EVALUATION OF DIATOMITE-BASED PYRETHRIN INSECTICIDES.

by

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Evaluation of Diatomite-Based Pyrethrin Insecticides

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ABSTRACT

Diacide^{R} and Fossil Flower^{R} are two commercial insecticides (Pest Control Products Act Nos. 14,074 and 14,013), formulated with natural pyrethrins and piperonyl butoxide in diatomaceous earth (diatomite). They have been marketed in British Columbia as "natural insecticides" on the basis that they exert their insecticidal effects by physical means, namely by dehydration. The toxicities of these, with time, to the adult flour beetle, Tribolium castaneum, were tested at high (80%) and low (10%) relative humidities, and compared with those of a talcum-based pyrethrum dust (PCP Act No. 13,074) and insecticide-free diatomite.

At 80% relative humidity, Diacide^{R} was significantly more effective than Fossil Flower^{R} and the talcum-based product. At 10% relative humidity, the talcum-based product acted more quickly than the diatomite-based ones, but all produced nearly 100% mortality after 68 hours. Neither diatomite nor talcum alone gave any mortality at experimental concentrations. For diatomite alone, no significant mortality (greater than 5%) was observed at four times the recommended rate for Diacide^{R} after 72 hours at 10% relative humidity.

The modes of insecticidal action of diatomite and pyrethrum are reviewed and discussed in the light of the experimental results.

Finally, the economic viability of the diatomite-based product Diacide^{R} is discussed and suggestions for further investigation are made.

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PREFACE

The use of chemicals to control pest insects is often controversial. In addition to being insecticidally effective, an acceptable chemical should be so specific as to make it harmless to non-target organisms. Additionally, the residues of the compound must dissipate at such a rate that hazardous accumulations do not occur and, of course, the product as marketed must be economically viable. This paper examines one type of insecticidal formulation which is supposed to meet most of these criteria.

The 'Diacide' and 'Fossil Flower' products (PCP Act Nos 14,074 and 14,013) are formulations of natural pyrethrins and piperonyl butoxide in a diatomite base. The products have been marketed in British Columbia as "natural insecticides" on the basis that they exert their insecticidal effects by physical means, namely by dehydration.

This paper reviews the literature pertaining to the modes of insecticidal action of both diatomite and pyrethrum and presents the results of experiments testing the efficacy of the diatomite-based products. The mode of action, safety and residual properties of the products are discussed in the light of the review and the experimental work. Finally the economic viability of the products are discussed and suggestions for further research are made.

A. Literature Review

This part of the paper is intended to present a basic outline of current knowledge of dust and pyrethrin insecticides in order to provide a background for the discussion of the experimental results.

I. Dusts as Insecticides

Introduction

It has long been known that some chemically inert materials are harmful to certain arthropods and, in many parts of the world, locally available materials such as dusts and ashes are still used to protect stored grain from insect attack (Golob & Webley 1980). Since the introduction of synthetic pesticides, however, the major use of dusts has been as carriers of toxicants rather than as active ingredients.

Types of Dust and their Modes of Action

Hockenyos (1933) found abnormal loss of body moisture to be the cause of death of oriental cockroaches (*Blatta orientalis*) treated with fine particles of magnesium carbonate. Alexander *et al.* (1944) found dusts of hard materials (e.g. diamond, quartz) to be more effective at killing grain weevils (*Calandra granaria*) than softer substances (e.g. talc, china clay). They also found that good adherence of the dust to the insect body was essential and that adherence was inversely proportional to particle size.

Alexander *et al.* (1944) also found that effective dusts absorbed no water from the insect and they proposed that death was due to the dust breaking down the water resistance of the epicuticle. Beament (1945) and Wigglesworth (1945) reported that inert dusts produced no water loss from motionless or dead insects apart from the cockroach and for many years thereafter the erroneous belief prevailed that for most insects, abrasion was the only mechanism by which dusts removed the epicuticular wax to the extent of causing desiccation (Ebeling 1971).

In fact, in 1944 Alexander *et al.* had proposed that sorption rather than abrasion might be responsible for breaking down the water resistance of the cuticle but it was about fifteen years before researchers, notably Walter Ebeling at the University of California, Los Angeles, began investigating the differences between dusts. Ebeling and co-workers pointed out

that inert dusts can be divided into those that exert their insecticidal effects by abrasion and those that kill through sorption. In both cases, the result is abnormal water loss from the body leading to death. Examples illustrating the different properties of the two types of dust are given in Table I.

The insecticidal efficacy of non-sorptive dusts depends mainly on abrasiveness and size whereas for sorptive dusts it is closely correlated with specific surface, provided the pores in the dust are sufficiently large to admit the molecules of epicuticular wax whose disruption causes the breakdown of water resistance (Ebeling 1971). In his extensive review of insecticidal sorptive dusts Ebeling (1971) presented a very impressive photograph of absorption of a lipid from the dorsal surface of the elytron of a carabid beetle by a silica aerogel.

Fluorinated silica aerogels were the most insecticidal sorptive dusts used in a test of 110 different dusts against the German cockroach (Blattella germanica) by Tarshis (1961). Silica gels are absorbent, dehydrating agents that are formed by reacting sodium silicate with sulphuric acid; those of the smallest particle size are called aerogels and ammonium fluosilicate is often added to prevent caking. One of these gels, SG-67, is marketed as an insecticide under the trade name Dri-Die 67. This type of compound owes its outstanding insecticidal efficacy to its large specific surface (300 m^2/g), adequate pore diameter (115 A) and low sorptivity for water (Ebeling 1971).

TABLE I

Some Properties of Selected Dusts.[a/]

PROPERTY	DUST TYPE			
	Silica Aerogel (SG - 67)	Diatomite (Celite 209)	Ground Silica (RD - 1)	Talcum (Cohutta)
Hardness (Moh's)	2.5	4 - 6	5.5 - 6.5	1
Sorptivity				
Oil, % b.w.	300	172	30	39
Water, % b.w.	-	205	38	40
Bulk Density, kg/m^3	72	128	480	1,120
Specific Surface, m^2/g	300	15 - 25	4	3

[a/] Sources: Ebeling 1971; Tarshis 1961; Watkins & Norton 1955.

Other sorptive dusts include montmorillonite, bentonite, attapulgite and diatomite. Diatomite is both abrasive and sorptive and consists of the fossilised siliceous remains of diatoms, which are single-celled aquatic algae. It apparently acts principally as an abrasive since its specific surface is relatively small at about 20 m^2/g (Watkins & Norton 1955)[1] and when tested for rate of absorption of beeswax it performed very poorly in comparison to silica aerogels (Ebeling 1971). Examination of Table I indicates that diatomite has a relatively high sorptivity for oil and thus it must be supposed that its relatively low insecticidal efficacy is due to other constraints such as insufficiently large pore size for admission of epicuticular lipids and high sorptivity for water.

Talcum, the other dust used in the experiments described in part B, is not significantly sorptive or abrasive (see Table I).

Uses

In 1924 Headlee described the use of dusts for the protection of stored seeds and to this day the major use of dusts as insecticides is probably for the protection of stored products. In the United States several different types of dust have been tested in large bins (Carlson & Ball 1962) and one diatomite product has had limited commercial use (Ebeling 1971).

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[1]Ebeling (1971) gives a figure of 3 m^2/gm but I have been unable to confirm such a low value for any commercially available diatomite product.

Dusts are also used for the treatment of drywood termite infestations and, according to Ebeling (1971), some termite control operators refuse to fumigate buildings without a subsequent treatment with a sorptive dust, e.g. Dri-Die. Tarshis (1961) successfully used Dri-Die to control human and animal ectoparasites and Schull (1932) controlled the cattle louse, Bovicola bovis, with diatomite. Dri-Die has also been used successfully to control cockroaches especially when followed by an application of Drione (TM) (1.0% pyrethrins, 10% piperonyl butoxide, 38.12% amorphous silica aerogel, 1.88% ammonium fluosilicate, 49% petroleum oil base). The effect of the pyrethrins in Drione lasts much longer than it does in most other formulations (see also page 15 for pyrethrin stability), apparently because the oil-pyrethrin-piperonyl butoxide solution is protected from air and light by its position in the pores of the silica particles (Ebeling 1971).

Sorptive dusts have not been used as much as other dust formulations in agriculture mainly because they drift uncontrollably beyond the treated areas. Diatomite has a more suitable bulk density than the silica aerogels (Table I) and has been proposed for use as a crop protectant (Ross 1981) but because of its relatively low insecticidal efficacy four applications of 25 lbs/1500 ft[2] during the growing season might be required for adequate control (DeCrosta 1979).[2]

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[2]Equivalent to about 2,800 lbs/acre, or 3,136 kg/ha.

Overall, the use of dusts as insecticidal agents is not widespread, probably because of the availability of synthetic insecticides and the belief that dusts are only effective at low humidities - a belief which, in one case at least, has been demonstrated to be incorrect (Ebeling & Wagner 1959).

II. Natural Pyrethrin Insecticides

Introduction

Natural pyrethrins are insecticidal substances contained in certain pyrethrum flowers, mostly Chrysanthemum cinerariaefolium Trev., a tropical member of the family Compositae. Accounts of the discovery of the insecticidal properties of the plant are not clear, but it seems certain that the dried flower heads of pyrethrum were in use in Europe as an insecticide by 1828 and in Persia considerably earlier (Matsui & Yamamoto 1971).

The insecticidal activity of pyrethrum is attributed to six esters formed by the combination of two acids, chrysanthemic and pyrethric, and three alcohols, pyrethrolone, cinerolone and jasmololene (Casida 1980)[1]. These compounds are known as Pyrethrin I and II, Cinerin I and II and Jasmolin I and II, the suffix referring to the acid moiety. Thus Cinerin II is formed from pyrethric acid and cinerolone whereas Cinerin I is formed from chrysanthemic acid.

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[1]Some authors (e.g. Matsumura 1975, Murphy 1980) do not consider the jasmololene esters to be active ingredients.

Mode of insecticidal action

One of the most important attributes of the pyrethroid[2] insecticides is that they disable insects very rapidly, this is known as their "knock-down" effect. Even in small doses knock-down occurs almost at once although the effect is usually only temporary. The dosage required to kill insects is usually much higher than that required for paralysis (Matsui & Yamamoto 1971) and, in fact, the processes of knockdown, paralysis and death may be wholly or partially independent (Camougis 1973).

At the proximate level, the mode of action is undoubtedly by interference with nerve impulse conduction, almost certainly by the binding or dissolving of pyrethroids in the lipid layer of the nerve cell membrane. Pyrethroids are known to retard closure of sodium gates on the nerve axon, probably by inhibition of certain calcium-dependent ATPases, and thereby to oppose membrane repolarization (Beeman 1982). However, pyrethroids also stimulate the central and peripheral nervous systems at lower concentrations than those required to block nervous transmission. It has been suggested that this stimulatory effect of pyrethroid action knocks the insect down as soon as the sensory structures associated with the exoskeleton are affected and that paralysis results only as the poison accumulates to the required blocking level (Camougis

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[2]"Pyrethroid" is a general term that encompasses both the natural pyrethrins and the synthetic pyrethroids.

1973).

The ultimate cause of death of the insect is quite a different matter, however, and little attention seems to have been paid to this subject in the literature. It may be that fatal lesions are caused by the action of pyrethroids within ganglia as has been suggested by Burt & Goodchild (1971). But studies on the effect of pyrethroids on nerve-muscle preparations (e.g. Adams & Miller 1979, 1980) show a positive temperature coefficient, whereas pyrethroids show a negative correlation between temperature and insecticidal effectiveness (Hassall 1982). It is thus quite possible that there may be other, less esoteric, reasons for death under some conditions.

Several insecticides, including pyrethroids, have been shown to cause abnormal water loss through the cuticle in the cockroach, Periplaneta americana (Chattoraj & Sharma 1964). Ingram (1955) induced water loss from nymphal cockroaches (Periplaneta americana) by abdominal injection or topical application of natural pyrethrins. He found that abdominal injections caused less water loss than topical applications and that visible droplets of water appeared on the surface of poisoned cockroaches, these droplets not being confined to the area of application. Ingram's conclusion was that the water loss was caused by a toxic action of the pyrethrins on secretory activity by the epidermal cells. Hewlett & Gostick (1955) treated flour beetles (Tribolium castaneum) with pyrethrins and observed rapid weight loss apparently through loss of water. The

pyrethrin content of the solutions used appeared to account so precisely for the observed weight loss that a method of bioassay for pyrethrins was proposed, based on this loss. Irradiation of the pyrethrum solutions that destroyed their toxicity also destroyed their weight-reducing properties.

It is suggested that, in some insects at least, the ultimate cause of death from pyrethrum poisoning is desiccation. Wigglesworth proposed this explanation in 1941, but only investigated the possibility that the desiccation might be due to interference with spiracular control, a hypothesis that he found to be incorrect. Desiccation through the cuticle, however, seems to be eminently possible when coupled with the observation that the great difference in the water content of the blood and the cuticle must be maintained actively, perhaps by some epidermal 'water pump' (Winston 1967, Winston & Beament 1969)[3]. Gerolt (1976) found that contact with any of the four main groups of insecticides affecting neural function caused accelerated water loss from all major parts of the integument of the housefly and suggested that this reaction was the primary cause of eventual death. An active mechanism of water conservation is, as pointed out by Ingram (1955), a prime target for interference by neurotoxic substances such as pyrethrins, especially if, as has been suggested, the mechanism is under some type of neuroendocrine control (Treherne & Willmer 1975,

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[3]There is some dispute about this model (e.g. Machin 1979, 1980) but it seems likely that water loss through the cuticle is actively controlled (Edney 1980).

Wharton & Richards 1978, Gerolt 1976, 1983). Miller & Adams (1982) think it reasonable "to assume that pyrethroids can cause changes in the hormonal state of insects through direct actions on neurosecretory neurons" and Gerolt (1983) has advanced a general hypothesis for the mode of action of all neurotoxic insecticides, based on the proposition that induced water loss through the integument causes lethal dehydration of the CNS. The results of the experimental work presented in part B are most easily (and perhaps solely) explicable on this basis.

Metabolism, Synergism and Toxicology

For many years it was considered that hydrolysis of the ester linkage was the major detoxification mechanism for pyrethrins in insects but the discovery of effective synergistic actions by methylene dioxyphenyl compounds, such as piperonyl butoxide, indicated that oxidation of the pyrethrin molecule might be the major metabolic route (Matsumura 1975). It now seems certain that metabolic detoxification is a major limiting factor in the insecticidal activity of pyrethrins and that this is, in fact, achieved by oxidation of methyl groups in the isobutenyl substituent of the corresponding carboxylic acid moiety (Casida 1980). These reactions are effected by the microsomal mixed function oxidase (MFO) system (with NADPH as co-factor) and the methylene dioxyphenyl compounds mentioned above appear to inhibit competitively the action of this system on pyrethrins.

The acute oral toxicity of pyrethrins to rats is between 584 and 900 mg/kg (Spencer 1982) but the intravenous toxicity is very high, e.g., 6-8 mg/kg is lethal to dogs (Matsui & Yamamoto 1971). Mammals also metabolise pyrethrins by oxidation and ester cleavage, but fortunately piperonyl butoxide as normally used gives little if any increase in toxicity (Casida 1980). However, synergists should be used with care, not least because the MFO system is involved in many mammalian metabolic processes and undesirable interactions can occur. Similarly, structural

modifications to stabilize the pyrethrins to insect metabolism, as in the case of the synthetic pyrethroids, could produce very hazardous compounds if they responded in the same way in mammalian systems. Fortunately, all the synthetic pyrethrin analogues so far used have structural modifications that enhance photostability and insecticidal activity whilst remaining rapidly biodegradable in mammals (Casida 1980).

The instability of the natural pyrethrins[4] is a great advantage from the environmental viewpoint since residues will not accumulate (Mrak 1973), and they are well suited for indoor, domestic and stored product use. However, as insecticides for outdoor use, greater persistence in the natural pyrethrins would be desirable; in fact, the possibility of increased persistence was one of the stimuli for the development of light-stable synthetic pyrethroids.

Various formulating techniques have been used to achieve greater stability including the use of uv (290-320 nm) screening agents and antioxidants (Miskus & Andrews 1972). The best result achieved in this way was an increased persistence of at least 4 h in sunlight (Miskus & Lyon 1973).

Another approach to the problem of photodecomposition is to use controlled release formulations. A microencapsulated pyrethrin formulation, SECTROL[5], has recently been marketed. It

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[4]In the presence of sunlight and air there is no detectable residue remaining after 12 to 24 hours (Moore 1973).

[5]Trademark of 3M company, St. Paul, MN 55144, U.S.A.

is claimed that Sectrol releases natural pyrethrins for up to 60 days (Bennett & Lund 1977). It will be seen in parts B and C that the diatomite used in formulations tested as part of this research also seems to prolong the release of pyrethrins.

Finally, there is currently concern in Canada about the toxicity of synthetic pyrethroids to fish (Khan 1983). Natural pyrethrins are also extremely toxic to fish despite being rapidly degraded in aquatic systems, giving 96-h LC50s of between 18 and 149 PPB (ug/L, nearest whole number, 95% confidence limits, Mauck & Olson 1976). The toxicity of slow release formulations of natural pyrethrins in aquatic ecosystems does not appear to have been studied, but there is certainly a possibility that these formulations may even increase the toxicity to the level of the more stable synthetic pyrethroids (less than 1 PPB, Mauck & Olson 1976).

B. Experimental Work

The experimental work described in this section was carried out to test the relative efficacy of three commercial pyrethrin formulations. The major technique used was *in vitro* bioassay. Gas-liquid chromatography (GLC) and scanning electron-microscopy (SEM) were employed in supporting experiments.

I. Bioassays

Materials and Methods

Insects

The test insects were adult (3-5 weeks) red flour beetles, Tribolium castaneum Herbst, raised on whole wheat and 5% brewer's yeast at 26-28 C and 30-60% RH in a 12-hour photoperiod.

Insecticides

Details of the formulations tested in the bioassays are given in Table II.

Experimental Equipment

All bioassays were carried out in a specially constructed environmental chamber. The chamber was of stainless steel and measured 91.2 x 69.4 x 50.6 cm, giving a volume of approximately 320 litres. The lid had attached to its perimeter a soft tubular silicone rubber gasket which sealed against a 2.5 cm horizontal ledge around the inside of the top of the chamber. The lid was secured by eight retaining clamps.

TABLE II

Details of Pyrethrum Dust Formulations Tested in the Bioassays.[a/]
Each Formulation Guaranteed[b/] to Contain 0.2% Pyrethrins and 1.0% Piperonyl Butoxide.

Formulation	Trade Name	Registration Number[b/]	Diluent	Manufacturer	Source
A	Household Diacide	14074	Diatomite	Diatom Holdings Ltd. White Rock, B.C. Canada	Retail
B	Household Diacide	14074	Diatomite	Diatom Holdings Ltd. White Rock, B.C. Canada	Manufacturer
F	Fossil Flower	14013	Diatomite	Fossil Flower Co. Toronto, Ont. Canada	Retail
L	Later's Pyrethrin Dust	13074	Talcum	Later Chemicals Ltd, Richmond, B.C. Canada	Retail

[a/] For control experiments, insecticide-free diatomite and talcum were obtained, respectively, from Diatom Holdings Ltd. and from Later Chemicals Ltd. [b/] Pest Control Products Act, Canada.

One of the long sides of the chamber had a sealed polyacrylamide observation window through which the environmental conditions could be monitored from a recording thermohygrograph (Fuess, Model 79t). The other side of the chamber had a removable polyacrylamide window (64 x 18 cm) through which trays (40 x 35 cm), containing test insects in Petri dishes, could be inserted or removed.

Humidity was controlled within the chamber by a closed circuit system driven by an extractor fan mounted at one end of the chamber. The fan circulated air through 3-cm diameter plastic tubing (Tygon No. 3603) into either a drying vessel containing $CaSO_4$ (Drierite TM) or a counter-current water flow system and thence back into the opposite end of the chamber. Temperature was controlled by a combination of the thermostatically controlled heating system of the room in which the experiments were carried out and the buffering properties of the sealed system.

A second recording thermohygrograph was used to monitor temperature and humidity outside the chamber. The chamber was maintained at 25C (+/- 1C) and 10% or 80% RH (+/- 5%) throughout the bioassays.

Exposure of Beetles

The formulations were tested at 10% and 80% RH. Bioassays consisted of exposing beetles for up to 84 hours on Whatman No.1 filter paper in 15-cm glass Petri dishes covered by insect-proof

plastic mesh. Fifty insects were tested in each dish which contained 13.8 mg[1] of commercial product evenly distributed on the filter paper by vibration. Two dishes, i.e. 100 insects, were used per treatment. The controls were 100 insects in two dishes, treated with 13.8 mg of diatomite or talcum carrier only, and 50 insects remaining untreated.

Bioassay of insecticide-free diatomite was carried out at 10% RH as above except that the amounts of commercial product used per dish were 19.8, 39.6, 59.4, 79.2 and 99 mg.[2] Fifty insects were left untreated as controls.

Mortality counts were made at set intervals (see Tables III and IV, pp. 26 & 27) throughout the bioassays. Dishes were removed from the chamber through the detachable side window for observation. A beetle was counted as dead if, when viewed under low power magnification, it neither moved spontaneously nor responded by reflex movement when touched by a probe (Hewlett 1974). Dead beetles were not removed from the dishes nor were any of the beetles disturbed more than necessary; mortality counts were conducted as quickly as possible to minimise the time spent outside the controlled environmental conditions. Certain features of the technique were worked out in preliminary experiments.

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[1]Equivalent to 7 lbs/acre - the recommended application rate of 'Diacide' on homogeneous terrain.

[2]Equivalent to 10, 20, 30, 40 and 50 lbs/acre respectively.

Statistical Analysis

At first sight there seems little difference between the data produced by time-mortality studies such as this, and those produced by dose-mortality experiments, e.g. LD50 determinations. However, each observation in a dose-mortality series is made with different sample of organisms, whereas in time-mortality studies this is not usually the case, although given a sufficient supply of test organisms and facilities, it could be so arranged. The problem is succinctly outlined by Bliss (1937):

> "Each observation in a dosage-mortality curve represents a different set of organisms, so that the individuals at the successive dosages are unrelated in susceptibility. It is possible for the mortality to be less at a longer than at a shorter period if by chance the first group of organisms should contain a large proportion of more resistant individuals. But this can never happen in a time-mortality curve since all of the points are merely different observations on a single set of animals and the percentage mortality at a given time can never be less than that recorded earlier in the experiment. The successive observations are strongly correlated with each other and in consequence the methods of computation that have been described for the dosage-mortality curve are not applicable here."

The computational methods to which Bliss refers are those of probit analysis, that is, the procedure of testing the curve obtained by a probit transformation for goodness of fit to a straight line and concluding that, should the line prove adequate, the original data were normally distributed. This is quite invalid for correlated observations (Sampford 1952).

To test time-mortality data for goodness of fit to a normal distribution the values of the third and fourth sample moments

were considered (Sampford 1952). These calculations were performed according to the method given by Bliss (1937) for the data of Formulation A, 80% RH (Table IV, p27) using log time as the response time metameter.[3] The constants g1 and g2 were obtained as 1.172 and 22.58 respectively, indicating that although the data give a distribution of acceptable symmetry, the distribution is not normal and a probit transformation would be inapplicable since it would cause a large overcorrection for the only slightly sigmoid nature of the distribution.[4]

Since at least part of the data was not suitable for standard log-probit analysis and since all of the observations were truncated to some degree, it was decided that another type of analysis might be employed. Additionally, since the insects were exposed continuously to the insecticides throughout the experiments, the times to death are combinations of time as a dose factor and time as a response (which two factors are inextricably mixed) and thus any descriptive statistic (e.g., LT50) would not be particularly useful.

The non-parametric method of 'survival analysis' (BMDP 1981) was employed. This computer analysis was designed to

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[3]This time transformation is often found to give a normal distribution (Hewlett & Plackett 1979).

[4]It is interesting to note that some methods of treatment of time-mortality data (e.g. Litchfield 1949) recommend the use of graphic log-probit analysis for rapidity; certainly, the data of Formulation A used above do give, to the eye, an exceptionally 'good' straight line (especially if one is used to dealing with dose-mortality data) and it may be that, despite statistical principles, the log-probit method would give useful and meaningful results.

quantify the survival (time to response) of hospital patients who had been observed for varying periods. The equality of different survival distributions could be tested by two non-parametric rank tests which are appropriate even if the distributions are truncated, i.e. if some of the data are censored. The tests available are those proposed by Mantel (1966) and Breslow (1970). The Breslow test was chosen because it tends to give more weight to early times to response (BMDP 1981) and is more powerful when the hazard ratios are non-constant (Tarone & Ware 1977).

The median time given in Table V (p30) is simply the time to the midpoint of the interval in which the 50th beetle died (since a non-parametric method, by definition, presupposes no particular form of distribution) and the mean survival time, although calculable, is limited to the times to death of the beetles that died in the course of the experiments and so gives little basis for comparison. Despite these limitations, however, I considered that 'survival analysis' fulfilled the requirement of comparing the responses to different treatments without becoming involved in detailed and complex statistical theory.

Results

The results of the comparative bioassays are given in Tables III and IV and are presented graphically in Figure 1. There was no mortality in any of the controls and there was no significant difference between replicates. In the bioassay of Formulation A, 80% RH, one beetle was observed to be completely immobile after four hours; it was decided not to record mortality at this early stage since it was considered that that particular beetle was atypical and may have been dead from the start of the bioassay. In the bioassays of insecticide-free diatomite at 10% relative humidity, no mortality occurred at realistic concentrations (equivalent to 10 lbs/acre); in fact no significant mortality (> 5%) was observed after 72 hours at concentrations below the equivalent of 30 lbs/acre.

Analysis

Tables V and VI summarise the results of the BMDP survival analysis (see page 23) of the bioassay results.

TABLE III

Mortality[a/], in Cumulative Percentage, of Adult Flour Beetles, Tribolium castaneum, With Time of Exposure to Four Pyrethrum Dust Formulations at 25C and 10% RH.

Hours of Exposure	FORMULATION[b/] A	B	F	L
12	-	-	-	4
16	-	-	-	21
20	8	5	5	39
24	13	15	12	51
28	22	23	18	-
32	38	39	28	-
36	-	-	-	89
40	60	63	54	91
44	67	75	65	-
48	79	80	74	-
52	85	90	81	-
56	88	91	85	-
68	99	99	98	

[a/] There was no mortality among the control beetles. [b/] vide Table II.

TABLE IV

Mortality[a/], in Cumulative Percentage, of Adult Flour Beetles, Tribolium castaneum, With Time of Exposure to Four Pyrethrum Dust Formulations at 25C and 80% RH.

Hours of Exposure	FORMULATION[b/] A	B	F	L
36	6	5	3	4
40	12	10	5	8
44	18	16	8	15
48	29	30	17	26
60	53	55	25	28
64	59	62	27	29
68	61	65	30	29
72	66	69	31	30
84	81	74	38	32

[a/] There was no mortality among the control beetles. [b/] vide Table II.

Figure 1.

Mortality, in Cumulative Percentage, of Adult Flour Beetles, Tribolium castaneum, With Time of Exposure to Four Pyrethrum Dust Formulations at 25C and 10% or 80% RH.

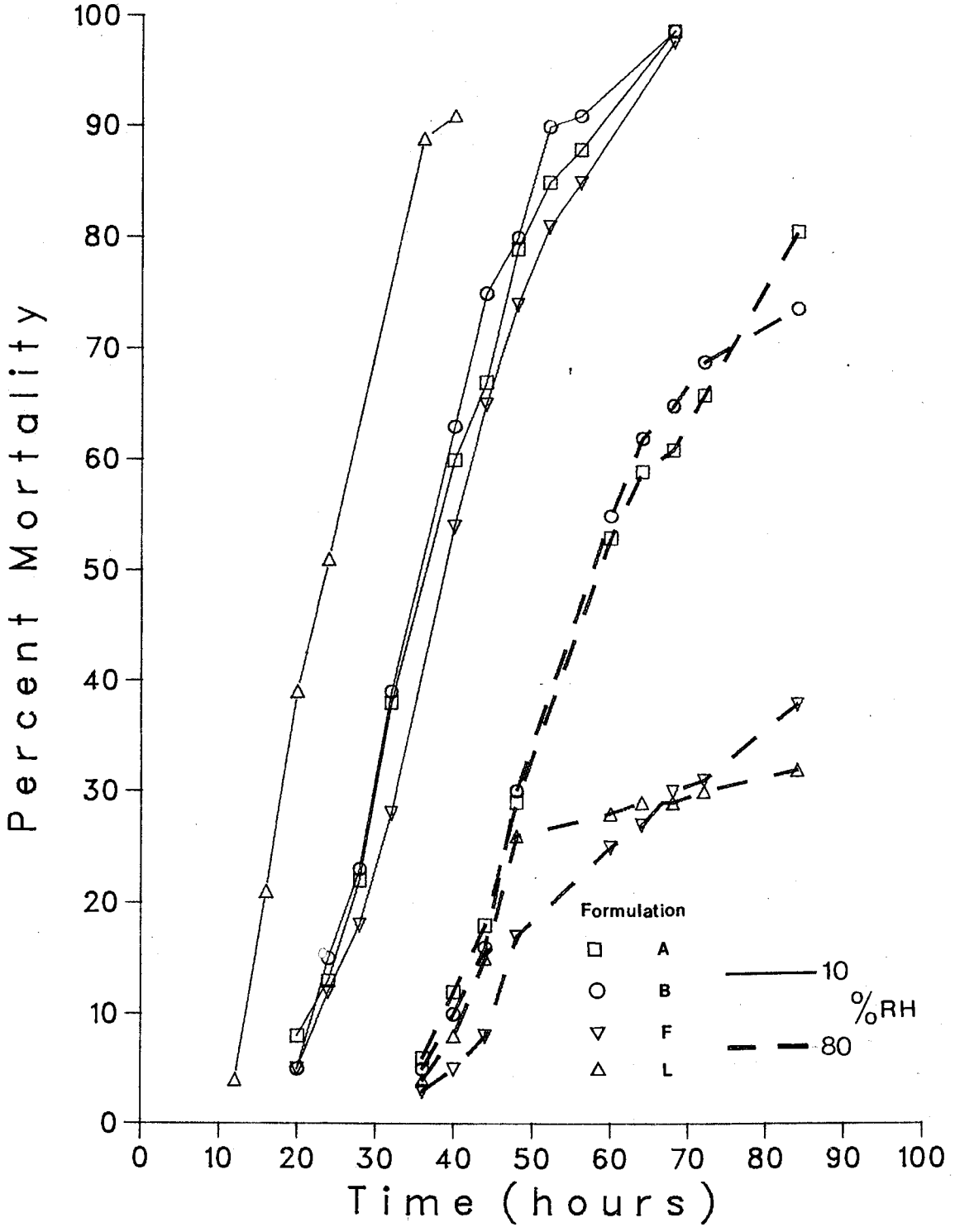
Percent Mortality
Time (hours)
0
10
20
30
40
50
60
70
80
90
100
Formulation
A
B
F
L
10
80
%RH

TABLE V

Parameters of Time-Mortality Distributions for the Flour Beetle, Tribolium castaneum, Exposed to Four Pyrethrum Dust Formulations at Two Relative Humidities; Data from Tables III and IV.

	10% Relative Humidity		80% Relative Humidity	
Treatment	Median, h [a]	Mean, h [b]	Median, h [a]	Mean, h [b]
A	36	38.0/68	54	60.7/84
B	36	37.1/68	54	60.6/84
F	36	40.3/68	N/A [c]	72.7/84
L	22	24.4/40	N/A [c]	71.7/84

[a] Time to death of 50% of the test animals. [b] Mean time to death during period indicated by denominator. [c] Less than 50% mortality.

TABLE VI

Probabilities That Two Groups of Flour Beetles, Tribolium castaneum, Exposed to Different Pyrethrum Dust Formulations at Two Relative Humidities, Show the Same Survival Distribution; Data From Tables III and IV.

	10% Rel. Humidity	80% Rel. Humidity
T e s t	Probability	Probability
A v. B	0.67	0.90
C[a] v. L	< 0.01	< 0.01
C v. L + 13[b]	0.35	—
C v. F	0.08	< 0.01
L v. F	< 0.01	0.85
L + 16[c] v. F	0.58	—

[a] C: pooled values of A and B. [b] Differences, in h, between means of C and L; and [c] between means of L and F.

II. GLC Analysis of Commercial Products

Materials and Methods

Standards

The standards used in the analysis of the commercial products were;

1. MGK Premium Pyrocide lot # 7839, EPA Regn. 1021/24, concentrated pyrethrum extract containing 20% pyrethrins.
2. EPA #5940, containing Pyrethrin I 9.54%, Pyrethrin II 10.46%.

Instrument

Tracor MT 220, equipped with a flame ionization detector, using hydrogen flow at 60 ml/min and air at 250 ml/min. Glass column, 1.2M x 4mm i.d., packed with 6% OV25 + 4% OV101 on Gaschrom Q, 60/80 mesh. Oven temperature 210 C. Carrier gas: nitrogen at 80 ml/min.

Sample extraction

1 gm of commercial product was suspended in 10 ml of glass-distilled acetone and sonicated (Bransonic 52

ultrasonicator) for 10 minutes. The suspension was centrifuged at approximately 1,600 g. for 15 minutes after which the supernatant was collected and the residue resuspended in 10 ml of acetone. The extraction procedure was then repeated. The 20 ml of supernatant finally collected were flash evaporated just to dryness and the residue was picked up in 4 ml of acetone.

Analysis

1. The pyrethrin content of each sample was analysed against the MGK Premium Pyrocide standard containing 20% total pyrethrins. The detector responses caused by injection of 4 ul of 0.02%, 0.04% and 0.08% MGK standard in acetone were recorded. The response caused by injection of 4 ul of each sample was then recorded and the constancy of the system was checked by another injection of 0.08% standard at the end of the run. A standard curve was constructed for each run from the means of the standard responses; as is usual in pyrethrin analysis, only the responses representing Pyrethrin I and Pyrethrin II were considered for quantification (Head 1973).
2. The EPA standard was used to identify the 'fingerprint' of the MGK standard and to identify and quantify its Pyrethrins I and II content.

Results

1. Relative to the MGK Premium Pyrocide standard, the Pyrethrum content of the commercial products based on their Pyrethrins I and II content were:

   A - 'Household Diacide' from retailer - 0.19%.

   B - 'Household Diacide' from manufacturer - 0.22%.

   F - 'Fossil Flower' from retailer - 0.14%.[1]

   L - 'Later's Pyrethrin Dust' from retailer - 0.18%.

2. Using the EPA standard of known Pyrethrins I and II content (9.54% and 10.46% respectively), the MGK standard was found to contain 4.6% Pyrethrin I and 3.8% Pyrethrin II.

3. The analysis showed that the diatomite-based products contained a compound, responding to flame ionization detection and extractable with acetone, with a retention time that was not characteristic of any of the pyrethrum esters. This compound was not identified.

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[1]This low value is most likely to have been caused by decomposition of the pyrethrins during extended storage.

III. SEM Examination of Commercial Products

Materials and Methods

Dusts

The dusts examined were:

'Household Diacide' from manufacturer.

'Fossil Flower' from retailer.

'Diatomaceous Earth, Grade II' from Sigma Chemical Company.

Talcum, from manufacturer of 'Later's Pyrethrin Dust'.

Preparation

A dust density appropriate for examination was produced by the following technique; in each case the dust was applied liberally to a piece of double-sided photographic mounting tape which had been stuck onto a specimen stub. A second stub with attached tape was then pressed onto the tape of the first stub so that some of the dust was removed and this process was repeated with a third stub. This gave a 'dilution' of dust densities across the three stubs.

The junctions between the tape and the stubs were covered with a conducting paint and the specimens were then gold coated in an NRC-3115 vacuum evaporator.

SEM examination

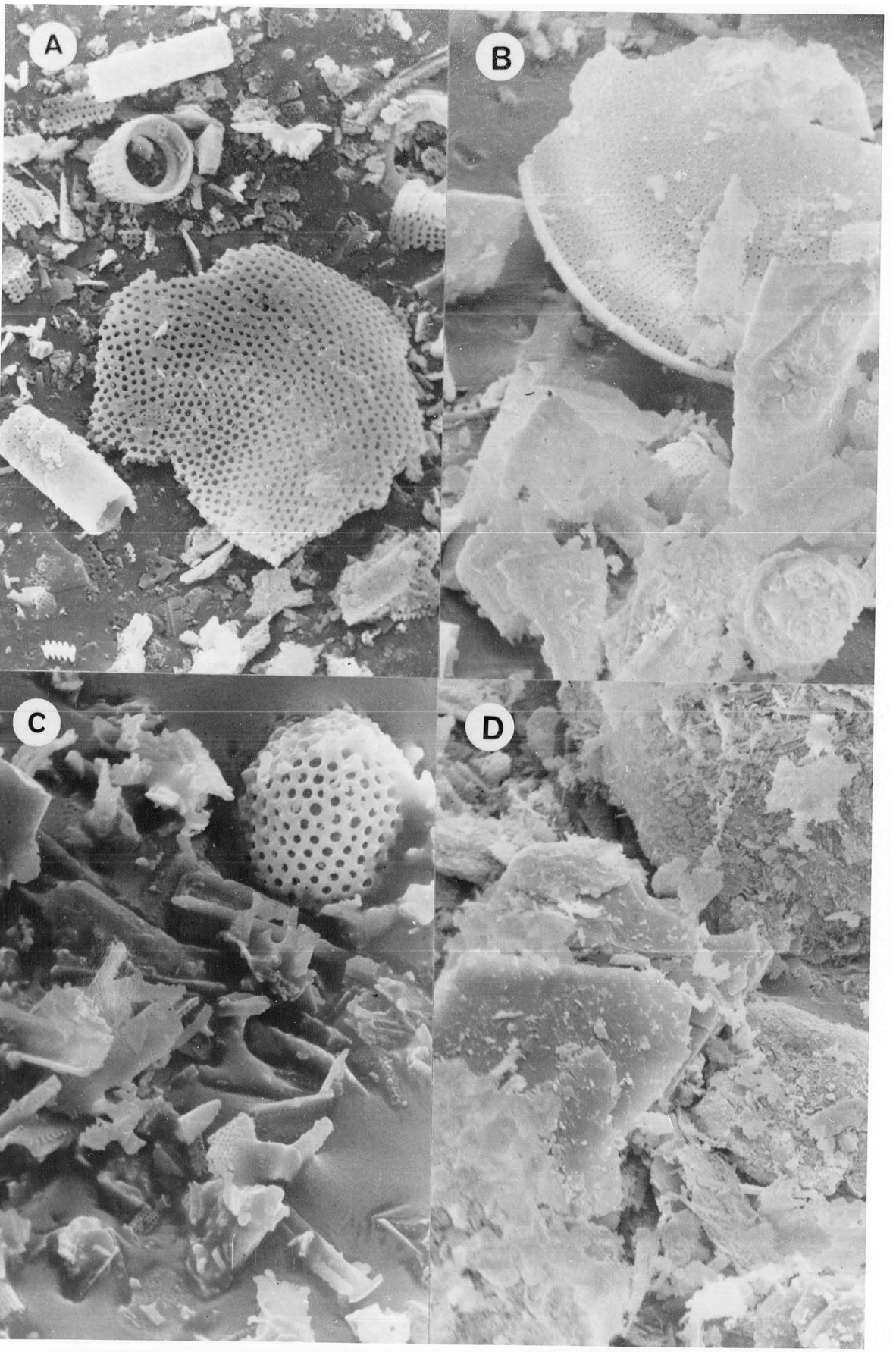
All specimens were observed with an ETEC Autoscan SEM. For each dust, the stub with the most suitable coverage for examination of individual particles was selected and thoroughly scanned for uncharacteristic or anomalous particles. No quantitative analysis was attempted.

Results

The examination did not reveal any qualitative difference in composition between the two diatomite formulations and the diatomite obtained from the chemical supply company. The talcum examined was, of course, substantially different in structure from the diatomite products. Representative electronmicrographs of the four dusts examined are presented in Figure 2.

Figure 2.

Electronmicrographs (x1000) of Four Different Dusts Prepared as Described (p34). A. 'Household Diacide' (Formulation B). B. 'Fossil Flower' (Formulation F). C. 'Sigma Chemical Co. Diatomaceous Earth Grade II'. D. Talcum.


A
B
C
D

C. Discussion and Conclusions

I. Discussion

The absence of mortality in the controls of the comparative bioassays indicates that, for the amounts used (7 lbs/acre equiv.), neither diatomite nor talcum was lethal to adult flour beetles at either 10% or 80% RH.

The comparative bioassay results (Figure 1, p28) suggest that the time-mortality distributions fall into four distinct "efficacy groups" by formulation, viz;

1. L - talcum formulation - 10% RH.
2. A, B and F - diatomite formulations - 10% RH.
3. A and B - diatomite formulations - 80% RH.
4. F and L - diatomite and talcum formulations - 80% RH.

These apparent groupings were supported by the survival analysis statistics given in Table VI, p30 ($p<0.01$ that the groups are drawn from the same survival distribution).

The results demonstrated that all formulations were considerably more effective at 10% RH than at 80%. Comparison of the time-mortality distributions for A, B and L suggests that, although the diatomite formulations took effect more slowly than the talcum-based product at 10% RH, they were effective at 80% RH, whilst L was not. F was anomalous in that, although it was grouped with the other diatomite formulations at 10% RH, at 80% it produced a mortality distribution similar to that of L, the talcum-based product.

According to the GLC analyses (p33) A, B and L contained 0.19%, 0.22% and 0.18% pyrethrins respectively, but F contained 0.14% only. All formulations were guaranteed to contain 0.2% total pyrethrins (Table II, p19).

The SEM examination, although not quantitative, gave no indication that the formulations contained any particulate matter other than either diatomite or talcum.

Relative Humidity

The greater efficacy of all formulations at low (10%) humidity could be due either to an effect of humidity on the formulation, for example causing an increase in pyrethrin volatility and therefore penetration through the spiracles, or to an effect of humidity on the insect itself.

According to Dalton's law of partial pressures, the volatility of the pyrethrum esters should be independent of the water vapour content of the air. However, pyrethrin-carrier interactions could possibly be affected by water vapour absorbed by the carrier. For instance, humidity-dependent differences in the pyrethrin-carrier relationships of talcum, as opposed to diatomite, may partly account for the formulations' differences in efficacy at low and high humidities.

Alternatively, high humidity could alter the distribution of the carrier on a treated surface, for example by causing caking, and thus affect the efficacy of the formulation.

It seems likely, however, that the differences in the time-mortality distributions at low and high humidities are due mainly to a direct effect on the insect. If, as suggested earlier (p12), pyrethrins exert their insecticidal effects by rendering the integument permeable to water, then it is clear that relative humidity will profoundly affect the time to death after poisoning. The water activity of the haemolymph of most terrestrial insects is 0.99 (equivalent to 99% RH) and the integument, especially the wax layer, is a primary waterproofing unit (Wharton & Richards 1978); any disruption of this waterproofing will therefore lead to transpiration across the integument at a rate related to the difference between the water activity of the haemolymph and that of the ambient air. Gerolt (1983) in fact envisages an insecticide-induced, active, "extrusion of fluid from the epidermis into the cuticle and beyond, fluid lost from the epidermal cell layer being replaced from the haemolymph and internal tissues". In either case, it is clear that the rate at which a lethal water deficit[1] is incurred will be influenced by the rate of evaporation into the air, this being directly related to the relative humidity. It is therefore suggested that differential water-loss rates from the insects at low and high humidities account for the observed differences in the time-mortality distributions to a greater extent than effects of humidity on the formulations.

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[1]Houseflies can recover from a 27% loss of body water (Sun & Johnson 1972).

Formulation

As discussed earlier (p15), natural pyrethrins are unstable in the presence of sunlight and air and thus a formulation which released pyrethrins at a slow, but still effective, rate could produce higher mortality than a fast-release formulation under conditions of high humidity. This is because the insecticidal activity of the pyrethrins, in this case induction of water-loss, will be prolonged beyond the point of lethal water deficit. At low humidity, however, a slower release of pyrethrins could delay the onset of water-loss and thus the time to eventual death. It is therefore suggested that the mortality differences between formulations at the same humidity are related to the rate of release of pyrethrins from the formulations.

This interpretation effectively explains the results if it is assumed that the talcum formulation (L) released pyrethrins faster than the diatomite formulations (A, B and F). To return to the "efficacy groups" referred to on page 39, the talcum formulation at 10% RH produced mortality more rapidly than the diatomite formulations, the difference between mean times to death being about 13 hours (Table V, p30). However, as far as can be determined by survival analysis, the talcum formulation did not cause a significantly faster rate of mortality once initiated (CvL+13, Table VI, p30), as would be expected if, once the insect cuticle became permeable to water, the time to death

was dependent not on concentration of insecticide (above a certain threshold) but on the difference in water potential between the insect's haemolymph and the ambient air.

At 80% RH, the difference in water potential is much smaller than at 10% RH, and if the pyrethrins were depleted, either because of an initially low concentration or a high rate of release, before a lethal water deficit was incurred, then the insects should survive. Indeed, this phenomenon was observed in the high (80%) humidity bioassays of F and L. Initially, up to about 50 hours, the patterns of mortality for F and, especially, L resembled those for A and B (Figure 1, p28). But thereafter (48-84 h), L caused very little additional mortality and the rate of mortality caused by F decreased, so that at the conclusion of the experiment, F and L, respectively, had produced 38% and 32% cumulative mortalities only (Table IV, p27), suggesting that pyrethrins from these formulations were greatly (F) or completely (L) depleted after about 50 hours under the experimental conditions. The abrupt change in the slope of the high-humidity time-mortality distribution for L seems to illustrate particularly well the point at which the pyrethrin concentration fell below the threshold level essential to sustain efficacy.

The survival analysis technique was not able to distinguish the distribution for L from that of F at high humidity (Table VI, p30). However, the anomalous, seemingly contradictory behaviour of F may have other reasonable explanations. Unlike A

and B, F is manufactured by a different company (Table II, p19). Consequently, the diatomites used may be of different origins and thus perhaps possess different properties.[2] Moreover, differences in manufacturing processes may differently affect the nature of the diatomite-pyrethrin association (absorption, protection of pyrethrins etc.). It is possible that, at 80% RH, such differences reduce the persistence of pyrethrin availability from F, as from L, but not from A and B. At 10% RH, on the other hand, these differences do not become apparent by bioassay and F gives rise to a mortality distribution similar to that of A and B.

In other words, it is suggested that diatomite, relative to talcum, retards the release of pyrethrins regardless of its origin and manufacturing process, and that differences between A/B and F were not apparent for up to about 50 hours, enough time to kill the insects at 10% RH. To kill at 80% RH, however, pyrethrin must "last longer" (vide supra) than 50 hours and neither talcum nor the diatomite of F seem to be able to provide this extended release. However, close scrutiny of the distributions of mortalites due to F and L at 80% RH beyond about 50 hours still suggests some release of pyrethrins from F (Figure 1, p28), and the pyrethrin deficiency of F, mentioned earlier, may have contributed to the apparent similarity between

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[2]For example, Smart & Anderson (1952) reported that, with respect to pneumoconiosis, there are significant differences in toxicity to humans between marine and freshwater diatomite deposits.

the distributions of F and L. In fact, the actual performance of F at 80% RH, had it contained the guaranteed amount of pyrethrins, might well have been intermediate.

II. Conclusions

The role of diatomite

Comparison of the specific surface and sorptivity of diatomite and talcum (Table I, p5) and examination of the electronmicrographs of the dusts (Figure 2, p36) indicate that, as suggested, diatomite could provide slower release of pyrethrins than talcum and consequently improve the insecticidal properties of the diatomite-based dusts under conditions of high RH. Ebeling (1971) attributed the prolonged effect of a silica aerogel-pyrethrin formulation to protection of the pyrethrins from light and air by the position of the toxicant in the pores of the silica particles; there is no reason to suppose that this protection could not also be afforded by diatomite.

The possibility of a synergism between the water-loss-inducing effects of pyrethrins and the sorptive properties of diatomite should not be overlooked. However, the results of the bioassays, as discussed above, indicate that the importance of this phenomenon, if it occurs, is by far outweighed by the differences in release rates between carriers. Nevertheless, the abrasive and sorptive properties of diatomite could well become important when dealing with a less 'hardy' insect or insect larva.

Potential of diatomite-based products

To date, no extensive field tests of the diatomite-based products have been conducted. There is obviously a market for domestic and indoor use of controlled-release pyrethrum products as evidenced by the widespread use of the described micro-encapsulated product (p15), but it is not known if the diatomite-based products would be competitive. However, the "natural" origin of the products may give them an advantage in certain markets.

For agricultural and forestry use, controlled release of a non-persistent pesticide would seem to have potential, especially if electrostatic charging of the dust enables enhanced coverage of foliage. According to the manufacturers of 'Diacide', agricultural use of the dust compares favourably with commonly-used insecticides on a cost-per-acre basis. However, I have not analysed this aspect and the whole issue may be confounded by the high toxicity of pyrethrins to fish (p16).

At present, the 'Diacide' product would seem to be best suited to the home garden market because of its effectiveness at low and high humidity, its low mammalian toxicity and its "organic" nature (pace piperonyl butoxide).

Suggestions for further research

1. Controlled release hypothesis.

   A test of the hypothesis should be conducted using GLC analysis (as part B II) to monitor the residual pyrethrins content of the talcum- and diatomite-based formulations after exposure to light and air for varying periods of time at different temperatures and humidities. U.V. illumination (290-320 nm, p15) could be used to accelerate the rate of pyrethrin breakdown.

2. Efficacy testing.

   The products should be tested at varying temperatures and humidities on a range of insects, concentrating especially on those of known pest status and using species of different 'hardiness' to desiccation. Resources permitting, large scale (e.g. 5,000 insects) LT50 tests might also be conducted.

3. Pyrethrum mode of action.

   Further studies, including LD50 tests, on pyrethrum efficacy at varying humidities should provide support for the new hypothesis of pyrethrum mode of action (Gerolt 1983).

4. Environmental toxicology.

   As suggested on p16, the toxicity of slow-release pyrethrum formulations to wildlife, especially fish, should be considered further.

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