LABORATORY EVALUATION OF INSECTICIDES AS A POTENTIAL CONTROL OF WHITE GRUBS, PHYLLOPHAGA SPP. (COLEOPTERA: SCARABAEIDAE).

by

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ABSTRACT

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LABORATORY EVALUATION OF INSECTICIDES AS A POTENTIAL CONTROL OF WHITE GRUBS, PHYLLOPHAGA SPP. (COLEOPTERA: SCARABAEIDAE).

Laboratory insecticide bioassay testing was conducted on field-collected *Phyllophaga* spp. during 1978 and 1979. Adult *Phyllophaga* spp. were shown to be highly susceptible to cypermethrin and fenvalerate. Six insecticides: isofenphos, carbofuran, diazinon, fensulfothion, fonofos and chlorpyrifos were used both as contact insecticides and incorporated into muck, sand, clay and sandy-loam soils for controlling *Phyllophaga* spp. larvae. Chlorpyrifos was the most effective, of the six tested, in controlling larvae both as a contact and soil insecticide. Fonofos, except when incorporated into muck soil, also provided good control. Two methods evaluated for assessing white grub mortality in bioassay testing, showed mortalities based on lack of reflex movements to be a more accurate indication of insecticide toxicities.

RESUME

M.Sc. Glenn J. Letendre Entomologie

Des tests bioassay furent effectués en laboratoire sur Phyllophaga spp. recueillis dans le champ au cours des années 1978 et 1979. Phyllophaga spp. adulte a démontré une grande susceptibilité à la cyperméthrine et au fenvalerate. Six insecticides: isofenphos, carbofuran, diazinon, fensulfothion, fonofos et chlorpyrifos ont été utilisés comme insecticides de contact et incorporés dans des sols organiques, sableux, argileux et sableux-limoneux pour contrôler les larves de Phyllophaga spp. Chlorpyrifos fut le plus efficace des six insecticides pour le contrôle des larves en tant qu'insecticide de contact et incorporé au sol. Fonofos montre un bon contrôle, excepté lorsqu'incorporé dans un sol organique. Deux méthodes évaluées, pour estimer le taux de mortalité des vers blancs dans les tests bioassay, montrent que le taux de mortalité basé sur l'inexistence de réflexes est une indication plus exacte de la toxicité des insecticides.

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I. GENERAL INTRODUCTION

June beetles, Phyllophaga spp., are minor defoliating pests of deciduous trees such as elm, willow and oak. The immature larvae, commonly known as white grubs, are major soil-dwelling pests of such agricultural crops as grass, hay, pasture, corn, potatoes, strawberries and young nursery trees (Metcalf et al., 1962).

The most common species, and consequently the one causing the heaviest losses in the province of Quebec, is Phyllophaga anxia (LeConte)(Hammond, 1940; Lim et al., 1979). Despite soil treatment with chlordane, crop losses to white grubs have been reported in the Nicolet area for potatoes (Morrison, 1971), strawberries (Anonymous, 1981a), and widespread white grub infestations have been observed in Quebec pastures. At present, no chemical recommendation for white grub control exists in Quebec (Anonymous, 1979, 1981b); preventative cultural and mechanical practices are recommended for farmers instead. Chlorinated hydrocarbon insecticides such as BHC, aldrin, dieldrin and heptachlor were recommended for grub control in soil up to the early 1970's, and generally provided good protection of crops (Hammond, 1947, 1949, 1952, 1960; Shenfelt and Sinkover, 1951; Pass, 1964; Fowler and Wilson 1971b, 1974). However, many of these persistent chemicals have since been de-registered for agricultural use, and studies have shown several cases of the development of white grub resistance to chlorinated hydrocarbon insecticides (Frankie et al.,1973; Teetes 1973, 1975; Fuchs et al., 1974; Pike et al., 1978).

Hence, interest is currently focused on finding less persistent alternative insecticides, mainly in the organophosphoros and carbamate groups, for white grub control (Frankie et al., 1973; Teetes, 1973, 1975; Fuchs et al., 1974; Rivers et al., 1977; Pike et al., 1978; Reinert, 1979). The research described in this study was undertaken to test several potential insecticides on Quebec populations of white grubs, in order to supplement local crop protection recommendations wtih a chemical control.

II. LITERATURE REVIEW

White grubs of the genus *Phyllophaga* have caused losses in agricultural crops since before the turn of the century (Pike *et al*., 1976). Although *Phyllophaga anxia* is by far the most abundant species in Quebec (Hammond, 1940; Lim *et al*., 1979), this review included information on other species of *Phyllophaga*, commonly known as white grubs or "hannetons" to Quebec farmers.

A. TAXONOMY

The common name white grub is given to larvae of many chafer beetle species of economic importance. The common June beetle, *Phyllophaga anxia* is native to North America (Hammond, 1948; Ritcher, 1949; Neiswander, 1963), and is one of 152 species of *Phyllophaga* found there (Luginbill and Painter, 1953). *P. anxia* was first described by LeConte (1850), and until a revision by Glasgow in 1916, *Lachnosterna* was used as the genus name. At this time *Lachnosterna* was found to be synonymous with *Phyllophaga* Harris, 1827, and was therefore replaced. The following is a list of synonyms for *P. anxia* provided by Luginbill and Painter (1953).

Lachnosterna anxia LeConte 1850
Ancylonycha brevicollis Blanchard 1850
Ancylonycha puncticollis Blanchard 1850
Lachnosterna cephalica LeConte 1856
Ancylonycha uninotata Walker 1866

Lachnosterna dubia Smith 1889

Lachnosterna insperata Linell 1897

Phyllophaga anxia Glasgow 1916

The taxonomic position of *P. anxia* (LeConte) is as follows (Ritcher, 1966):

Order: Coleoptera

Suborder: Polyphaga

Superfamily: Scarabaeoidea

Family: Scarabaeidae

Subfamily: Melolonthinae

Tribe: Melolonthini

Genus: *Phyllophaga*

Subgenus: *Phyllophaga*

Species: *anxia*

Original keys were based on external morphological characters such as the form of the clypeus, antennae, pronotum, spur and the hind tibia of males, and the structure of the abdominal sterna (Nairn and Wong, 1965). Further taxonomic work used the structure of the male and female genitalia (Langston, 1927; Ritcher, 1940; Boving, 1942; Luginbill and Painter, 1953). Keys for identification of white grubs of *Phyllophaga* have considerable overlaps and variations within species, making positive identification difficult (Böving, 1942; Ritcher, 1940, 1966). Although the insects in the following work were field collected in areas where Phyllophaga anxia was the predominant species (Lim et al., 1979), large scale identifications of individual insects were not made.

B. LIFE CYCLE OF PHYLLOPHAGA ANXIA

Phyllophaga anxia has a development period of three years in eastern Canada (Forbes, 1916; Hammond, 1931, 1940, 1948a; Hammond and Maheux, 1934; Jarvis, 1966). The adults emerge from the soil about the middle of May and fly at dusk to neighbouring trees where feeding and mating take place. The daylight period is spent hidden in the soil or beneath ground cover, and this diurnal pattern of activity continues over a period of up to two months, with peak flight activity occurring in June (Criddle, 1918; Maheux and Gauthier, 1944; Hammond, 1948; Sutton and Stone, 1974; Lim et al., 1979). Eggs are laid in grassy areas at a depth of approximately 10 cm in the soil, some ten days after mating has taken place (Maheux and Gauthier, 1944). First-instar grubs emerge about 30 days later and consume decaying organic matter and small roots (Maheux and Gauthier, 1944; Hammond, 1948). Moulting to the second-instar larva occurs 6 to 8 weeks later, and thereafter grubs feed on living plant roots exclusively. As soil temperatures decrease (September-October), the second-instar larvae migrate to various depths in the soil to overwinter (Hammond, 1948). During early spring of the second year, the second-instar grubs return to the surface to feed for a short period, and the final larval moult into the third-instar occurs in July. It is this final stage (second-year, third-instar grubs) which causes most damage to crops, for the grubs are voracious feeders till the coming fall (Hammond, 1948). Little feeding is done in the third year of development because the grubs remain relatively inactive. Pupation occurs in mid-July and emerged teneral adults remain in the soil till spring of the following year (Hammond, 1948; Lim et al., 1979).

C. OCCURRENCE AND DAMAGE

White grubs are amongst the most destructive of soil insects. Infestations may go undetected until the situation is beyond corrective control because larvae live in the soil and the adults fly at night.

White grubs have been reported to attack a variety of agricultural crops. Oats (Bigger and Flint, 1939), wheat (Fenton, 1939; Burkardt, 1955; Daniels, 1971; Teetes, 1973), grain sorghum (Daniels, 1971; Teetes, 1973), and sugarcane (Fuchs et al., 1974) have suffered economic losses to white grubs in the United States. Bluegrass pasture in the south, and rough pasture, hay and turf crops in Quebec, support heavy infestations of white grubs, resulting in widespread deterioration of grass and pasture (Graber et al., 1931; Fluke et al., 1932; Fuelleman and Graber, 1937; Burcalow et al., 1940; Pass, 1964). White grubs are a major concern in corn in the United States (Fuelleman and Graber, 1937; Bigger and Blanchard, 1955; Rivers et al., 1977, Pike et al., 1978). Corn fields attacked by grubs appear patchy, with plants reaching heights of less than 2 feet (60 cm)(Metcalf et al., 1962). Potato roots are consumed by white grubs, and holes measuring from 7 to 14 mm deep are made in the tubers (Anonymous, 1979). Losses to potato growers in the United States (Hodgson et al., 1974) as well as in Quebec (Morrison, 1971; Perron, 1972), have necessitated the development of new control measures. Strawberries and sugar beets are also attacked in the Province of Quebec (Anonymous, 1977, 1981a), strawberries showing symptoms similar to those caused by drought conditions.

White grubs consume the smaller roots of red pine (Fowler and Wilson, 1971a, 1974) and eventually girdle the larger ones, reducing growth, weakening, and finally killing the young seedling. Recommendations to avoid planting pine seedling in areas having more than 0.5 grubs per square foot (900 sq cm) indicate the severity of the pest. Watts and Hatcher (1954) reported white grub damage in plantations in the Carolinas. Young hemlock, in the state of New York, progressively yellowed and died within a month when attacked by as few as 3 to 4 second-year grubs per tree (Schwardt, 1942). Massive root girdling may also kill shrubs and saplings when infestations are heavy (Hammond, 1960).

As adults, *Phyllophaga* spp. have been reported to defoliate oak in Wisconsin (Fuelleman and Graber, 1937). Other deciduous trees such as elm (Davis, 1916; Hammond, 1947), willow (Chamberlain et al., 1938; Travis and Decker, 1939; Sanderson, 1944), hickory, ash (Davis, 1916; Sanderson, 1944), and poplar are favoured diets of the adult beetles. Shrubs and bushes such as lilac, rose, pecan, walnut, butternut, chinese elm, wild plum, blackberry, and apple are also fed upon (Ritcher, 1940; Sanderson, 1944; Hammond, 1948).

In the past, eastern Canadian producers have suffered losses amounting to several hundreds of thousands of dollars (Hammond, 1960). A survey of 45 farms conducted in the province of Quebec in 1935, showed an estimated loss caused by white grubs, to be at approximately $216 per farm. An outbreak of grubs in eastern Ontario and western Quebec in 1933, caused an average loss of $188 per farm (Hammond, 1940). White grub infestations of 78 farms in the Eastern Townships, Quebec, caused damages estimated at $108 per farm in 1938 (Maheux and Gauthier, 1944).

White grubs are more abundant in light soils such as sand and sandy-loam and are commonly found in pasture fields with large amounts of timothy (Anonymous, 1981b). Hammond (1940) reported white grubs to be of economic importance in large areas of agricultural land in Quebec south of Montreal. These included the counties of Huntington, Chateauguay, St. Jean, Iberville, Rouville, Shefford, Brome, and Missisiquoi, and light soils in Jacques Cartier, Laval, Two Mountains, western Terrebonne counties were also infested. Infestations continued eastward along the St. Lawrence River through the St. Maurice, Champlain, and Portneuf counties. More recently, outbreaks in potato fields in Nicolet county were reported to Macdonald College (Morrison, 1971). Perron (1972) stated that white grubs were a problem in potatoes and corn in Quebec. White grub damage was found by the author in the Mirabel region in 1978 and 1979, in grassy fields grown for sod production. Also in the Nicolet region, a strawberry field was totally destroyed in 1979 by third instar grubs. White grubs were found in adjacent potato fields as well as in other strawberry fields in the area (C. Ritchot, personal communication).

D. CULTURAL CONTROL OF WHITE GRUBS

Damage by white grubs to susceptible agricultural crops such as potatoes, corn, strawberries, and nursery stock (trees) can be avoided by not planting them in freshly-ploughed pasture or neglected fields (Pettit, 1930; Hammond, 1940; Fowler and Wilson, 1971a; Sutton and Stone, 1974; Anonymous, 1977, 1979; 1981, a, b, c, e).

Ploughing of infested fields followed by repeated cultivations using a disc-harrow has been shown to reduce population levels of white grubs (Davis, 1916; Criddle, 1918; Drake et al., 1932; Hammond, 1933, 1940, 1948, 1960; Maheux and Gauthier, 1938, 1944; Bourqui et al., 1950; Hodgson et al., 1974), but timing of the operation is critical for maximum effect. Destruction of first-year grubs is best done during the period from late July to late September (Davis, 1916; Hammond, 1933, 1960; Hodgson et al., 1974). Second-year grubs are most susceptible to mechanical destruction from early May to early July (Hodgson et al., 1974), and Maheux and Gauthier (1938) found the most susceptible period to be during the pupation period from mid-June to early July of the third year. During these periods, the grubs are living sufficiently near the soil surface to be killed by a combination of physical injuries and exposure to adverse climatic conditions and natural enemies.

Crop rotation has been recommended for the control of white grubs. Davis (1916) noted that crop rotations based on a knowledge of the life cycle of Phyllophaga, could be effective in protecting crops from damage. A rotation of corn or clover and small grains was one recommendation; the clover or corn being planted during a flight year (Davis, 1918). Plants resistant to grub damage, such as white clover, red clover, and alfalfa have also been recommended for growing during flight years (Hammond, 1940; Chamberlin and Fluke, 1947). Pasture fields of grass, when planted in combination with sweet clover, red clover or alfalfa, demonstrated some repellent effects to adults, and reduced oviposition (Fluke et al., 1932; Fuelleman and Graber, 1937).

E. CHEMICAL CONTROL OF WHITE GRUBS

Attempts have been made since the 1930's to find an effective chemical control for white grubs and June beetles. Initially, control was directed at the adults while they fed on foliage during the spring. Trees preferred by the beetles such as elm, willow, and oak were sprayed with inorganic insecticides including lead arsenate, calcium arsenate, sodium fluosilicate, and paris green (Fluke and Ritcher, 1935; Travis 1936; Andre and Pratt, 1936; Andre 1937; Travis and Decker, 1939; Hammond, 1940). Lead arsenate provided better control of the adult in most cases, and its efficacy against white grubs also led to its recommendation for use on turf (Luginbill and Chamberlin, 1938; Neiswander, 1951). When lead arsenate was mixed with sand, protection of strawberries was achieved with little phytotoxicity (Kerr, 1939, 1940, 1941; Hammond, 1940; Marshall, 1951). A similar mixture provided control of white grubs in young hemlock plantations (Schwardt, 1942).

Control of Phyllophaga spp. grubs using soil fumigants was studied as a possible alternative to lead arsenate. Fumigants such as dichlorethylether, methyl bromide, ethylene dibromide, carbon disulfide, chloropicrin, and paradichlorobenzene were used on turf and in nurseries (Johnston and Eaton, 1942; Ritcher and Jewett, 1942; Schwardt, 1942; Hammond, 1945, 1946). Chloropicrin provided good grub control but its cost and phytotoxicity made it unfavorable (Hammond, 1946). Paradichlorobenzene was cheaper to apply, but reaction times were slower (Johnston and Eaton, 1942). The development of benzene hexachloride (BHC) and DDT as insecticides in the 1940's saw a new series of studies using them as foliar sprays to control the adults, and also as soil insecticides against the grub. Both chemicals, when applied to the foliage independently, provided good beetle control (Hammond, 1947, 1952; Marshall, 1951). Control of white grubs in pasture, using BHC, was achieved in studies conducted by Hammond (1947, 1949). However, similar trials in Switzerland on cockchafer grubs produced opposite results (Bourqui et al., 1950). Aldrin was used successfully to control grubs in bluegrass lawns (Pass, 1964) and red pine nurseries (Fowler and Wilson, 1971b, 1974). Other chlorinated hydrocarbon insecticides such as dieldrin, heptachlor and endrin were also found to be effective on bluegrass lawns (Pass, 1964) and wheat (Burkardt, 1955; Daniels, 1966, 1971). Chlordane was first used against white grubs in 1948, but was found to be ineffective in various soils (Marshall, 1951; Hammond, 1952).

Recent legislative restrictions on the use of persistent chlorinated hydrocarbon insecticides for crop protection have reduced the number of chemicals available for white grub control, and testing of alternatives has been mainly with carbamate and organophosphoros insecticides. Certain organophosphoros insecticides used by Pass (1964) showed comparable control effectiveness to some of the better chlorinated hydrocarbons. Diazinon, when applied to clay-loam planted with wheat and grain sorghum, showed a lower control level than the chlorinated hydrocarbon insecticides (Daniel, 1966, 1971). Contrary to these findings Frankie et al. (1973) recommended the use of diazinon in a granular formulation for white grubs in lawn infestations. Effective control was achieved in grain sorghum and wheat using applications of fensulfothion, diazinon and carbofuran (Teetes, 1973). Chlordane and heptachlor were not effective, possibly due to a degree of resistance having developed (Teetes, 1973, 1975). A new technique developed by Fuchs et al. (1974) for determining insecticide efficacy against white grubs, *Phyllophaga crinita*, indicated effective control using fensulfothion, diazinon, fonofos and carbofuran. An experimental organophosphoroate, CGA 12223, was reported to be the most effective insecticide in greenhouse testing by Rivers et al. (1977).[1] Pike et al. (1978) demonstrated resistance of white grubs to chlorinated hydrocarbons and showed carbofuran to be the most toxic soil insecticide tested against second and third- instar grubs. Recent field work on Bermuda grass in Florida, by Reinert (1979), showed consistent control of white grubs with carbofuran, fonofos and isazofos. Lim et al. (1980) found

[1] See appendix N.

fensulfothion, fonofos, isofenphos and WL 24073 to be most toxic to third-instar grubs, Phyllophaga spp.[1] Fensulfothion, applied to established fescue and Kentucky bluegrass for control of European chafer (Rhizotrogus majalis), controlled populations 100% the first year and between 70 and 100% the following year (Tashiro et al., 1981). Diazinon, though not significantly diffe-rent from the fensulofothion treatments, reduced populations by 89%.

F. PRESENT CONTROL RECOMMENDATIONS IN EASTERN CANADA

Both Quebec and Ontario Ministries of Agriculture presently recommend the use of preventive measures to avoid heavy losses caused by white grubs. Producers are warned not to plant vegetables on land that has been in grass sod for two years or more, especially in the year following a flight year (Anonymous, 1979, 1981a, b, c, d, e). The statement made by the Quebec government (Anonymous, 1981a), that there is potentially no damage to strawberries nor a need to treat fields previously worked over and used for a cultivated crop, could be misleading. A case reported by Kerr (1940) showed the presence of a white grub infestation (Phyllophaga spp.) in strawberries which had been planted,in a field previously used for a number of crops planted in rotation for two years. White potatoes, sweet potatoes and wax beans were planted with a cover crop of rye prior to the strawberries. Severe damage to corn planted in two fields which contained soybean the previous year, and also a field that had been planted in corn two consecutive years, was caused by white grubs in Illinois (Bigger and Blanchard, 1955). A local case occurred

[1] See appendix N.

during the summer of 1979, at Pierreville, Quebec. Second year, third-instar grubs were present in damaging numbers in a strawberry field that was in its third year of growth. The above cases would indicate that eggs were laid in fields other than those with a grass cover.

Ontario presently recommends the application of chlordane, a broadcast application at a rate of 5.6 to 9.0 kg AI/ha, if vegetables must be planted in newly ploughed land (Anonymous, 1981e). Higher rates are required in heavy soil or when populations are higher. However, chlordane may not be effective especially in heavy infestations, as was the case in many strawberry fields in the Pierreville region during the summer of 1979. Chlordane had been applied as recommended by the Quebec government for the protection of strawberries at a rate of 9.0 kg AI/ha a few days prior to planting and immediately after, to a depth of 10 to 15 cm (Anonymous, 1981a). In the state of New York, diazinon applied at a rate of 6.7 kg AI/ha, or 2.2 kg AI/ha of chlorpyrifos are recommended against white grubs in turf.

III. FIELD COLLECTION OF ADULTS AND GRUBS OF *PHYLLOPHAGA* SPP.

A. INTRODUCTION

Large numbers of test organisms of maximum uniformity are required for conducting accurate biological assay (bioassay), where the potency of a stimulus can be measured by recording the occurrence of a pre-determined response in a test population on a graded exposure to the stimulus. Since *Phyllophaga* spp. have a three year life cycle, laboratory culturing of large, uniform populations for bioassay is difficult and largely impractical. Attempts at rearing white grubs individually in the field and in the laboratory have all given poor results (Miner, 1948, 1952;Desai and Patel, 1965;Ritcher, 1940; Reinhard, 1946; Toohey, 1977 unpubl. thesis). Consequently, the large number of specimens needed to carry out the experiments described in the following chapters had to be collected from wild field populations.

B. COLLECTION OF WHITE GRUBS FROM TURF FARMS

Phyllophaga grubs are found to occur sporadically in areas of pasture and abandoned farm land, where they feed on the roots of grasses. Randomly over-turning of sods and analizing of the roots and soil to a depth of approximately 10 cm, in an attempt to collect sufficient numbers of grubs, was tedious and often unproductive. A method for collecting larger numbers of grubs was attempted by K.P. Lim and W.N. Yule in the summer of 1977 (Lim, *et al.*

1979). A tractor-mounted plough was used to over-turn the sod on a field known to be infested at Nicolet, but it was found that the plough destroyed or mutilated many grubs and exposed them to sunlight and predators in the period between ploughing and collecting. This greatly reduced the number of healthy grubs that could be collected using this method.

Surveying for adult beetles using light traps distributed in the Montreal area during the spring of 1978, revealed the presence of large localized populations of June beetles, and further investigation of the soils of several large turf farms in the Mirabel region revealed infestations of second-instar white grubs in fields not treated with insecticides. Large numbers of larvae were easily collected without injury as they were exposed by a mechanical turf cutter (Figures 1 and 2), as it cut and rolled the top 4 cm of sod and soil. The grubs were collected by hand and placed in a cooler to protect them from excessive heat and direct sunlight. This technique was found to have the following advantages and was used for collecting grubs early in the summers of 1978 and 1979.

1) Since white grubs are found in sporadic groupings throughout any particular field when infestations are moderate (Lim, 1979), the mechanized removal of turf over large areas exposed pockets of grubs, which might not have been located using random digging.

2) The turf was cut just above the level at which the grubs were feeding and was rolled up mechanically, thus exposing uninjured grubs on the fresh soil surface (Figure 2).

FIGURE 1: A small turf cutter used at Mirabel.

FIGURE 2: A field at Mirabel where white grubs were collected as turf was removed.

3) Large numbers of grubs (approx. 300-400/day) could be collected by only two workers, and fewer grubs were destroyed than with a plough.

4) The turf cutter advanced at a pace slightly faster than one could walk, making it possible to collect the grubs as they were exposed, thereby reducing the time they were in direct sunlight.

5) Turf grass is normally irrigated during periods of dry weather to ensure that it is in good condition for easy cutting, and also to sustain it in delivery. This provides an ideal environment for white grub survival near the surface.

C. COLLECTION OF WHITE GRUBS IN STRAWBERRY FIELDS

Later in the summer of 1979, a 1.0 ha strawberry field (Figure 3) located 8 km north of Pierreville, Quebec, was found to be infested with second year third-instar white grubs (personal communication, C. Ritchot and C. Turmel, Agr.). The hand-pulling of strawberry plants revealed the presence of as many as 6 grubs feeding on each plant (Figure 4). A visual comparison between healthy plants (Figure 7) and those attacked by white grubs (Figure 8), showed a scorching of the leaves, the absence of the many secondary roots, and in advanced cases, loss of the main root and crown which caused the death of the plant. Grubs were found in the top 10 cm of soil and were easily turned up using fork-spades and 3-pronged hand cultivators (Figure 5). Grubs were placed in soil-filled flats and kept in a cool place until transferred to boxes for transportation to the laboratory.

FIGURE 3: The strawberry field at Pierreville where white grubs were collected in 1979.

FIGURE 4: As many as six third-instar white grubs were found feeding on the roots and crowns of strawberry plants, Pierreville, 1979.

FIGURE 5: Grubs were uncovered using cultivators and fork-spades.

FIGURE 6: Grubs were placed in flats and covered with soil to protect them from the sun during collection.

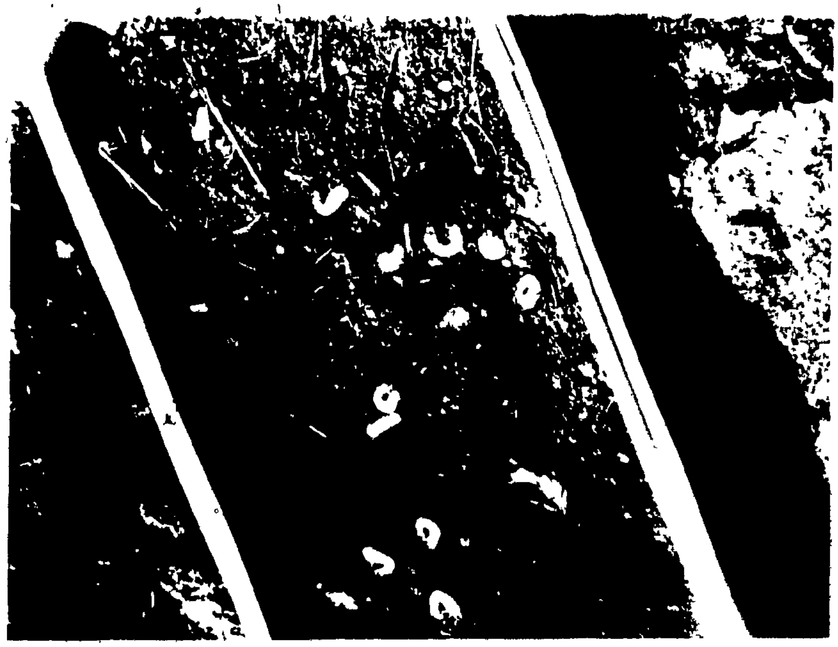


FIGURE 7: A healthy strawberry plant.

FIGURE 8: A strawberry plant showing heavy damage caused by feeding of third-instar white grubs.

D. COLLECTION OF ADULT JUNE BEETLES

Blacklight traps were used to collect adult June beetles. Ward's 4-baffled insect traps (Ward's Natural Science Establishment, Inc., Rochester, N.Y.), were fitted with 8 watt blacklight fluorescent tubes. A galvanized steel collecting funnel (upper diameter: 30 cm, funnel opening: 3.5 cm) was attached to the base of the collecting trap and a cone was tied to the top to prevent rain from entering (Lim, 1979). A nylon bag was attached to the funnel base to collect the beetles alive. The traps were suspended at approximately 1 m from the ground when in operation. In 1978, traps were located in the Ste. Anne de Bellevue area, and in 1979 traps were operated in the county of Nicolet. The bags were removed every morning and placed in a cool place till removed to the laboratory.

IV. INSTALLATION AND CALIBRATION OF A POTTER SPRAY TOWER

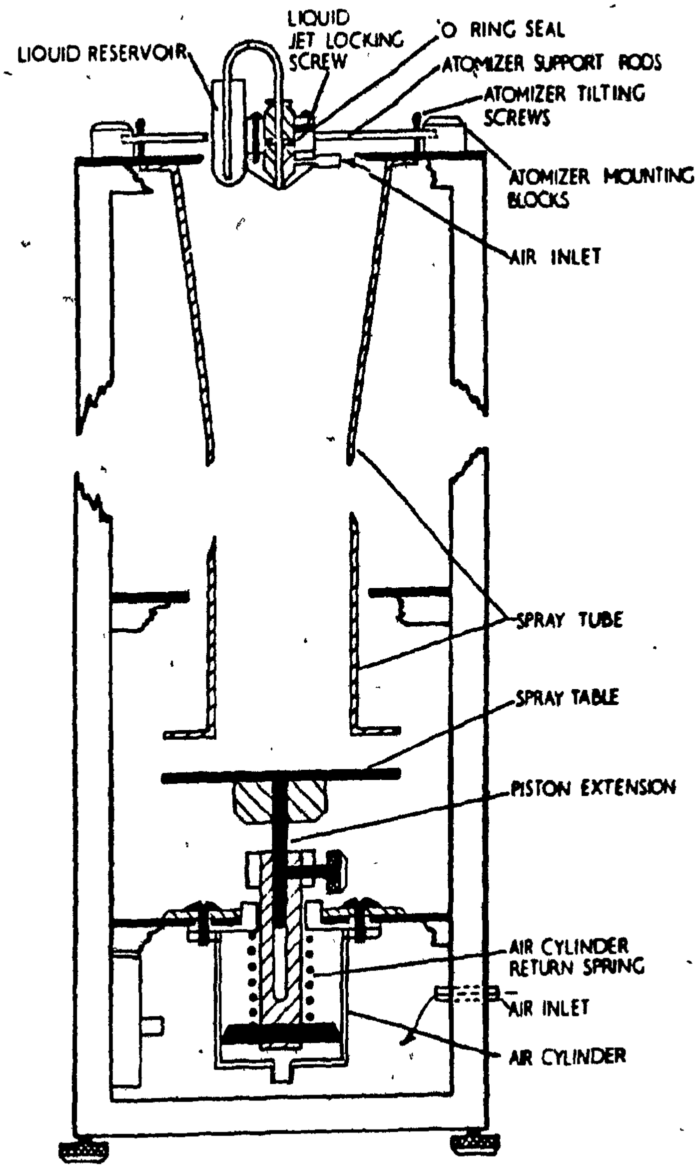
A. INSTALLATION

A Potter spray tower was purchased from Burkard Manufacturing Co., Ltd., Rickmanworth, Herts., England, for laboratory screening of insecticides. Installation and operation methods were studied during a visit to the Soil Laboratory, Pesticide Research Institute, Agriculture Canada, London, Ontario in 1978 by kind arrangement with Dr. C.R. Harris. The tower was installed within a fume hood (Figure 9) to remove toxic spray and fumes given off during operation. The apparatus was set up for use according to the instructions of its designer (Potter, 1952). Adjustments were carried out to level and centralize the spray tube, spray table, and atomizer, to performance specifications of the makers (Diagram 1).

Studies by Potter (1952) showed the apparatus capable of repeatedly giving an even distribution of spray deposit over a target 9 cm in diameter, using both distilled water and a light petroleum oil. Similar tests were carried out, and minor adjustments made periodically to calibrate and check for uniform distribution of insecticide on the surface exposed below the tower. A central compressed air line was connected to the tower's air cylinder and atomizer, and line pressure was found to be relatively constant,(80 ± 0.5 cm mercury) measured with an open-end mercury manometer and a pressure gauge supplied on the tower. Air supplied to the atomizer was filtered and pressure

FIGURE 9: Potter spray tower installed in a fume hood to remove toxic spray and fumes when the tower was operated.

DIAGRAM 1: Potter Spray Tower.


LIQUID RESERVOIR
LIQUID JET LOCKING SCREW
'O RING SEAL
ATOMIZER SUPPORT RODS
ATOMIZER TILTING SCREWS
ATOMIZER MOUNTING BLOCKS
AIR INLET
SPRAY TUBE
SPRAY TABLE
PISTON EXTENSION
AIR CYLINDER RETURN SPRING
AIR INLET
AIR CYLINDER

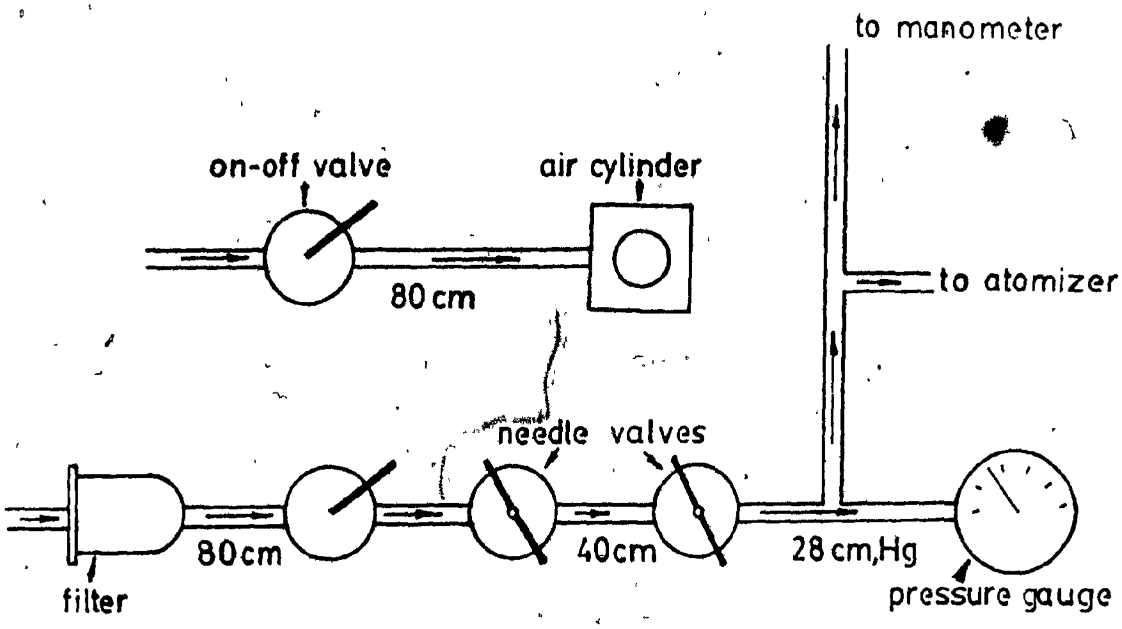
was reduced twice using needle valves, from line pressure of 80 cm of mercury to approximately 40 cm and finally to 28 cm (nozzle operating pressure) as shown in Diagram 3. A modification to the air line system (Diagram 3) was made to ensure a constant, reproducible pressure when spraying. The platform cylinder's air line was separated from the nozzle line to prevent a slight drop in pressure occurring when the spray table was raised. An on-off toggle valve was also installed in the atomizer line (Diagram 2) to provide a rapid release of air and thereby reduce any gradual build-up of pressure when spraying started, which could change the droplet spectrum emitted.

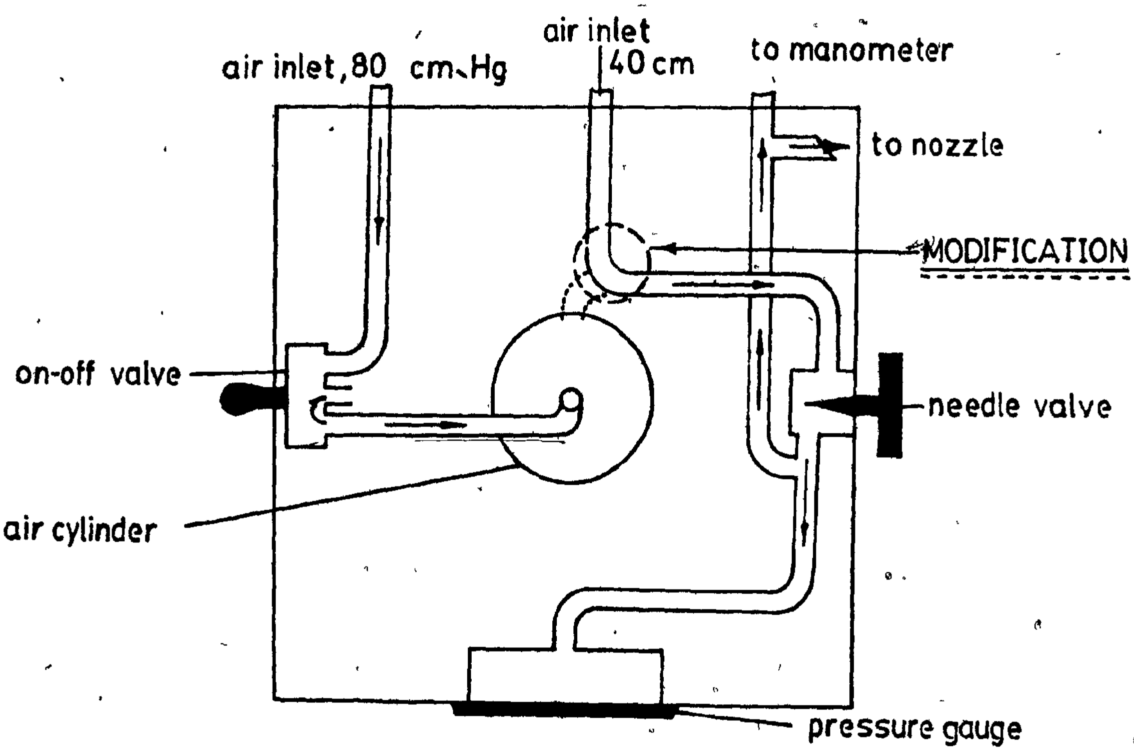
B. CALIBRATION

The air pressure level was set at 28 cm of mercury (approximately 5 PSI). The test fluid was measured using a 5 mL pipette into the reservoir of the atomizer. The spray table was lowered and a 9 cm diameter Petri dish containing 4- 2.56 cm diameter cover slips was placed on it and returned to the spray position. The atomizing air stream was turned on using the toggle valve, and the fluid was sprayed down the cylinder. When all had been atomized, the compressed air was turned off and the table lowered to remove the cover slips after 15 seconds had elapsed. The position of the individual cover slips was recorded, and the weight of deposited fluid was calculated by subtracting the weight of the preweighed cover slip from the total. Standard errors (Steel and Torrie, 1960) were calculated on each single deposit, mean of the deposits

DIAGRAM 2: Pressure line supplying tower.

DIAGRAM 3: Bottom view of tower.


to manometer
on-off valve
air cylinder
to atomizer
80 cm
needle valves
80cm
40cm
28 cm,Hg
filter
pressure gauge


air inlet
air inlet, 80 cm, Hg
40 cm
to manometer
to nozzle
MODIFICATION
on-off valve
needle valve
air cylinder
pressure gauge

at one position, and of the mean of the deposits in one test. Values were obtained using distilled water and also a mixture of olive oil and acetone in a ratio of 1:1. In the case of olive oil and acetone, the weights observed were that of the oil deposit only because of the rapid evaporation of the acetone. The standard errors were acceptably low (less than 5 percent), indicating that deposit uniformity and reproductibility were within the specifications recommended by Potter (1952), and were consistent with both water and olive oil-acetone. Positional weights as well as calculated standard errors are given in Tables 1 to 5.

TABLE 1: Distribution of deposit using water.

Measured by obtaining the weight of water deposited on 25.6 mm cover slips placed in a 9 cm glass Petri dish. Gap, 1.25 cm, 5 mL of water sprayed at a pressure of 48 cm of mercury.

Test number	Position of cover slip				Mean (mg)
	Rear	Right front	Left front	Centre	
1	3.4	3.1	2.6	2.7	2.95
2	3.1	2.7	3.0	4.1	3.23
3	3.4	3.8	3.0	4.0	3.55
4	3.6	3.0	4.1	3.6	3.58
5	3.4	3.8	3.3	3.2	3.43
Means	3.38	3.28	3.20	3.52	3.35

Standard error (S.E.) of a single deposit = 0.10 = 3.07% of mean.

S.E. of the mean of five deposits at one position = 0.07 = 2.06%

S.E. of the mean of four deposits on one test = 0.12 = 3.49%.

TABLE 2: Distribution of deposit using water.

Measured by obtaining the weight of water deposited on 25.6 mm cover slips placed in a 9 cm glass Petri dish. Gap, 1.25 cm, 5 mL of water sprayed at a pressure of 48 cm of mercury.

Test Number	Position of cover slip				Mean (mg)
	Rear	Right front	Left front	Centre	
1	4.1	3.7	4.0	4.4	4.05
2	3.8	2.6	3.4	3.7	3.38
3	4.0	2.5	3.4	3.8	3.43
4	3.4	3.5	2.9	4.1	3.48
5	2.6	3.5	3.9	4.1	3.50
6	3.9	3.1	3.1	3.9	3.50
Means	3.62	3.15	3.45	4.00	3.56

S.E. of a single deposit = 0.29 = 3.07%

S.E. of the mean of six deposits at one position = 0.12 = 4.98%

S.E. of the mean of four deposits on one test = 0.10 = 2.82%

TABLE 3: Distribution of deposit using water

Same conditions as described in Tables 1 and 2.

	Position of cover slip				
Test number	Rear	Right front	Left front	Centre	Mean (mg)
1	3.4	2.5	3.4	3.4	3.18
2	3.1	2.4	2.8	3.2	2.88
3	2.8	2.7	2.5	3.2	2.80
4	2.9	2.7	2.6	3.4	2.80
5	3.0	2.4	2.6	3.4	2.85
6	3.4	3.3	3.0	3.5	3.30
Means	3.10	2.67	2.82	3.28	2.97

S.E. of the mean of a single deposit = 0.07 = 2.49%.

S.E. of the mean of six deposits at one position = 0.14 = 4.62%.

S.E. of the mean of four deposits on one test = 0.09 = 2.97%.

TABLE 4: Distribution of deposit using water.

Measured by obtaining the weight of water deposited on cover slips placed in a 9 cm glass Petri dish. Gap, 1.25 cm, 5 mL of water sprayed at a pressure of 25.5 cm of mercury.

	Position of cover slip				
Test number	Rear	Right front	Left front	Centre	Mean (mg)
1	11.6	10.5	12.3	12.7	11.78
2	12.1	11.8	10.9	12.2	11.75
3	12.1	11.6	11.4	12.5	11.90
4	12.6	12.1	11.5	12.3	12.13
5	11.8	10.5	11.9	13.0	11.80
6	11.8	11.3	11.2	12.1	11.60
7	12.7	11.0	12.2	12.4	12.08
8	12.3	11.6	11.4	12.7	12.00
9	12.2	11.3	11.8	12.9	12.05
10	12.1	12.1	12.3	13.2	12.43
<u>Means</u>	12.13	11.38	11.69	12.6	11.95

S.E. of a single deposit = 0.10 = 0.84%.

S.E. of the mean of ten deposits at one position = 0.27 = 2.22%.

S.E. of the mean of four deposits on one test = 0.08 = 0.63%.

TABLE 5: Distribution of deposit using acetone and olive oil.

Measured by obtaining the weight of olive oil (applied in a 1:1 ratio with acetone) deposited on 25.6 mm cover slips placed in a 9 cm glass Petri dish. Gap, 1.25 cm, 5 mL of solvent sprayed at a pressure of 27 cm of mercury.

Test number	Position of cover slip: Rear	Right front	Left front	Centre	Mean (mg)
1	1.6	1.4	1.0	1.3	1.33
2	1.3	1.5	1.1	1.1	1.25
3	1.4	1.6	1.5	1.6	1.53
4	1.6	1.4	1.4	1.5	1.48
5	1.3	1.0	1.3	1.3	1.23
6	1.7	1.5	1.2	1.5	1.48
7	1.5	1.2	1.4	1.4	1.38
8	1.5	1.3	1.2	1.4	1.35
9	1.2	1.3	1.6	1.3	1.35
10	1.2	1.4	1.2	1.2	1.25
Means	1.43	1.36	1.29	1.26	1.36

S.E. of the mean of ten deposits at one position = 0.03 = 2.10%.

S.E. of the mean of four deposits on one test = 0.034 = 2.45%.

V. CONTACT TOXICITY OF INSECTICIDES TO FIELD-COLLECTED JUNE BEETLES

A. INTRODUCTION

Early attempts to control white grubs were directed at the adult stage, or June beetle. Andre (1937) demonstrated that, in field applications, Paris green caused higher mortalities of beetles than did acid lead arsenate, calcium arsenate, and sodium fluosilicate. Evidence was found by Fluke and Ritcher (1935) that lead arsenate sprays provided protection from defoliation of oak in Wisconsin. This was substantiated by laboratory tests using leaf sandwiches containing lead arsenate (Travis, 1936), and later in field tests conducted by Travis and Decker (1939), where lead arsenate applied to foliage caused an 80% mortality compared to 30% with calcium arsenate. Hammond (1947) studied the effectiveness of DDT sprays and dusts, BHC, and a combination of DDT and BHC applied to both the soil and foliage. DDT sprays were shown to be the most effective. Contrary to observations by Andre and Pratt (1939), indicating a higher susceptibility in males, Hammond (1947) found no differences in mortality with the sexes. Further tests by Hammond (1952) showed BHC was effective in eliminating first-year white grubs when applied to the soil near and under trees, at a rate of 20 pounds per acre.

Recent restrictions on the use of chlorinated hydrocarbon insecticides have stimulated efforts to find alternative controlling chemicals. However, the feasibility of controlling June beetles is questionable due to their wide range of food sources (Clark and Hoveland, 1938), and their low population densities (H. Tashiro, personal communication). Recent work by Bindra and Singh (1971) on Holotricha conquinea, an oriental species closely related to the genus Phyllophaga, showed carbaryl at 0.1 and 0.2%, and fenitrothion at 0.05 and 0.1%, to be effective control agents when applied to foliage. Lack of information concerning the toxicity of other currently available insecticides on Phyllophaga prompted the present investigation, which included the adult stage for completeness.

B. MATERIALS AND METHODS

Two experiments were conducted in the summers of 1978 and 1979 using June beetles. The beetles were trapped alive using a modified Ward's light trap (Lim, 1979) and were stored at 5°C until sufficient numbers of insects were accumulated for a test to be made.

Experiment 1: Adult stage Phyllophaga spp. were collected in the area surrounding Ste. Anne de Bellevue, Quebec, during the first weeks of June 1978. The beetles were refrigerated at 5°C then transported by air in a cooler by the author to the Soil Laboratory Pesticide Research Institute, Agriculture Canada, London, Ontario, where they were sprayed June 21. The beetles, upon arrival, were placed in cages, supplied with water and held overnight at 21°C. The 14 premium grade technical insecticides listed in Table 6 were dissolved in a mixture of acetone-olive oil (19:1) and applied topically using a Potter Spray Tower (Harris and Mazurek, 1961). Two replicates of 5 June beetles were sprayed with 5 mL of a 0.01% weight/volume solution of each insecticide at a pressure of 48 cm mercury. Duplicate controls of acetone-olive oil only were included in the test. Treated insects were placed in cardboard cartons and held 24 hours at 27°C, 65 ± 5% RH and 24 hours of light. Each beetle was inverted, placed under a bright light and prodded gently with pointed forceps, and any showing a movement, response of any appendage, was counted as alive.

Experiment 2: Beetles were collected during the last week of May and the first week of June, 1979, in Nicolet county, Quebec. The accumulated beetles were stored in the same manner as described for experiment 1. The beetles were sprayed June 9 at Macdonald Campus using a Potter Tower at a pressure of 28 cm mercury. Four replicates of 5 insects were sprayed with a series of five concentrations of diazinon, fenvalerate, and cypermethrin as well as an acetone-olive oil control. The concentrations used were 0.005, 0.1, 0.2, 0.4 and 0.8% weight/volume.

Mortalities were determined at 24 and 48-hour post-treatment (Appendix A and B), and values were corrected for natural mortality in the controls using Abbott's formula (Abbott, 1925). Because June beetles feign death, only insects that attempted to grasp or push away a probing instrument were considered alive after exposure for 3 minutes under a 150 watt incandescent lamp.

C. RESULTS AND DISCUSSION

Only 5 of the 14 insecticides tested on the adult stage in 1978 produced mortalities greater than 60% at a concentration of 0.01% weight/volume (Table 6). The experimental chemical WL 43775 killed 100% of the test beetles after 24 hours. The insecticide CGA 12223 also produced a high mortality of 87.5%, but has since been withdrawn from the market. Fensulfothion and chlorpyrifos showed similar contact toxicity (75%) and the remainder of the chemicals showed lower toxicities at the 0.001% concentration tested. Determination of LD 50 values could not be made in the 1978 test due to insufficient numbers of beetles.

Natural mortalities in treatments were corrected using Abbott's formula (Abbott, 1925) and a regression line for each chemical tested was calculated. Mean lethal doses of LD 50 were calculated with fiducial limits at the 95% confidence level. The mortality response was plotted on probit log-dose paper. Probit analysis was carried out with the aid of a computer program available from Statistical Analysis Systems at the McGill Computer Centre. Using data obtained from bioassay tests, the program generated the equation of the best line, giving the slope and Y intercept (example: Appendix L and M).

Within 15 minutes after treatment, beetles sprayed with the pyrethroids (Table 7) showed accelerated movement and regurgitation. Results from topical application of diazinon, cypermethrin, and fenvalerate produced significantly large Chi-square values for heterogeneity. It is believed that the variable

TABLE 6: Percentage mortalities produced by 14 insecticides tested at a concentration of 0.1% w/v on June beetles, Phyllophaga spp., 24 hours post-treatment, 1978.

Insecticide	% mortality[a]	Insecticide	% mortality[a]
WL 43775	100.0	Terbufos	25.0
CGA 12223	87.5	Fonofos	25.0
Fensulfothion	75.0	Isofenphos	25.0
Chlorpyrifos	75.0	Dieldrin	25.0
Diazinon	63.5	WL 41706	25.0
WL 43467	50.0	Permethrin	12.5
Chlorfenvinphos	37.5	WL 24073	0.0

a average mortality of two replicates, corrected according to Abbott's formula (Abbott, 1925).

(Refer to Appendix N for chemical structures and trade names) response to standardized treatment shown by the beetles (Appendix A and B), which inflated the Chi-square values, was possibly caused by several factors. Beetles were collected over several days from field populations thereby affecting their physical condition, feeding status prior to capture, age, and sex. Because of the erratic response, variances were multiplied by a heterogeneity factor. Results of this experiment are questionable because of the large Chi-square values. A larger population of beetles tested against a broader concentration range of chemicals would be required to distinguish the causes of the heterogeneity.

The adult population used in 1979 was highly susceptible to all three chemicals tested; LD 50 values were calculated where possible. Because of high toxicity levels produced by cypermethrin, an accurate LD 50 value could not be calculated. However, LD 50 values were determined for diazinon and fenvalerate at 0.1 and 0.04% respectively (Figure 10), and concentrations required to kill 95% of the population using diazinon and fenvalerate were approximately equal (Table 7). This clearly shows that these new pyrethroids are very effective in killing <u>Phyllophaga</u> spp. adults. Results 48 hours post-treatment showed variability in mortality response, and consequently higher Chi-square values. Calculated LD 50 values 48 hours post-treatment showed a decrease in the dosage required to control the adults (Table 8).

The feasibility of controlling June beetles is small due to: 1) large feeding range; 2) varied host preferences; and 3) low population densities. As shown by the results obtained by this experiment, the three insecticides

FIGURE 10: Log-dose/probit lines for topical applications of diazinon and fenvalerate to adult beetles 24 hours post-treatment (1979, see also Table 7).

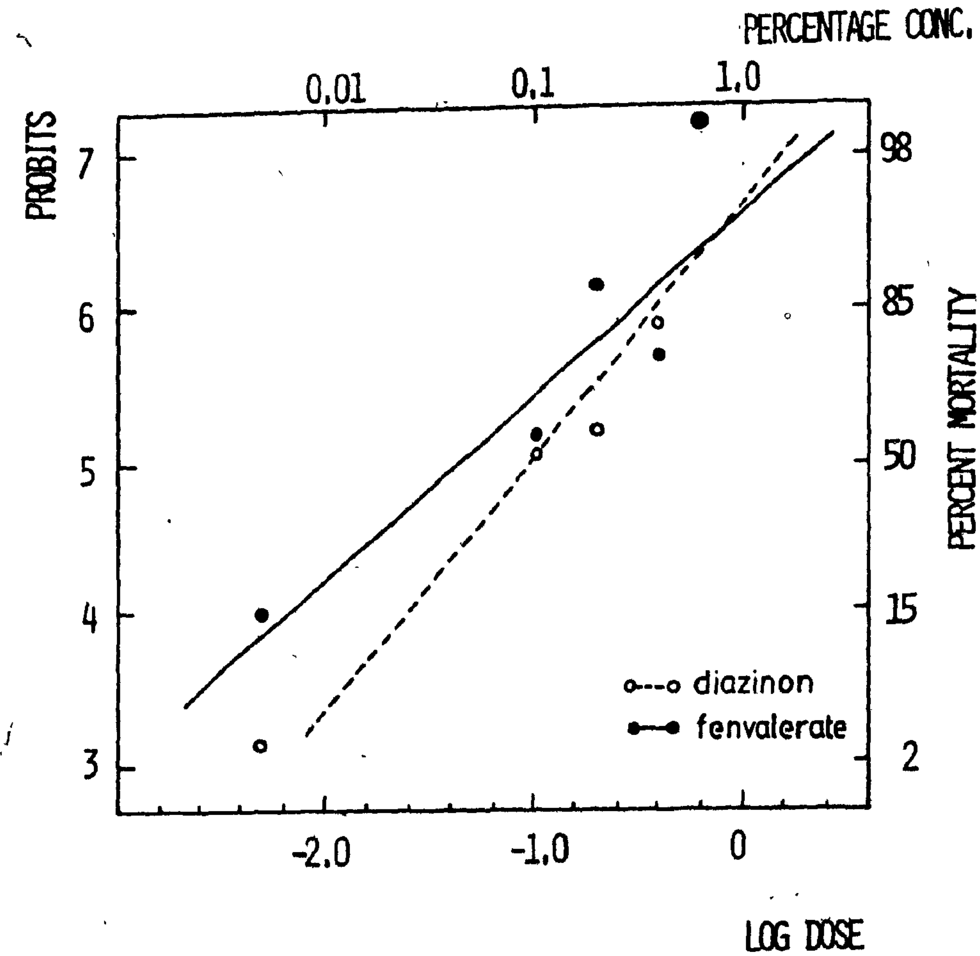

PERCENTAGE CONC.
0.01
0.1
1.0
PROBITS
7
6
5
4
3
98
85
50
15
2
PERCENT MORTALITY
o---o diazinon
•—• fenvalerate
-2.0
-1.0
0
LOG DOSE

TABLE 7: Mortality date on Phyllophaga spp. sprayed with 3 insecticides diluted with acetone-olive oil and applied using a Potter spray tower at a pressure of 28 cm of mercury, 1979. (Refer to Appendix A and B)

Insecticide	Period HOURS	Chi-square	df	Prob.	Slope	LD 50 % conc.	Fiducial limits Upper	Lower	LD 95 % conc.
Fenvalerate	24	5.599	3	0.133	1.180	0.04	0.08	0.02	1.09
t = 1.96									
Diazinon	24	3.144	3	0.370	1.626	0.10	0.16	0.05	1.03
t = 1.96									
Cypermethrin	24	37.642	3	0.0001	0.854	0.02	--	--	1.78
t = 3.18									
H = 12.55									

Prob.: Probability greater than Chi-square.

t = value used to compute fiducial limits at 95% confidence level.

H = heterogeneity factor.

TABLE 8: LD 50 values calculated from date on sprayed adult Phyllophaga.

(Refer to Appendix A and B)

Insecticide	Period HOURS	Chi-square	LD 50 values % conc.
Fenvalerate	24	5.599	0.044
	48	8.666	0.003
Diazinon	24	3.144	0.100
	48	10.644	0.057

tested provided very good control of the adults treated, however further testing using larger populations and in the case of cypermethrin lower concentrations, would permit a more accurate dose-mortality response determination.

VI. LABORATORY EVALUATION OF CANDIDATE INSECTICIDES FOR CONTROL OF SECOND-INSTAR WHITE GRUBS

A. INTRODUCTION

Laboratory and field insecticide studies have been conducted in the past to assess the efficacy of arsenicals and organochlorines against various white grub populations (Kerr, 1941; Hammond, 1949, 1952; Marshall, 1951; Burkardt, 1955; Fowler and Wilson, 1974). Apparent resistance of certain *Phyllophaga* spp. to chlorinated hydrocarbons has been demonstrated (Teetes, 1973, 1975; Fuchs *et al.*, 1974; Pike *et al.*, 1978). Environmental persistence of chlorinated hydrocarbons and the development of resistence, has resulted in their removal from the registration list for white grub control in crops. Little research has been done on white grub control using organophosphoros and carbamate insecticides. Field testing in sorghum and wheat, conducted by Teetes (1973, 1975), showed diazinon, fensulfothion and carbofuran to be effective in reducing grub populations. Artificial infestations in sugarcane plots were used by Fuchs *et al.* (1974) to determine the activity of several soil insecticides. In addition to diazinon and carbofuran, they found fonofos effective in controlling grubs. Relative toxicity tests of soil insecticides on white grubs showed carbofuran to be the most toxic to second and third-intar grubs (Pike *et al.*, 1978). Application of carbofuran, fonofos and isazofos to Bermuda grass reduced population levels in a white grub complex tested in Florida (Reinert, 1979).

Topical toxicities do not necessarily reflect insecticide activity in soils. The influence of soil type and moisture on the toxicities of insecticides applied to soils was demonstrated by Harris and Mazurek (1966); Harris (1972). Further studies demonstrated a general reduction of a chemical's activity when applied to the soil, as opposed to direct contact activity (Harris and Mazurek, 1966). These differences in efficacy are studies by comparing topical and soil applications. The assessment of the activity of candidate materials in the soil can provide information towards choosing an effective insecticide for the control of white grubs under different field conditions.

B. MATERIALS AND METHODS

I. First Year, Second instar white grubs.

First year, second instar grubs were collected from a sod field in Mirabel during the summer of 1978. Field microplots, covered with turf, were seeded with the collected grubs by perforating the root mass with a sharpened stick and dropping a white grub in each hole. This procedure was undertaken to maintain healthy grubs for testing. The grubs were removed from the microplots using a soil screen and were then moved to the laboratory for testing.

a) Topical application

Two replicates of 10 insects each were sprayed with a 0.1% solution of the following insecticides: diazinon, isofenphos, fenvalerate, fensulfothion, azinphosmethyl and permethrin. The solvent used was a mixture of olive oil and acetone in a ratio of 1:19. Because the technical grades of the various insecticides available were not of consistent purity, equivalent proportions of stock insecticides were used to ensure a 0.1% w/v concentration of active ingredient. Healthy insects were selected for testing; ten white grubs were placed in a 9 cm Petri dish lined with a filter paper, and sprayed under a Potter spray tower. Five ml of 0.1% solutions were sprayed on each dish at a pressure of 28 cm of mercury. Mortality counts, using reflex response to a probe, were taken at 24 and 48 H (Table 9).

b) Soil incorporation

Upland sand was treated with 50 ppm of the following insecticides: diazinon, isofenphos, fenvalerate, fensulfothion, azinphosmethyl and permethrin. The insecticides were diluted in a solvent containing pentane and acetone in a ratio of 1:1. The insecticide was pipetted onto the soil surface of 50 g of sand contained in 400 ml glass jars and tumble mixed for 5 min. (Harris and Mazurek, 1966). The jars were allowed to ventilate for 1 hour to prevent mortalities caused by solvent vapors. Ten grubs were then added to the soil and allowed to burrow down. The soil soil moisture level was maintained at 10% by weight. Mortality counts were made 24, 48 and 72 H post-treatment (Table 10).

II. Second-year, second-instar white grubs.

Second-year, second-instar white grubs were collected from a sod field in Mirabel in the spring of 1979. Due to the small number of grubs available for testing, only two short tests were undertaken.

TABLE 9: Mortalities of second-instar grubs treated with insecticides applied using a Potter spray tower. Percentage mortality of both replicates combined (20 insects).

Insecticide	% Mortality [a]	
Period (H)	24	48
Fensulfothion	40	87.5
Azinphosmethyl	30	62.5
Fenvalerate	35	47.3
Diazinon	15	25.0
Isofenphos	10	12.5
Permethrin	5	0

[a] Mortalities corrected using Abbott's formula (Abbott, 1925)

TABLE 10: Mortalities of second-instar grubs treated with insecticides applied to the soil (sand) containing 10% moisture. Two replicates of 20 grubs, soil containing 50 ppm. Percentage mortality of both replicates combined.

Insecticide	% Mortality [a]		
Period (H)	24	48	72
Fensulfothion	0	15	42.1
Azinphosmethyl	0	25	36.8
Fenvalerate	0	0	0
Diazinon	5	20	36.8
Isofenphos	0	5	26.3
Permethrin	0	0	0

[a] Mortalities corrected using Abbott's formula (Abbott, 1925)

Two replicates of five white grubs each were sprayed with diazinon, fensulfothion, fonofos and chlorpyrifos. Three concentrations were applied; 0.01, 0.1 and 1.0%. The technical grade insecticides were diluted with a mixture of olive oil-acetone (Ratio 1:19). Mortalities were obtained 24 and 48 H post-treatment (Table 11).

Diazinon and fensulfothion were sprayed on late second-instar grubs using the concentrations indicated in Table 4. Lethal dose 50 values were calculated for both insecticides using observations 24 and 48 H post-treatment.

C. RESULTS AND DISCUSSION

Second-instar grubs sprayed with 0.1% insecticide solutions showed greatest susceptibility to fensulfothion (Table 9). When applied to sand, its activity was relatively slow. Only after a period of 72 H did fensulfothion kill more grubs than the other treatments. A test using fensulfothion in sand by Lim (1980), showed a similar reduction in activity. Azinphos-methyl was two times more effective than diazinon when applied directly to the grubs, however their potencies were similar; 72 H post-treatment when applied to the sand. Fenvalerate, although controlling approximately 50% of the test insects when used as a contact insecticide, was de-activated when when applied to the sand. Isofenphos was the least effective of the four organophosphates tested on second instar larvae. Permethrin was ineffective on second-instars both as a contact and soil insecticide.

TABLE 11: Mortalities of second-instar grubs treated with diazinon, fensulfothion, fonofos and chlorpyrifos. Two replicates of 5 insects each sprayed with 5 mL of solution using a Potter spray tower at air pressure 28 cm of mercury.

Insecticide	Period HOURS	Conc.%	% Mortality [a]
Diazinon	24	0.01	12.5
Fensulfothion			0
Fonofos			0
Chlorpyrifos			0
Diazinon	24	0.1	25.0
Fensulfothion			62.5
Fonofos			0
Chlorpyrifos			37.5
Diazinon	24	1.0	87.5
Fensulfothion			100.0
Fonofos			37.5
Chlorpyrifos			75.0
Diazinon	48	0.01	5.2
Fensulfothion			0
Fonofos			5.2
Chlorpyrifos			0
Diazinon	48	0.1	36.8
Fensulfothion			68.4
Fonofos			21.0
Chlorpyrifos			68.4
Diazinon	48	1.0	100.0
Fensulfothion			100.0
Fonofos			68.4
Chlorpyrifos			100.0

a Mortalities corrected using Abbott's formula (Abbott, 1925).

Control mortality 24 hours = 20.0%.

Control mortality 48 hours = 36.7%.

Contact testing using late second-instar (second year), again showed fensulfothion to be the most effective (Table 11). Only diazinon showed insecticidal activity at the 0.01% concentration 24 H post-treatment. Chlorpyrifos at 0.1% was more effective than diazinon but at 1.0% diazinon's efficacy was greater. This trend continued 48 H post-treatment, however at the 1.0% concentration, 100% mortality was achieved using both. Fonofos showed no activity 48 H post-treatment at the 0.01% level. Approximately 70% of the grubs tested using a 1.0% concentration, were killed.

The high contact toxicity of fensulfothion agreed with results reported by Pike et al. (1978) and Lim et al. (1980). It has also been shown to be an effective soil insecticide for controlling P. crinita (Teetes, 1973; Fuchs et al., 1974). Control using fensulfothion required a longer period of time compared to the other effective organophosphates tested.

Lethal dosage 50 values were calculated for diazinon and fensulfothion 24 H post-treatment using them as contact insecticides on late second-instar grubs. The LD 50 for fensulfothion, 0.09%, was three times less that of diazinon. The LD 95 value for fensulfothion was six times less that of diazinon (Table 12). The LD 50 value for fensulfothion 48 H post-treatment remained relatively unchanged, however the LD 95 decreased. A delay in toxic action was apparent, supported by the decrease in LD 50 to 0.07% and LD 95 to 1.02%

Comparisons of mortalities caused by 0.1% concentrations of diazinon and fensulfothion between first and second-year, second-instar grubs, showed

TABLE 12: Mortalities of late second-instar grubs. Two replicates of 10 late second-instar grubs were sprayed with each concentration using a Potter spray tower at 28 cm of mercury. Five mL of solution were used in each treatment. Diazinon was applied at 0.05, 0.1, 0.2, 0.4, 0.8% (w/v). Fensulfothion was applied at 0.025, 0.05, 0.1, 0.2, 0.4% (w/v).

Insecticide	Period HOURS	LD 50 % conc.	Upper Limits	Lower (LD 50)	LD 95 % conc.
Diazinon	24	0.31	2.09	0.13	8.44
Fensulfothion	24	0.09	0.20	0.04	1.39
Diazinon	48	0.07	0.36	0.01	1.02
Fensulfothion	48	0.07	0.03	0.03	0.82

first-year grubs slightly more susceptible to fensulfothion than diazinon, and the reverse with second-year grubs.

VII. LABORATORY EVALUATION OF CANDIDATE INSECTICIDE FOR CONTROL OF THIRD-INSTAR WHITE GRUBS

A. INTRODUCTION

Early third-instar white grubs are voracious feeders and are responsible for extensive damage to crops. Because of their increased size from that of second-instar grubs, a study was undertaken to determine their susceptibility to several insecticides presently used for soil insect treatment.

B. MATERIALS AND METHODS

Third-instar larvae were collected using fork-spades in a strawberry field approximately 8 km north of Pierreville, Quebec, during the last week of August and the first week of September, 1979. The white grubs were placed in cardboard boxes with a large proportion of soil taken from the field. Because of the aggressive nature of third-instar larvae, numbers of grubs in each box were kept low to reduce mutilation and further loss of specimens to disease. The boxes were transported to the laboratory and stored at 5°C in order to slow the metabolic rate of the grubs and eliminate feeding. Mortalities caused by disease were also reduced using this method. The soil was kept relatively moist to avoid desiccation and other stresses in the stock populations of grubs.

The boxes were removed from cold storage approximately one hour prior to treatment (Figures 11 and 12), and healthy-looking grubs (free from injury) and external signs of disease) were selected. Acclimatization was kept to a minimum to reduce the period of contact between grubs and thus reducing injury due to cannibalism. The grubs responded quickly to this increase in ambient temperature (ca. 20°C), becoming active in a matter of minutes.

a). Topical Application of insecticides

Healthy insects were selected at random and placed 5 to a 9 cm Petri dish for treatment using a Potter spray tower (Figure 13). Technical grade insecticides were used in formulating the concentrations, and each formulation contained the same relative amount of active ingredient. Concentrations of 0.5, 1.0 and 1.5% were applied to four replicates of 5 insects each. The insecticides were diluted in a solution of re-distilled acetone and olive oil (19:1) on a weight/volume basis. Five milliliters of insecticide were atomized at 28 cm of mercury (air pressure) onto each Petri dish containing the 5 grubs. The insects were transferred to 400 mL glass jars containing 100 g of sand (Figure 14) having a 5% water content (the % water based on oven-dry weight of soil after 24 H at 105°C). The jars were covered with a glass plate and held at 20°C for post-treatment observations at 24 and 48 H periods (Figures 15 and 16). Six insecticides; fensulfothion, fonofos, diazinon, isofenphos, chlorpyrifos and carbofuran, were sprayed at three concentrations.

FIGURE 11: Third-instar white grubs being sorted from soil after storage at 5°C.

FIGURE 12: Healthy white grubs selected for toxicity tests.

FIGURE 13: White grubs sorted into Petri dishes for spraying in the Potter tower.

FIGURE 14: White grubs were placed in jars containing sand with 5% moisture content after being sprayed.

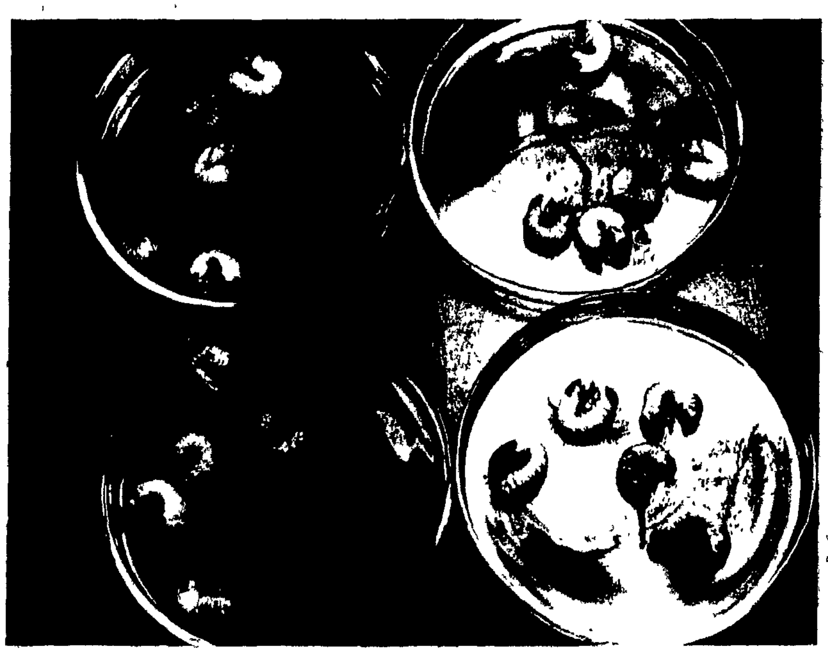
Insecticide incorporation into soils were conducted using the same type of jars.

FIGURE 15: Standard form used for recording mortalities.

FIGURE 16: White grubs were removed from soil for mortality determination.

DATE: DESCRIPTION: [illegible] TIME:

INSECTICIDE	CONCENTRATION	REPLICATE	NO. DEAD	% [illegible]	[illegible] CORR



b). Soil Incorporation of Insecticides

Insecticide activity in three soil types, Upland sand, Ste. Rosalie clay and muck, was assessed by procedures similar to those used by Harris and Mazurek (1966). The insecticides listed above were dissolved in distilled n-pentane and pipetted onto 500 g of soil contained in 910 mL Mason jars. The quantity of insecticide applied depended on the required concentration, and additional pentane was added to each treatment to ensure that each received the same volume of solvent. Concentrations of 25, 50 and 100 ppm were used in the sand and clay tests; 50, 100 and 200 ppm were applied to the muck soil. The jars were hand shaken for 5 minutes. One hundred grams of treated soil were transferred to 400 mL jars and allowed to ventilate for one hour to avoid mortality due to a fumigant effect of the solvents present in the soil. The moisture levels of sand, clay and muck (oven-dry weight) were ca. 10, 20 and 40% respectively. Five healthy white grubs were placed in each jar and allowed to burrow into the soil. Grubs that failed to burrow within 0.5 hours were replaced. The jars were covered to prevent moisture loss. Mortalities were recorded 24 H post-treatment.

In both experiments, grubs were removed from the jars and exposed for 3 minutes to an incandescent lamp (150 watts) to facilitate mortality determination of grubs. Grubs were considered dead if they failed to: 1) move about freely and, or 2) demonstrate reflex action towards the abdominal region when touched with forceps. Controls comprised of solvent-treated soils and grubs exposed to the same handling and holding procedures.

C. RESULTS AND CONCLUSIONS

a). Topical Application of Insecticides

Based on mortalities produced by the topical application of six insecticides on the third-instar white grubs (Appendix C and D), fensulfothion was the most toxic with a LD 50 of 0.65% after 24 hours (Figure 21, Table 13). White grubs reacted to diazinon and chlorpyrifos in a similar manner, as shown by the slopes of the regression lines (Figures 17 and 19) and comparable LD 50 values of 1.21 and 1.28% respectively (Table 13). Although fonofos was only slightly less toxic than either of the above, the shallower slope (Figure 18) indicated a lower mortality response to dosage increases. The shallow slope of the line representing the response of the grubs to isofenphos (Figure 20) demonstrated poor control performance. Carbofuran caused a 50% mortality at all the concentrations tested 24 hours post-treatment.

Mortalities 48 hours post-treatment, using fensulfothion, were too high to enable the calculation of a LD 50 (see Appendix D). Although the LD 50 value of diazinon was not the lowest after 48 hours (Figure 22, Table 13), the slope was the steepest of all those calculated, resulting in a higher percentage mortalities at lower rates than occurred with the remaining chemicals tested. Both carbofuran and chlorpyrifos (Figures 24 and 25) had similar LD 50 values, 0.58 and 0.56% respectively as well as slopes 2.76 and 2.57 respectively. Fonofos was only slightly less toxic than carbofuran and chlorpyrifos

(Figure 23), having a LD 50 of 0.83%. Isofenphos was the least toxic to the white grubs, causing only 25% mortality at 1.5% concentration 28 hours post-treatment (Appendix D).

In conclusion, the six chemicals were effective in controlling third instar white grubs, when applied topically, in the following order: fensulfothion>diazinon>carbofuran and chlorpyrifos>fonofos>isofenphos.

b). Soil Incorporation of Insecticides

Harris and Mazurek (1966) demonstrated the influence of soil types on insecticide activities. In this present study, the application of fensulfothion to sand showed a marked delay in activity in relation to the other five insecticides screened (see Appendix E). A concentration of 100 ppm caused only 15% mortality in the grubs tested. Chlorpyrifos and diazinon were the most toxic materials at 24 hours post-treatment, when applied to sand, having LD 50 values of 17 and 14 ppm respectively (Table 14). Although both isofenphos and fonofos showed similar mortality to dose responses, indicated by their slopes (Table 14), fonofos was the more toxic having a LD 50 of 68 ppm compared to 108 ppm of isofenphos. Carbofuran was the least toxic of the six insecticides tested, with an LD 50 of 250 ppm 24 hours post-treatment and a final percentage mortality of 56% (corrected using Abbott's formula (Abbott, 1925)) after 48 hours when treated with 100 ppm. Variability in the mortality response of carbofuran applied to sand supported results obtained in the topical application indicating heterogeneity in the population to carbofuran. Due to the high mortality rate at all the doses tested, LD 50 values were not

TABLE 13: Topical application of insecticides to third-instar white grubs using a Potter spray tower at air pressure of 28 cm of mercury. Five mL of solution were applied to each replicate.

(Refer to Appendix C and D)

Insecticide	Period HOURS	LD 50 % Conc.	Upper Limits	Lower (LD 50)
Diazinon	24	1.21	1.01	1.53
Fonofos	24	1.45	1.01	12.35
Chlorpyrifos	24	1.28	1.05	1.78
Fensulfothion	24	0.65	0.45	0.82
Isofenphos	24	9.84	2.08	--
Diazinon	48	0.82	0.67	0.98
Fonofos	48	0.83	0.54	1.13
Chlorpyrifos	48	0.56	0.19	0.77
Carbofuran	48	0.58	0.25	0.78

Fiducial limits (95% confidence level) calculated using T = 1.96

FIGURE 17: Mortality response of third-instar grubs sprayed with Diazinon. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

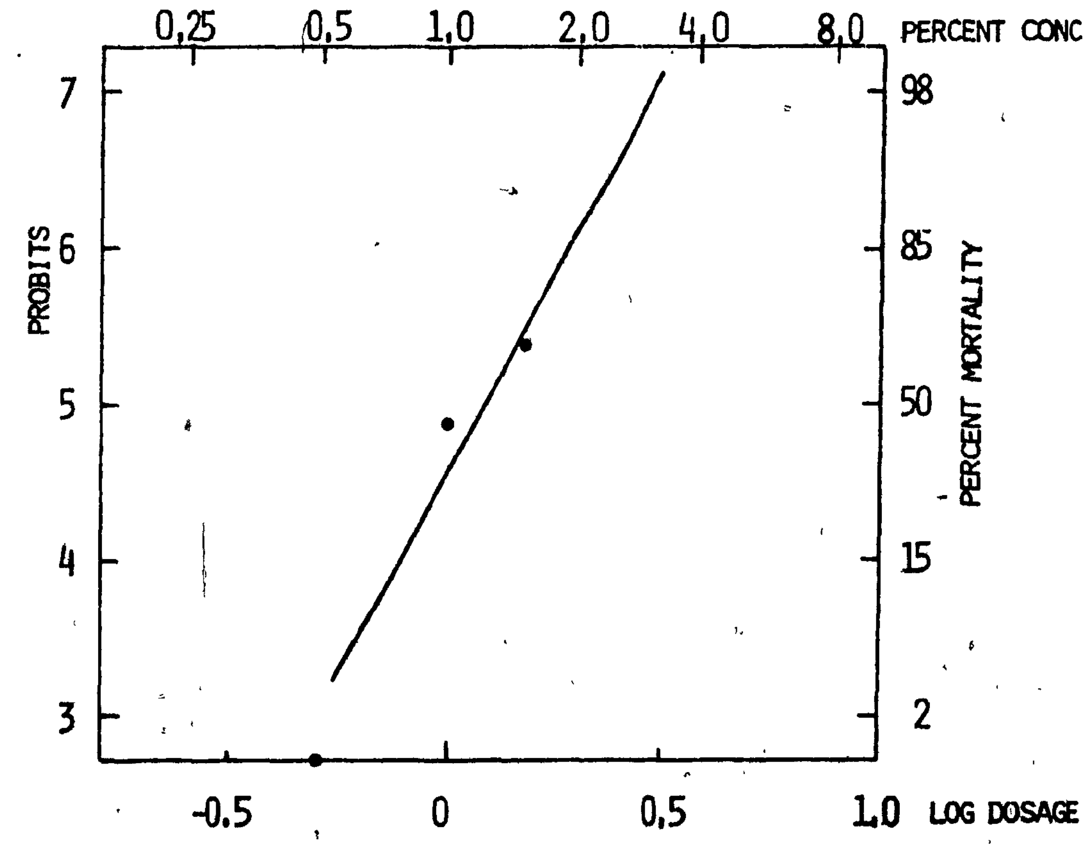
(24 HOURS)

Regression line	Y = 4.589 + 5.074X
Chi-square	0.7276
Degrees of freedom	1
Chi-square probability	0.3937
Log LD 50	0.081
LD 50	1.21%
Fiducial limits	1.53% Upper
(t = 1.96)	1.01% Lower

FIGURE 18: Mortality response of third-instar grubs sprayed with fonofos. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

(24 HOURS)

Regression line	Y = 4.652 + 2.163X
Chi-square	0.0479
Degrees of freedom	1
Chi-square probability	0.8268
Log LD 50	0.161
LD 50	1.45%
Fiducial limits	12.35% Upper
(t = 1.96)	1.01% Lower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PERCENT CONC.
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LOG DOSAGE

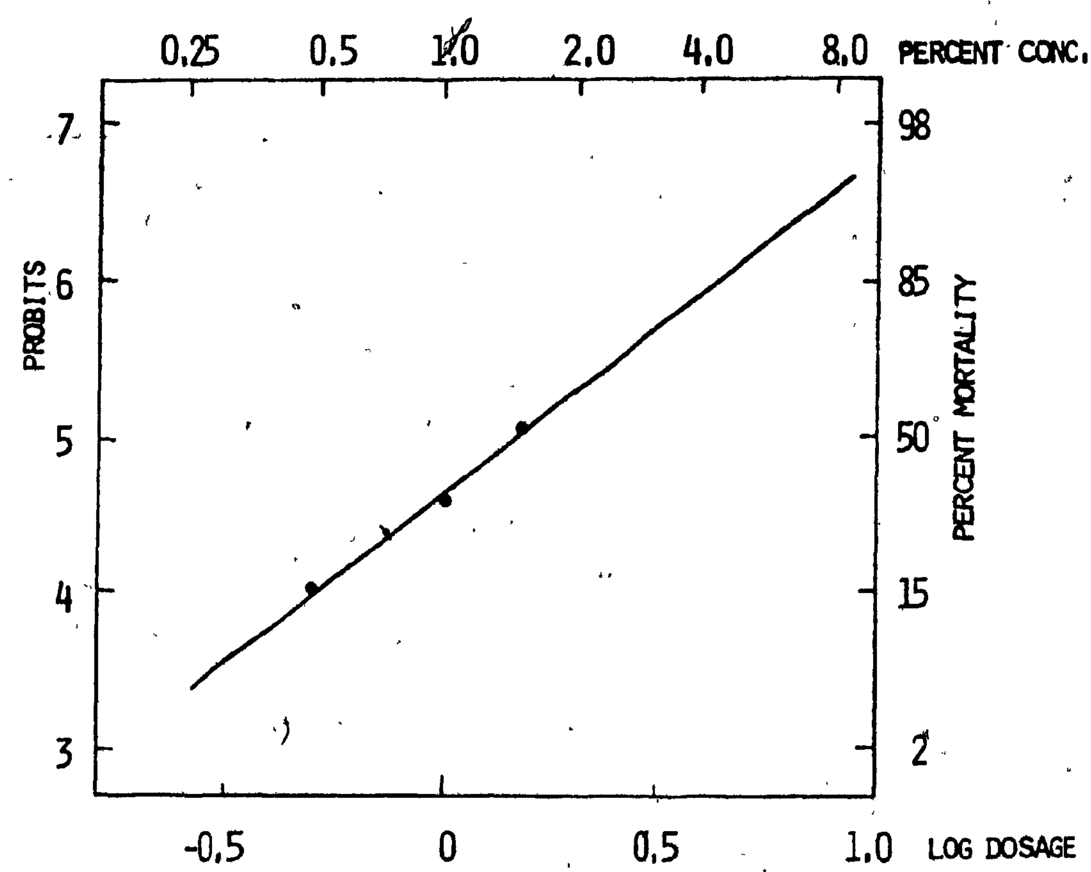
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FIGURE 19: Mortality response of third-instar grubs sprayed with chlorpyrifos. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

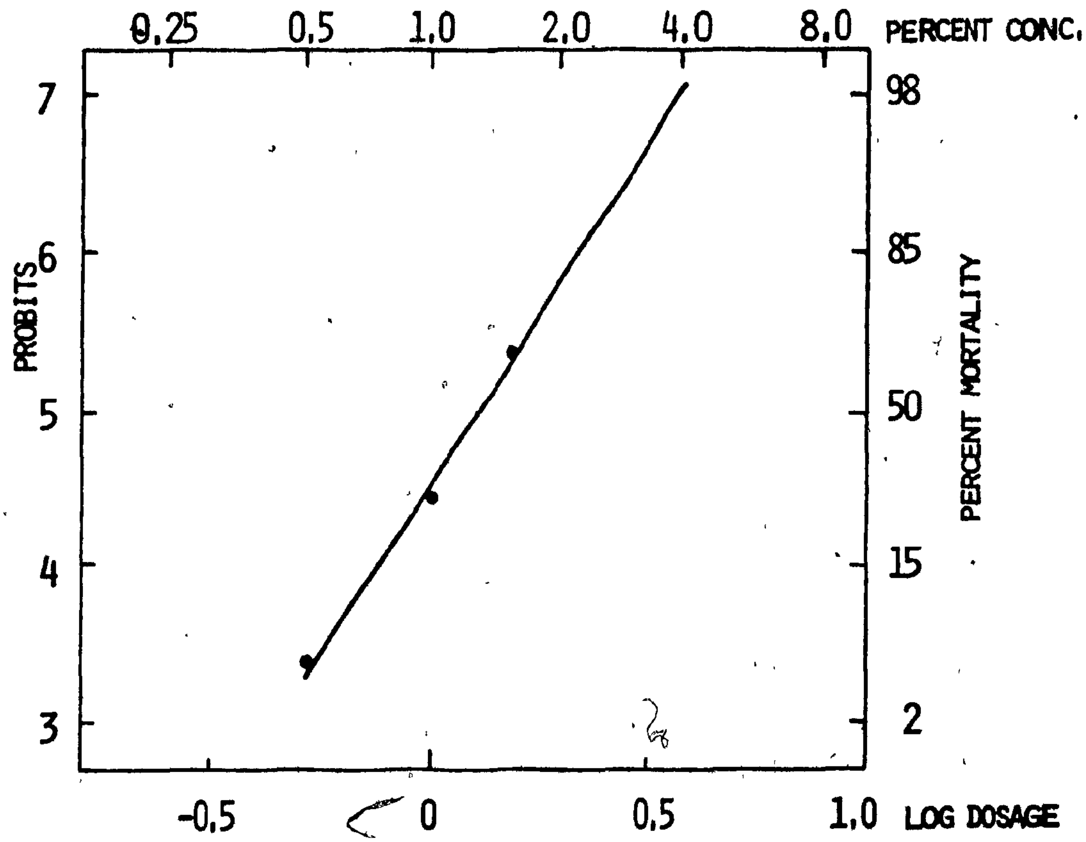
(24 HOURS)

Regression line	Y = 4.544 + 4.323X	
Chi-square	0.2527	
Degrees of freedom	1	
Chi-square probability	0.6152	
Log LD 50	0.1055	
LD 50	1.28%	
Fiducial limits	1.78%	Upper
(t = 1.96)	1.05%	Lower

FIGURE 20: Mortality response of third-instar grubs sprayed with isofenphos. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

(24 HOURS)

Regression line	Y = 3.732 + 1.277X	
Chi-square	0.0019	
Degrees of freedom	1	
Chi-square probability	0.9653	
Log LD 50	0.993	
LD 50	9.84%	
Fiducial limits	------	Upper
(t = 1.96)	2.08%	Lower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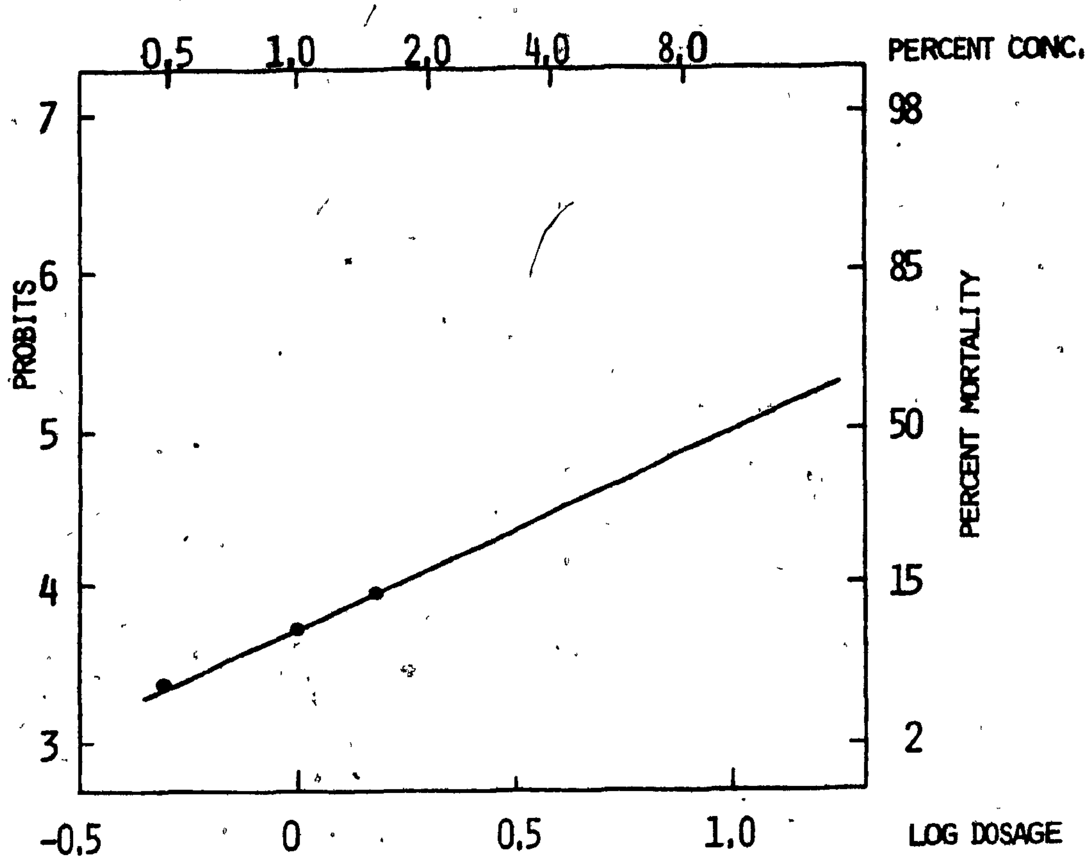
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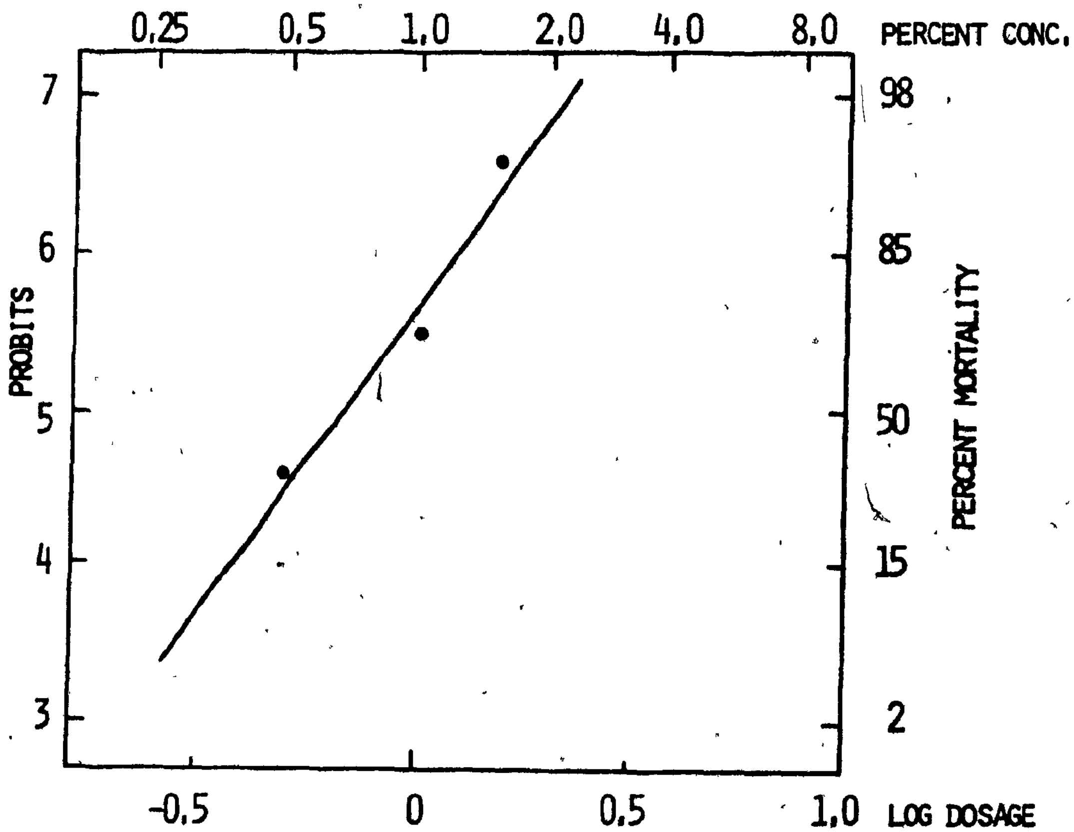
LOG DOSAGE

FIGURE 21: Mortality response of third-instar grubs sprayed with fensulfothion. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

(24 HOURS)

Regression line	Y = 5.710 + 3.850 X	
Chi-square	0.7403	
Degrees of freedom	1	
Chi-square probability	0.3896	
Log LD 50	-0.184	
LD 50	0.65%	
Fiducial limits	0.82%	Upper
(t = 1.96)	0.45%	Lower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PERCENT MORTALITY
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LOG DOSAGE

FIGURE 22: Mortality response of third-instar grubs sprayed with diazinon. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

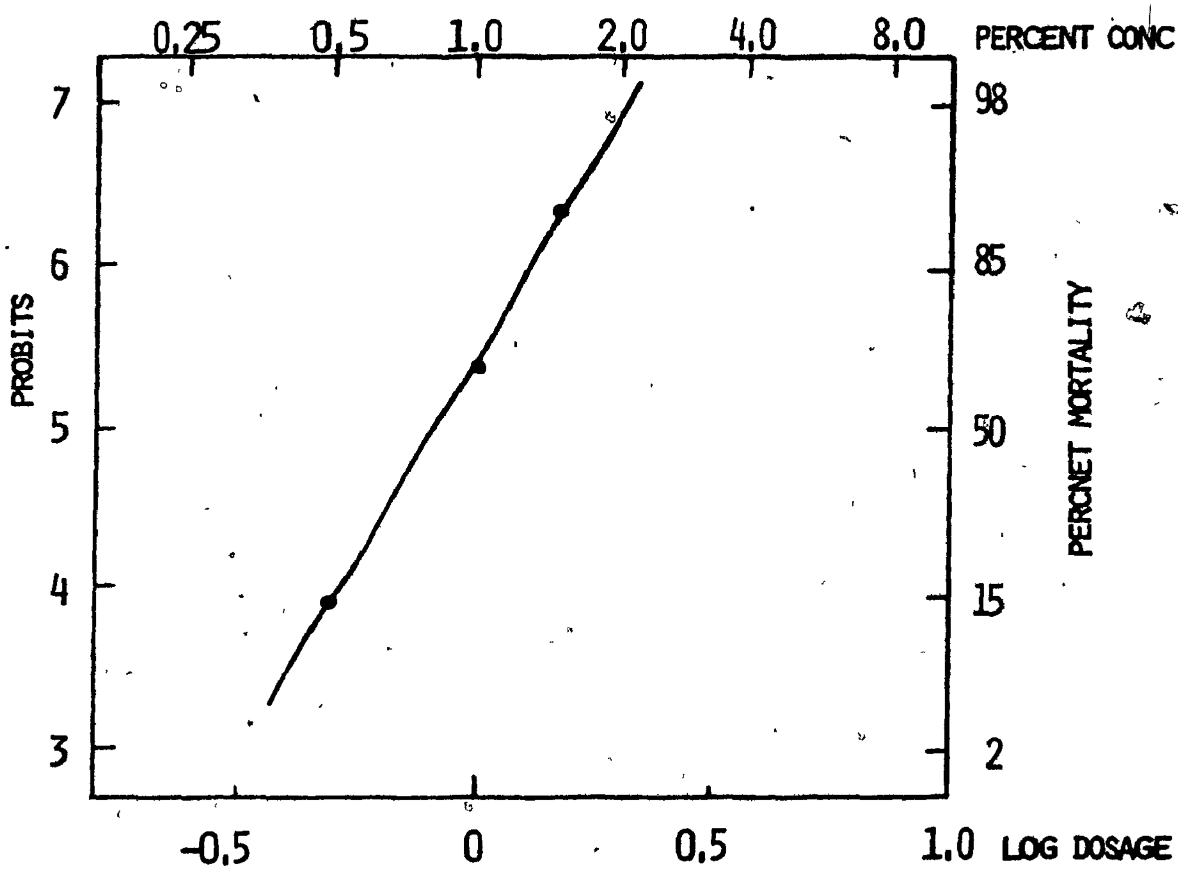
(48 HOURS)

Regression line	Y = 5.434 + 5.092X	
Chi-square	0.0284	
Degrees of freedom	1	
Chi-square probability	0.8663	
Log LD 50	-0.085	
LD 50	0.82%	
Fiducial limits	0.98%	Upper
(t = 1.96)	0.67%	Lower

FIGURE 23: Mortality response of third-instar grubs sprayed with fonofos. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

(48 HOURS)

Regression line	Y = 5.234 + 2.795X	
Chi-square	1.5603	
Degrees of freedom	1	
Chi-square probability	0.2116	
Log LD 50	-0.084	
LD 50	0.83%	
Fiducial limits	1.13%	Upper
(t = 1.96)	0.54%	Lower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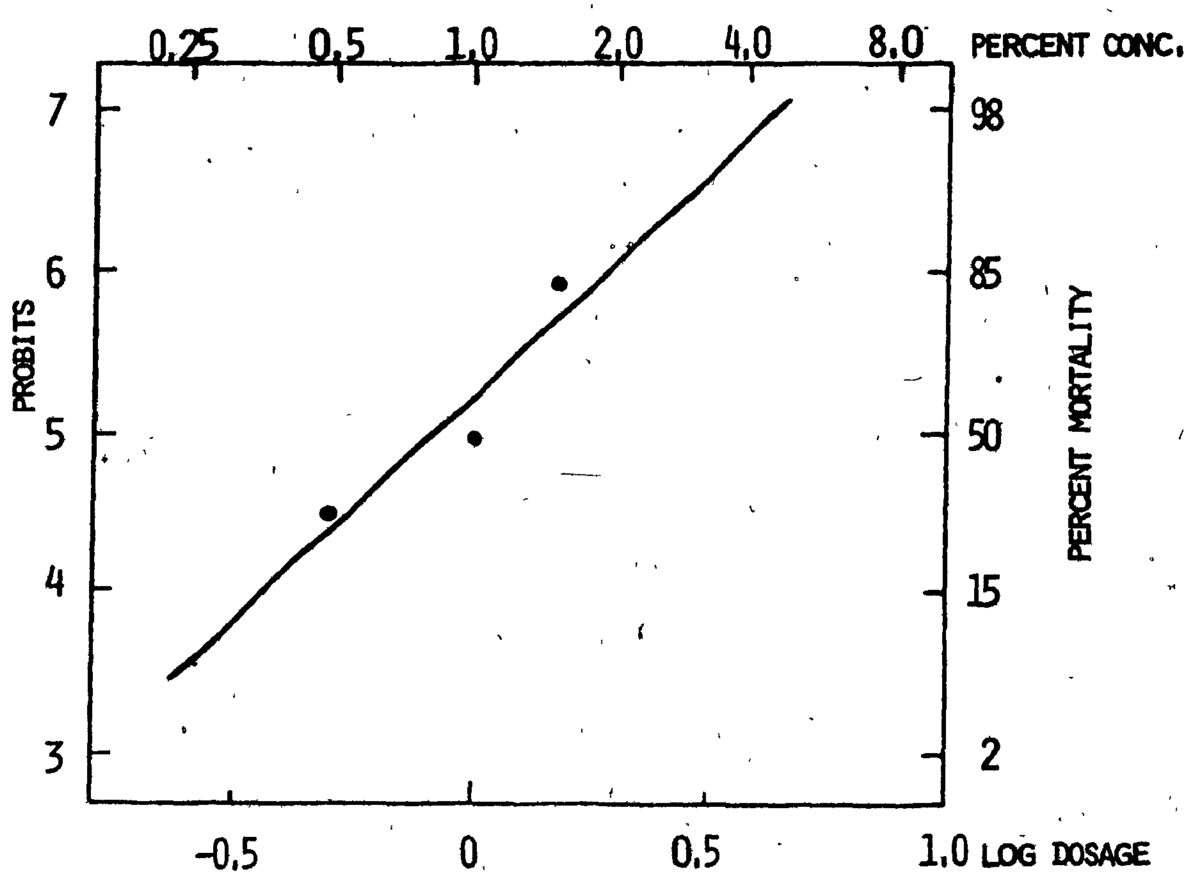
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FIGURE 24: Mortality response of third-instar grubs sprayed with chlorpyrifos. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

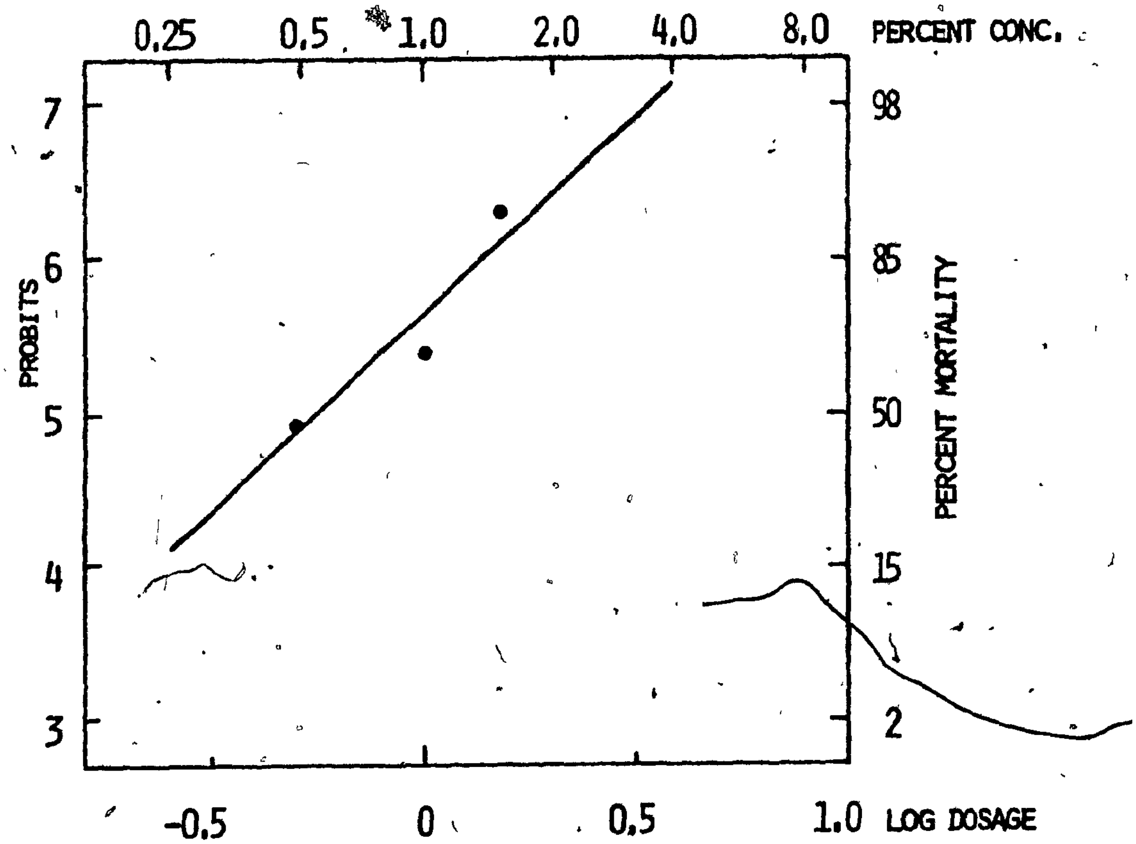
(48 HOURS)

Regression line	Y = 5.651 + 2.570X	
Chi-square	1.3187	
Degrees of freedom	1	
Chi-square probability	0.2508	
Log LD 50	-0.253	
LD 50	0.56%	
Fiducial limits	0.77%	Upper
(t = 1.96)	0.19%	Lower

FIGURE 25: Mortality response of third-instar grubs sprayed with carbofuran. Probit-log dose regression line generated by computer from observed mortalities, indicated by dots on the graph (refer to Table 1, Appendix C and D).

(48 HOURS)

Regression line	Y = 5.660 + 2.760X	
Chi-square	0.3648	
Degrees of freedom	1	
Chi-square probability	0.5458	
Log LD 50	-0.239	
LD 50	0.58%	
Fiducial limits	0.78%	Upper
(t = 1.96)	0.25%	Lower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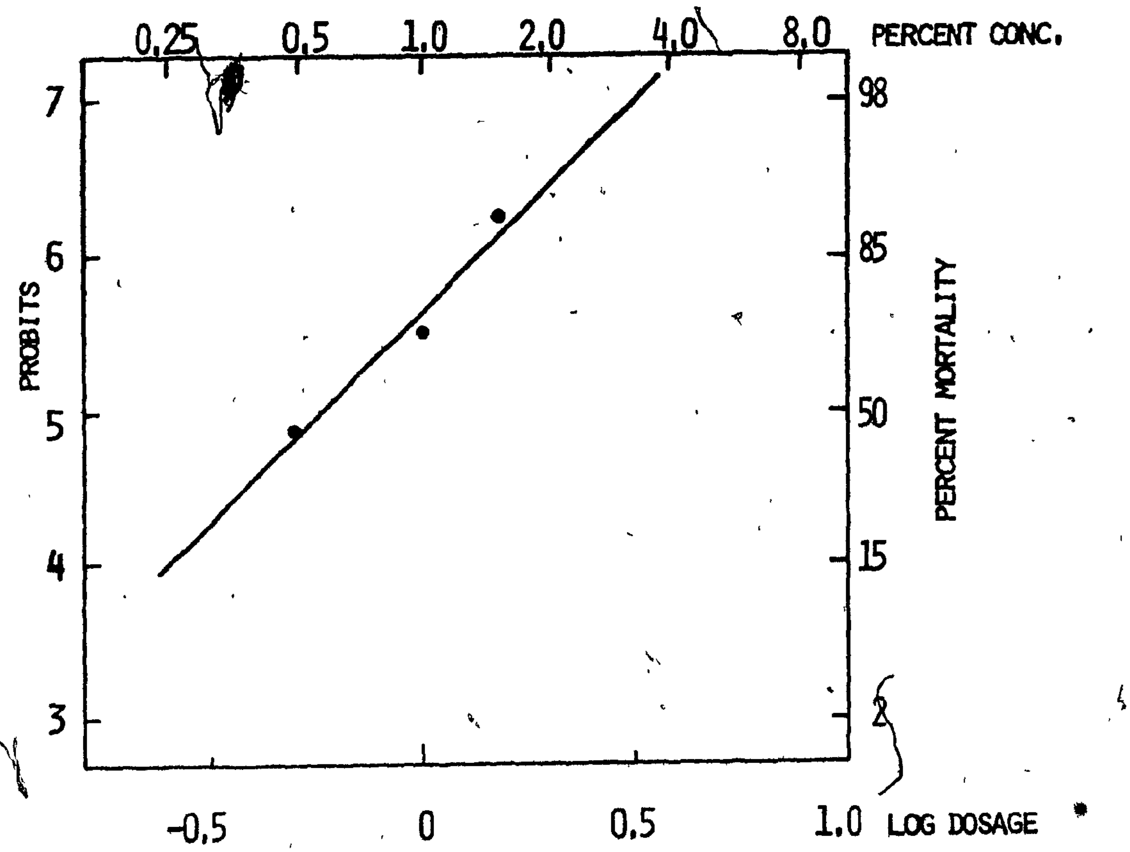
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LOG DOSAGE

TABLE 14: Insecticides applied to sand containing 10% water. LD 50 values calculated 24 hours post-treatment unless indicated (refer to Appendix E).

Insecticide	Chi-square	Slope	LD 50	Upper Limits	Lower (ppm)
Diazinon	0.1634	1.28	14	27	3
Chlorpyrifos [a]	3.3624	1.08	17	--	--
Carbofuran	0.1793	1.54	250	335	109
Isofenphos	1.2431	2.37	108	386	72
Fonofos	0.1790	2.31	68	118	46
Isofenphos [b]	0.0089	2.09	35	53	21
Fensulfothion [b]	1.5444	1.83	49	86	30

Fiducial limits calculated using T = 1.96

a Because Chi-square ($P < 0.10$) T value of 12.71 used to calculate fiducial limits.

b LD 50 values calculated using observations 48 hours post-treatment.

calculated for diazinon, chlorpyrifos and fonofos. Fensulfothion, though slower in killing the grubs, was effective 48 hours post-treatment with a LD 50 of 35 ppm. Isofenphos was only slightly active 24 hours post-treatment but increased in efficacy after 48 hours, surpassing fensulfothion with an LD 50 of 35 ppm and a steeper slope of regression line (Table 14).

Comparisons made with diazinon, chlorpyrifos and fonofos to the other chemicals at 48 hours post-treatment were based on the percentage mortality obtained using the three concentrations of chemicals. Chlorpyrifos was the most active in sand, causing 89% mortality in test grubs at a concentration of 10 ppm. Diazinon was slightly less toxic at the same concentrations controlling 73% of the population tested. Fonofos, though superior to fensulfothion, isofenphos and carbofuran, showed only a 30% control at 10 ppm. At rates of 50 and 100 ppm, diazinon, chlorpyrifos and fonofos proved to be very toxic, killing 95 to 100% of the test grubs (see Appendix F).

The effect of adding the chemicals to sand as opposed to direct contact application can be shown by the change in order of toxicity to white grubs: chlorpyrifos > diazinon > fonofos > isofenphos > fensulfothion > carbofuran.

When applied to clay, chlorpyrifos, diazinon and fonofos were found to have very similar LD 50 values after 24 hours (see Table 15). On considering the slopes however, chlorpyrifos had a greater mortality/dose response followed by diazinon and fonofos. Carbofuran was more effective in clay than in sand, having an LD 50 of 50 ppm 24 hours post-treatment. However, as indicated by the large Chi-square value, variability in susceptibility or a possible

TABLE 15: Insecticides applied to clay soil containing 20% water. LD 50 values calculated 24 hours post-treatment. (Refer to Appendix G)

Insecticide	Chi-square	Slope	LD 50 (ppm)	Upper Limits	Lower (ppm)
Diazinon	0.3207	2.11	25	39	1
Chlorpyrifos	0.6069	2.71	26	37	7
Carbofuran	3.3762	2.49	50	--	--
Isofenphos	0.0003	0.92	11260	--	90
Fonofos	0.1738	1.62	21	38	--
Fensulfothion	1.8093	0.61	113	--	2

Fiducial limits calculated using T value of 1.96.

TABLE 16: Insecticides applied to muck soil containing 40% water. LD 50 values calculated 24 hours post-treatment. (Refer to Appendix H)

Insecticide	Chi-square	Slope	LD 50 (ppm)	Upper Limits	Lower (ppm)
Diazinon	------	----	---	---	---
Chlorpyrifos [a]	2.7291	2.64	70	---	---
Carbofuran [b]	2.4874	2.84	140	225	105
Isofenphos	------	----	---	---	---
Fonofos [a]	3.4336	3.33	104	---	---
Fensulfothion [b]	2.3735	5.94	59	71	46

[a] Because Chi-square ($P < 0.10$) T value of 12.71 used to calculate fiducial limits.

[b] Chi-square small ($P > 0.10$), fiducial limits calculated using T value of 1.96.

incipient resistence to the chemical, reduced its relative efficacy and reliability for field control purposes. Observations made 48 hours post-treatment, showed carbofuran to control only 80% of the grubs when treated with 100 ppm. After 48 hours chlorpyrifos caused 100% kill at 25 ppm. Diazinon and fonofos, at the same rates, gave good control (see Appendix H). At 100 ppm, diazinon, chlorpyrifos and fonofos gave 100% control.

After 48 hours the order of efficacy of the chemicals tested with clay soil was as follows: chlorpyrifos>diazinon and fonofos>carbofuran isofenphos>fensulfothion.

Insecticides applied to muck soil generally show a decrease in activity (Harris and Mazurek, 1964). Diazinon was almost totally inactivated in muck soil. At a concentration of 200 ppm, only 25% of the insects treated were killed after 48 hours. This would indicate a strong adsorption of diazinon in muck soils. Isofenphos showed no insecticidal activity at concentrations lower than 200 ppm. Carbofuran and chlorpyrifos LD 50 values showed a decrease in efficacy of three times when compared to values obtained with clay (Table 16). Fonofos was five times more toxic in clay than in muck. Fensulfothion showed greater efficacy in muck soil than in clay. A concentration of 100 ppm was adequate to produce a 95% mortality in test grubs. Chlorpyrifos gave the highest control, 94% at 50 ppm after 48 hours. Variability in mortality response, indicated by the high values of Chi-square, is thought to have been caused by a de-activation of the chemical by the muck soil, affecting the availability of the chemical to the grubs.

Based on the fact that low concentrations of chlorpyrifos were effective in all the tests and soils used here, it was considered the most toxic to third instar white grubs. Although diazinon was as effective as chlorpyrifos when applied directly to the grubs, its inactivation in organic soils would limit its application to soils containing low percentages of organic matter. Insecticidal activity of carbofuran on the population of white grubs tested, showed variability in response, which may be an indication of incipient resistence within the population studied. Fonofos gave consistent results in all soil types; however concentrations needed to obtain a high mortality were greater, based on the response rates of the tests. Fensulfothion was the most toxic as a contact insecticide and showed good activity in muck soil. Its activity however in sand was slow and it was almost de-activated in clay.

These experiments, although somewhat approximate in that LD 50 values were determined on a small population sample, indicate the general high toxicity level of the insecticides tested, and also that chlorpyrifos followed by fonofos, should be considered for further testing under field conditions as possible insecticides for white grub control.

VIII. LABORATORY BIOASSAY OF SOIL INSECTICIDE FORMULATIONS USING FIELD-COLLECTED WHITE GRUBS

A. INTRODUCTION

Increasing problems with resistance-development and mounting concern over residues of chlorinated hydrocarbons present in foodstuffs and the environment, have produced a need for extensive testing of alternative materials for white grub control. Recent field testing of insecticides in grain sorghum and wheat (Teetes, 1973, 1975) and on Phyllophaga infestations of Bermuda grass (Reinert, 1979), have not established the efficacy of several insecticides and their formulations when applied to newly cultivated fields. Consequently, the microplot technique developed by Tashiro and Kuhr (1978) was utilized in this series of tests, whereby interference due to variations in soil types,sod penetration and weather are eliminated. A more representative evaluation of the efficacy of both granular and emulsified insecticides as potential chemical control agents of white grubs in the field should be achieved using this technique.

The susceptibility of white grubs to insecticides has been assessed using a variety of criteria for the determination of death in white grubs. Pike et al. (1978), in tests involving topical toxicity to field-collected Phyllophaga spp., based mortality-assessment on the movement response of the grub to thoracic tactile stimulus. Grubs were considered 'dead' if incapable of laterally twisting the head and forelegs towards the stimulus. In bioassay tests on the European chafer conducted by Tashiro and Kuhr (1978), it was considered that any movement, regardless of the degree of mobility, was an indication of a 'live' grub. In this present work, mortalities due to soil treatments, using two formulations of four insecticides, were assessed using both these criteria, to determine which would evaluate the toxicity of insecticides more reliably, and lead to a more accurate selection of chemical formulations for practical field application.

B. MATERIALS AND METHODS

Second-year, third-instar white grubs were collected from a strawberry field located 8 kilometers north of Pierreville, Quebec, using fork-spades to gently turn over the top 15 cm of soil. The grubs were transported in boxes containing liberal amounts of soil taken from the field, to reduce losses due to injury caused by cannibalism. The boxes were stored at 5°C, a temperature level which immobilised the grubs and eliminated the need to provide them with food, when kept for short periods of time.

A sandy-loam soil containing 1.3% organic matter was passed through a 0.625 cm mesh screen and allowed to air dry by spreading out on the laboratory bench for several days. The moisture level was determined by weighing 100 g samples after oven-drying at 105°C for 12 hours. The difference in weight divided by the oven-dry weight multiplied by 100 gave the percentage soil moisture content. Thereafter, the soil moisture level was maintained at 15% throughout each experiment by the addition of calculated amounts of distilled water, to prevent desiccation of the grubs and to provide moisture for grass seed germination. The soil was contained in microplots constructed of wood and measuring 25 by 25 cm with a depth of 10 cm (inside measurements) and waterproofed with an acrylic paint (Tashiro, personal communication).

Two formulations of chlorpyrifos, diazinon, fonofos and isofenphos were incorporated by hand-mixing into 4.5 kg of sandy-loam to provide a concentration of 5 ppm of active ingredient per treatment. The actual formulations used are given in Table 1. Granular formulations were sprinkled directly on the soil surface and 50 mL of water were added to each treatment before it was hand mixed and placed in a microplot. Liquid formulations were diluted in 50 mL of water and added to the soil surface prior to mixing. During the preparation of each treatment, 50 g of pre-soaked grass seed were added as a source of food for the grubs. The mixture contained Kentucky Bluegrass, creeping red fescue and ryegrass. Pre-soaking of the seeds was done to reduce any moisture loss from soils, and to accelerate germination. Control treatments received only 50 mL of water to bring the moisture content to 15%, and the 50 g of grass seeds. Each insecticide formulation treatment was replicated three times. The microplots containing the treated soils were allowed to stand overnight in a controlled environment chamber at 27°C and relative humidity of ca. 90% to allow any difference in soil moisture to equilibriate. After grubs were added, the microplots were held under these conditions for the remainder of the experiment. The weights of the microplots were recorded daily and distilled water was added to maintain the 15% moisture level.

White grubs were brought to room temperature, and only active, healthy-looking grubs were selected for the test. The microplots were each infested with 15 field-collected third-instar grubs placed on the soil surface. Grubs that failed to burrow down from the surface within 30 minutes were replaced (Tashiro and Kuhr, 1978). The microplots were returned to the controlled environment for the remainder of the experiment.

Early indications of poisoning were shown by the return of grubs to the soil surface. Mortalities were determined using both criteria of Pike et al. (1978) and Tashiro and Kuhr (1978), and were recorded 72, 120 and 168 hours post-treatment. Grubs were removed from the soil by hand extraction, and followed by visual examination after exposure to an incandescent lamp for 3 minutes prior to counting (Figure 26).

C. RESULTS AND DISCUSSION

Grub migration to the soil surface of the microplots gave an early indication of poisoning (Figure 27). Mean migrations of three replicates are given in Table 17. Chlorpyrifos 15 G, isofenphos 6 E and fonofos 10 G showed a significantly greater number of migrations than the other formulations or the controls. This difference however gave no indication of representing the relative toxicities of the chemicals tested (Tables 18 and 19, see also Appendix K), and cannot therefore be accurately used as a means

FIGURE 26: White grubs were removed from soil in microplots and exposed to a bright light prior to being probed for reflex response.

FIGURE 27: White grubs that burrowed up to the surface, an early symptom of poisoning.

TABLE 17: Number of white grubs on soil surface after 72 hours. Sandy-loam soil with a moisture content of 15%, treated with 5 ppm of active ingredient.

Chemical and formulation	Means[1,2]		
Lorsban 15 G Chlorpyrifos	7.67 a	Basudin 5 G Diazinon	3.00 de
Amaze 6 E Isofenphos	7.33 ab	Basudin 50 EC Diazinon	3.00 de
Dyfonate 10 G Fonofos	6.00 abc	Amaze 15 G Isofenphos	1.67 de
Dyfonate 4 E Fonofos	4.33 bcd	Control	0.33 e
Dursban 2 E Chlorpyrifos	3.00 de		0.00 e

[1] Means in colums not followed by same letter are significantly different (P = 0.05) by Duncan's multiple range test (Steel and Torries, 1962).

[2] Analysis of variance indicates significant difference of means of treatments at 0.05 and 0.01 level.

Chlorpyrifos: 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate
- Dursban 2 E: 2 lb. AI/gal., emulsifiable concentrate
- Lorsban 15 G: 15% of weight AI, granules.

Diazinon: 0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate.
- Basudin 50 EC: 50% of liquid AI.
- Basidin 5 G: 5% of weight AI.

Isofenphos: 1-methylethyl 2-[[ethoxy (1-methylethyl) amino phosphinothioyl]-oxy]benzoate.
- Amaze 6 E: 6 lb. AI/gal.
- Amaze 15 G: 15% of weight AI.
- also known as Bay 92114, Bay sra 12869, Oftanol

Fonofos: 0-ethyl s-phenyl ethylphosphonodithioate
- Dyfonate 4 E: 4 lb. AI/gal.
- Dyfonate 15 G: 15% of weight AI.

of evaluating efficacy. This reaction might however, be useful in increasing total effectiveness in a field control situation.

Observed mortalities, using both methods, are given in Tables 18 and 19. Mortalities based on lack of reflex action (Table 18) showed a significant difference in the means of the treatments. After 72 hours, the granular formulations, except that of isofenphos showed higher toxicities than the liquids when applied to the soil. Mortalities 120 and 168 hours post-treatment indicated similar activity of diazinon 5 G and 50 EC, chlorpyrifos 15 G and 2 E, and fonofos 10 G. Both formulations of diazinon and chlorpyrifos gave 100% control at 5 ppm. Fonofos 10 G and isofenphos 6 E reduced microplot populations by 90%. Based on the rapidity of control, the insecticides diazinon, fonofos, and chlorpyrifos surpassed isofenphos. However, after an exposure of one week, no treatment tested showed a significant difference in efficacy.

When comparing mortalities at the 72-hour period based on absence of any movement (Table 19), both formulations of fonofos were significantly better than all the other treatments. No significant difference was observed amongst the other treatments including mortalities in the controls. Mortalities based on absence of movement after 120 and 168 hours post-treatment showed no significant difference in chemical efficacy, regardless of the formulation.

Losses in the control treatments (less than 25%) were similar using either criteria for determining death. The mean values of dead grubs were significantly different from the insecticide-treated mean values when mortality

TABLE 18: Mean number of white grubs found dead in microplots treated with insecticides based on an absence of reflexes (3 replicates of 15 insects). Concentration: 5 ppm; soil type, sandy-loam; moisture content 15%.

Chemical	Means [1] Post-treatment	(Mortalities based on absence of reflexes, Pike et al., 1978)	
	72 hours [2]	120 hours [2]	168 hours [2]
Basudin 50 EC	12.7 a	13.7 a	15.0 a
Basudin 5 G	12.7 a	14.0 a	15.0 a
Dyfonate 10 G	11.7 ab	11.7 ab	14.0 ab
Dyfonate 4 E	11.3 ab	12.7 a	11.7 b
Lorsban 15 G	10.7 ab	14.0 a	15.0 a
Dursban 2 E	10.3 b	13.0 a	15.0 a
Amaze 6 E	10.0 b	10.3 b	14.0 ab
Amaze 15 G	5.3 c	10.0 b	12.7 ab
Control	3.0 d	3.7 c	5.0 c
	3.0 d	4.3 c	4.7 c

[1] Means in a column not followed by the same letter are significantly different (P = 0.05) by Duncan's multiple range test.

[2] Analysis of variance indicates significant difference in means of treatments at 0.01 level.

TABLE 19: Mean number of white grubs found dead in microplots treated with insecticides based on absence of any movement (3 replicates of 15 insects). Concentration: 5 ppm; soil type, sandy-loam; moisture content 15%.

Chemical	Means [1] post-treatment	(Mortalities based on absence of any movement, Tashiro and Kuhr, 1978).	
	72 hours [2]	120 hours [3]	168 hours [3]
Dyfonate 4 E	8.7 a	7.0 a	8.3 a
Dyfonate 10 G	6.0 ab	6.0 a	8.0 a
Basudin 50 EC	5.0 bc	6.0 a	8.0 a
Amaze 6 E	4.3 c	6.7 a	9.7 a
Lorsban 15 G	4.0 c	5.7 a	7.3 a
Dursban 2 E	4.0 c	6.0 a	7.0 a
Amaze 15 G	3.7 c	6.7 a	6.7 a
Basudin 5 G	3.3 c	6.3 a	8.3 a
Control	3.0 c	3.0 a	4.7 b
	3.0 c	3.7 a	4.7 b

[1] Means in column not followed by the same letter are significantly different (P = 0.05) by Duncan's multiple range test.

[2] Analysis of variance indicates significant difference in means of treatments at 0.05 level.

[3] Analysis of variance indicates no significant difference in means of treatments.

was based on the lack of reflexes. White grubs from the control treatments clearly showed a different response between dead and healthy individuals. When exposed to the light and gently probed, insects demonstrated a reflex movement or no movement at all. Intoxicated grubs showed an inability to react to the stimulus. This demonstrates that the reflex action may be a more realistic and accurate criterion for representing chemical toxicity than would lack of movement. Grubs capable of only slight movement and showing advanced intoxication, would be unlikely to recover in the field. It is desirable that the efficacy of a chemical be determined while control mortalities are low, and the use of lack of reflexes permits earlier evaluation.

IX. SUMMARY AND CONCLUSIONS

Adult Phyllophaga spp. were captured using blacklight traps located in areas of Quebec, west of Montreal in 1978, and Nicolet in 1979.

Topical applications of fourteen insectcicdes, using a Potter spray tower, in London, Ontario, in 1978, showed adults to be highly susceptible to WL 43775, fensulfothion and chlorpyrifos.[1] Adults treated in 1979, at Macdonald Campus of McGill, showed a high susceptibility to pyrethroids cypermethrin and fenvalerate. Such a control strategem under field conditions would however have questionable value due to low population densities and a varied host range of the adults.

Second-instar white grubs were collected near Mirabel, Quebec in 1978, and third-instar grubs were collected from both Mirabel and Pierreville in 1979. Specimens were maintained in humid soil and stored at $5^{0}C$ until used for biological testing of insecticides to avoid cannabalism and need for feeding.

A short bioassay on second-instar white grubs, using six insecticides showed fensulfothion,as both a contact and soil treatment, to cause the highest mortality.

Both contact and soil incorporation tests using isofenphos, carbofuran, diazinon, fensulfothion, fonofos and chlorpyrifos, were conducted on

[1] See appendix N.

third-instar larvae in order to establish relative toxicity levels. Values for LD 50 of each insceticide were determined when possible for contact activity, and when incorporated in sand, clay and muck soils.

Mortalities obtained with third-instar larvae when treated topically showed fensulfothion > diazinon > chlorpyrifos and carbofuran > fonofos > isfenphos in efficacy, 48 hours post-treatment. When incorporated into sand, chlorpyrifos was most toxic to third-instar larvae, followed by diazinon fonofos isofenphos carbofuran. In clay, chlorpyrifos remained the most toxic, followed by diazinon and fonofos > carbofuran > isofenphos > fensulfothion. Chlorpyrifos was also the most effective in much soil, controlling 94% of the test population at a rate of 50 ppm.

Chlorpyrifos proved the most effective insecticide for controlling white grubs in the different tests and soil types that were used. Fonofos, to a lesser degree, consistently controlled white grubs in all but much soils.

Two methods of assessing white grub mortality were evaluated using microplots filled with sandy-loam soil and held under laboratory conditions. Granular and liquid formulations, at a concentration of 5 ppm of chlorpyrifos, isofenphos, fonofos and diazinon were incorporated and compared. Mortality-determination based on a lack of reflex action was found to be a more accurate method for assessing insecticidal toxicity to white grubs. Granular formulations appeared to produce a faster control of white grubs in soil. Diazinon, fonofos and chlorpyrifos showed a more rapid control effect than isofenphos. No insecticide or formulation, at the concentration tested, provided a superior control after one week.

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APPENDIX (Containing raw data used in computations)

APPENDIX A

Adults (June beetles) sprayed with 5 mL of insecticide solution using a Potter spray tower at a pressure of 28 cm of mercury.

Insecticide	Conc. %	Period HOURS	Number Treated	% Dead *
Fenvalerate	0.005	24	20	15.4
Cypermethrin	0.005			33.5
Diazinon	0.005			3.3
Fenvalerate	0.1	24	20	57.7
Cypermethrin	0.1			87.9
Diazinon	0.1			51.6
Fenvalerate	0.2	24	20	87.9
Cypermethrin	0.2			81.8
Diazinon	0.2			57.7
Fenvalerate	0.4	24	20	75.8
Cypermethrin	0.4			100.0
Diazinon	0.4			81.8
Fenvalerate	0.8	24	20	100.0
Cypermethrin	0.8			100.0
Diazinon	0.8			100.0
CONTROL	0	24	80	17.3

* Mortalities corrected using Abbott's formula (Abbott, 1925)

APPENDIX B

Adults (June beetles) sprayed with 5 mL of insecticide solution using a Potter spray tower at a pressure of 28 cm of mercury.

Insecticide	Conc. %	Period HOURS	Number Treated	% Dead *
Fenvalerate	0.005	48	20	57.0
Cypermethrin	0.005			57.0
Diazinon	0.005			14.5
Fenvalerate	0.1	48	20	68.0
Cypermethrin	0.1			100.0
Diazinon	0.1			68.0
Fenvalerate	0.2	48	20	89.0
Cypermethrin	0.2			89.0
Diazinon	0.2			46.5
Fenvalerate	0.4	48	20	68.0
Cypermethrin	0.4			100.0
Diazinon	0.4			89.0
Fenvalerate	0.8	48	20	100.0
Cypermethrin	0.8			100.0
Diazinon	0.8			100.0
CONTROL	0	48	20	33.0

* Mortalities corrected using Abbott's formula (Abbott, 1925)

APPENDIX C

Insecticides applied to third instar larvae with 5 mL of solution using a Potter spray tower at a pressure of 28 cm of mercury.

Insecticide	Conc. %	Period HOURS	Number Treated	% Dead
Fensulfothion	0.5	24	20	35.00
Chlorpyrifos	0.5			20.00
Diazinon	0.5			10.00
Fonofos	0.5			30.00
Carbofuran	0.5			45.00
Isofenphos	0.5			5.00
Fensulfothion	1.0	24	20	70.00
Chlorpyrifos	1.0			40.00
Diazinon	1.0			50.00
Fonofos	1.0			45.00
Carbofuran	1.0			40.00
Isofenphos	1.0			10.00
Fensulfothion	1.5	24	20	95.00
Chlorpyrifos	1.5			70.00
Diazinon	1.5			70.00
Fonofos	1.5			60.00
Carbofuran	1.5			45.00
Isofenphos	1.5			15.00
CONTROL	0	24	45	16.00

APPENDIX D

Insecticides applied to third instar larvae with 5 mL of solution using a Potter spray tower at a pressure of 28 cm of mercury.

Insecticide	Conc. %	Period HOURS	Number Treated	% Dead
Fensulfothion	0.5	48	20	60.00
Chlorpyrifos	0.5			70.00
Diazinon	0.5			50.00
Fonofos	0.5			60.00
Carbofuran	0.5			45.00
Isofenphos	0.5			20.00
Fensulfothion	1.0	48	20	95.00
Chlorpyrifos	1.0			80.00
Diazinon	1.0			80.00
Fonofos	1.0			70.00
Carbofuran	1.0			70.00
Isofenphos	1.0			20.00
Fensulfothion	1.5	48	20	95.00
Chlorpyrifos	1.5			95.00
Diazinon	1.5			95.00
Fonofos	1.5			90.00
Carbofuran	1.5			90.00
Isofenphos	1.5			25.00
CONTROL	0	48	45	42.00

APPENDIX E (Refer to Table 14)

Insecticides applied to sand. Moisture content = 10%, third instar, second year white grubs.

Insecticide	Conc. PPM	Period HOURS	Number Treated	% Dead
Fensulfothion	10	24	20	10.00
Diazinon	10			45.00
Chlorpyrifos	10			40.00
Fonofos	10			5.00
Carbofuran	10			0.00
Isofenphos	10			5.00
Fensulfothion	50	24	20	5.00
Diazinon	50			80.00
Chlorpyrifos	50			85.00
Fonofos	50			45.00
Carbofuran	50			22.22
Isofenphos	50			20.00
Fensulfothion	100	24	20	20.00
Diazinon	100			85.00
Chlorpyrifos	100			72.22
Fonofos	100			65.00
Carbofuran	100			30.00
Isofenphos	100			55.00
CONTROL	0	24	80	6.67

APPENDIX F (Refer to Table 14)

Insecticides applied to sand. Moisture content = 10%, third instar, second year white grubs.

Insecticide	Conc. PPM	Period HOURS	Number Treated	% Dead
Fensulfothion	10	48	20	20.00
Diazinon	10			75.00
Chlorpyrifos	10			90.00
Fonofos	10			35.00
Carbofuran	10			15.00
Isofenphos	10			20.00
Fensulfothion	50	48	20	45.00
Diazinon	50			95.00
Chlorpyrifos	50			95.00
Fonofos	50			100.00
Carbofuran	50			72.22
Isofenphos	50			65.00
Fensulfothion	100	48	20	80.00
Diazinon	100			95.00
Chlorpyrifos	100			100.00
Fonofos	100			100.00
Carbofuran	100			60.00
Isofenphos	100			85.00
CONTROL	0	48	80	8.00

APPENDIX G (Refer to Table 15)

Insecticides applied to clay soil. Moisture content - 20%, third instar, second year white grubs.

Insecticide	Conc. PPM	Period HOURS	Number Treated	% Dead
Fensulfothion	25	24	15	13.33
Diazinon	25			60.00
Chlorpyrifos	25			53.33
Fonofos	25			60.00
Carbofuran	25			26.67
Isofenphos	25			26.67
Fensulfothion	50	24	15	26.67
Diazinon	50			73.33
Chlorpyrifos	50			86.67
Fonofos	50			80.00
Carbofuran	50			73.33
Isofenphos	50			33.33
Fensulfothion	100	24	15	20.00
Diazinon	100			93.33
Chlorpyrifos	100			93.33
Fonofos	100			86.67
Carbofuran	100			73.33
Isofenphos	100			40.00
CONTROL	0	24	60	15.00

APPENDIX H

Insecticides applied to clay soil. Moisture content = 20%, third instar, second year white grubs.

Insecticide	Conc. PPM	Period HOURS	Number Treated	% Dead
Fensulfothion	25	48	15	26.67
Diazinon	25			86.67
Chlorpyrifos	25			100.00
Fonofos	25			86.67
Carbofuran	25			40.00
Isofenphos	25			66.67
Fensulfothion	50	48	15	46.67
Diazinon	50			86.67
Chlorpyrifos	50			100.00
Fonofos	50			80.00
Carbofuran	50			86.67
Isofenphos	50			60.00
Fensulfothion	100	48	15	40.00
Diazinon	100			100.00
Chlorpyrifos	100			100.00
Fonofos	100			100.00
Carbofuran	100			86.67
Isofenphos	100			86.67
CONTROL	0	48	60	27.27

APPENDIX I (Refer to Table 16)

Insecticides applied to muck soil. Moisture content = 40%, third instar, second year white grubs.

Insecticides	Conc. PPM	Period HOURS	Number Treated	% Dead
Fensulfothion	50	24	20	35.00
Diazinon	50			10.00
Chlorpyrifos	50			45.00
Fonofos	50			25.00
Carbofuran	50			20.00
Isofenphos	50			10.00
Fensulfothion	100	24	20	95.00
Diazinon	100			10.00
Chlorpyrifos	100			55.00
Fonofos	100			35.00
Carbofuran	100			25.00
Isofenphos	100			0.00
Fensulfothion	200	24	20	100.00
Diazinon	200			15.00
Chlorpyrifos	200			95.00
Fonofos	200			90.00
Carbofuran	200			75.00
Isofenphos	200			35.00
CONTROL	0	24	80	5.33

APPENDIX J (Refer to

Insecticides applied to muck soil. Moisture content = 40%, third instar, second year white grubs.

Insecticide	Conc. PPM	Period	Number Treated	% Dead
Fensulfothion	50	48	20	75.00
Diazinon	50			35.00
Chlorpyrifos	50			95.00
Fonofos	50			45.00
Carbofuran	50			25.00
Isofenphos	50			20.00
Fensulfothion	100	48	20	90.00
Diazinon	100			25.00
Chlorpyrifos	100			100.00
Fonofos	100			70.00
Carbofuran	100			65.00
Isofenphos	100			10.00
Fensulfothion	200	48	20	100.00
Diazinon	200			40.00
Chlorpyrifos	200			100.00
Fonofos	200			95.00
Carbofuran	200			85.00
Isofenphos	200			50.00
CONTROL	0	48	80	20.00

APPENDIX K (Refer to Table 17, 18 and 19)

Microplot experiment: three replicates containing 15 grubs were treated with the following insecticides (5 ppm) and held for one week. The moisture content was kept constant at 15%.

Insecticide	Period HOURS	#'s showing no movement	#'s showing no reflexes	avg. % mort. no movement	avg. % mort. no reflexes
Amaze 6 EC	72	5	12	28.9	66.7
		2	10		
		6	8		
15 G		5	5	24.4	35.5
		3	5		
		3	6		
6 EC	120	8	9	44.4	68.9
		5	11		
		7	11		
15 G		7	11	44.5	66.7
		4	9		
		9	10		
6 EC	168	10	14	64.5	93.3
		9	15		
		10	13		
15 G		7	15	44.5	84.4
		6	11		
		7	12		

APPENDIX K (continued)

Insecticide	Period HOURS	#'s showing no movement	#'s showing no reflexes	avg. % mort. no movement	avg. % mort. no reflexes
Basudin 50 EC	72	8	12	33.3	84.4
		2	14		
		5	12		
5 G		4	14	22.2	84.4
		1	12		
		5	12		
50 EC	120	8	12	42.2	93.3
		3	14		
		7	15		
5 G		8	15		
		4	13		
		7	14		
50 EC	168	10	15	53.3	100.0
		6	15		
		8	15		
5 G		10	15	55.6	100.0
		6	15		
		9	15		

APPENDIX K (continued)

Insecticide	Period* HOURS	#'s showing no movement	#'s showing no reflexes	avg. % mort. no movement	avg. % mort. no reflexes
Dursban 2 E	72	2	10	26.7	68.9
		3	11		
		7	10		
Lorsban 15 G		4	10	26.7	71.1
		5	10		
		3	12		
2 E	120	4	13	40.0	86.7
		5	13		
		9	13		
15 G		4	14	37.8	93.3
		9	15		
		4	13		
2 E	168	4	15	46.7	100.0
		6	15		
		11	15		
15 G		5	15	48.7	100.0
		9	15		
		8	15		

APPENDIX K, (continued)

Insecticide	Period HOURS	#'s showing no movement	#'s showing no reflexes	avg. % mort. no movement	avg. % mort. no reflexes
Dyfonate 4 E	72	6	11	57.8	75.5
		7	11		
		13	12		
10 G		6	12	40.0	77.8
		6	12		
		6	11		
4 E	120	6	11	46.7	84.4
		8	15		
		7	12		
10 G		6	12	40.0	77.8
		6	12		
		6	11		
4 E	168	6	9	55.5	77.8
		11	14		
		8	12		
10 G		6	15	53.3	93.3
		12	15		
		6	12		

APPENDIX K (continued)

Insecticide	Period HOURS	#'s showing no movement	#'s showing no reflexes	avg. % mort. no movement	avg. % mort. no reflexes
Control	72	3	3	20.0	20.0
		5	5		
		2	2		
		4	4		
		2	2		
		2	2		
	120	3	3	22.2	26.7
		6	6		
		4	5		
		3	5		
		2	3		
		2	2		
	168	6	6	31.1	32.2
		7	7		
		4	5		
		6	6		
		2	2		
		3	3		

STATISTICAL ANALYSIS SYSTEM

15:31 WEDNESDAY, JANUARY 30, 19[illegible]

PROBIT ANALYSIS ON LOG10(DOSE)

ITERATION	INTERCEPT	SLOPE	MU	SIGMA
0	4.50959132	5.79237304	0.08466455	0.17264081
1	4.60200894	4.95372924	0.08034171	0.20186812
2	4.58944571	5.07075330	0.08096515	0.19720936
3	4.58913163	5.07368915	0.08098029	0.19709524
4	4.58913144	5.07369095	0.08098021	0.19709517

COVARIANCE MATRIX

	INTERCEPT	SLOPE
INTERCEPT	0.04167977	-0.08826815
SLOPE	-0.08826815	1.82992472

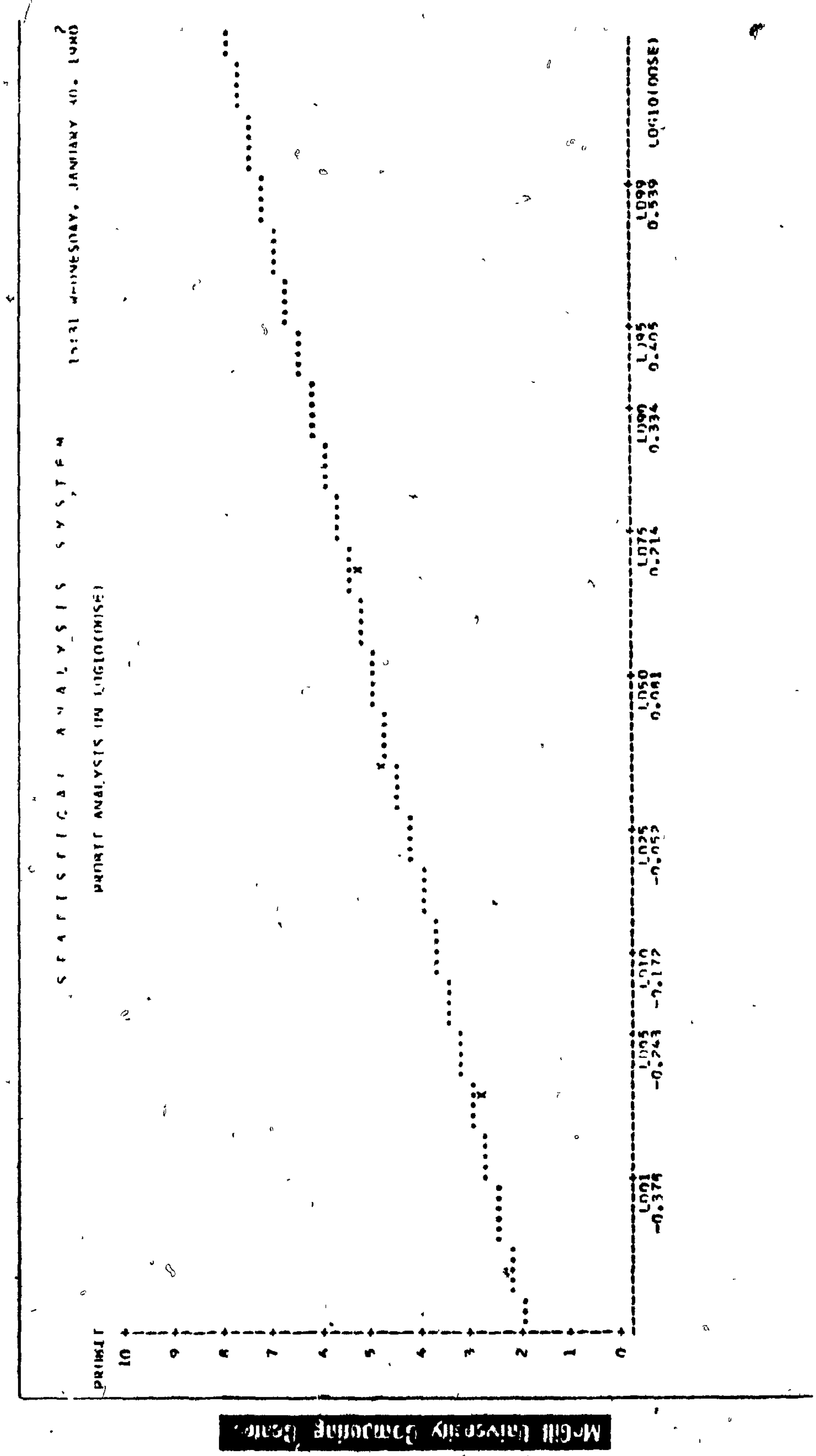
COVARIANCE MATRIX

	MU	SIGMA
MU	0.00152993	0.00045877
SIGMA	0.00045877	0.00276149

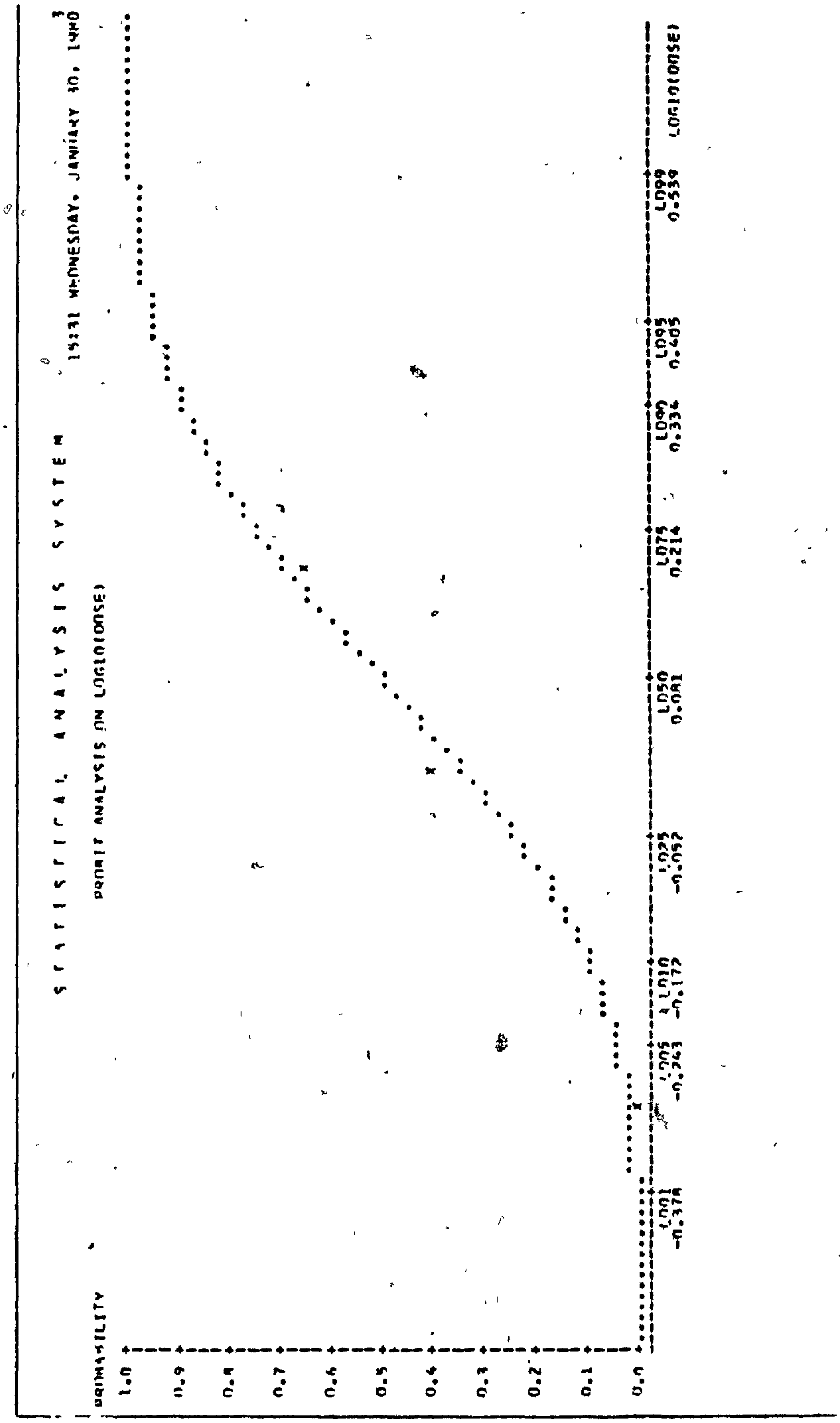
CHI-SQ = 0.7276 WITH 1 DF PROB > CHI-SQ = 0.3937

NOTE: SINCE THE CHI-SQUARE IS SMALL (P > 0.10), FIDUCIAL LIMITS WILL BE COMPUTED USING A T VALUE OF 1.96 .

APPENDIX L


STATISTICAL ANALYSIS SYSTEM
15:31 WEDNESDAY, JANUARY 30, 1980
2
PROBIT ANALYSIS ON LOG10(DOSE)
PROBIT
10
9
8
7
6
5
4
3
2
1
0
LD01 -0.379
LD05 -0.243
LD10 -0.172
LD25 -0.052
LD50 0.081
LD75 0.214
LD90 0.334
LD95 0.405
LD99 0.539
LOG10(DOSE)
McGill University Computing Centre.

APPENDIX L


S T A T I S T I C A L   A N A L Y S I S   S Y S T E M
15:31 WEDNESDAY, JANUARY 30, 1980
PROBIT ANALYSIS ON LOG10(DOSE)
PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
LD01 -0.378
LD05 -0.243
LD10 -0.172
LD25 -0.052
LD50 0.081
LD75 0.214
LD90 0.334
LD95 0.405
LD99 0.539
LOG10(DOSE)

APPENDIX L

STATISTICAL ANALYSIS SYSTEM   15:31 WEDNESDAY, JANUARY 30, 19[illegible]

PROBIT ANALYSIS ON LOG10(DOSE)

PROBABILITY	LOG10(DOSE)	95 PERCENT FIDUCIAL LIMITS LOWER	UPPER	DOSE	95 PERCENT FIDUCIAL LIMITS LOWER	UPPER
0.01	-0.37753173	-0.85584694	-0.21009797	0.41974537	0.13935837	0.60391241
0.02	-0.32380379	-0.74502343	-0.18211499	0.47445429	0.17947739	0.65748070
0.03	-0.28971513	-0.67495377	-0.15939476	0.51319797	0.21137140	0.69438965
0.04	-0.26407157	-0.62241676	-0.14037929	0.54441293	0.23455210	0.72340355
0.05	-0.24321250	-0.57781822	-0.12558720	0.57119904	0.26313692	0.74888098
0.06	-0.22545816	-0.54367[illegible]4	-0.11278133	0.59503408	0.28497256	0.77111414
0.07	-0.20969109	-0.51208829	-0.10163816	0.61674955	0.30754715	0.79133767
0.08	-0.19595241	-0.48380974	-0.09147708	0.63686501	0.32817104	0.81007073
0.09	-0.18327414	-0.45835195	-0.08214752	0.65572827	0.34805522	0.82766097
0.10	-0.17160742	-0.43491955	-0.07347507	0.67358527	0.36715035	0.84435471
0.15	-0.12329581	-0.33902640	-0.03644596	0.75284751	0.45811403	0.91950444
0.20	-0.08489927	-0.26469566	-0.00513431	0.82243333	0.54363115	0.98824741
0.25	-0.05195846	-0.20304845	0.02385043	0.88724085	0.62654368	1.05645362
0.30	-0.02237660	-0.15019316	0.05238502	0.94978083	0.70763107	1.12819720
0.35	0.00503541	-0.10418395	0.08179584	1.01166193	0.78671268	1.20724619
0.40	0.03104672	-0.06392809	0.11310636	1.07410495	0.86312145	1.29749701
0.45	0.05621797	-0.02862809	0.14704757	1.13818529	0.93620706	1.40296737
0.50	0.08098021	0.00252510	0.18603770	1.20498103	1.00543129	1.53764898
0.55	0.10574746	0.03044092	0.22426539	1.27569677	1.07260773	1.67598670
0.60	0.13091370	0.05407144	0.26787607	1.35180392	1.13781443	1.85300279
0.65	0.15692501	0.08033535	0.31517845	1.43524160	1.20319314	2.06622897
0.70	0.18433792	0.10410597	0.36682790	1.52875194	1.27088417	2.32716889
0.75	0.21391888	0.12827885	0.42404515	1.63651033	1.34342739	2.65488155
0.80	0.24685949	0.15393241	0.48902315	1.76546734	1.42538641	3.08335230
0.85	0.28525423	0.18268439	0.56591376	1.92866247	1.52245560	3.68055484
0.90	0.33354786	0.21749474	0.66382567	2.15550933	1.65080106	4.61132431
0.91	0.34523456	0.22600973	0.68761542	2.21430049	1.68271177	4.87096946
0.92	0.35791303	0.23499310	0.71350951	2.27988548	1.71748109	5.17022578
0.93	0.37185150	0.24481714	0.74203510	2.35424415	1.75718359	5.52122055
0.94	0.38741858	0.25572988	0.77395288	2.44016154	1.80189667	5.94227684
0.95	0.40517292	0.26810967	0.81042253	2.54198446	1.85399548	6.46282791
0.96	0.42693199	0.28257240	0.85334944	2.67305508	1.91678056	7.13426424
0.97	0.45167555	0.30025271	0.90622360	2.82927755	1.99462368	8.05793210
0.98	0.48576421	0.32341240	0.97665381	3.06030145	2.13674703	9.47667753
0.99	0.53949215	0.34016984	1.08792085	3.46331423	2.29176372	12.24393064

S T A T I S T I C A L   A N A L Y S I S   S Y S T E M

15:41 WEDNESDAY, JANUARY 30, 1980 1

PROBIT ANALYSIS ON LOG10(DOSE)

ITERATION	INTERCEPT	SLOPE	MU	SIGMA
0	5.44330275	5.11585697	-0.08665269	0.19547067
1	5.43375784	5.09227606	-0.08517956	0.19637584
2	5.43378551	5.09240768	-0.08518279	0.19637077
3	5.43378551	5.09240769	-0.08518279	0.19637077

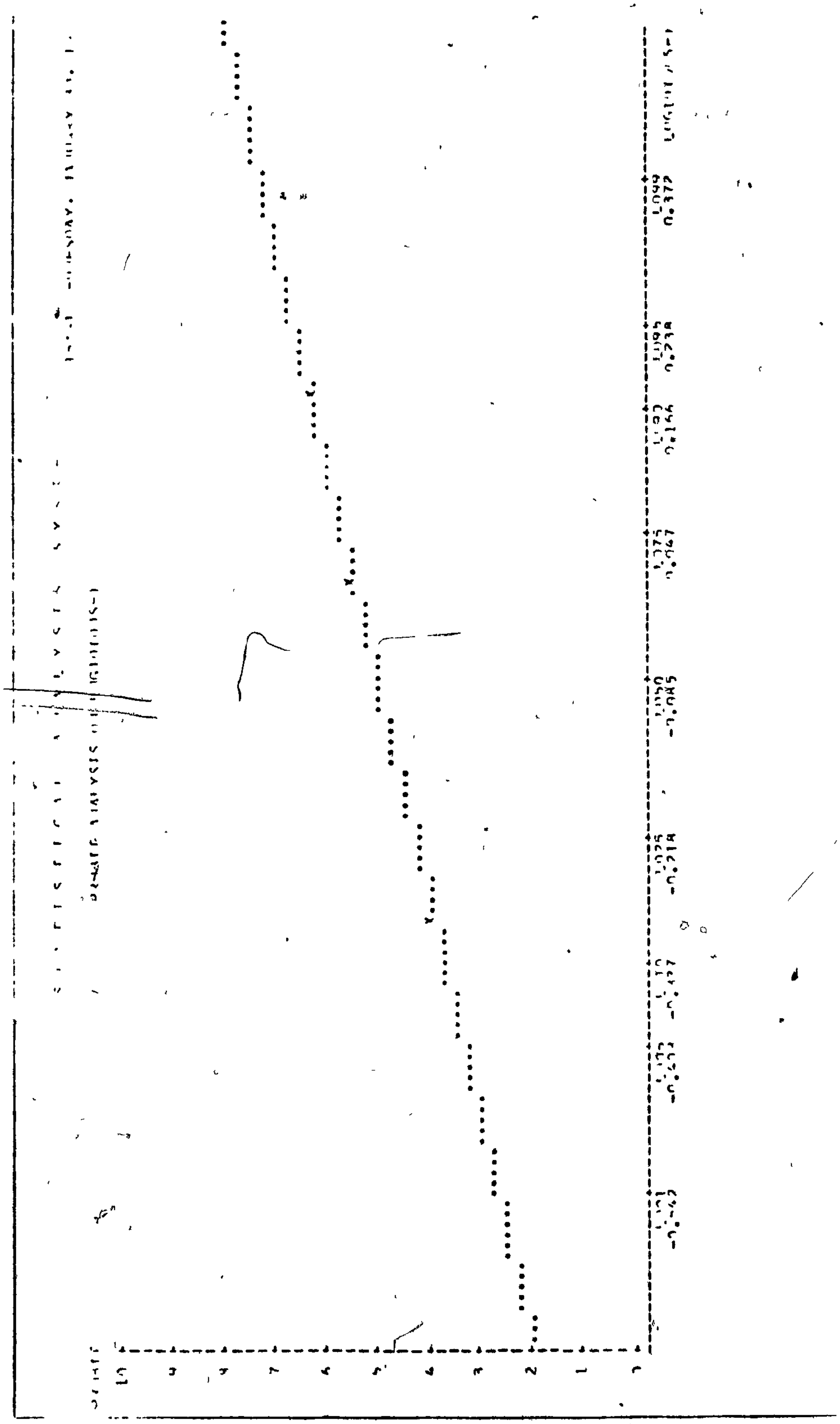
COVARIANCE MATRIX	INTERCEPT	SLOPE
INTERCEPT	0.04055519	0.05700286
SLOPE	0.05700286	1.14723007

COVARIANCE MATRIX	MU	SIGMA
MU	0.00151039	-0.00030836
SIGMA	-0.00030836	0.00170592

CHI-SQ = 0.0286 WITH 1 DF PROB > CHI-SQ = 0.8663

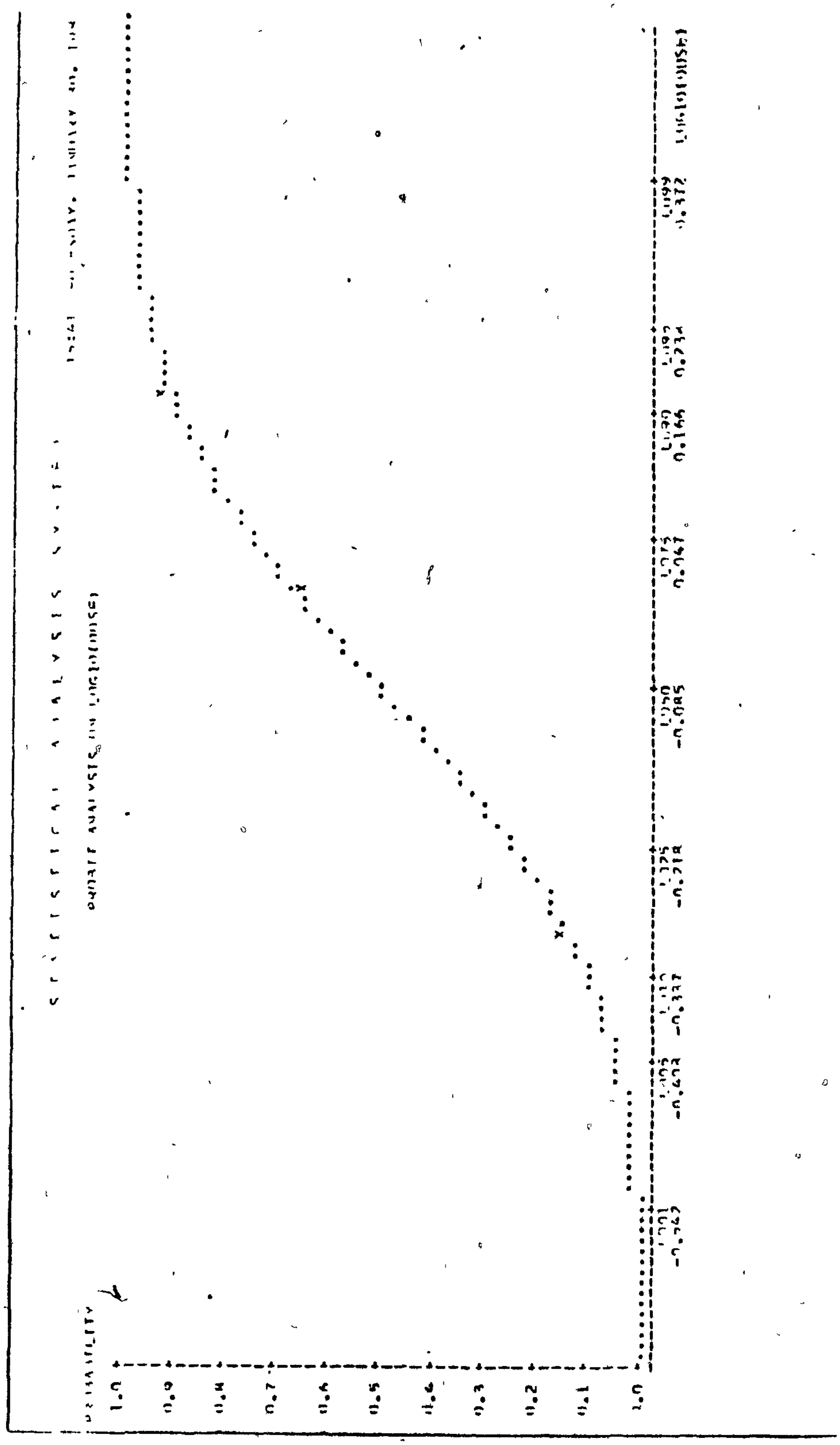
NOTE: SINCE THE CHI-SQUARE IS SMALL (P > 0.10), FIDUCIAL LIMITS WILL BE COMPUTED USING A T VALUE OF 1.96 .

APPENDIX M



McGill University Computing Centre

APPENDIX M



APPENDIX M

STATISTICAL ANALYSIS SYSTEM

15:41 [illegible]

PROBIT ANALYSIS ON LOG10(DOSE)

PROBABILITY	LOG10(DOSE)	95 PERCENT FIDUCIAL LIMITS LOWER	UPPER	[illegible]	95 PERCENT FIDUCIAL LIMITS LOWER	UPPER
0.01	-0.54200951	-0.90072231	-0.38489666	0.28707177	0.12564333	0.41214574
0.02	-0.48947904	-0.81111365	-0.36545731	0.32477891	0.15446119	0.45134040
0.03	-0.45451548	-0.75655457	-0.35124697	0.35114325	0.17597109	0.47836019
0.04	-0.42806636	-0.71274673	-0.30115714	0.37242577	0.19405028	0.49485364
0.05	-0.40814396	-0.67762471	-0.29553423	0.39067534	0.21017451	0.51815629
0.06	-0.39049488	-0.64837497	-0.27217133	0.41401434	0.22471136	0.53435351
0.07	-0.37498501	-0.62274116	-0.26034630	0.42171175	0.24334763	0.54405228
0.08	-0.36139777	-0.59933606	-0.24377710	0.43541344	0.25122512	0.56262968
0.09	-0.34864740	-0.57427566	-0.24007731	0.44926215	0.26370844	0.57533750
0.10	-0.33634206	-0.54717705	-0.23110043	0.46042394	0.27541505	0.58735351
0.15	-0.28870401	-0.48105746	-0.19333022	0.51438937	0.32446549	0.64070893
0.20	-0.25045260	-0.42068709	-0.16242415	0.56175550	0.37458760	0.68797372
0.25	-0.21763286	-0.36002149	-0.13501588	0.60595293	0.42756173	0.73279774
0.30	-0.18815472	-0.32357188	-0.10465032	0.64839592	0.47470972	0.77723022
0.35	-0.16084847	-0.28250344	-0.08471261	0.69048068	0.52174097	0.82278693
0.40	-0.13493276	-0.24471597	-0.06005650	0.73293307	0.56922509	0.87085029
0.45	-0.10985901	-0.20950236	-0.03485571	0.77669914	0.61730194	0.92287905
0.50	-0.08518279	-0.17537349	-0.00852705	0.82189664	0.66623354	0.98055724
0.55	-0.06050658	-0.14494827	0.01959678	0.86994925	0.71422472	1.04593521
0.60	-0.03543283	-0.11485992	0.04984133	0.92165743	0.76759137	1.12160859
0.65	-0.00951712	-0.08573396	0.08314995	0.97832439	0.82045426	1.21101334
0.70	0.01779414	-0.05772112	0.12024226	1.04192367	0.87695419	1.31899224
0.75	0.04726728	-0.02801870	0.16225501	1.11409051	0.93757164	1.45296452
0.80	0.08008701	0.00231917	0.21099590	1.20250836	1.00539437	1.62553342
0.85	0.11834742	0.03571245	0.26977918	1.31323493	1.08570752	1.86113630
0.90	0.16647547	0.07561198	0.34595744	1.46715459	1.19017417	2.21744841
0.91	0.17810230	0.08408655	0.36449517	1.50496199	1.21614434	2.31470243
0.92	0.19073218	0.09507773	0.38483554	1.55143007	1.24473739	2.42569137
0.93	0.20461942	0.10607322	0.40730119	1.60186104	1.27665401	2.55447225
0.94	0.22012929	0.11826207	0.43250227	1.66003103	1.31293424	2.70708735
0.95	0.23781837	0.13199747	0.46136941	1.72003303	1.35518151	2.89313975
0.96	0.25860077	0.14800345	0.49543736	1.81384757	1.40607812	3.12919310
0.97	0.28415004	0.16750830	0.53744479	1.92375445	1.47066651	3.44742073
0.98	0.31811345	0.19314758	0.59366933	2.08024095	1.56015439	3.92346047
0.99	0.37164302	0.23314209	0.68267583	2.35311914	1.71057487	4.81588191

APPENDIX N

Chlorfenvinphos:	Birlane® = 2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate.
Chlorpyrifos:	Lorsban®, Dursban® = 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate.
CGA 12223:	0-(5-chloro-1-isopropyl-1,2,4-triazol-3-yl) phosphorothioate.
Diazinon:	Diazinon®, Basudin® = 0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate.
Dieldrin:	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a-5,6,7,8a-octahydro-1,4-endo-exo-5,8-di-methanonaphthalene.
Fensulfothion:	Dasanit® = 0,0-diethyl 0-[p-(methyl-sufinyl) phenyl] phosphorothioate.
Fonofos:	Dyfonate® = 0-ethyl s-phenyl ethylphosphonodithioate.
Isazofos:	0,0-diethyl-0-(5-chlor-1-iso-propyl-1,2,4-trizol-3-yl)-phosphorthioate.
Isofenphos:	Amaze® = 1-methylethyl 2-[ethoxy (1-methylethyl) amino] phosphinothioyl.
Permethrin:	Ambush® = 3-phenoxybenzyl (±) cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclo = propanecarboxylate.
Terbufos:	Counter® = s-[tert-butylthio methyl]0,0-diethyl phosphordithioate.
WL 24073:	0-2[-chloro-1-(2,5-dichlorophenyl) vinyl] 0-methyl-ethylphosphorothioate.
WL 41706:	Unknown.
WL 43467:	α-cyano-3-phenoxybenzyl-2,2-dimethyl-3-2-(2,2-dichlorovinyl) cyclopropane carboxylate.
WL 43775:	α-cyano-3-phenoxybenzyl 2-(4-chlorphenyl)-3-methyl butyrate.