per cent in four hours, and 88 per cent in eight hours.

Rate of Wax Adsorption with Successive Exposures

It was believed that successive exposures of the same wax layer or film to fresh portions of sorptive powder might reveal information on the nature of wax adsorption that could not be ascertained by the exposure of the wax to only one application of the powder. Plastic vials 2.5 cm. wide and 7.5 cm. long were dipped into melted yellow beeswax, and others were dipped into paraffin wax, so that layers of wax were formed on their outer surfaces. The layers of wax were about 65μ thick on the vials dipped into beeswax and about 75μ thick on those dipped into paraffin wax (Parowax). The vials were then weighed, and

half of them were coated with the silica aerogel SG-67, after which the excess powder was removed by light tapping, and the vials were then kept at room temperatures ranging from 23° to 27° C. (average, 24.4° C.) or in an oven at a constant temperature of 48° C. The other half were kept under the same temperature conditions but not in contact with powder. In this and the remaining experiments discussed in the present paper, three vials were used for each test.

At twenty-four-hour intervals, the vials were swabbed with cotton under a stream of cold water. They were then air-dried and weighed, in order to determine the loss in weight caused by the adsorption of wax. Loss of weight with untreated checks was extremely small but was nevertheless subtracted from the loss with treated vials. By wiping the inside of the vial with a clean cloth just before weighing, variation caused by differences in degree of moisture sorption on the inner (unwaxed) surfaces of the vials was eliminated.

The results are shown in figure 4. The loss of weight of beeswax at 24.4° C. or at 48° C. (fig. 4, *A* and *C*), or of paraffin at 24.4° C. (*B*), was negligible. However, both dusted beeswax at 24.4° C. (*D*) and dusted paraffin at 24.4° C. (*E*) were readily adsorbed by silica aerogel. The amount of wax adsorbed in sixteen days was about 5.5 times greater for dusted beeswax in contact with silica aerogel at 48° C. (*F*) than at 24.4° C. (*D*).

The weight-loss curve for paraffin at 48° C. (*G*) would not have been so great during the first four or five days if it had not been for the fact that some of the wax softened enough to flow off the vials to the bottom of the container in which the vials were standing in a vertical position. This was not the case, however, when the wax film was covered with silica aerogel (*H*), for the wax was adsorbed so rapidly that it did not flow.

Plotting the loss in weight of wax films at 48° C., or wax films in silica aerogel at either 48° C. or room temperature, resulted in all cases in an exponential curve, as one would expect from first-order reaction kinetics. This is the type of curve that characterizes the selective removal of certain kinds of molecules, which constitute a progressively lower percentage of the remaining mass with each successive removal.

Figure 5 shows the loss in weight of waxed vials placed in eight-ounce jars that were half full of powder and then agitated mechanically, as mentioned previously, for successive periods of one hour, using a fresh batch of powder each time. After each period, the vials were washed and weighed as in the experiments depicted in figure 4. Five of the vials were agitated moderately and uniformly, but the sixth, indicated by the words "severe abrasion" in figure 5, was shaken with greater force and rapidity. The uppermost of the two curves for carborundum depicts the more severe abrasive action.

With carborundum, the first weighing of a series must be rejected because of a gain in weight caused by particles lodged in the wax which are not removable by the washing technique used in these experiments. The quantity appears to be compensable, however, for after the first washing, the wax film underwent a continual reduction in weight as a result of the successive abrasions.

As in the experiment depicted in figure 4, the curves in figure 5, showing the amount of beeswax removed with successive shakings of the waxed vials,

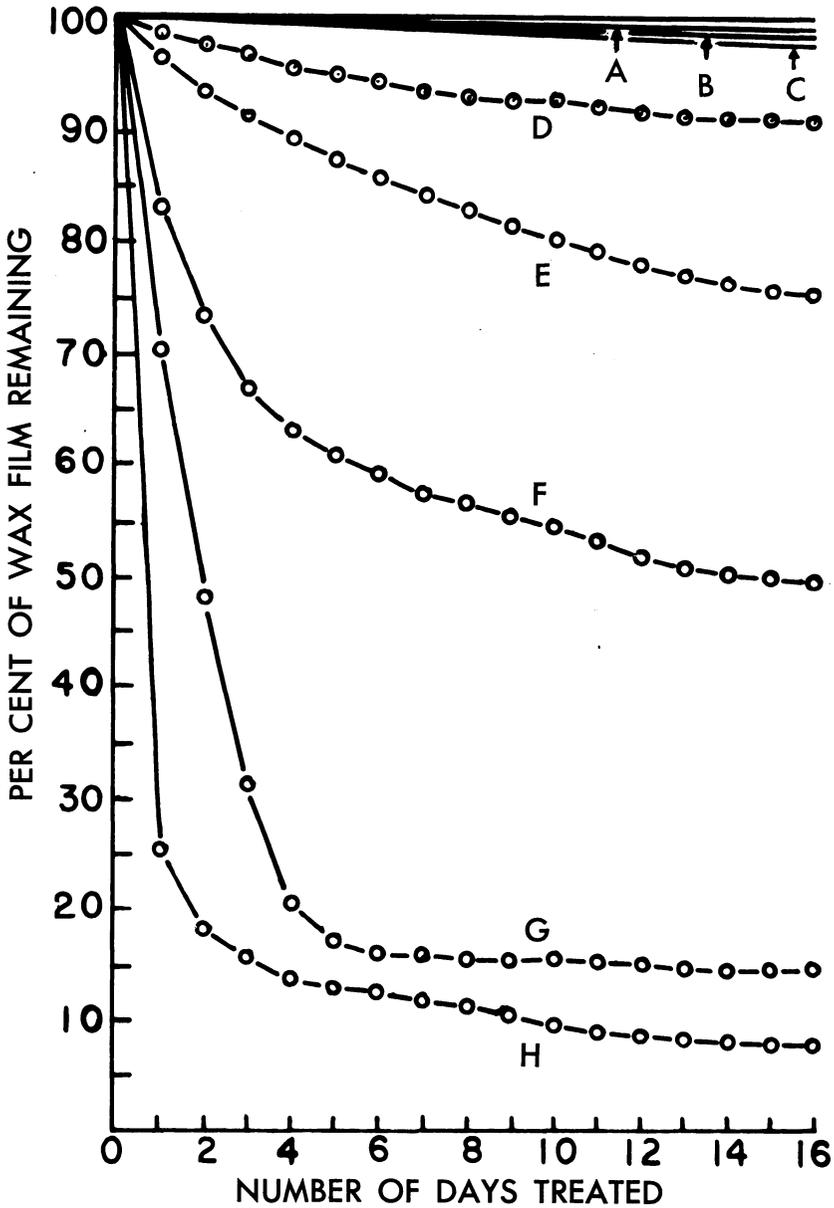


Fig. 4. Per cent decrease in weight of films of beeswax and paraffin on plastic vials, half of which were dusted with silica aerogel SG-67. All vials remained at 24.4° C. or 47.8° C. for twenty-four-hour periods. *A*, beeswax at 24.4° C.; *B*, paraffin at 24.4° C.; *C*, beeswax at 48° C.; *D*, dusted beeswax at 24.4° C.; *E*, dusted paraffin at 24.4° C.; *F*, dusted beeswax at 48° C.; *G*, paraffin at 48° C.; *H*, dusted paraffin at 48° C.

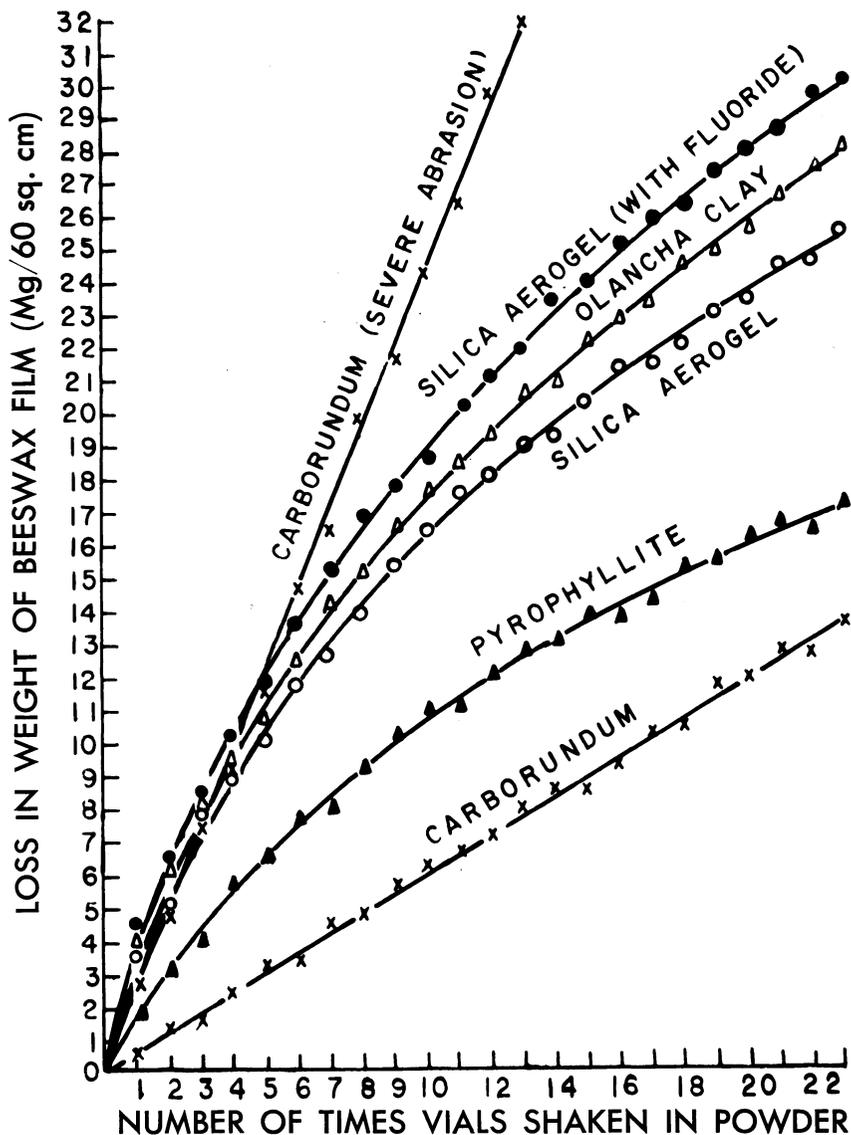


Fig. 5. Weight losses of 65- μ -thick beeswax films on plastic vials repeatedly immersed in various powders and simultaneously mechanically shaken for periods of one hour, except for the one marked "severe abrasion," which was shaken in a more violent manner, separately from the others.

were exponential in character whenever the removal of the wax was caused primarily by adsorption. With carborundum, however, the beeswax was removed from the vials primarily by abrasion. This was indicated by the opaque and dull appearance of the wax, in sharp contrast to the smooth and translucent appearance of the films on the vials shaken in the relatively

nonabrasive powders. The appearance caused by the carborundum is characteristic of the action of abrasive powders when their particles have been in motion against the wax film. Powders whose action is predominantly adsorptive do not change the appearance of the wax film, even though their ability to remove wax is greater than that of the abrasive powders.

The points representing weight loss of wax from shaking the waxed vials in carborundum form straight lines. This indicates that abrasive powders are nonselective, removing the wax molecules indiscriminately, layer after layer, until all the wax is removed. On the other hand, powders that are primarily sorptive remove only those molecules which are amenable to removal by sorption. Plotting of the weight loss of the wax film when such powders are used, whether the particles of the powder are moving or motionless, invariably results in an exponential curve reaching an asymptote. Regardless of the thickness of the wax film, a point is reached when no more wax can be removed by successive adsorptions, unless the remaining wax is melted or dissolved away with a solvent and redeposited on the vial.

Figure 6 shows the loss in weight of films consisting of white beeswax (*A*) and the yellow beeswax of commerce (*B*) when the waxed vials were shaken in silica aerogel SG-67 for successive ten-minute periods and for one twenty-four-hour period. In this experiment, glass vials 2 cm. in diameter and 8.5 cm. long were dipped in a 3 per cent solution of beeswax in carbon tetrachloride, so that the wax film was 1 μ in thickness. A strip of wax about 1 cm. in width was then removed from both ends of each vial, to facilitate handling without touching the wax. It is interesting to note that the adsorption curves reached their asymptote when about 20 per cent (curve *A*) and 24 per cent (curve *B*) of the wax was removed. It was found throughout this investigation that the shorter the successive periods of adsorption, the smaller the ultimate yield of wax. Further removal of wax can be accomplished only by long, continuous contact of the wax film with the powder, whether the powder is in motion or not. This is shown by the curves in figure 6.

Further data on this subject were obtained by immersing waxed glass vials in silica aerogel SG-67 in five successive series of immersions, each consisting of four hourly periods followed by a forty-four-hour period of continuous immersion. No agitation of the vials was involved in this test. The results are shown in table 3. In the first trial, the sum of the quantities of wax adsorbed during the first four hourly immersions was 79 per cent as great as for the following forty-four hours of continuous adsorption. However, the fifth time this comparison was made, the corresponding percentage was 32. Over the ten-day period, the decrease in the yield for four consecutive hour-long adsorptions was 80 per cent, compared with 47 per cent for the forty-four-hour periods of continuous adsorption. The significance of these data appears to be that, as the adsorbed molecules are being withdrawn increasingly farther from the surface with successive adsorptions, the advantage of long, continuous periods of adsorption becomes progressively greater.

Figure 7 refers to an experiment in which glass vials the size of those used in the experiment depicted in figure 6 were dipped in 3 per cent beeswax in carbon tetrachloride, so that the film of wax was approximately 1 μ in thickness. The waxed vials were buried in silica aerogel SG-67 for successive

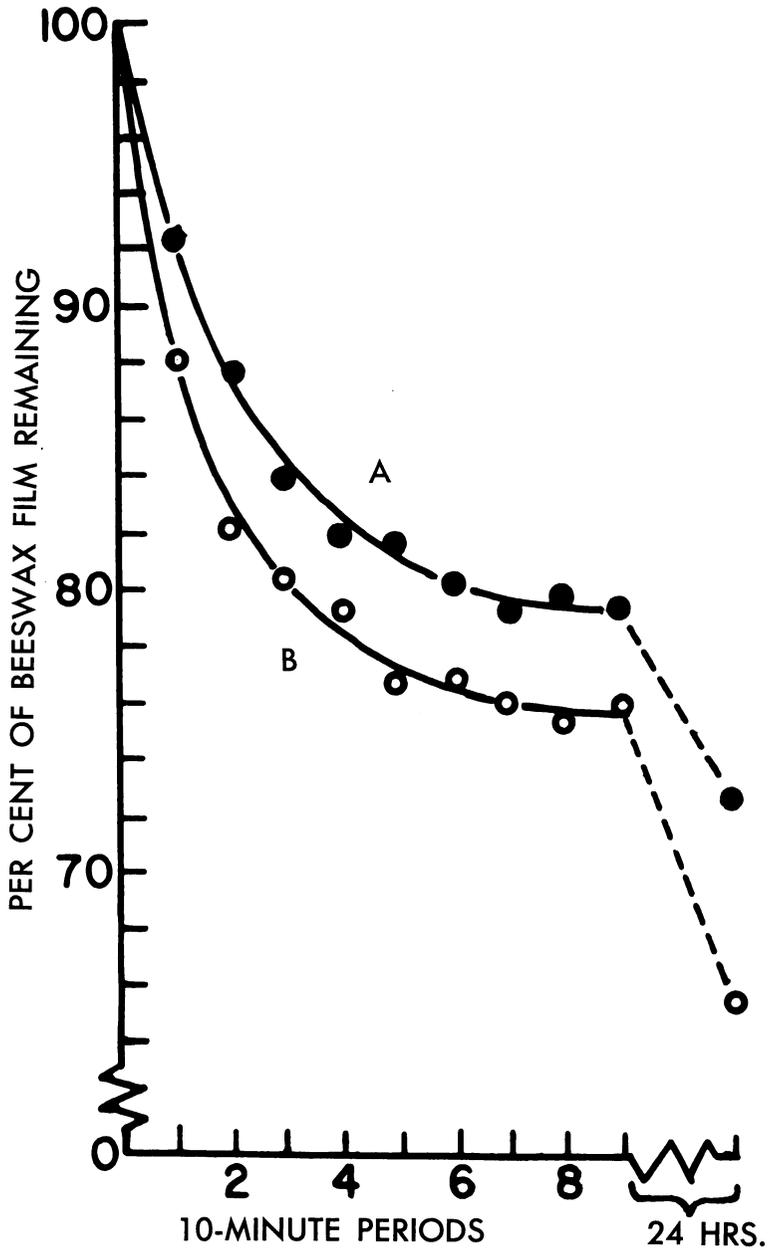


Fig. 6. Per cent decrease in weight of beeswax films on plastic vials repeatedly immersed in silica aerogel SG-67, and mechanically shaken for nine ten-minute periods and one twenty-four-hour period. *A*, white beeswax; *B*, commercial yellow beeswax.

TABLE 3
 DECLINE IN YIELD OF WAX THROUGH ADSORPTION BY
 SILICA AEROGEL SG-67 IN FIVE CONSECUTIVE TRIALS
 OF FOUR ONE-HOUR PERIODS FOLLOWED BY
 ONE FORTY-FOUR-HOUR PERIOD

Consecutive trials	Quantity of beeswax adsorbed (mg.)	
	Four one-hour periods*	One forty-four-hour period†
1.....	2.08	2.62
2.....	1.31	2.44
3.....	0.64	1.68
4.....	0.54	1.47
5.....	0.44	1.39

* Decrease in four-hour yields, 80 per cent.
 † Decrease in forty-four-hour yields, 47 per cent.

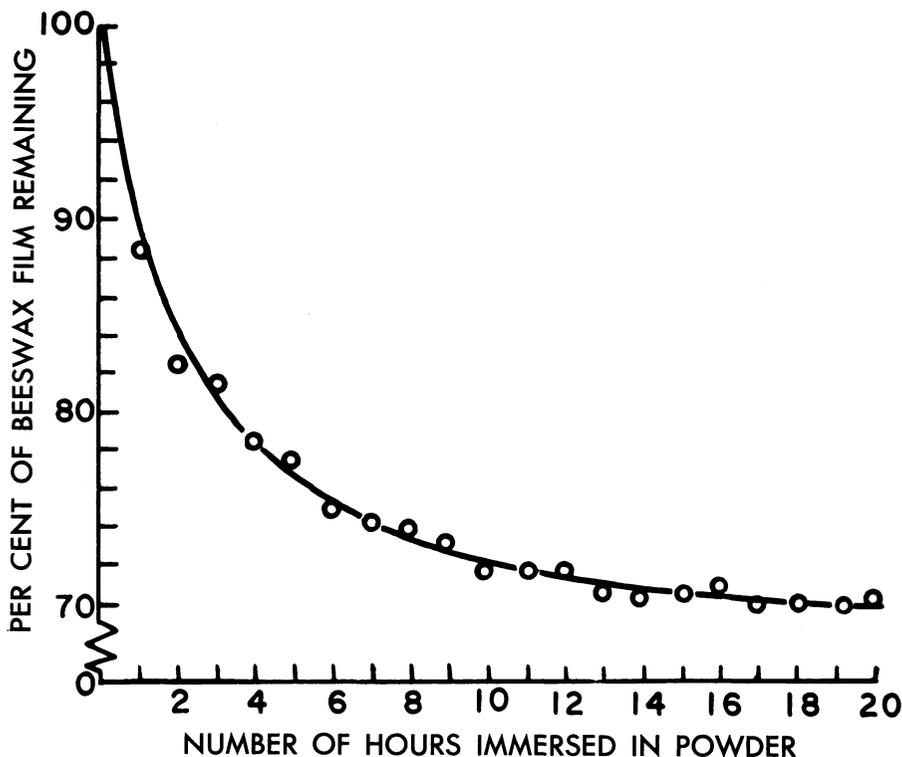


Fig. 7. Per cent decrease in weight of 1- μ -thick beeswax films on glass vials when repeatedly immersed in fresh silica aerogel SG-67 for periods of one hour.

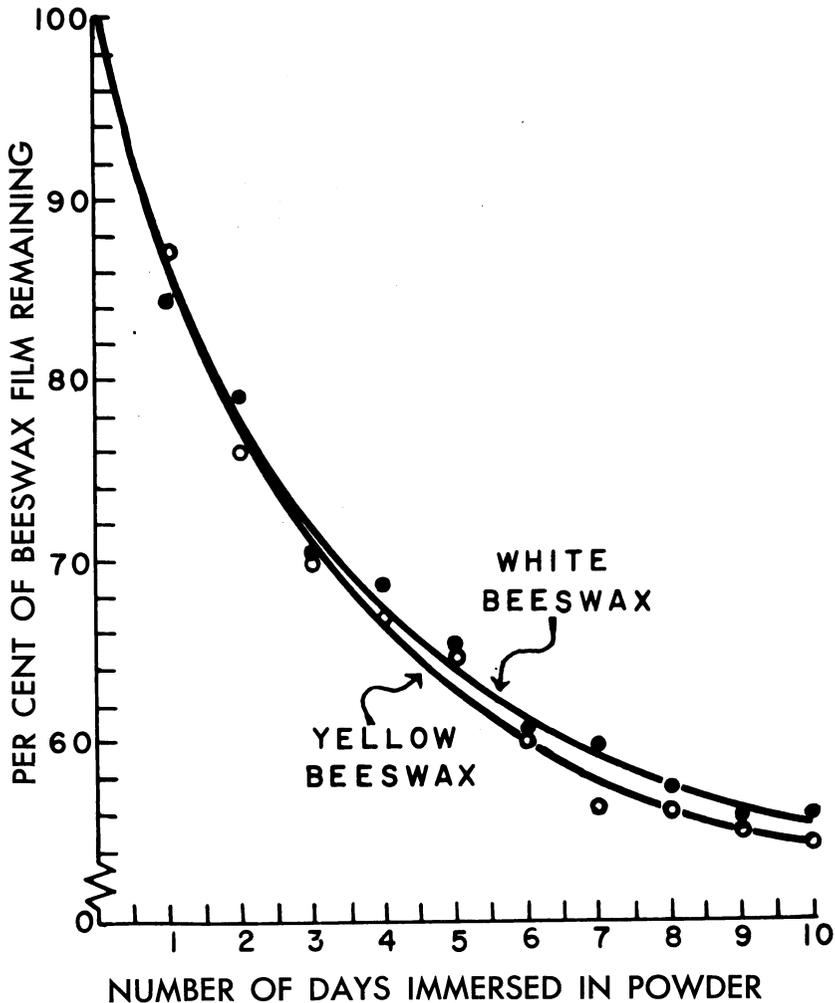


Fig. 8. Per cent decrease in weight of $1\text{-}\mu$ -thick beeswax films on glass vials when repeatedly immersed in fresh silica aerogel SG-67 for periods of twenty-four hours.

periods of one hour. The vials remained motionless, and care was taken to avoid movement of powder against their surfaces. As in previous experiments, the powder was removed at the end of each period of immersion.

The curves in figure 8 should be compared with the one in figure 7. The experiment was made in the same way, except that the waxed vials were immersed in the silica aerogel for twenty-four-hour periods instead of one-hour periods. White beeswax was compared with yellow beeswax, and no significant difference was found. Again the films of wax were $1\ \mu$ thick. Note that figure 7 shows about the same amount of wax adsorbed in four one-hour periods as in the first two twenty-four-hour periods of the experiment shown in figure 8. However, there was no further adsorption of wax after about 29

per cent had been removed. On the other hand, figure 8 shows continued losses of wax from the vials until about 46 per cent had been removed. This again illustrates the necessity for long, continuous periods of adsorption for the highest yields of wax.

Adsorption experiments were also made with much thinner layers of wax, down to a thickness of 0.32μ —well within the range of the thickness of the lipid layer on insects. However, the data were much more variable, and the wax layer had a tendency to flake off in a few places before the experiment had been terminated. Nevertheless, it appeared that about 40 per cent of the wax in these very thin layers could be adsorbed, and the data, despite the greater scattering of points, resulted in curves similar to those shown for $1\text{-}\mu$ wax layers (figs. 7 and 8).

Percentage of Wax Removed to Effect Water Permeability

To obtain an estimate of the approximate percentage of wax that must be removed to enable water to penetrate the protective wax layers on insects, glass vials were dipped into a hot 3 per cent solution of white beeswax in carbon tetrachloride. The layers formed were approximately 0.3μ in thickness. Since the C-C distance is 1.54 \AA , this figure was used as the basis for calculation of the lengths of typical molecules. The linear component of the distance between the zigzagging carbon atoms can be calculated to be 1.27 \AA . Since the acids, hydrocarbons, and the longest branch of the V-shaped esters contain 30–33 carbon atoms, their length was calculated to range from 36.8 to 40.6 \AA . Assuming the molecules to be unbranched and placed end to end and uncoiled, a $0.3\text{-}\mu$ layer of beeswax should be between 74 and 81 molecules in depth.

The glass vials with these very thin layers of beeswax were slowly but continuously moved about in revolving jars of silica aerogel SG-67 for thirty minutes. This is the period required to cause a knockdown, by desiccation, of honeybees crawling about in silica aerogel or highly sorptive clay. About 16 per cent of the wax was removed in this period. It appears that some such small percentage of loss of amorphous-wax molecules suffices to make certain insects susceptible to an abnormal and lethal rate of desiccation.

DISCUSSION

It has been shown by Beament (1945) that the layer of oriented wax molecules resting on the tanned protein of the insect epicuticle provides practically all the protection against water loss. Since the wax must be secreted with sufficient solvent to insure its mobility, it not only can pass through the hardened cuticle during the process of repair, but it is also temporarily in a form readily amenable to orientation. Considering the relative polarities of the components of insect wax (acids, alcohols, esters, and hydrocarbons), one might expect that the first layer of molecules at the interface between the lipid and its hydrophylic substrate might reasonably consist principally of wax acids and alcohols. Since the nonpolar ends of these molecules would extend away from the interface, the next layer of molecules might contain many esters and hydrocarbons.

The oriented layer of molecules must be considered to be closely packed, for the intramolecular spaces are normally impervious to water. Apparently,

the removal of only a small percentage of these molecules enables the relatively small water molecules to pass through the widened intramolecular spaces. The present investigation indicated that, with honeybees, only about 16 per cent of the wax need be removed, and that this may be accomplished in as brief a period as thirty minutes. This would explain the rapid effect of sorptive powders on certain small insects such as *Drosophila*, ants, and hymenopterous parasites. However, merely a disorganization of the layer of oriented molecules could also have the effect of making it penetrable by water.

The physical chemistry of the removal of wax from the insect epicuticle by adsorption must, for the time being, remain speculative. X-ray patterns of white beeswax rodlets, 1 mm. in diameter, showed that the crystallites that remained after eight twenty-four-hour adsorptions with silica aerogel SG-67 were identical to those in the original wax. If the wax consisted entirely of crystallites and these were adsorbed by the silica powder, one would expect that, in the case of a wax film, the entire film could be removed and that the rate of adsorption would be a straight-line function. However, since no more than 46 per cent of the wax was ever removed by adsorption in the present series of experiments, it was concluded that the adsorbed material was amorphous (see p. 536) and that the crystallites could not be removed.

Apparently, when a nonadsorbed wax film is dissolved in a suitable solvent, a random redistribution of molecules occurs, so that, when a new film is formed, approximately the same percentage of amorphous material is again present and amenable to adsorption.

Infrared spectra of beeswax showed a higher peak for wax acids before contact of the wax with silica aerogel than after repeated contacts. It is conceivable that the acids of the amorphous wax might be preferentially adsorbed by the polar silica particles, but since the acids constitute less than 15 per cent of the wax film, this would account for only a small part of the adsorbed wax. It is most likely that the exponential nature of the adsorption curve resulted primarily from the preferential adsorption of the amorphous wax in general. With each successive adsorption, less material was removed from the film, until only the unadsorbable crystallites remained.

As amorphous-wax molecules were removed from the surface layers, those farther down would take their places, and so on down through the wax film. Eventually, minute channels would be formed through the remaining crystallite matrix. In addition, a sufficient number of molecules would be removed from the closely packed and oriented layer at the lipid/protein interface to result in a significant increase in the intramolecular spacing in this layer. This would, in turn, result in the escape of the relatively small water molecules from the water-bearing protein substrate.

It is realized, of course, that in the insect epicuticle the presence or absence of the "tectocuticle," and its consistency or location, are factors that would alter the process of wax removal from the ideal representation afforded by a homogeneous layer of beeswax. However, experience indicates that such factors affect only the speed of the process, rather than its essential mechanism.

With adsorption, it is not necessary to remove all the wax at a given area of the insect's epicuticle in order to cause water loss at that point. As explained above, desiccation begins when only a small fraction of the wax has

been removed. In contrast, with abrasion the wax must be completely removed, down to the water-bearing tanned protein of the insect's epicuticle, in order to cause water loss at a given point.

The epicuticle has no pores visible, even with the aid of an electron microscope (see Richards and Anderson, 1942), but water is copiously supplied to this thin hydrophilic layer (*circa* $1\ \mu$) by means of myriads of pore canals extending through the procuticle. Pore canals commonly have a density of about 1,000,000 per sq. mm. (Richards, 1958). In certain species, these eventually become plugged with cuticular material, but obviously the "solid" procuticle is readily permeable to water. Thus, the mechanism exists for the rapid passage of water out of the insect's body at an abnormal rate under a rather wide variety of conditions: (1) when a certain percentage of the molecules in the wax layer on the epicuticle has been removed by the process of selective adsorption of molecules described in the present paper; (2) by the removal of wax the entire distance down to the lipid/protein interface by abrasion provided by the experimenter, as with an emery cloth, or when the insect's habits are conducive to the abrasive action of dusts; (3) by an increase in temperature to the point at which the oriented molecules of the organized portion of the lipid protective layer are disorganized (Beament, 1958); (4) by the application of oil or solvents (Wigglesworth, 1942, 1945; Hurst, 1943; Beament, 1945; and Ebeling and Wagner, 1959), particularly those with the most suitable balance of hydrophilic and lipophilic characteristics; and (5) by the application of any of a wide variety of insecticides (Ebeling and Pence, 1957; Kitaoka and Yajima, 1958; and Ebeling and Wagner, 1959).

SUMMARY

Beeswax is adsorbed at room temperature by silica aerogel and other sorptive powders and subsequently migrates from particle to particle to saturate an entire aggregate of powder. This was demonstrated by allowing silica aerogel powder to settle on beeswax layers from a cloud of dust and then observing the change in color of the aerogel from a translucent gray to an opaque white as it gradually adsorbed the wax. The observation of adsorption was facilitated by the use of a dyed beeswax or the dyed cuticles of various species of insects.

A study was made of the correlation of certain physical characteristics of finely divided powders, such as particle size, pore size, specific surface, abrasiveness, and ability to adsorb beeswax, with their ability to cause knock-down or death by desiccation of four species of insects. The only characteristics of the powders that were generally correlated with high insecticidal effectiveness were adequate pore size, large specific surface, and the resulting high degree of wax sorption. Porous powders, even those with a large specific surface, were generally ineffective if their pores were below 20 Å in diameter. Such powders might have a high sorptivity for water and the smaller organic molecules, but their pores appeared to be too small to adsorb adequate quantities of the larger wax molecules (*circa* C_{30}) that are characteristic of insect waxes. However, the lipid of the cockroach epicuticle, which contains a large quantity of relatively small molecules of the order of C_8 - C_{12} , can be adsorbed in sufficient quantity by perfectly dry Molecular Sieves, with a pore diameter of 13 Å, to result in death by desiccation in seventy-two minutes.

One silica gel (AL-1) resulted in a knockdown of German cockroaches in two minutes. Apparently, these insects lost water at such a rapid rate that they were put into a state of shock about a half-hour before they had lost enough water to result in death.

The above statements refer to dry powders. Some of these adsorb too much water under ordinary atmospheric conditions and do not retain their ability to adsorb wax. Others adsorb very little water until high humidities are reached; these are the only powders that are of practical usefulness in pest control.

The rate of weight loss of wax films on glass or plastic vials from successive adsorptions with powders was an exponential function, indicating that the adsorption was selective, removing only certain wax constituents. This was true, whether the waxed vials were agitated or were allowed to remain completely motionless in the powder. Since X-ray patterns indicated that beeswax was only partially crystalline, it was concluded that amorphous material was preferentially removed.

Infrared spectra indicated a preferential adsorption of wax acids from 1- μ films of beeswax by silica aerogel powder. However, acids constituted less than 15 per cent of the total, and as much as 46 per cent of the wax was adsorbed. Probably, any molecules in amorphous wax can be adsorbed.

When the remaining wax was redissolved and new films were thus formed, the adsorption of wax by silica aerogel was almost as rapid as before. This indicates that when the remaining layer of crystallites was reorganized by dissolving in a solvent and preparing a new layer, amorphous wax again appeared, and adsorption by means of silica aerogel powder could again be accomplished.

When agitated with carborundum, which is abrasive but nonsorptive, the rate of weight loss of beeswax was a straight-line function, indicating an indiscriminate and nonselective removal of wax.

When glass vials with 1- μ films of beeswax were allowed to remain in silica aerogel for successive periods of one hour, the weight-loss curve reached an asymptote when about 29 per cent of the wax was adsorbed. When left in silica aerogel for successive periods of twenty-four hours, about 46 per cent of the wax was eventually adsorbed.

Decrease in the opacity of waxed glass slides, when immersed in various powders for six successive twenty-four-hour periods, was approximately proportional to the loss of weight of wax and depended on the adsorbency of the powders.

It is believed that solid wax can be removed from wax films by adsorption of surface molecules unattached to crystal lattices and a progressive displacement of successive layers of similarly unattached molecules as they fill the spaces left by those removed in the layer above. With insects, this process would begin at the surface and proceed progressively downward to the interface of the wax layer and its water-bearing substrate. It appears that, with some insects, when only approximately a sixth of the wax film is removed by adsorption, minute channels are created in the matrix of remaining crystallites. Finally, a sufficient number of molecules are removed from the closely packed and oriented layer at the lipid/protein interface to result in a significant increase in the intramolecular spacing of this layer. This, in turn, allows

for the escape of the relatively small water molecules from the water-bearing protein substrate. The lipid film is transformed, in effect, into a "molecular sieve." The resulting rapid loss of water can cause the knockdown or death of some insects in as brief a period as ten or fifteen minutes.

In one test, German cockroaches lost 41.7 per cent of their weight and suffered a 100 per cent mortality when in contact with an unfluoridated silica aerogel (SG-68) at 100 per cent relative humidity and for a period of twenty-four hours. Weight loss in a water-saturated atmosphere takes place at this rate only if the insects are in constant contact with powder. The water appears to be released by the adsorption of the protective wax layer, even in a water-saturated atmosphere. Apparently, it must be continuously sponged away from the cuticle, by means of the powder, in order to allow for passage of more water.

The addition of a monomolecular layer (4.7 per cent) of ammonium fluosilicate to SG-68 causes the treated powder (SG-67) to be as effective against some insects at 100 per cent relative humidity as it is at normal humidities.

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LITERATURE CITED

- ALEXANDER, P., J. A. KITCHENER, and H. V. A. BRISCOE
1944. Inert dust insecticides. *Ann. Appl. Biol.* **31**:143-59.
- AZAROFF, L. V., and M. J. BUERGER
1958. The powder method in X-ray crystallography. McGraw-Hill Book Co., Inc., New York. 342 pp. *See specifically*: p. 266.
- BARTLETT, B. R.
1951. The action of certain "inert" dust materials on parasitic Hymenoptera. *Jour. Econ. Ent.* **44**:891-96.
- BEAMENT, J. W. L.
1945. The cuticular lipoids of insects. *Jour. Exptl. Biol.* **21**:115-31.
1955. Wax secretion in the cockroach. *Jour. Exptl. Biol.* **32**:514-38.
1958. Waterproofing mechanism of an insect. *Jour. Exptl. Biol.* **35**:494-515.
1959. The waterproofing mechanism of arthropods. I. The effect of temperature on cuticle permeability in terrestrial insects and ticks. *Jour. Exptl. Biol.* **36**:391-422.
- BIKERMAN, J. J.
1958. Surface chemistry. Second edition. Academic Press, Inc., New York. 501 pp.
- BISSON, C. V., G. H. VANSELL, and W. B. DYE
1940. Investigations on the physical and chemical properties of beeswax. U.S.D.A. Tech. Bul. **716**:1-24.
- CHIU, S. F.
1939. Toxicity studies of so-called "inert" materials with the bean weevil *Acanthoscelides obtectus*. *Jour. Econ. Ent.* **32**:240-48.
- COTTON, R. T., and J. C. FRANKENFELD
1949. Silica aerogel for protecting stored seed or milled cereal products from insects. *Jour. Econ. Ent.* **42**:553.
- DAVID, W. A. L., and B. O. C. GARDINER
1950. Factors influencing the action of dust insecticides. *Bul. Ent. Res.* **41**:1-61.
- EBELING, W., and R. J. PENCE
1957. The use of pesticides in the preparation of insects and mites for photography. *Ann. Ent. Soc. Amer.* **50**(6):637-38.
- EBELING, W., and R. E. WAGNER
1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. *Jour. Econ. Ent.* **52**:190-207.
- GERMAR, B.
1936. Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. *Zeitschr. angew. Ent.* **22**(4):603-30.
- GLYNNE JONES, G. D.
1955. The cuticular waterproofing mechanism of the worker honeybee. *Jour. Exptl. Biol.* **32**:95-109.
- HADAWAY, A. B., and F. BARLOW
1951. Sorption of solid insecticides by dried mud. *Nature (London)* **167**:854.
- HELVEY, T. C.
1952. Insecticidal effect of inert solid diluents. *Science* **116**:631-32.
- HOCKENYOS, G. L.
1933. Effect of dusts on the Oriental roach. *Jour. Econ. Ent.* **26**:792-94.
- HOLDGATE, M. W., and M. SEAL
1956. The epicuticular wax layers of the pupa of *Tenebrio molitor* L. *Jour. Exptl. Biol.* **33**:82-106.
- HUNT, C. R.
1947. Toxicity of insecticide dust diluents and carriers to larvae of the Mexican bean beetle. *Jour. Econ. Ent.* **40**:215-19.

HURST, H.

1943. Principles of insecticidal action as a guide to drug reactivity-phase distribution relationships. *Trans. Faraday Soc.* **39**:390-411.
1948. Asymmetrical behavior of insect cuticle in relation to water permeability. *Disc. Faraday Soc.* **3**:193-210. *See also*: General discussions, pp. 220-39; *specifically*, pp. 232-35.

KALMUS, H.

1944. Action of inert dusts on insects. *Nature (London)* **153**:714-15.

KITAOKA, S., and A. YAJIMA

1958. Effects of insecticides on ticks. II. Effect on loss of body weight and oviposition of ticks. *Japanese Jour. Appl. Ent. and Zool.* **2**(1)11-16.

MICKS, D. W.

1960. Susceptibility of mosquitoes to silica gel insecticides. *Jour. Econ. Ent.* **53**:915-18.

NAIR, M. R. G. K.

1957. Structure of waterproofing epicuticular layers in insects in relation to inert dust action. *Indian Jour. Ent.* **10**(1):37-49.

PARKIN, E. A.

1944. Control of the granary weevil with finely ground mineral dusts. *Ann. Appl. Biol.* **31**:84-88.

RICHARDS, A. G.

1951. The integument of arthropods. University of Minnesota Press, Minneapolis. 411 pp.
1957. Studies on arthropod cuticle. XIII. The penetration of dissolved oxygen and electrolytes in relation to the multiple barriers of the epicuticle. *Jour. Ins. Physiol.* **1**:23-39.
1958. The cuticle of arthropods. *Ergebnisse der Biologie* **20**:1-26.

RICHARDS, A. G., and T. F. ANDERSON

1942. Electron microscope studies of insect cuticle, with a discussion of the application of electron optics to the problem. *Jour. Morph.* **71**:135-83.

RIDEAL, E. K., and J. TADAYON

- 1954a. On overturning and anchoring of monolayers. I. Overturning and transfer. *Proc. Roy. Soc. (London)* **A225**:346-56.
- 1954b. On overturning and anchoring of monolayers. II. Surface diffusion. *Proc. Roy. Soc. (London)* **A225**:357-61.

TARSHIS, I. B.

1959. U.C.L.A. tests with desiccant dusts for roach control. *Pest Control* **27**:16-18, 20, 22, 24, 26-28, 30, 32.
1960. Control of the snake mite (*Ophionyssus natricis*), other mites, and certain insects with the sorptive dust, SG-67. *Jour. Econ. Ent.* **53**:903-8.
1961. Laboratory and field evaluation studies with sorptive dusts for the control of arthropods affecting man and animal. *Exptl. Parasitol.* **11**: February-March.

VOLMER, M.

1932. The migration of adsorbed molecules on surfaces of solids. *Trans. Faraday Soc.* **28**:359-63.

WAGNER, R. E., and W. EBELING

1959. Lethality of inert dust materials to *Kaloterms minor* Hagen and their role as preventives in structural pest control. *Jour. Econ. Ent.* **52**:207-12.

WARTH, A. H.

1956. The chemistry and technology of waxes. Reinhold Publishing Co., New York. 940 pp. *See specifically*: pp. 76-93.

WIGGLESWORTH, V. B.

1942. Some notes on the integument of insects in relation to the entry of contact insecticides. *Bul. Ent. Res.* **33**:205-19.
1944. Action of inert dusts on insects. *Nature (London)* **153**:493-94.
1945. Transpiration through the cuticle of insects. *Jour. Exptl. Biol.* **21**:97-114.

- 1947a. The site of action of inert dusts on certain beetles infesting stored products. Proc. Roy. Ent. Soc. (London), ser. A, **22**:65-69.
- 1947b. The epicuticle of an insect, *Rhodnius prolixus*. Proc. Roy. Ent. Soc. (London), ser. B, **134**:163-81.
1957. The physiology of insect cuticle. Ann. Rev. Ent. **2**:37-54.
1958. Abrasion of the insect cuticle by aqueous suspensions of small particles. Nature (London) **181**:779-80.
- ZACHER, F.
- 1937a. Eine neue Gruppe von Insektiziden. C. R. du XII Congrès Int. de Zool. (Lisbonne), 1935. Pages 2336-40.
- 1937b. Neue Untersuchungen über die Einwirkung oberflächenaktiver Pulver auf Insekten. Zool. Anz. **10**, Supplementband, pp. 264-71.
- ZACHER, F., and G. KÜNIGKE
1931. Untersuchungen über die insektizide Wirkung von Oxyden und Karbonaten. Arb. aus Biol. Reichsans. **18**:201-31.

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