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CONTROL OF INSECTS BY BACILLUS THURINGIENSIS

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Summary Spores and crystals of Bacillus thuringiensis form an absolutely safe, easily manufactured and stable microbial insecticide, which is atoxic to plants, virtually specific against lepidopterous larvae and compatible with all biological forms of control and most pesticidal chemicals. It is active only per os, stopping feeding within hours of ingestion. Midgut enzymes release from the crystal remarkably potent gut - paralytic and destructive toxins. It is effective only against surface-feeding larvae above ground. Even cover is essential. In the U.S.A., cost of control equals that with chemicals against four pests and effectiveness against 23 pests has been registered. Use is considerable and increasing, particularly on brassicas and tobacco. Use outside the U.S.A. is small, with little or none in the U.K., where it is recommended at present only against ermine moth in hedgerows. The most likely potential U.K. uses are reviewed. Research is active with possible hope of a lead to new approaches to insect control.

INTRODUCTION

In the 1920's, trials were conducted in Europe on a bacterium, Bacillus thuringiensis, capable of killing some lepidopterous larvae in a few hours. It was easy to produce on artificial media and just before the Second World War a commercial product was available in France. After the war four American companies started production at a time when the deep fermentation industry was developing rapidly. At present two companies in the U.S.A. are in regular, profitable production. In parallel with this commercial development, laboratory studies have revealed a pathology of exceptional interest and great research potential.

NATURE OF BACILLUS THURINGIENSIS

B. thuringiensis is a rod shaped, aerobic bacterium that grows vigorously in deep aerated fermentors on media incorporating cheap industrial by-products. During vegetative growth it produces low concentrations of water soluble exotoxin, toxic for many groups of insects. The exotoxin, after concentration and extraction,

Table 1.

Comparative potency (μg/g body wt) of the Bacillus thuringiensis crystal

Poison	Route	LD 50	Test species
B. thuringiensis crystal	oral	0.5	Bombyx mori larva
	oral	0.08-0.15	Pieris brassicae larva
DDT	topical	11.4	Musca domestica adult
Parathion	topical	3	Sitophilus granarius adult
Curare	injection	0.25	rabbit
Parathion	oral	6-15	rat

proved toxic to many insect groups, producing teratological effects at moulting and metamorphosis, and has some toxicity by injection to mammals. In present commercial products the effect of the exotoxin is avoided by discarding the supernatant of centrifugation or by using a bacterial strain that does not produce it. At sporulation each rod forms a spore and a bipyramidal crystal of protein, toxic specifically to lepidopterous larvae. Our main interest lies in this toxic crystal. Its potency against susceptible larvae, 0.08 to 0.5 µg pure crystal/gram of insect, matches that of potent poisons of mammals or insects (Table 1). It is present at about 0.1 to 1% in products after formulation as liquid spray concentrates, wettable powders or dust concentrates.

Commercial B. thuringiensis products are absolutely safe for man, domestic animals, wildlife, bees, predaceous and parasitic insects, since the crystal is specific to some lepidopterous larvae and the spore incapable of infecting most other invertebrates and all tested vertebrates. B. thuringiensis is registered in the U.S.A. without tolerance limits or other restrictions and similar registrations exist in the U.K. and many other countries.

MODE OF ACTION

The intact crystal is a protoxin, harmless on injection into the body cavity even of lepidopterous larvae. On oral ingestion, however, midgut enzymes of a susceptible larva break the crystal into smaller molecules which, after purification, proved toxic by haemocoelic injection. Within 20 min of eating a large dose of crystal the most susceptible species suffer buccal paralysis, which prevents further feeding (Heimpel and Angus, 1959). Within hours, the epithelium of the midgut is destroyed and gut contents seep into the body cavity, rapidly causing death. Slightly less susceptible species suffer buccal paralysis and injury to the epithelium, but not gross swamping of the blood, and death takes about 2-5 days largely from starvation or septicaemia. At low doses in these species, or in other species only slightly susceptible to pure crystal, the crystal acts as a conditioner, which alters gut conditions such that bacteria are able to grow from spores and eventually penetrate into the haemocoel. It is believed that toxin released from the crystal wrecks the balance of chemical exchange between gut and blood, and across cell membranes, possibly by interfering with the movement of potassium ions.

CHARACTERISTICS AS A CONTROL AGENT

B. thuringiensis usually kills only caterpillars in a pest complex. In parallel with this specificity, there is no direct effect on beneficial arthropods, the only effect being a reduction in numbers of hosts. Successful emergence of many parasites from diseased hosts has been recorded. Thus no immediate resurgence of the pest or replacement by another pest species is likely.

Resistance to the spore-crystal complex has not yet been recorded and if it does occur the existence of twelve varieties and many more sub-strains offers scope for circumvention.

If kept dry, B. thuringiensis powders store indefinitely in darkness at room temperature, and liquids at 5°C. At 32°C, potency of liquids against species susceptible to the crystal does not alter detectably in 6 months and at 45°C potency lasts for 1 month, although it gradually falls over longer periods. Initially, products were standardized by their viable spore content and this is still the official basis in the U.S.A. However, it is generally agreed that this is inadequate and should be replaced by toxicity units based on bioassay. One product now has a toxicity unit as well as spore count on its labels.

Unlike many insect pathogens, this bacterium has limited powers of spread and

so must be used as a microbial insecticide and applied in the same way as chemicals. Used in this way it is very effective, even against sparse populations. If applied within a few hours of mixing, B. thuringiensis is compatible with most pesticides, although even without additives it should never stand in diluted form for more than 12 hours. Extremes of pH should be avoided. To obtain good coverage, adequate wetting agent is often essential but only a minimum should be used, because it causes the product to run off the foliage and increases the washing action of rain and dew. In rainy or dewy periods, or with overhead irrigation, an adhesive is valuable. Infection or crystal poisoning can take place only when the bacterium is eaten: there is no contact action whatsoever. The bacterium is thus equivalent to a larval stomach poison. Consequently it is effective in the field only against defoliators or surface-feeding larvae. Good coverage of foliage is essential and the undersides of leaves, where the young larvae of many species feed, should be well covered particularly since young larvae are usually much more susceptible than older ones.

Residual activity on the plant lasts 2 weeks or more in good conditions, but under average conditions only 4 to 10 days on rapidly growing foliage. Strong sunlight kills spores. One product, 'Thuricide', contains an additive that protects them from ultra violet radiation. The manufacturer claims that the protected formulation can resist 17-24 h exposure to noon sun at 38°C. Solids present in dry formulations also give protection. The best time for application is after the sun falls. New foliage growing after treatment will not be protected and generally two applications at a lower dosage are more effective than one at high dosage.

Because of buccal paralysis, damage ceases immediately after application, but growers often do not appreciate this since larvae may remain on the plant without feeding for 2-5 days before death. Dead larvae generally shrivel, dry and fall off the plant. No unsightly residue remains. In some species, a few larvae may linger in a weakened state for up to 30 days. These stunted larvae do little or no damage and are particularly susceptible to natural controlling forces. The survival, activity, size and fecundity of adults maturing from sublethally infected larvae are impaired. There is no effect on eggs, pupae and adults that are present and healthy at the time of treatment.

Phytotoxicity has never been recorded. The deposit on foliage is usually invisible. No unsightly residues appear on flower blooms treated just before harvest unless these are affected by aqueous sprays. The deposit does not alter the odour, flavour, taste or appearance of cooked broccoli, fresh lettuce or the smoke from treated flue-cured tobacco leaf.

Consideration should be given to prevailing temperatures because the effect of B. thuringiensis is reduced by low temperature. For instance, air temperatures should reach 15°C daily for several hours to stimulate larvae of the oak tortrix moth, Tortrix viridana, to feed and acquire a lethal dose (Franz et al., 1967).

USE AS A CONTROL AGENT

About 150 species of Lepidoptera have so far proved susceptible and 23 are registered on various crops in the U.S.A. (Table 2), which means that state and federal agencies of the U.S.D.A. are satisfied with the efficacy of field trials. Data are being collected on a further 45 well known susceptible American species, many of them with a view to registration (Table 2). The species have been graded from very susceptible (1) to slightly so (4). However, many of the available data are based on early products and many species may be more susceptible to the improved modern products. For instance, a major improvement in the potency of one product was reported against the cotton boll worm (Table 2) late in 1968. Many species occurring outside the U.S.A. are susceptible.

Table 2.

Registered uses of 'Thuricide' and/or 'Biotrol' in the U.S.A. and the susceptibility (1 = very susceptible) of some N. American insects to Bacillus thuringiensis : data from the I.M.C. and Nutrilite Products Inc.

REGISTERED: COST COMPARABLE TO, OR EVEN LOWER THAN, THAT OF CHEMICALS

1	Colias eurytheme	alfalfa caterpillar	alfalfa
1	Pieris rapae	small cabbage white butterfly	brassicas
1	Protoparce quinquemaculata	tomato hornworm	tomato
1	Protoparce sexta	tobacco hornworm	tobacco

REGISTERED: COST LIKELY TO EXCEED THAT OF CHEMICALS

2	Alsophila pometaria	fall cankerworm	shade trees, ornamentals
2	Archips argyrospilus	fruit-tree leaf roller	orange
-	Crambus sperryillus	lawn moth	turf
2	Desmia funeralis	grape leaf folder	grape
2	Erannis tiliaria	Linden looper	forest
1	Estigmene acrea	salt marsh caterpillar	turf, flowers etc.
3	Heliothis virescens	tobacco budworm	tobacco
3	Heliothis zea	bollworm, earworm	-
2	Malacosoma fragile	tent caterpillar	forest
2	Paleacrita vernata	spring cankerworm	shade trees, ornamentals
1	Papilio cresphontes	orange dog	orange
2	Phryganidia californica	oak moth	forest
2	Platyptilia carduidactyla	artichoke plume moth	artichoke
1	Plutella maculipennis	diamond back moth	cabbage
3	Porthetria dispar	gypsy moth	trees, shrubs, flowers etc.
4	Proxenus mindara	rough skin cutworm	-
1	Pyrausta nubilalis	European corn borer	sweet corn
-	Thyridopteryx ephemeraeformis	bagworm	trees, shrubs
2	Trichoplusia ni	cabbage looper	many crops

A SELECTION FROM 45 SUSCEPTIBLE SPECIES NOT YET REGISTERED

4	Agrotis ipsilon	black cutworm
2	Archips rosaceanus	oblique-banded leaf roller
2	Choristoneura fumiferana	spruce budworm
2	Cydia pomonella	codling moth
2	Galleria mellonella	greater wax moth
2	Hyphantria cunea	fall webworm
2	Malacosoma americanum	tent caterpillar
2	Malacosoma pluvial	western tent caterpillar
2	Operophtera brumata	winter moth
3	Spodoptera exigua	beet armyworm

Field crop, pasture, garden and vineyard

B. thuringiensis controls four of the most susceptible species at a cost comparable to or even lower than that of chemical insecticides (Table 2). Many other species are controlled at a higher cost than that with one of the lower priced chemicals and so control becomes economic on valuable crops such as vegetables and tobacco (Table 2). B. thuringiensis is particularly valuable where edible crops, such as brassicas and lettuce, require protection up to harvest and afterwards. Economically it compares favourably with repeated applications of short-lived chemicals which are severely restricted by residue tolerances and cut-off dates. It is also valuable when it is necessary to avoid harming natural enemies of the pest with chemicals, which result in more serious infestation at a later date.

On vegetables, because large larvae are less susceptible than small ones and take longer to die, successive applications during the season from the time small larvae appear (if necessary with an aphicide) are often more effective than the initial use of chemicals followed by B. thuringiensis when residue regulations forbid chemicals. If large larvae are present the last application should be made in time for them to die, shrivel and fall from the plants before harvest. On vines, treatment against the grape leaf folder (Table 2) should start as soon as possible after hatching and before larvae are protected by leaf rolls.

Of the lepidopterous pests in the U.K. many, but not all, of the complex that attack brassicas can be controlled with B. thuringiensis. Larvae of the small white butterfly and the diamondback moth are very susceptible and both are registered in the U.S.A. (Table 2). The large white butterfly, Pieris brassicae, is also very susceptible and probably Pieris napae too. Some evidence was obtained by King (1968) that the garden pebble moth, Evergestis forficalis, was less susceptible than P. brassicae, but more study is needed. However, more seriously, the cabbage moth Mamestra brassicae (an important pest) is not susceptible although it can probably be controlled by a polyhedrosis virus if this becomes available commercially, or an attempt could be made to develop a strain of B. thuringiensis active against this pest. P. maculipennis and the pierids are heavily attacked by insect parasites which would not be killed by B. thuringiensis. The beet webworm is also very susceptible and registered in the U.S.A. (Table 2), but is not very important in the U.K.

In a single trial, an early product gave no control of the pea moth, Laspeyresia nigricana, probably because the larvae ingest only a little pod surface (Legowski and Gould, 1965). The parsnip moth has not been tested, but it might not feed on the surface enough to pick up a lethal dose. Four cutworms, Euxoa nigricans, Agrotis segetum, A. ipsilon, and A. exclamationis, cause most damage by cutting off young plants at ground level. They would be unlikely to acquire lethal doses except when feeding as young larvae on foliage. A. ipsilon is susceptible at grade 4.

Forest

B. thuringiensis is effective against a number of forest insects, e.g. those in Table 2, but it usually costs more than DDT. However, this comparison is a poorer yardstick of its value in the forest than in other applications, because it is necessary only to bring the pest population below economic or aesthetically acceptable values, and it is vital not to harm predators and parasites for fear of worse trouble later and also not to pollute watersheds. In Europe, B. thuringiensis has been used on a commercial scale to protect valuable oak forests in W. Germany against the oak tortricid, Tortrix viridana. Franz and Krieg (1967) review successes against several species in Europe and the U.S.S.R.

In the U.K., the only economically serious pest feeding exposed on the surface of leaves is the pine looper, Bupalus piniarius, against which a small field test with one of the early products was unsuccessful (Ronald M. Brown, personal communication, 1969), although the habits and feeding season appear to be suitable.

Orchard, hedgerow and ornamental plants

B. thuringiensis is effective only against defoliators feeding unprotected on leaves and the outside of fruit (Table 2). It must be applied early against leaf-folding species before the larvae form their shelters. Its harmlessness to bees and other pollinators, and freedom from restrictions are valuable assets.

In U.K. orchards, the surface-feeding Lepidoptera have been eliminated or greatly reduced by past spray practices. The most important Lepidoptera are protected feeders such as the codling moth, Cydia pomonella and the tortricid,

Archips podana, and as chemical control is effective only if broad-spectrum insecticides are used, beneficial insects suffer high mortality (G.H.L. Dicker, personal communication). B. thuringiensis is unlikely to be useful against the codling moth in orchards despite grade 2 susceptibility (Table 2), because the larvae burrow directly into the fruit and because the egg hatching season is protracted. Walnut, on which B. thuringiensis is promising in the U.S.A., may prove an exception, because larvae largely feed externally. It may prove worthwhile to spray the trunks of orchard trees, forcing spray into protected crevices, because larvae may acquire a lethal dose when they enlarge pupation sites at a time when they may prove very susceptible, i.e. during overwintering. The tortricid is likely to be controlled only during the short period of unprotected feeding after hatching.

In the past the two most important orchard surface feeders have been the winter moth and a small ermine moth (Yponomeuta malinella) (G.H.L. Dicker, personal communication). These are susceptible at or about grade 2 (Table 2; Burges et al., 1967). In orchards, good control of winter moth and ermine moth was obtained in France (Martouret and Milaire, 1963) nearly as good as with chemicals in Germany (Herfs, 1964a) and on a Prunus spinosa hedge control was as good as with DDT and BHC (Herfs, 1964b). Videnova (1963) obtained good control of ermine moth in Bulgaria. In a small trial in the U.K., there was no control of winter moth and tortricids (Gould, French and Vernon, 1967); further trials seem warranted. In Canada, Jaques (1965) obtained substantial control of apple-orchard pests without harming beneficial insects, but the injury to fruit was not reduced to an economically acceptable level.

The N.A.A.S. recommend B. thuringiensis against one of the small ermine moths, Yponomeuta padella, on hawthorne hedges, where chemicals must be avoided because of browsing dairy cattle. In trials modern products gave as good a control as trichlorfon, which was very effective, but the cost is about five times as great (N.C. O'Flanagan, personal communication).

Species of Archips, Erannis and Malacosoma occurring in the U.S.A. are susceptible at grade 2 (Table 2) and, despite protected feeding over at least part of the larval period, the bacillus is registered as effective against three of these in practice (Table 2), suggesting that their British relatives might be worthy of further study. Species with possibly suitable habits and some importance as pests are:- Archips podana (fruit, oak); A. rosana (rose); Ditula angustiorana (fruit); flax tortricid, Cnephasia virgaureana (herbaceous plants); mottled umber, Erannis defoliaria, and the March moth Alsophila aescularia (fruit, forest, ornamental trees and shrubs); lackey moth, Malacosoma neustria, and vapourer moth, Orgyia antiqua, against which a limited effect has been obtained in France (Martouret and Milaire, 1963) (fruit, hawthorne, ornamental trees and shrubs); clouded drab moth, Orthosia incerta (apple); ermine moths, Yponomeuta cagnagella (ornamental spindle trees), Y. evonymella (bird cherry) and Y. rorrella (willows). If it proves susceptible, the apple leaf skeletonizer, Eutromula pariana, might prove a particularly suitable insect because young larvae feed on the upper epidermis of leaves.

Protected crops

B. thuringiensis is effective against a number of cutworms which are very difficult to control with chemicals. The Mediterranean climbing cutworm, Spodoptera littoralis, attacks chrysanthemums in the Mediterranean area and is moderately susceptible. My recent work on the silver Y moth, Plusia gamma, indicates that this occasional pest of chrysanthemums is in the same category and the comparatively high cost of material may be acceptable in this high value crop. The susceptibility of the angleshades moth, Phlogophora meticulosa, the carnation tortrix, Cacoecimorpha pronubana, should also be established for use where biological control of other pests is contemplated.

Stored products

B. thuringiensis might be useful in a few situations where chemicals have failed to control moths in a pest complex, or have actually increased moth problems by killing natural enemies and eliminating such beetle pests as the flour beetles, Tribolium spp., which preyed on moth eggs and pupae, as in maize in Kenya. The continuing improvement of B. thuringiensis products might make this economical, but the desirability of adding spores of a bacterium that can reproduce saprophytically to raw foodstuffs like grain awaits assessment.

Honey bee hive

B. thuringiensis controls the greater wax moth, Galleria mellonella, in beecomb where contact insecticides cannot be used (Table 2; Burges and Bailey, 1968). Its value lies in its harmlessness to both bee and man, and the ease with which it can be incorporated into beeswax foundation in the factory. However, products now available commercially have proved unstable in the hive and the future depends on our current attempts at the P.I.L. to overcome the problem.

AMOUNT OF USE AND THE FUTURE

Very little or no B. thuringiensis is used commercially in the U.K. The greatest usage occurs in the U.S.A., where many years of costly research by industry, universities and government laboratories began to show a return in 1965, particularly for the control of the tobacco hornworm, tobacco budworm and cabbage looper (Table 2). For instance, it is gradually replacing chemicals against the tobacco budworm in N. Carolina. In 1965 and 1966 it was used on more than 60 per cent of the lettuce in Arizona and on more than 50 per cent of brassica acreage on the Southern Californian coast, also extensively on cauliflower on Long Island, N.Y. and on melon crops in several states. Usage in the U.S.A. is still growing. Exports from the U.S.A. are probably no more than 5% of total production and more or less equally divided between Europe, the Middle East and South America, with some to S.E. Asia, although it is doubtful whether any one area is using more than $100,000 worth. Franz et al. (1967) report its use to save valuable oak stands in Germany.

The future looks bright. It seems likely that the use of B. thuringiensis will continue to increase as products are improved and as integrated control, in which the bacterium is particularly valuable, becomes more widely practiced. Fundamental and applied research is active and there is a hope that study of the unique toxins may reveal some basic, important new approach to insect control.

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CONTROL OF INFESTATION IN STORED GRAIN BY AIRTIGHT STORAGE OR BY COOLING

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Summary Insect infestations in stored products can be controlled and mould growth prevented by storage in airtight containers. Dry grain is little affected by the storage, but changes occur in grain of over 17% moisture content that limit its use to animal feed. In structures with slight leaks light infestations can survive, and in damp grain there may be some mould growth, which can present hazards under practical conditions.

The life cycles of the important granivorous insects are interrupted by cooling the grain to below 17°C. In practice, in temperate climates, selected ambient air is forced through bulks of warm grain using low-power fans and widely spaced ducts to attain temperature of 5-10°C. Damp grain is also preserved by cooling but mould growth and mite infestations sometimes occur.

INTRODUCTION

The use of chemical substances - insecticides, fungicides - is and probably will remain the chief means of controlling harmful organisms, especially in field crops, where the pests are distributed over a wide area. Where the pest is more limited in its range, as are insects, mites and moulds attacking stored products, it is possible to achieve control by altering the environment so that the pests are either unable to survive or unable to multiply.

Two promising methods of non-chemical control are airtight storage, in which the insects and moulds die or become inactive through lack of oxygen, and cooling, in which the temperature of the grain is reduced to a level at which the development of the pest ceases or becomes so slow that damage does not occur. The two types of storage are described separately.

AIRTIGHT STORAGE

METHOD

Tests at the Pest Infestation Laboratory have been carried out on a laboratory scale in cans and jars and in larger structures, including bitumen-treated concrete pits, welded and bolted metal silos and flexible silos made of plastic or elastic material, supported in a metal cage. In the tests with insects on dry grain, measurements were made of the oxygen and carbon dioxide inside the container, populations of insects having been added at the time the grain was put into store. With damp grain, additional observations were made of the state of the grain, including presence of mould, germination, and certain chemical properties.

RESULTS (AIRTIGHT STORAGE)

Dry grain

At the moisture contents normally considered suitable for open storage, i.e., not exceeding 14%, there was little change in germination and other properties

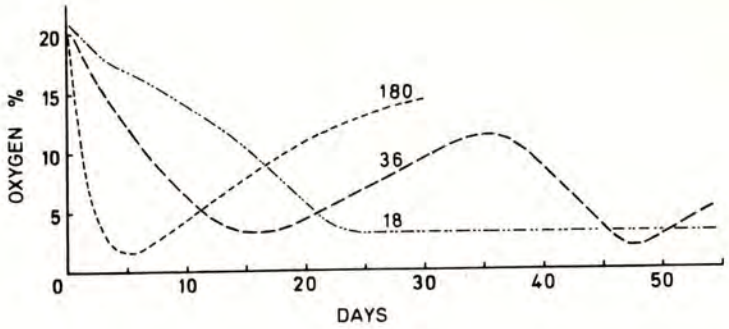

Figure 1. Concentration of oxygen in 1600 ml containers of dry wheat with a "standard" leak with 18, 36 and 180 adults of Sitophilus granarius per container.

during storage in an airtight container.

In infested grain, the oxygen in the intergranular air was used up by the insects, and carbon dioxide produced, at a rate which varied with the density of the insect population. When the concentration of oxygen fell to about 2% the insects were killed and, in a completely airtight container, the oxygen remained at the same low level. Tests with "controlled leaks" (Oxley, T.A. and Wickenden, G., 1963) showed (Figure 1) that whereas heavy infestations were killed, with subsequent re-enty of oxygen, with smaller numbers of insects some survived and continued as a low population in the non-lethal concentration of oxygen that was maintained by the leak. Further tests have shown that if the entry of oxygen cannot be entirely prevented, but is greatly restricted, i.e., to less than 0.5% of oxygen per day, the structure can give satisfactory results in practice, although there may not be 100% mortality of the insects.

Damp grain

When wheat or barley at moisture contents ranging from 16 to 24% was stored in sealed containers, the respiration of the grain and associated micro-organisms used up the oxygen and produced carbon dioxide. Anaerobic activity followed, resulting in further production of carbon dioxide, to an extent that depended on the moisture content of the grain. Although the moist grain remained bright and free-flowing, and without visible mould, it developed a characteristic sour-sweet smell and bitter taste, not always removed by subsequent drying (Hyde, M.B. and Oxley, T.A., 1960). Chemical changes affecting mainly the sugars and gluten made the grain unacceptable for bread-making, and the viability was reduced (Hyde, M.B. 1965), so that after a few weeks storage grain above about 20% moisture content was suitable only for animal feed.

At the time of the early experiments there was little interest in this aspect of using damp grain. A few years later, however, the practice of feeding rolled barley to livestock became widespread, and later experiments were carried out in "farm" silos, of bolted metal construction or of flexible materials such as butyl rubber, polythene or polyvinyl chloride.

In commercial metal silos the pressure resulting from the production of carbon dioxide is relieved by a valve or other device, and the high concentration of carbon

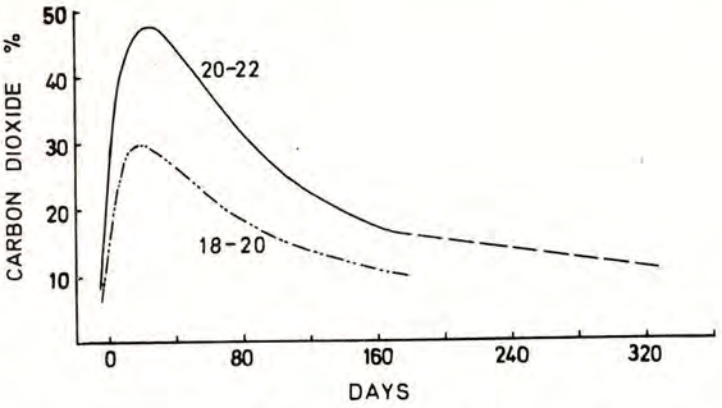

Figure 2. Concentration of carbon dioxide in a 60-ton bolted metal "sealed" silo, with barley at the moisture contents (%) shown.

dioxide was, therefore, not maintained (Figure 2). During the period when a silo was being emptied (dotted part of upper curve), the oxygen entering as grain was removed enabled the concentration of this gas to rise from its former level of 0.5 - 1.0% to over 5%, a concentration at which some moulds can grow.

These changes in the concentration of the intergranular atmosphere corresponded to the succession of micro-organisms found on the grain by the Laboratory's mycology unit (Clarke, J.H. et al., 1966, 1968). The microflora present at harvest (Clarke, J.H., 1968) declined early in the storage period, the decrease being more rapid the higher the moisture content. Then followed a phase characterised by few species, composed almost entirely of yeasts (Candida and Hansenula spp.), which persisted for 9-12 months. The fourth phase included the appearance of typical "storage fungi" (sensu Christensen, C.M., 1957). A fifth stage, involving "heating" of the grain, occurred in some of the silos examined and included fungi potentially pathogenic to animals , e.g. Aspergillus fumigatus, and Thermoactinomyces vulgaris, one of the organisms found to be responsible for farmer's -lung disease in mouldy fodder (Lacey, J., 1969).

DISCUSSION AND CONCLUSIONS (AIRTIGHT STORAGE)

Airtight silos are little used in this country for storing dry grain, although there is no reason why they should not be. They are of particular value in tropical countries, for long-term reserves. In Argentina, where pit-storage was developed extensively in the 1940s for war-time surpluses, about 2 million tons of grain are stored in airtight pits. It has been reported (Hall, D.W. & Hyde M.B., 1954) that wheat was removed in good condition after 7 years storage, during which time it received little or no attention. Within the last year seventy 1000-ton concrete semi-underground silos, of a type used in Cyprus for the past 14 years, have been erected in Kenya for storing reserve stocks of wheat and maize. Modern developments in butyl rubber silos have indicated that these could be useful for dry grain in tropical countries, if problems of migration of moisture can be overcome.

It is however with damp grain that there are more problems to be solved. It has been suggested that addition of carbon dioxide on filling may reduce the fermentation, but this has still to be confirmed; at present, it seems preferable to keep the method as simple as possible.

Much still remains to be found out about the nutritional properties to livestock of the slightly fermented grain, including the claims of increased palatability. In commercial silos it is very difficult to prevent some mould development, particularly as the grain is withdrawn. With year-round feeding to livestock, work is urgently needed on the possibly insidious, long-term effect of prolonged feeding of slightly mouldy grain.

COOLING

Damp grain is readily attacked by moulds, mites and insects but only insects will attack grain that has been dried. There is, however, an increasing number of farmers who store damp grain by cooling it with a small fan and widely-spaced air ducts, thus avoiding the high capital and power costs normally associated with drying but increasing the risk of attack by moulds and mites. The aim of the work described here was to observe changes occurring during ventilated storage and so to find the limitations of the method and the highest moisture content for safe farm storage with least expenditure of capital, labour and power.

METHOD (COOLING)

The investigations since 1960 have included laboratory experiments under controlled conditions, pilot-scale tests at the laboratory in bins of 8-20 tons capacity and also observations in farm and commercial stores from 12 tons to 30,000 tons. The tests covered ranges from -6°C to 20°C and from 12 to 30% moisture content. The bins of grain were cooled either by passing selected ambient air or refrigerated air through the grain. Only biological effects of ventilation are described in the present paper, although the work included engineering aspects, assessment of chemical changes and physical conditions such as the establishment of cooling patterns and changes in moisture profiles.

RESULTS (COOLING)

Effect of cool storage on insect populations

A. Two 8-ton bins of grain were artificially infested with adult Sitophilus granarius L. grain weevils (Burrell, N.J. and Laundon, J.H.J., 1967) and were kept for six months at a temperature range of 0-18°C (mean 7°C). During this period 97% of the insects migrated from the bins; 2.5% were found dead and only 0.5% survived the test within the bin. No breeding occurred.

B. A 100-ton bin of barley (Burrell, N.J., 1967) heavily infested throughout by Oryzaephilus surinamensis (mean infestation 300 insects per kg of grain) was ventilated during the winter. During aeration the maximum temperature was reduced from 38° to 16°C, so preventing breeding and limiting further damage. After ventilation ceased the infestation caused slight rewarming in areas remaining above 10°C but no further heating occurred in grain at a temperature of 8-9°C. The grain was finally fumigated to destroy the infestation.

In many industrial grain stores where insect infestations have previously arisen frequently, the introduction of a cooling system has normally prevented their development (Burges, H.D. and Burrell, N.J., 1964). Residual infestations in brickwork and crevices usually disappear during the first season of ventilation. Three exceptions have, however, occurred but these were due to inadequate ventilation and could have been avoided. In one, no ventilation was carried out until December, when an infestation had already appeared; in another instance air was lost through a bulkhead of sacks instead of passing up through the grain and, thirdly, at one grain store the ventilation system was planned to cool a grain heap one foot deep at the sides, but the following year the store was loaded to 12 ft deep at the sides without any adjustment to the duct system. Although aeration of bulk grain produces sufficient cooling to prevent insect infestations arising, ventilation cannot

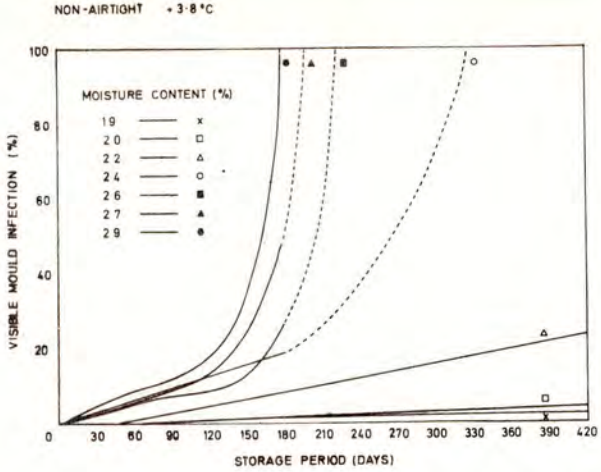

Figure 3. Percentage of grains visibly infected by mould colonies in samples of grain stored at 19-29% moisture content and 3.8°C in non-airtight glass tubes.

be used to destroy an existing infestation.

Effect of cool storage on mould growth in damp grain

Over the past 5-6 years it has often been stated, particularly by those who market grain refrigeration machinery, that grain cooled to below 10°C would not support mould growth, irrespective of the grain moisture content. Some published data were available to contradict such statements but the extent and rapidity of mould development had not been established. These factors have been determined by counting the percentage of grains visibly infected by mould colonies under controlled laboratory conditions and also in farm and other bins at wide ranges of moisture contents. Figure 3 shows an example of the rate of infection on grain at 19-29% moisture content and at 3.8°C. The main attack was by Penicillium spp. (Burrell, N.J. et al., 1965).

Storage at -6°C prevented visible mould growth on grain samples of up to 29% moisture content for over 6 months. Some anaerobic activity also occurred at -6°C as was shown by the increase in pressure and build-up of carbon dioxide in a series of airtight tubes. Comparisons made between mould growth in experimental bins of grain and in unsealed tubes buried within the grain bulks showed some differences, the mould growth in tubes being rather more rapid than in the bulks. It was also noticed that the moisture content of damp grain increased during storage in glass tubes where CO_2 was permitted to escape but where loss of water vapour was severely restricted by a polythene closure (Burrell, N.J. et al., 1968). After 6 months storage there was a direct relationship between the percentage of visibly infected grains and the initial moisture content (Table 1).

Table 1

Moisture increase, percentage mould infection, estimated dry weight losses and mean heat production in barley stored in tubes buried in a ventilated grain bulk for 6 months at temperatures reducing from 17°C to 5°C

Initial moisture content (%)	18	20	21	22	23	24	25	26
Moisture increase (%)	0	0.2	0.6	1.1	1.8	2.8	3.8	5.0
Infected grains (%)	0	5	10	15	20	25	30	34
Dry Weight loss (%)	0	0.3	1.0	2.0	3.2	4.7	6.5	8.5
Mean heat production (kcal/ton/day)	-	65	194	356	583	907	1,230	1,620

By investigating hundreds of samples taken at intervals from experimental and farm grain stores we arrived at the following conclusions:- under British conditions a musty odour due to mould activity usually occurs on grain over 18% moisture content after 6-8 months ventilated storage. The development of the odour is sometimes delayed by prolonged or frequent periods of ventilation. A low temperature alone does not prevent a musty odour, which occurs whether or not mites are present. At moisture contents of 20-21%, visible mould colonies usually appear on a small percentage of grains. Further mycological investigations are needed to identify the fungi growing at temperatures of 0-15°C and veterinary trials are also necessary to decide whether or not it is safe to encourage the use of this cheap and simple storage method for damp grain.

The effect of cool damp storage on infestation by storage mites

Our tests on farms led to the assumption that storage mites have always existed in small to large numbers in undried grain or in grain dried slowly in bins or on the floor. Although the problem is by no means new, present practices, e.g. low rate ventilation and low temperature drying, may increase the frequency or density of infestation. Work by Szwabowicz, A. et al. (1957, 1958) suggested that storage mites are not a serious hazard to animal health. However, further veterinary investigations are essential, for although infestations of storage mites are often reported to be associated with ill-health amongst stock, there is as yet no prima facie published evidence to show that mites, and not moulds, were the cause of loss of condition. The aim of our work on density and distribution of mites is to estimate the damage caused by them in grain bulks and to provide information for future veterinary tests which may conceivably set tolerance levels for mite populations.

Viability and dormancy of ventilated grain

The combined effect of temperature and moisture content on the viability of cereal grains has been studied by numerous workers (e.g. Kreyger, J. 1958, 1959). Our work has aimed at extending the existing information to higher temperatures applicable to tropical conditions (Burges, H.D. et al., 1963) and to lower temperatures for storage of damp grain under refrigerated or ventilated conditions (Burrell, N.J. et al., 1965). The dormancy of dried malting barley has also been studied. The dormancy period was found to be extended for long periods when the barley was cooled rapidly after drying, making such grain unsuitable for the malting process. To avoid prolonged dormancy it is now usual to store the dried barley in a warm condition for 2-3 weeks before cooling is started. Extended dormancy is also found with undried and ventilated damp grain, but the natural dormancy may be superseded by a secondary dormancy or "water-sensitivity" which may be due to mould growth.

DISCUSSION AND CONCLUSIONS (COOLING)

Ventilation of grain at a low rate of airflow (0.1-0.2 m^3/m^3grain/min) can affect the organisms within a grain bulk by altering the environmental temperature and possibly the relative humidity of the micro-climate between grains. Whether such air movement affects the behaviour of the organisms directly is uncertain although we hope to determine which of the environmental factors is responsible for the migration of Sitophilus granarius and other arthropods from cooled bulks of grain.

The number of mites occurring in a grain bulk seems to be reduced by frequent ventilation, irrespective of any cooling or drying that occurs. In the event of future tolerance levels for mite infestation being established, possibly as a result of veterinary trials or by the amount of damage produced by the mites, it is hoped that our work will enable farmers to keep infestation below these levels cheaply and simply.

If, in the future, the use of insecticides is restricted for any reason, cooling may be a practical means of preventing infestations, and in tropical countries this may involve some use of refrigeration. Work at the Laboratory has shown that the advantages of using refrigeration as a means of storing damp grain under temperate conditions are only marginally greater than those of ventilation with selected ambient air, and of course refrigeration is far more costly. However, the use of refrigeration for insect control in dry grain in tropical countries may prove to be far more economical since rapid cooling is not essential and, once cool, the low thermal conductivity of the grain should help to maintain a low temperature, particularly in large bulks.

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STORAGE LOSSES OF APPLES IN THE WEST MIDLANDS DURING 1968-69

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Summary A survey of storage losses of apples was carried out during 1968-69 at a large Herefordshire store and packing plant. Weekly visits were made to the plant to examine representative samples of fruit rejected as unsaleable during the normal grading procedures. An assessment was made of total losses and of major factors responsible for rejection of fruit.

Samples from twenty-seven consignments of fruit were examined. 8.4% of the total bulk of these consignments was rejected. The mean loss was found to be 10.3% and individual losses varied from 1.3% to 40.4%. The most important causes of rejection were russeting and weather injury in cold-stored fruit, Gloeosporium and other storage rots in fruit from scrubbed, gas stores.

Results of a field trial in 1968 suggested that dichlorophen applied three times at low concentration can give better control of Gloeosporium rot than an equivalent number of late-summer captan sprays.

INTRODUCTION

During the last ten years the growth in number and capacity of both refrigerated and controlled-atmosphere stores has been a major factor in the development of the apple industry in the West Midlands. The adoption of modern storage techniques has provided the opportunity for growers to store their produce for four or five months prior to marketing it. Significant losses of fruit have been shown to occur during prolonged storage as a result of both disease and various forms of damage.

On visits to Herefordshire during 1967 and 1968 attention was drawn to the lack of detailed information on storage losses in the county. The present survey was designed to acquire this information. The survey was carried out at a large store and packing plant at Ledbury, Herefordshire. The plant is part of a co-operative involving twenty-four growers and nine hundred and forty acres of apples in Herefordshire, Worcestershire and the Newent area of Gloucestershire.

The aim of the survey was to examine consignments of stored fruit from various farms and orchards, to assess losses of apples during storage and to determine the major factors responsible for these losses. It was hoped that a survey of this nature would identify problem orchards and act later as a springboard for advisory work.

METHOD AND MATERIALS

Survey Weekly visits were made to Ledbury from 24th September to 6th March. On each visit, representative samples of rejected fruit from consignments graded on that day were examined. Rejected fruit consisted of unsaleable, completely rotted fruit, "culls" (partially damaged or rotted fruit, often assigned to cider

production) and under-sized apples. On any one visit to Ledbury three to five hundred rejected fruit were examined from each consignment and the reasons for rejection of individual apples recorded. In assessing an apple affected with two or more diseases or forms of damage, each factor was recorded and considered to be equally responsible for its rejection and loss.

At the time of each visit the total weight of an examined consignment and the weight of fruit rejected from this bulk were recorded. Results were finally expressed as the percentage of a consignment rejected due to each disease or form of damage. Weekly results were issued to the management of the co-operative and to growers whose fruit had been examined.

Experimental Following reports of heavy losses of fruit due to Gloeosporium rot at Ledbury during 1967-68 a spray trial was carried out in a commercial orchard at Tillington, Herefordshire in 1968 to attempt control of this disease. The orchard was of Cox's Orange Pippin trees, approximately twenty years old. Five treatments were employed, each treatment applied to a single plot of twelve trees. The treatments were as follows:-

1. 1% dichlorophen applied at early pink bud (30th April).

2. 0.5% dichlorophen applied at early pink bud + 0.25% dichlorophen applied 14 days after petal fall (12th June) and again 28 days after petal fall (26th June).

3. As treatment 2 above + captan 2 lb a.i./100 gal applied three times (2nd August, 22nd August and 5th September).

4. Captan 2 lb a.i./100 gal applied three times as in treatment 3 above.

5. Control, not sprayed for Gloeosporium.

Fruit was picked on 1st October and stored in a scrubbed, gas store. Five hundred apples from each treatment were examined for Gloeosporium rot on 7th March, 1969.

RESULTS

Survey During storage season 1968-69 samples of twenty-seven consignments of fruit were examined. These consignments represented a bulk of 567 tons of apples. Sixteen of the consignments were of Cox's Orange Pippin, the remainder of Egremont Russet, Laxton's Fortune, Laxton's Superb, Lord Lambourne, Spartan and Worcester Pearmain. Cold-stored fruit were examined up to 20th November, all subsequent consignments were from gas stores.

The mean losses of fruit due to various disease and damage factors are given in Table 1. Losses from individual consignments and the major reasons for rejection of early marketed cultivars are given in Table 2 and of Cox's Orange Pippin in Table 3.

The overall loss of fruit through rejection was 8.4% by weight of the twenty-seven consignments. The mean loss was 10.3% and losses from individual farms or orchards varied from 1.3% to 40.4%.

The most important factors responsible for rejection of apples were russeting and weather injury in cold-stored fruit, Gloeosporium and other storage rots in fruit from gas stores.

Table 1

Mean percentage loss of fruit during 1968-69

Condition	Mean % of twenty-seven consignments of fruit
Marketable fruit	89.7
Rejected fruit	10.3
Losses of fruit due to:-	
Bitter pit	1.2
Gloeosporium rot	2.0
Pest damage	0.7
Pre-storage mechanical damage	0.7
Rots (Brown rot, Botrytis and Penicillium rots)	2.7
Russeting and cracking	2.0
Scab	0.2
Weather injury	1.6

Weather injury consisted of frost and hail damage. Hail damage in 1968 was a result of severe hailstorms which affected many orchards around Hereford during the first week of July. With the prospect of rapid deterioration during storage most fruit from hail-affected orchards was marketed by mid-November.

Russeting and cracking caused losses ranging from 0.2% to 18.5%. Russeting was most severe in and around the stalk cavity. From early December onwards a marked association was noted between stalk end russet and development of Gloeosporium rot.

Bitter pit commonly accounted for losses of between 1% and 3% of individual consignments although the very wet growing season in 1968 did not favour the disorder. Other physiological conditions such as core flush, low-temperature breakdown and senescent breakdown were only rarely recorded and were of no significance in rejection of fruit.

Losses as a result of pest damage varied from nil to 2.7%. These losses were due mainly to damage by birds, dock sawfly (Ametastegia glabrata), earwig (Forficula auricularia) and tortrix caterpillars (mainly Archips podana and Pammene rhediella).

Figures for mechanical damage incurred prior to storage were generally low but much damage will inevitably have been masked by secondary rots.

Scab (Venturia inaequalis) and eye-rots due to Botrytis cinerea and Nectria galligena were found at low levels in almost all samples of fruit examined, but were of little importance as causes of rejection.

Brown rot (Sclerotinia fructigena) and secondary rots, mainly Botrytis and Penicillium, were the most important storage diseases of early-marketed cultivars. Gloeosporium rot was the major disease of Cox's Orange Pippin.

Experimental Results of the orchard trial at Tillington to attempt control of Gloeosporium are presented in Table 4.

Table 2

Storage losses of early-marketed cultivars 1968-69

Date of visit	Total loss as % of consignment	% loss due to: Bitter pit	Gloeosporium rot	Pest damage	Russeting and cracking	Rots (Brown rot, Botrytis, Penicillium)	Weather injury	Other factors
Egremont Russet								
Nov. 20th	10.2	5.5	0	0.7	0	0.5	0.2	4.8
Nov. 20th	6.0	2.4	0	0.2	0	0.2	0.1	4.3
Laxton's Fortune								
Dec. 18th	2.6	0	0.2	0.1	0.2	1.4	0.5	0.8
Laxton's Superb								
Oct. 23rd	8.1	0	0	0.7	0.4	0.2	0.8	6.0
Lord Lambourne								
Oct. 9th	7.5	0.4	0	0.5	3.5	0.2	2.2	1.3
Oct. 16th	13.7	2.9	0	1.5	3.6	2.0	3.1	4.1
Oct. 16th	15.7	0.2	0	2.4	2.6	4.8	8.7	4.4
Nov. 27th	9.1	0.2	1.4	0.8	1.8	1.3	2.6	2.9
Spartan								
Dec. 4th	1.3	0	0	0.2	0	0.3	0	0.8
Worcester Pearmain								
Sept. 24th	10.7	0	0	1.3	4.4	0	3.1	1.3
Oct. 30th	9.3	0.3	0	0.3	1.5	1.0	1.1	5.4

Table 3

Storage losses of Cox's Orange Pippin 1968-69

Date of visit	Total loss as % of consignment	% loss due to						
		Bitter pit	Gloeosporium rot	Pest damage	Russeting and cracking	Rots (Brown rot, Botrytis, Penicillium)	Weather injury	Other factors
Nov. 6th	5.3	0.2	0.1	1.0	0.8	1.3	1.9	1.5
Nov. 13th	6.2	2.1	0	0.7	1.7	1.7	0.6	1.3
Dec. 12th	40.4	0.5	17.8	2.7	18.5	2.5	9.4	10.5
Dec. 18th	12.9	1.5	2.2	0.7	0.7	6.2	0.1	2.5
Jan. 1st	3.6	1.8	1.3	0.3	0.5	1.5	0.7	0.8
Jan. 8th	3.8	0	0.3	0	0.2	0.6	0.1	2.8
Jan. 15th	9.9	2.8	1.2	0.8	1.2	3.3	0.7	5.5
Jan. 15th	19.4	1.8	1.3	1.2	1.0	12.4	0.2	1.9
Jan. 22nd	6.1	1.4	2.3	0.2	1.6	1.3	2.4	0.7
Jan. 22nd	2.4	0.1	0.1	0	0.1	0.4	0.1	1.8
Jan. 28th	5.1	0.3	1.3	0.1	0.6	2.6	1.3	0.8
Feb. 19th	21.0	3.0	4.5	0.2	1.5	9.5	0.3	2.3
Feb. 19th	9.0	0	4.2	0.3	1.9	4.1	0.1	1.2
Feb. 25th	5.6	0.2	0.6	0.8	0.6	3.5	0	1.7
Feb. 25th	19.7	2.4	9.9	0.9	2.7	5.5	3.3	1.5
Mar. 6th	12.4	1.1	6.1	0.6	2.1	4.1	0.6	1.4

Table 4

A comparison of captan and dichlorophen for control of Gloeosporium

Treatment	% fruit infected with Gloeosporium
1. 1% dichlorophen	13.0
2. 0.5% dichlorophen + 2 sprays of 0.25% dichlorophen	6.8
3. As 2 above + 3 sprays of captan at 2 lb a.i./100 gal	5.2
4. Three sprays of captan at 2 lb a.i./100 gal	10.0
5. Control, not sprayed for Gloeosporium	12.4

Three applications of dichlorophen were more effective in controlling Gloeosporium than an equivalent number of captan sprays. The most effective control was obtained when three dichlorophen sprays were reinforced by a similar number of late-summer applications of captan.

DISCUSSION

The mean loss from consignments of fruit examined in the West Midlands during 1968-69 was 10.3%. The mean loss of Cox's Orange Pippin during the same period was 11.4%. These figures are very much higher than equivalent losses recorded by N.A.A.S. plant pathologists in a national survey carried out during 1961-65 (Preece, 1967). The highest national mean loss recorded for Cox's in that study was 7.4% in 1963-64.

Although the mechanics of storage have been greatly improved in the last decade, it is apparent from the results of the present survey that serious losses of fruit still occur under optimum storage conditions. These losses undoubtedly account for substantial reductions in the profitability of many orchards. They also focus attention on the need for more effective control of disease in the orchard, and for the reduction of damage to fruit as a result of russet and pre-storage handling.

The survey confirmed the importance of Gloeosporium as the major storage disease of Cox's Orange Pippin in the West Midlands. At present many of the Herefordshire orchards are relatively young and not severely infected with the disease. In the absence of an effective form of chemical control there is every likelihood of the disease building-up to serious proportions as the trees age.

Although one year's data must be viewed with caution, results of the orchard trial at Tillington show that it is possible for early summer applications of dichlorophen to reduce Gloeosporium rotting. A similar control of the disease to that achieved by Corke, Edney and Hamer (1965) was obtained when a dichlorophen programme was reinforced by late-summer applications of captan. The trial is to be

repeated in similar form in 1969. In addition, the performance of captan and dichlorophen will be compared in a number of orchards identified as having a Gloeosporium problem in the 1968-69 survey.

Acknowledgments

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A METHOD OF ASSESSING ROSE MILDEW, (SPHAEROTHECA PANNOSA) ON ROSA MULTIFLORA ROOTSTOCKS

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Summary Rose mildew, Sphaerotheca pannosa can cause stunting of Rosa multiflora seedlings resulting in a loss of crop when grown as rootstocks. Infected transplanted rootstocks can act as a source of infection in rose nurseries. Assessment of infection using a rating scale 0 - 5, no infection to severe infection, was not regarded as satisfactory. Assessment of infection and fungicidal control by measuring shoot length, shoot infection, leaf and leaflet infection showed that leaf and leaflet measurements were best and that since they were closely correlated no benefit was gained by going to the extra trouble of measuring leaflet infection.

INTRODUCTION

The production of rose bushes depends on budding cultivated varieties on species rose rootstocks. It has been variously estimated in the popular press that more than 40 million rose rootstocks are imported into Great Britain each year and that commercial rose growers are entirely dependent on these imports. Research has been initiated into the possibility of producing suitable rose rootstocks in Great Britain and among the problems identified in N. Ireland was the extensive infection of Rosa multiflora rootstocks with rose mildew, Sphaerotheca pannosa. Examination of 12,000 home produced plants at the Horticultural Centre, Loughgall showed only 200 not infected with rose mildew and in the following year only 16 of the 200 remained uninfected.

Rose rootstocks are produced from seed as an annual crop and being woody plants require sustained growth for as long as possible. Infection by mildew at between the first and fourth true leaf stage can cause permanent stunting and complete failure of the crop. Infection after this stage appears to reduce the vigour of the seedlings and hence the number of saleable rootstocks. As a result of infection in the rootstocks the disease can be carried over to the rose fields in the following year. The leaves of seedling Rosa multiflora are softer in texture and appearance than those of modern hybrid tea or floribunda roses which are reputed to have been bred from mildew resistant parents. Infected leaves at first show discrete patches of almost translucent white to grey mycelium which enlarge and coalesce becoming opaque and more white than grey. With the production of conidia the infected leaves take on the typical white powdery appearance of powdery mildew infection. Extension growth continues from apparently uninfected apical buds but infection appears within a few days of the leaves expanding. Cleistocarps form in the white, felted mycelium on the stems and bases of spines, producing ascospores in the following year.

Screening of a wide range of fungicides produced no effective control of rose mildew on Rosa multiflora rootstocks (Cartwright 1965, 1966, 1967). It did show however, that assessment using a rating scale from 0 - 5, no infection to very severe infection, was not a satisfactory method because in assessing the degree of infection of the experimental plots, 3 yd by 1 yd, the picture presented was of extensive infection where the mildew altered the basic overall green colour of the leaves to a grey/green colour. Fungicidal control could only be measured in terms of shades of grey/green on the assumption that the more green the plots appeared the greater the degree of mildew control. Where the assessment was made on between

thirty and forty plots with very slight differences in treated plots compared with the untreated control the assessors very quickly became confused in differentiating the degree of infection.

This paper describes possible alternative methods of assessing mildew infection on Rosa multiflora seedling and transplanted rootstocks.

METHOD AND MATERIALS

Seedling rootstocks were produced in two rows 6 in apart on flattened ridges at 26 in centres. The experimental design consisted of 3 yd long plots with six treatments replicated five times in randomised blocks, see Table 1.

The transplanted rootstocks were planted 6 in apart in rows 2 ft 6 in apart and designed to give a randomised layout of eight blocks of six treatments, see Table 2. Fungicide treatments were applied to the point of run off by a manually operated, pressure-retaining, knapsack sprayer, on 1, 8, 17 and 25th July, 2, 8, 20, 28th August and 4th September.

Records were made on 28th August to 4th September, from 50 shoots selected at random in each treatment plot, in terms of length, whether the shoot was infected or not, the number of leaves infected in the first three fully expanded leaves and the number of leaflets infected in the first three fully expanded leaves.

Treatments

1. Control, no spray.
2. Milcol, 40 percent col. at 2 pt/100 gal
3. Karathane, 25 percent W.P. at 2 lb/100 gal
4. M 2452, 50 percent W.P. at 1 lb/100 gal
5. W 1263, 20 percent M.L. at 1 pt/100 gal
6. Benlate, 50 percent W.P. at 0.5 lb/100 gal + Surfactant F.
7. - 12. As above + dimethyl sulphoxide 5 percent V/V.

RESULTS

Table 1

The Effect of Rose Mildew Infection on Rosa Multiflora Seedlings

Treatment	Mean Shoot Length Per 50 Plants in Inches	Mean Numbers with Infection* Shoot	Leaf	Leaflet
Control	327.3	50.0	145.2	819.6
Milcol	360.9	49.8	129.6	676.0
Karathane	337.0	45.0	123.2	650.8
M 2452	395.8	50.0	139.6	738.0
W 1263	368.5	46.4	92.8	381.0
Benlate	362.5	50.0	138.2	735.0
Control + DMSO	314.5	45.0	128.6	707.0
Milcol + DMSO	376.1	50.0	129.4	679.6
Karathane + DMSO	373.4	50.0	141.4	740.6
M 2452 + DMSO	371.1	50.0	134.8	691.0
W 1263 + DMSO	393.3	47.4	91.4	406.8
Benlate + DMSO	296.7	44.0	116.4	560.6
L.S.D. P = 0.05	94.88	10.25	30.88	255.56

*Mean of five replicates

Table 2

The Effect of Rose Mildew Infection on Rosa Multiflora Transplants

Treatment	Mean Shoot Length Per 50 Plants in Inches	Mean Number with Infection* Shoot	Leaf	Leaflet
Control	658.6	19.6	150.0	870.1
Milcol	625.8	18.7	119.8	582.5
Karathane	634.6	19.7	160.2	920.7
M 2452	635.7	19.3	128.7	673.6
W 1263	632.7	17.7	90.0	384.7
Benlate	615.6	19.7	144.7	767.1
Control + DMSO	641.3	19.3	150.0	920.6
Milcol + DMSO	616.1	18.8	113.5	550.1
Karathane + DMSO	641.6	19.8	150.0	841.0
M 2452 + DMSO	640.5	18.0	115.1	578.0
W 1263 + DMSO	643.3	17.2	79.3	343.6
Benlate + DMSO	612.3	19.2	128.5	622.1
L.S.D. P = 0.05	46.04	2.44	27.11	201.85

*Mean of eight replicates

The only fungicides which showed consistent effect on infection of the leaves and leaflets were W 1263 for seedlings and transplants and Milcol for transplants only. The correlations between the leaf and leaflet assessments were high at 0.94 for seedlings and 0.96 for transplants. Dimethyl sulphoxide was added to the standard spray programmes to investigate if its penetrant properties would enhance the fungicidal properties of the sprays. The addition of DMSO had no significant effect on efficacy of the fungicides.

DISCUSSION

Extension growth of the rootstocks was not continuous but seems to occur in flushes. As the growing season progressed three distinct phases of growth could be observed in both the seedling and transplanted rootstocks. The growing tips consisted of vegetative buds and unexpanded leaves, were bronze/green in colour. The first three or four fully expanded leaves had a uniform green colour and the older more mature leaves were dull and paler green in colour. Infection by rose mildew was first seen as discrete, grey, translucent patches on the newly expanded leaflets. Within a few days these patches increased in size, coalesced and gave the leaflets an overall grey/green appearance. This colouring became evident over the whole crop. No infection was observed on the growing tip unless the plant was stunted in the early stages of growth by mildew. Infection of the stems did not appear until later in the season and was not noticeable until it appeared as white felted mycelium on the internodes and bases of spines. As the leaves matured the grey green colour was lost and the leaves became dull, puckered and showed brown and purple blotches. Infection appeared to be continuous throughout the growing season from June until September with each flush of newly expanded leaves showing the grey green infection. Even when in isolation from possible infection from wild roses which may have been infected with mildew the cultivated seedling and transplanted rootstocks were so severely infected that within ten to fourteen days of the first signs of infection the whole crop changed colour to grey/green. It was not determined if subsequent infections were caused by re-infection from diseased plants within the crop or from the original unknown source or a combination of these.

The results in Tables 1 and 2 showed that the effect of fungicide treatments was not shown by either the length of stem or by the number of stems showing infection. If the effect of the fungicides on infection of the stems had been significant a further qualitative assessment would have been required to account for the wide variation in degree of infection. The quantitative assessment based on leaves and leaflets required a further qualification to account for the loss of the overall grey infection colour. Preliminary examination of the leaflets showed wide variation from no infection through one or two infected patches to occasional overall infection. In view of the rapidity with which infection spreads on leaflets, a leaf or leaflet with one infected patch was recorded as an infected leaf or leaflet. Effective fungicidal control was related to the increased number of leaves or leaflets with no infection.

Correlation between the leaf and leaflet assessments was high enough to justify an assessment based on leaf counts alone, saving considerable time since these are only one-seventh of the leaflet counts.

Acknowledgements

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FURTHER OBSERVATIONS ON THE CONTROL OF BOTRYTIS ROT OF STRAWBERRIES IN THE WEST MIDLANDS

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Summary In 1967 two early sprays of dichlofluanid at the highest rate of application (3 lb 50% w.p.)/100 gal (H.V.) resulted in the best yield of marketable fruit. However, three sprays at the intermediate rate (2 lb 50% w.p.)/100 gal (H.V.) gave the best control of Botrytis in a trial where Botrytis rotting was of low incidence.

In 1968, a year when Botrytis fruit rotting was of high incidence, three or more sprays at the lower and intermediate rates of application (1½ to 2 lb 50% w.p.)/100 gal (H.V.) gave the best disease control and highest yields.

INTRODUCTION

Wiggell & Croxall (1965) indicated the need for field trials on the strengths and times of application of dichlofluanid in order to formulate a programme for Botrytis control in strawberries with no threat of taint or off-flavours in the produce. To this end the trials now reported on were carried out in 1967 and 1968.

METHODS AND MATERIALS

The trials were made on the variety Cambridge Favourite in its 5th and 6th fruiting years. The experimental area contained plants of even vigour in rows 3 ft apart. Treatments were replicated four times in four equal blocks. Plots were approximately 1/200th acre in area and yield data were obtained by cropping the plants in the centre row of each plot.

1. Control - 1 lb/100 gal 25% dinocap.
2. Dichlofluanid - 2 sprays 3 lb (50% W.P.)/100 gal (+1 lb/100 gal 25% dinocap).
3. Dichlofluanid - 3 sprays 2 lb (50% W.P.)/100 gal (+1 lb/100 gal 25% dinocap).
4. Dichlofluanid - 4 sprays 1½ lb (50% W.P.)/*100 gal (+1 lb/100 gal 25% dinocap).

* In 1967, starting at 25% open flower did not allow sufficient time for the intended four sprays to be applied.
In 1968 starting at 5% open flower allowed completion of 4 applications just before the crop reached the 50% "white fruit" stage.

Dinocap was applied each year to all four treatments on the first two dates only. All sprays were applied at the rate of 200 gal/acre.

RESULTS

Table 1.

Yields of Fruit and Incidence of Botrytis

Treatment	1967 Yield (ton/acre)		% Weight of total		
	Total	Marketable	Marketable	Unmarketable Botrytis	Unmarketable Other Causes
1. Control	4.61	4.23	91.76	4.34	3.90
2. Dichlofluanid, 2 sprays 3lb/100 gal.	6.04	5.74	95.04	1.32	3.64
3. Dichlofluanid, 3 sprays 2lb/100 gal.	5.07	4.83	95.27	0.39	4.34
4. Dichlofluanid, *3 sprays 1½lb/100 gal.	5.24	4.96	94.66	1.53	3.81

* In 1967, only three sprays at 1½lb/100 gal were applied.

Table 2.

Yields of Fruit and Incidence of Botrytis

	1968 Yield (ton/acre)		% Weight of Total		
	Total	Marketable	Marketable	Unmarketable Botrytis	Unmarketable Other Causes
1. Control	2.76	0.62	22.29	68.00	9.71
2. Dichlofluanid, 2 sprays 3lb/100 gal.	5.50	2.50	46.35	41.70	11.95
3. Dichlofluanid, 3 sprays 2lb/100 gal.	6.15	3.60	58.32	25.79	15.89
4. Dichlofluanid, 4 sprays 1½lb/100 gal.	5.24	3.50	65.87	15.05	19.08

DISCUSSION

The trials described were carried out in a commercial crop and the results shown in Tables 1 and 2 are therefore quite realistic. Comparison of the figures reveals a striking seasonal difference in the amount of Botrytis rotting. 1967 was virtually a "no Botrytis" year at the time of harvesting the crop; nevertheless, there was a marked increase in the total yields in the sprayed plots - particularly in treatment 2.

This increase in yield indicates a much higher fruit-set in the sprayed plots and it seems reasonable to suggest that the fungicidal sprays reduced Botrytis-induced flower abortion with a consequent boost to the number of fruits reaching maturity.

The 1968 season favoured Botrytis and losses were high even on the most frequently sprayed plots whilst those in the control plots were disastrous.

It is of interest that in 1968 total loss of fruit from causes other than Botrytis rotting was very high and it appears odd that it ranges from 10% in the control to nearly 20% in treatment 4. The explanation would seem to be that the control plots were picked over once only but the greater fruit set in the treated plots necessitated three pickings. Traversing the treated plots three times led to increased mechanical damage by bruising and squashing of the berries which in this season were in a very soft watery condition.

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"Development work with methiocarb in Great Britain"

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Summary Methiocarb, a new molluscicidal carbamate, and some of the results from fundamental experiments and field trials to test its effectiveness in Great Britain are presented. A blue pelleted formulation containing 4% methiocarb was compared with standard metaldehyde pellets in trapping experiments. Significantly greater numbers of slugs were trapped by methiocarb pellets even when the quantity per trap was considerably less than the standard metaldehyde formulation. Some differences in the species composition of the catch were detected. Baiting experiments confirmed that, under damp conditions, many metaldehyde baited slugs were able to recover, whereas the mortality of slugs baited with 4% methiocarb pellets was 94.9% and 100%. Results of experiments to test the effect of methiocarb on earthworms, and other soil fauna suggest that the chemical is unlikely to present any far-reaching hazards to the natural soil population.

Field trials in a range of agricultural and horticultural crops, substantiated methiocarb's efficiency. At the same time wild-life trials and observations indicated that the material should not present a significant hazard to birds. The blue colour is an added safeguard since birds respond least readily to blue and violet, and tests confirmed that the blue dye does not stain plant foliage.

INTRODUCTION

The ever present problem of slugs and snails as pests to agricultural and horticultural crops in Great Britain has already been discussed in this session (Hunter, 1969). In the past, inorganic materials such as copper sulphate and arsenic compounds have been used to control slugs and snails (Anderson and Taylor, 1926; Martin, 1940), but during the last two decades the material of choice has been metaldehyde as a bait formulation. A number of studies have been made into its mode of action and best method of use (Gimingham and Newton, 1946; Stringer, 1946; Thomas, 1948; Van den Bruel and Moens, 1960; Webley, 1962, 1965). An important disadvantage of the material is its uncertainty due to the recovery of slugs in damp conditions. Various additives have been tested by Webley (1962, 1965); aldrin, dimethoate, dazomet and zineb repelled slugs. Only carbaryl, a carbamate, appeared to improve the catch.

The purpose of this paper is to outline the work carried out in Great Britain to develop another carbamate, methiocarb, as a molluscicide. Some of the aspects dealt with below have already been published in some detail (Martin and Forrest, 1969). More recent findings are incorporated here, and the information is as current as possible.

Methiocarb (Bayer 37344)

Methiocarb is the common chemical name for 4-(methylthio)-3, 5-xylyl methyl carbamate. The insecticidal and acaricidal properties of this compound have been described by Unterstenhofer (1962). It is an odourless, white crystalline powder, insoluble in water but soluble in organic solvents. The molecular weight is 225.3, the melting point 121.5°C, and the vapour pressure almost nil. It is stable under normal conditions and temperatures up to about 120°C.

Toxicology

The acute oral toxicity (LD_{50}) to rats and guinea pigs has been determined as

follows: female rats 135 mg/kg; male rats 130 mg/kg; male guinea pigs 40mg/kg (Du Bois and Raymund, 1961). It is considerably more toxic to certain birds, and De Cino (1964) determined LD_{50} values as follows: starlings, 12-15 mg/kg; red winged blackbirds, 11-20 mg/kg; pigeons, 50 mg/kg; ring-bill gull, 5-10 mg/kg. The estimated LD_{50} for hens was 175 mg/kg (Du Bois, 1962) and Zeck (1966, 1967) estimated the LD_{50} to pheasants as 225 mg/kg.

Tests were carried out on various fish by Walker (1964); rainbow trout, goldfish, and black bullheads survived 1 ppm but not 10 ppm.

FUNDAMENTAL EXPERIMENTS

The first slug baiting experiment with methiocarb was prompted by the work of Webley (1962) and Rappel (1959) which indicated the molluscicidal activity of carbaryl. "Home-made" bran baits were compared by placing small heaps of each bait in ten random positions near the edge of a wood (Table 1).

Table 1

Slug baiting experiment, Rowhill, Kent, 1962

Type of bait	Total slugs per 10 traps	
	Agriolimax reticulatus	Arion hortensis
Metaldehyde bran 2%	7	10
Metaldehyde 2% + methiocarb 1% bran	59	27
Methiocarb bran 1%	59	17
Methiocarb bran 3%	92	56

This experiment indicated an excellent molluscicidal effect, but no further experiments were conducted in Great Britain until the advent of a pelleted bait, coded 5622b, containing 4% methiocarb, a blue dye, a conserving agent, and a food material. Tests were then initiated in West Midland gardens using simple traps which consisted of bait covered by a tile, supported by three short pegs, approximately 1-1½ inches from the surface of closely mown swards. Ten traps of each bait were set out in straight lines, 3 ft apart, in areas of reasonable ecological uniformity. Each morning slugs were collected from the traps and numbers and species recorded in the laboratory. The results are given in Tables 2 and 3.

The following slug species were trapped during these experiments: Agriolimax reticulatus, Arion hortensis, A. ater, A. subfuscus, Milax budapestensis, M. sowerbyi, and Limax maximus. Analysis of the results from two of these experiments showed that M. sowerbyi was the only species caught in significantly greater numbers by metaldehyde and consequently constituted a considerably greater proportion of the metaldehyde catch. On the other hand methiocarb caught a significantly greater number of A. hortensis than metaldehyde ($P=0.01$), and the same can be said of the composite numbers of A. ater, A. subfuscus, and A. fasciatus. In one experiment A. reticulatus was trapped in significantly greater numbers ($P=0.05$) by methiocarb than by metaldehyde.

Interesting results were obtained when the slugs taken from the traps in the experiment at Piccadily Orchard (Table 2), were kept in recovery chambers (Table 4).

Table 2

Trapping experiments in gardens - 1968

Mean number of slugs per trap

Type of Bait	Site	Piccadily Orchard Bromyard (10 days)	Cranford Bromyard (5 days)	Piccadily Orchard Bromyard (5 days)
Methiocarb 0.4 g		43.5		52.7
Methiocarb 0.2 g			131.0	47.0
Metaldehyde pellets 10 g		31.0		
Metaldehyde pellets 17.6 g			100.2	
Metaldehyde pellets 28 g				29.5
1% L.S.D.		8.4	28.2	14.1

Table 3

The Knoll, Whitbourne, Worcestershire, 1968

Numbers of slugs in six traps

Type of bait	Day number 1	2	3	4	5	Total
Methiocarb 0.2 g	27	3	13	34	11	93
Metaldehyde pellets (A) 5 g	17	10	3	4	5	39
Metaldehyde pellets (B) 5 g	4	2	0	4	3	13
*Methiocarb 0.2 g	30	27	24	21	12	114
*Metaldehyde 0.2 g	13	4	6	1	4	28
*Carbaryl 0.2 g	5	3	10	5	2	25

Following discussion with Dr. P. J. Hunter an experiment was carried out to try to test the relative attractiveness of a number of methiocarb pelleted formulations including 5622b, and a commercially available metaldehyde pellet. These were applied, to netted plots, with a distribution approximating to that obtained when they are used at the recommended rate per acre. Slugs were released in the evening, at the centre of each plot, and the following morning the distance travelled from the centre point was measured for every slug. Recaptured slugs were placed in recovery chambers so that relative mortalities could be obtained. The results obtained only

with 4% methiocarb pellets (5622b) and metaldehyde pellets are given in Table 5.

Table 4

Results of experiment to assess the mortality of trapped slugs after ten days in recovery chambers, 1968

Type of bait	Number of slugs trapped after 8 days	Number of slugs surviving	% mortality
Methiocarb 0.2 g	410	21	94.9
Metaldehyde pellets 10 g	293	141	51.9

Table 5

Comparison of attractiveness and efficiency of methiocarb and metaldehyde to slugs, 1969

	Mean distance travelled from plot centre (in)	Mean number of slugs recaptured (%)	Degree of attractiveness *		% mortality of recaptured slugs
4% methiocarb pellets	14.7	93.3	6.4		100
Metaldehyde pellets	12.2	82.0	4.8		35
Statistical significance	-	-	NS	1% LSD	51.1

* Degree of attractiveness = $\frac{\text{Mean distance travelled}}{\text{Mean number of slugs recaptured}}$ (%)

Other fauna

In all trapping experiments other fauna were killed in small numbers by methiocarb including: earthworms (Lumbricidae), beetles (Carabidae and Staphylinidae), harvestmen (Phalangidae), leatherjackets (Tipulidae), and woodlice (Isopoda). In a field trial a dead field mouse (Apodemus sylvaticus) was found.

Simple tests were carried out in order to study the ecological significance of the effect upon earthworms observed in trapping experiments and field trials. The numbers of earthworms lying on the surface of treated areas were recorded for an arbitrary number of days. An estimate of the population using a solution of potassium permanganate indicated a very small overall effect. Only a rate of 0.8 lb methiocarb per acre produced a significant reduction in the extracted population.

Two laboratory experiments were carried out to study the effect on earthworms of direct contact with methiocarb pellets. Mixed species populations of earthworms were allowed contact with moist pellets for periods of 10, 30 or 120 seconds. They were then placed on the surface of moistened potting compost in closed containers and kept in dim light.

Table 6

Effect of contact with methiocarb pellets on the burrowing ability of earthworms

	Time after contact (hours)	Per cent of earthworms buried in compost 10 sec	30 sec	120 sec	Control
Experiment 1	24	88	48	8	80
	48	88	64	32	84
	168	92	92	76	92
Experiment 2	2	87	47	13	87
	20	93	73	33	93
	92	93	87	87	93

Whilst 10 second contact had little effect, contact for the longer periods of 30 and 120 seconds disturbed the worms' burrowing ability. In the field these would be further immobilised by desiccation and ultra violet light.

Plots of 150 yd^2 were treated with methiocarb pellets at rates equivalent to 0.2 lb, 0.4 lb and 0.8 lb/acre. Potential changes in the population of Collembola, Acarina, and Protura were traced by extracting soil cores using Tullgren funnels.

Table 7

Numbers of Collembola, Acarina, and Protura extracted from 15 x 2 in cores per plot

	Weeks after treatment														
	Collembola					Acarina					Protura				
	1	2	3	8	12	1	2	3	8	12	1	2	3	8	12
Methiocarb 0.2 lb	35	102	173	107	9	4	10	9	7	5	4	0	2	0	0
Methiocarb 0.4 lb	48	87	150	184	-	14	3	10	4	-	1	4	5	0	0
Methiocarb 0.8 lb	53	92	141	96	-	7	12	5	3	-	2	3	7	0	-
Control untreated	57	79	165	70	17	18	12	16	8	2	1	3	2	0	0

The technique of Way and Scopes (1968), which involved the study of the rate of breakdown of buried sycamore leaf discs, was also used. No differences between treatments were observed.

Foliage staining

Moist and dry methiocarb pellets (5622b) were caged for 10 days on old and young foliage of: lettuce cv. Great Lakes; cabbage cv. Christmas Drumhead; Brussels sprouts cv. Sanda; strawberries cv. Talisman. The leaves were then washed and examined but no staining or phytotoxicity was observed.

FIELD TRIALS

Trials have been conducted in a range of crops including: cereals, cabbages, Brussels sprouts, dahlias, lettuces, pyrethrums, strawberries and hops. Results substantiated methiocarb's efficiency when compared with metaldehyde. In some of the trials on cereals, weather conditions were not conducive to slug activity and demonstrative results were not obtained. However, in a Worcestershire trial in which the molluscicidal pellets were applied to the soil surface a week after drilling the following results were obtained (Table 8).

Table 8

Winter wheat trial, Kinnersley, Worcestershire, 1968

Treatment	Slugs per $\frac{1}{2}$ yd^2	Plants per 2 ft row	Damaged plants per 2 ft row	% damaged plants	% Control
Methiocarb 0.2 lb per acre	4.3	33.48	2.48	7.83	81.8
Methiocarb 0.4 lb per acre	7.7	34.25	2.38	6.93	82.9
Metaldehyde pellets 28 lb per acre	2.7	33.08	5.70	17.30	57.3
Control	0	31.78	12.80	40.45	-
L.S.D. 5.0%	1.38		-	-	
1.0%	2.83	NS	3.28	-	
0.1%	-		4.83	10.20	

In a Norfolk trial during the autumn of 1967, methiocarb pellets were applied both pre- and post-drilling compared with metaldehyde pellets. A considerable reduction in numbers of hollowed grains was obtained with all treatments. In trials against slugs in direct drilled brassicae a response in braird was observed.

Other Pests

In other cereal trials during 1969 very promising results were obtained with methiocarb pellets for the control of leatherjackets. In one of these a significant correlation was obtained between dead leatherjackets on the soil surface and subsequent plant numbers. Trials were also conducted in strawberries to study the efficiency of methiocarb pellets in controlling seed beetle (Harpalus rufipes) damage

to the berries. In one trial at Rowhill Experimental Farm, damage on plots treated with 0.2 lb methiocarb (as 5622b) amounted to 7.5%, comparitive figures for 0.4 lb methiocarb, standard malathion bait, aldrin spray, and untreated were 2.4%, 6.7%, 1.4% and 25.6% respectively.

Wild life trials

In order to support the notification of 4% methiocarb pellets (5622b) to the Pesticides Safety Precautions Scheme a number of trials and experiments have been conducted to test any likely hazards to birds. Trials in Herefordshire and Suffolk consisted of pre- and post-treatment surveys of the bird populations in and around fields chosen for their proximity to bird habitats. Cage tests were conducted with pheasants and young pullets to study acceptability and possible toxicity. In other experiments poisoned slugs were fed to young pullets, affected earthworms fed to blackbirds, and studies were made into the acceptability, to garden birds, of pellets when mixed with seed. In a series of garden trials though moles, rabbits, a hedgehog, a toad, dogs and cats were observed on and around treated areas, no adverse effects were noted. This work indicates that birds reject the pellets and they should not present a significant hazard to wild life.

Commercial Usage

During 1969, considerable commercial experience has been gained with 4% methiocarb pellets, their small size (60 pellets/g) means that a distribution of approximately 30 pellets/yd^2 is obtained using the recommended rate of 5 lb/ac; whereas 28 lb/ac of a standard metaldehyde pellet provides approximately 20 pellets/yd^2. A number of fertiliser distributors and dust and granule applicators have been calibrated to apply the material, and considerable success with aircraft application has already been obtained. Crops on which these pellets have already been used in commercial practice include: Brussels sprouts, winter wheat, potatoes, lettuce, celery, direct drilled brassicas, strawberries, blackcurrants, hops, pyrethrums and other flower crops.

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We are grateful to a number of our colleagues for producing information from field trials and particularly to B. Eddy who conducted the experiments concerning the attractiveness of various bait pellets to slugs and the effect of methiocarb on soil fauna. We thank those scientists of the National Agricultural Advisory Service with whom we have had many useful discussions.

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A COMPARISON OF METHIOCARB AND METALDEHYDE BAITS FOR THE CONTROL OF FOUR SPECIES OF SLUGS

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Summary Metaldehyde (4%) and methiocarb (4%) pellets were compared on 50 separate occasions. significant improvement in catch was obtained of the large black slug (Arion ater) with methiocarb and this compound was slightly more effective for the control of the garden slug (Arion hortensis) and the white-soled slug (Arion fasciatus). There was little difference between the two compounds for the control of the grey field slug (Agriolimax reticulatus).

INTRODUCTION

One of the problems in comparing slug baits is that the catch is related to the animals' activity and not necessarily to the total slug population. Attempts have been made to remove some of the variances due to meterological factors (Webley, 1964) to give more real population estimates, but these are related only to the active field population and take no account of the animals' response to the baits at different times in their life history. Even comparisons of total numbers by soil sampling around the baits might not measure baits differences because the animal is not static and the manner and degree of colonisation of an area is unknown. Since this is the case, the only valid assessments of bait performance must be a continual observation of their catches over long periods of time. The present trial is an attempt to compare methiocarb and metaldehyde pellets.

METHODS

Twenty plots, ($2\frac{1}{2}$ ft x $\frac{1}{2}$ ft) with an interval of 6 ft between each, were marked with stakes at the beginning of the experiment. Ten pellets of methiocarb (4% a.i.) were stuck on small cards and five of metaldehyde (4% a.i.) on others, and these were placed at random at the centre of each marked plot, to give ten replicates of each compound. On the following four mornings the slugs at each bait were counted, identified, removed and destroyed, the cards were also removed on the last morning. The sites were left vacant for three days and fresh baits were set out on the same plots and the counts continued. Fifty separate experiments have been completed to date starting from September 1968.

RESULTS

The daily figures from each plot were totalled over ten experiments, transformed to $\sqrt{n + 0.5}$ and analysed to give five separate periods, the first covering the autumn of 1968, the second, the winter of '68/9, the third, the spring of '69 and fourth and fifth, the summer of the same year.

Table 1 shows the result of the analysis.

Table 1.

Mean number of slugs ($\sqrt{n + 0.5}$) caught on methiocarb and metaldehyde baits (1968-69)

Period	Species							
	Arion ater		Agriolimax reticulatus		Arion hortensis		Arion fasciatus	
	Methio	Meta	Methio	Meta	Methio	Meta	Methio	Meta
1 Autumn 1968	3.2	3.3	3.9	3.9	2.5	2.4	2.6*	1.8
2 Winter 68/69	4.0*	2.0	2.7	3.9*	3.5	3.9	2.6	2.7
3 Spring 1969	4.7*	2.7	2.2	3.4*	2.2	3.2*	1.6	1.7
4 Summer 1969	9.5*	8.1	4.0	5.0*	3.1	4.1*	1.6	2.7*
5 Summer 1969	5.0	7.0*	2.5	3.2*	1.1	1.4	0.8	0.9
S.E.	0.84		0.79		0.77		0.47	

* = significantly greater at P=0.05

With the exception of the white-soled slug (Arion fasciatus), where the catch of the methiocarb was significantly greater, the species showed no difference in catch between the compounds in the first period. In periods 2, 3 and 4 the catch of the large black slug (Arion ater) was greater on the methiocarb plots while there were significantly more grey field slugs (Agriolimax reticulatus) on the metaldehyde baits. In the 5th period there were more large black slugs trapped by metaldehyde. The garden slugs (Arion hortensis) were found in greater numbers on the metaldehyde pellets in the 3rd and 4th periods, while the white-soled slug responded more to that compound in the 4th period.

Observations at the time of counting showed that the response of the large black slug to methiocarb was mainly caused by the young immature forms while the metaldehyde successes were associated with mature forms. Godan (1966) has shown that another carbamate (Isolan) behaves in the opposite manner for the yellow slug (L. flavus). The present experiment shows that the catch obtained with baits can vary with the proportions of immature and mature individuals in the population: this must be borne in mind when testing a new chemical as a bait.

DISCUSSION

In practical terms the present results must be judged against the actual kill achieved. There has been few new assessments of the efficiency of meta as a bait since Thomas (1948) gave results ranging from 20 to 80 per cent kill. Webley (1965) has given a figure of 85 per cent for autumn trapping with metaldehyde (5% a.i.); and, unpublished, one of 94 per cent for methiocarb (4% a.i.). Martin (1969) gives a figure of 52 per cent for meta., against one of 95 per cent for methiocarb. It is obviously very difficult to arrive at a fair estimate without much more work since the percentage kill in the field might be affected by the animals' feeding habits as well as the weather conditions at the time of baiting. Although it is suspected that methiocarb is more efficient than metaldehyde under very wet conditions such as we experience in S. Wales, for the purpose of this paper a figure of 75 per cent kill is suggested for metaldehyde (used under reasonable baiting conditions) and one of 95 per cent for methiocarb. Based on these estimates, a significant improvement in catch and kill of the large black slug was obtained with methiocarb over metaldehyde in these experiments. It is doubtful if there is much difference between the compounds for the control of the grey field slug. In the case of

the garden slug and the white-soled slug the carbamate might be slightly more effective.

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THE SLUG PROBLEM IN PEAS FOR PROCESSING

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Summary Work started in 1968 to examine the conditions under which slugs are active on pea vine at harvest and how they might be kept off the plants in field crops.

The numbers of slugs present on the vine at harvest is greatly influenced by weather conditions. Where these were ideal 50% and 16% of the populations of mature and juvenile slugs, respectively, were actively feeding on the vine at harvest.

Slugs were virtually absent in crops grown on light soils in East Anglia. In 1969 more extensive field and factory surveys are being carried out on pea drillings grown on medium/heavy soils in North Lincolnshire.

In chemical control trials the best treatment, methiocarb bait applied in Spring (4% at 10 lb/acre), gave an immediate 90% reduction of slug activity. Further work is necessary to determine the best time to apply this and other molluscicide formulations.

INTRODUCTION

The complete removal of all slugs from vined peas in the processing factories is expensive and difficult and if not efficient could lead to consumer complaints. Methods are required to prevent the occurrence of slugs on the pea vine at harvest.

In 1968 a research project was started with the following three, interrelated objectives, to define more clearly the problems and the possibilities of solving them.

1. To study the relative efficacy of chemical control treatments in plot trials, to determine how molluscicides could be used in pea crops.

2. To assess the effect of climatic conditions on the numbers of slugs harvested on the vine.

3. To compare the populations and species of slugs in pea crops with the numbers present in factory loads after vining.

CHEMICAL CONTROL TRIALS

Methods

Trials were carried out in a series of 49 'microplots' arranged in a 7 x 7 square. Each microplot contained 1 yd^3 of soil with a surface area of 1 yd^2. Single-course brickwork enclosed each plot, down to a concrete base with a drainage hole. Brickwork above soil level was painted with bitumastic paint to restrict the movement of slugs between plots.

In the spring of 1968 90 grey field slugs (*Agriolimax reticulatus*) were placed in each plot to give high and relatively uniform populations of at least 435,600 per acre. The plots also contained a natural population of the garden slug (*Arion hortensis*).

To measure the relative efficacy of chemical treatments, comparative estimates of slug activity were obtained from counts of wheat grains eaten by slugs. For this method, devised by Dr. P. Hunter, Slug Research Unit, A.R.C., Cambridge, black cards measuring 6 in x 2 in each carrying ten wheat grains fixed by plastic cement were exposed in the plots for two or three days before recording. The wheat grains were protected from birds and field rodents by netting the microplots and laying a barrier of warfarin traps.

Unless stated otherwise the tabulated results refer to the mean numbers of wheat grains eaten out of 120 for each treatment.

Results

Trial 1 compared the four chemical treatments given in table 1, each treatment was replicated six times. The metaldehyde and methiocarb baits and the dinoseb amine spray were applied when the peas were at the three to four-node stage on 21 May, and the metaldehyde spray treatment at flowering on 12 June.

Table 1 gives the means, for numbers of wheat grains eaten, for all the post-treatment records and table 2 the means for the pre-harvest data. The post-treatment recording began 12 days after applying the treatments and the pre-harvest recording seven days before harvest, which started on 15 July. At harvest, slugs present on the vine were recorded (table 3) and counts of slugs in the soil were made by Dr. Hunter's staff at Cambridge, using a flooding technique. The estimates for the total slug populations present on each plot are given in table 4.

Reference to the sequential records in tables 1 to 4 shows no control of slugs by either the metaldehyde spray or the dinoseb amine spray. Methiocarb bait gave a reduction of 94% in damage to wheat grains 12 days after application (post treatment), and a substantial reduction (58%) was achieved with metaldehyde bait. Five weeks later (pre-harvest) only plots treated with methiocarb were still showing significantly less damage to wheat grains. The populations of slugs active on the vine at harvest were not reduced by any of the chemical treatments; similarly, none had apparently reduced the total populations of slugs counted on the plots at harvest.

This loss of control through time, particularly in the case of the most promising chemical methiocarb, was measured again on trial 2 and is dealt with later.

Table 1

Trial 1: Post-treatment records of numbers of wheat grains eaten

Treatment (lb a.i./ac)	Mean nos. of grains eaten Transformed (Sq. Rt.)	Untransformed	Percentage reduction of damage
Applied at 3-4 node stage			
Metaldehyde bait (0.84)	5.2	26.4	58
Methiocarb bait (0.40)	2.1	3.6	94
Dinoseb amine spray (1.85)	7.6	56.4	11
Untreated	8.0	63.6	0
Sig. diff. (P = 0.05)	1.2		
Coefficient of Variation	16.5%		
Applied at flowering			
Metaldehyde spray (2.50)	8.8	76.1	6
Untreated	9.0	80.8	0
Sig. diff. (P = 0.05)	1.3		
Coefficient of Variation	9.8%		

Table 2

Trial 1: Pre-harvest records of numbers of wheat grains eaten

Treatment	Mean nos. of grains eaten Transformed (Sq.Rt.)	Untransformed	Percentage reduction of damage
Metaldehyde bait	7.2	50.3	25
Methiocarb bait	5.8	32.6	51
Metaldehyde spray	8.3	67.4	0
Dinoseb amine spray	7.0	48.3	28
Untreated	8.3	67.2	0
Sig. diff. (P = 0.05)	1.3		
Coefficient of variation	15.2%		

Table 3

Trial 1: Numbers of slugs on harvested vine

Treatment	Mean nos. of slugs Transformed (Sq.Rt.)	Untransformed
Metaldehyde bait	3.6	13.4
Methiocarb bait	3.1	10.0
Metaldehyde spray	4.6	22.1
Dinoseb amine spray	4.1	17.3
Untreated	3.3	11.6
Sig. diff. (P = 0.05)	1.7	
Coefficient of variation	36.7%	

Table 4

Trial 1: Numbers of slugs per yd^2 of soil at harvest

Treatment	Mean nos. of slugs Transformed (Sq.Rt.)	Untransformed
Metaldehyde bait	12.0	149.3
Methiocarb bait	11.8	142.9
Metaldehyde spray	13.5	186.6
Dinoseb amine spray	10.2	108.7
Untreated	12.8	168.9
Sig. diff. (P = 0.05)	2.7	
Coefficient of variation	18.3%	

Results from trial 1 at harvest showed that slug populations were at the same level on 36 of the microplots, and these were used for a second trial. In trial 1 methiocarb bait at 0.4 lb a.i./ac was effective in suppressing slug populations but would cost 90/- per acre. Lower rates (table 5) were tested in trial 2 and their effectiveness through time examined.

The methiocarb treatments, each replicated six times, were applied on 2 Aug and their effects checked 12 days later by recording the number of wheat grains eaten, out of 160 per plot (40 put down on each of four successive days). The results in table 5 show that a rate of methiocarb of not less than 0.4 lb/ac was necessary to reduce the seed damage by over 90%, the same control being achieved with this rate in trial 1 (table 1).

Table 5

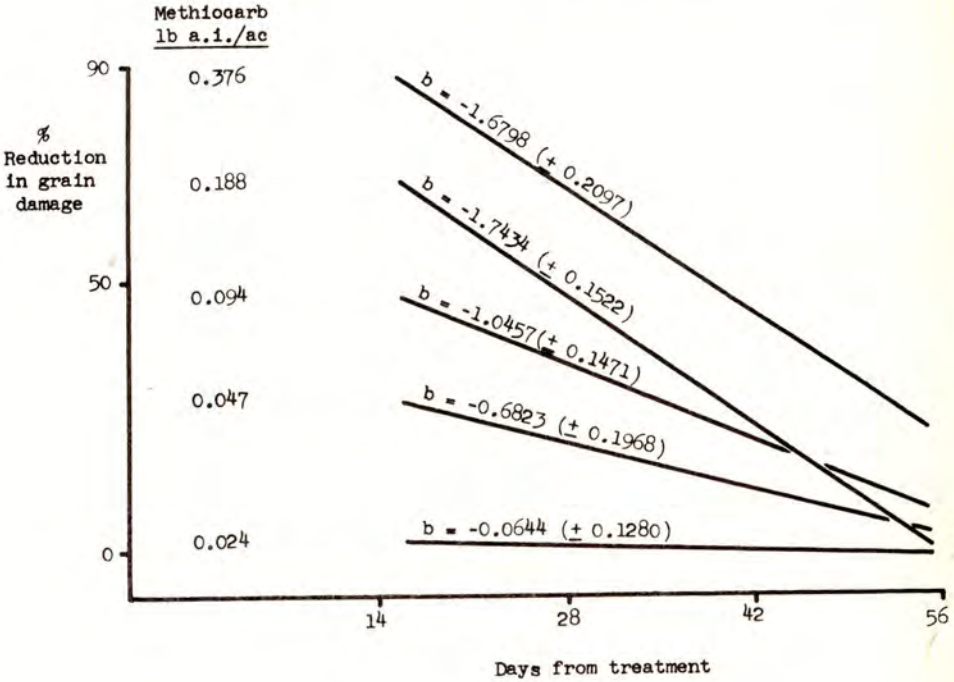
Trial 2: Effect of 5 rates of methiocarb bait in preventing damage to wheat grains 12 days after application

Treatment (lb a.i./ac)	No. of bait pellets per ft^2	Percentage reduction of damage
Methiocarb (0.024)	0.5	3
" (0.047)	1.0	27
" (0.094)	2.0	52
" (0.188)	4.0	74
" (0.376)	8.0	91

A further eight assessments were made over the period 17 Sep to 11 Oct, recording on each occasion the number of grains eaten out of 60 left down in each plot for 24 hours. The mean proportion of grains eaten on the untreated plots over the period covered by the ten assessments (including one pre-treatment) remained constant. This suggests that the numbers of slugs on untreated plots in trial 1 also remained the same during the eight weeks, as virtually the same number of grains were eaten at the beginning and end of the period of this trial.

Figure 1 illustrates the regression lines for percentage reduction in numbers of wheat grains eaten compared with the control, over the period of the first eight post-treatment assessments in trial 2. The regression coefficients for each methiocarb treatment are significant at $p = 0.001$ (46 d.f.) except for the lowest rate of 0.024 lb/ac which is not significant. The figure shows that eight weeks after application little control remained from any methiocarb treatment, a result first seen in trial 1.

Fig. 1. Trial 2: Percentage reduction in numbers of wheat grains eaten by slugs, from 12 to 55 days after applying Methiocarb treatments



The chemical control trials have shown that methiocarb applied at 0.4 lb a.i./acre gives an immediate control of slug damage exceeding 90%. Methiocarb is not an ovicide and applications on these trials were made within the peak periods for breeding of the grey field slug and the garden slug (Hunter 1966). Methiocarb bait pellets are dissipated under wet conditions in about one week and the apparent loss of control through time in trials 1 and 2 is probably due to hatches from eggs in the soil. As one grey field slug can lay up to 200 eggs a rapid increase in populations of juvenile slugs can obviously occur by harvest, eight weeks after applying the methiocarb.

The rate of increase varies (Fig. 1) according to the amount of methiocarb used and differences in the initial kill of mature slugs. This would result in differences in population pressures on plots which had received the different rates of chemical. The eventual effect, irrespective of treatment rates, is for populations to converge on the optimum which the square yard plots can support. This optimum is the population in the control plots which remained static at about 800,000 per acre - here the population pressures have probably resulted in natural mortality balancing the hatching of new slugs to keep the population constant.

This hypothesis infers that methiocarb is a highly effective molluscicide but that the timing of its application was incorrect. There is no point in considering applications earlier in the spring before breeding starts because soil and air temperatures are too low for slugs to emerge and feed on bait. The alternative is to apply chemicals to peas much nearer to harvest leaving no time for populations to increase between application and harvest.

EFFECT OF CLIMATIC CONDITIONS AT HARVEST

At maturity one block of Trial 1 was harvested on each of six days to get some indication of the effect of different weather conditions on the numbers of slugs harvested with the vine.

Table 6 gives the weather conditions at the time of harvesting and table 7 shows the effect of harvest time on the numbers and proportions of slugs harvested on the vine.

Table 6

Trial 1: Weather conditions at harvest

Harvest	Date	R.H. over 90% Period	Hours	Harvest time and conditions
1	15/7	2000* - 1200 hrs	16	0830 hrs. Haulm very wet, overcast, raining.
2	16/7	0300 - 0830 "	5	1030 hrs. Haulm dry, overcast, windy.
3	17/7	1630** - 1100 "	18½	1400 hrs. Haulm wet, overcast, raining, very windy.
4	18/7	0400 - 0600 "	2	1400 hrs. Haulm dry, overcast.
5	19/7	0100 - 0900 "	8	1400 hrs. Haulm dry, sunny.
6	20/7	0145 - 0215 "	½	1400 hrs. Haulm dry, overcast.

* 2000 hrs. on 14/7/68.

** 1630 hrs. on 16/7.

Table 7

Trial 1: Numbers of slugs on harvested vine

Harvests	Mean nos. of slugs per plot Transformed (Sq. Rt.)	Untransformed	As % of population	Approx. nos. of slugs per acre Harvested	Total
1	7.1	49.9	25	242,000	968,000
2	2.4	4.8	4	23,000	575,000
3	4.8	22.4	18	108,000	600,000
4	2.8	7.1	9	34,000	377,000
5	2.9	7.5	6	36,000	601,000
6	2.3	4.4	3	21,000	699,000
Sig. diff. (P=0.05)	1.9				

Coefficient of variation 36.7%

At harvests 1 and 3, 25% and 18%, respectively, of all slugs were still on the vine, significantly more than at any other harvest. Both were associated with very long periods of high humidity during the previous night and up to mid-day. The inference from this data is that, as might be expected, more slugs will be harvested with crops cut and vined at night but given the right climatic conditions for slug activity this probability extends also to crops harvested during the day.

A total of 522 slugs were harvested on the vine in Trial 1 comprising 155 grey field slugs and 367 garden slugs. 36% of all slugs on the vine were small (< 1 cm in length) but this represented only a mean 6% of all the small slugs, the highest proportion recorded being 16% in harvest 1. In contrast, slugs over 1 cm in length on the vine represented 65% of all the slugs and a mean 31% of all the large slugs, a proportion which did not fall below 11% (harvest 6) and reached 50% in harvest 1 and 49% in harvest 3.

As most of the small slugs are not active on pea vine when it is cut, even under ideal climatic conditions, it may not be necessary to aim at delaying chemical applications such that there will be no juvenile slugs present at harvest. Applications round about flowering may be sufficient to ensure that all larger slugs are killed and the numbers of juveniles at harvest so low that an insignificant proportion will be active on the vine.

FIELD AND FACTORY SURVEYS

A total of 13 pea crops in Norfolk were surveyed in 1968 for the numbers of slugs present at crop emergence, close to harvest, and in the vined peas.

In the field surveys slug counts were made using traps of 18 in x 6 in boards laid in the interrows, with a high concentration of methiocarb bait pellets under each board. For each drilling, traps were laid in six blocks distributed at random over the crop area with six traps placed 2 ft apart in each block. During each of the two assessment periods traps were inspected every three or four days over two to three weeks, the numbers of dead slugs recorded and the bait replenished; seven inspections were made during emergence and early growth and five during the flowering period.

Slug counts in the harvested peas were taken at a static vining station on a special inspection line set up by the Development Department of Birds Eye Foods Ltd. A minimum number of six tanks of peas from each of the surveyed crops was inspected.

No slugs were found in seven of the crops and five yielded a total of only nine and one slug during the first and second trapping periods respectively. In the remaining drilling seven and 32 slugs were found during each period.

Similar results were obtained in the survey of vined peas from these drillings, carried out by Birds Eye staff at the central vining station; a total of only 39 slugs were found in 286 tanks of peas examined.

All the drillings were on 'light' soils of the fine, sandy loam to loam type, on which about 80% of the peas are grown in this area.

The inference from these limited surveys is that a major slug contamination problem of vined peas is not originating from pea crops grown on the lighter soils in East Anglia. More refined techniques will allow for more extensive field surveys to be carried out in 1969 on pea crops grown on medium/heavy soils in Lincolnshire.

CONCLUSIONS

1. Slug activity in peas can be reduced by over 90% as recorded 12 days after applying methiocarb bait. If applied too early in crop growth subsequent hatches of eggs in the soil can still result in large numbers of slugs at harvest. Pre-harvest chemical treatment is probably necessary and is being examined in 1969.

2. Weather conditions are shown to have a marked effect on the numbers of slugs harvested with the vine. Using relative humidity as a guide crops harvested under conditions of over 90% R.H. are likely to contain a substantial proportion of the slug population. In general this will occur at night but it is impractical to stop night vining.

Under warm, humid conditions over 50% of all large slugs may be feeding on the vine when it is cut. Juvenile slugs are much less active on the vine. It may not be necessary to aim at delaying pre-harvest chemical applications so that there will be no young slugs present at harvest; applications round about flowering may be sufficient.

3. Knowledge of the relationship between field populations and the numbers of slugs in vined peas is still incomplete. The field and factory surveys suggest that a major, pack-contamination problem is not originating from the large acreages of peas grown on light lands in East Anglia. More extensive and detailed surveys of crops grown on the medium/heavy soils in North Lincolnshire have been carried out in 1969.

Acknowledgements

We had the benefit of valuable discussions with Dr. P.J. Hunter during these studies.

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POPULATION AND CONTROL OF SNAILS IN BLACKCURRANT PLANTATIONS

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Summary The dominant species on a blackcurrant plantation infested with snails were the Common Snail (Helix aspersa), and the Banded Snails (Cepaea nemoralis and C. hortensis). The weed cover, on a well managed herbicide controlled plantation, was still sufficient to support a total population of over 12000 snails/ac. It is suggested that the ovicidal effects of post-picking copper fungicides may have a long term controlling action. The new molluscicide methiocarb is an effective snail killer, and although a promising replacement for metaldehyde further investigations are needed concerning its persistence, timing of application and the behaviour of snails in relation to it as a bait.

INTRODUCTION

The increase over the last ten years in the number of snails in blackcurrant plantations presents a growing problem for both grower and jam manufacturer. The presence of snails in picked fruit may lead to contamination of jam because it is difficult to separate them from the fruit (especially when they are of berry size) after being kept in cold storage. Previous observations (Stringer, 1966) had suggested that the size of the snail population was dependent upon the amount of weed cover; clean cultivation together with the use of metaldehyde bait was therefore recommended as a control measure. It was found later however that several well-maintained plantations, using the recommended control measures, still had a perennial snail problem. Since the snail populations in the blackcurrant plantations at Long Ashton are very low, further work was started at the end of May 1967 in a highly infested plantation at Highnam, near Gloucester. The present paper describes the population counts of snails and preliminary control experiments.

EXPERIMENTAL

The experiments were carried out on a 15 ac orchard planted with Baldwins with 10 ft alleys and 4 ft between bushes. The plantation is surrounded by good thick hawthorn hedges, headlands with a lush growth of grass and weeds, and with drainage ditches running down east and west sides. The plantation is irrigated during periods of drought. The fungicide spray programme was based on zineb and prior to 1968 no copper fungicides had been used. The weeds were controlled by the use of herbicides, simazine and chlorthiamid. Metaldehyde slug pellets, at the rate of 28 lb/ac, had been used regularly in late spring or early summer with only partial control and the snail population remained relatively large.

The degree of infestation by weeds was estimated as a percentage cover and, using an 18 inch quadrat as a sample unit, 200 samples were taken at random in the alleys and 200 samples in the bush line (i.e. a strip 2 ft each side of the bushes).

The snail counts were made during the day when the snails were relatively immobile and were either resting in the bushes or concealed on the ground. For the purposes of the count a bush was treated as the rectangle of ground 4 ft wide and 10 ft long with the bush itself in the centre. Each bush was searched carefully for about 15 min, and the number of snails belonging to the family Helicidae (Typical snails) were counted. Litter species, such as the Glass snails (Oxychilus cellarius and O. allarius) were not recorded.

In 1968 the first trials were made to observe the effect of copper on snail populations. On 17 April and again on 29 May sub-plots, 40 ft wide and 44 ft long, were sprayed with a proprietary copper oxychloride fungicide and a copper sequestrene

(Na_2Cu, the chelate contains 13% Cu) to cover the ground and the butts of the bushes, at rates of 0.6 lb and 2.4 lb of copper/ac using both compounds in 100 gal/ac. The snail counts were made on 29 July. Later the grower sprayed a post-picking copper oxychloride fungicide (3 lb/ac) over the whole plantation.

In July 1969 a trial was carried out with the molluscicide methiocarb, as a proprietary pelleted bait formulation containing 4% w/w methiocarb. The bait was spread with a hand rotary granule applicator on sub-plots of approximately 1/20 acre at rates of 5 lb, 10 lb and 20 lb/ac. At an average of 20,000 pellets/lb the recommended dosage is approximately 20 pellets/yd^2. Mortality counts were made at 4, 7 and 14 days after application. The weather was hot and dry over the test period and the plots were irrigated the first day after application of the methiocarb with the equivalent of 1 in of rain.

RESULTS

Table 1 gives the results of the weed assessments for August and November 1967 and March 1968. The weed cover in May 1967, when the plantation was first visited was extremely low, probably 1 or 2% and no count was made. The dominant species of weeds in mid-summer were common orache and lesser bindweed, and in winter and spring groundsel and annual poa. The other species of weed present were mainly common horsetail, dandelion, chickweed, nettle, hogweed and couch. The dead vegetation in November was still often recognisable as orache and bindweed.

Table 1

Percentage weed cover

Weed species	Alley			Bush-line		
	18 Aug	20 Nov	26 Mar	18 Aug	20 Nov	26 Mar
Common orache	24.8	-	-	1.8	0.1	-
Lesser bindweed	16.8	0.1	-	6.4	-	-
Groundsel	0.6	5.1	6.3	0.7	1.8	4.5
Annual poa	0.2	4.2	5.4	0.2	0.6	0.7
Other species	5.4	0.9	2.4	0.2	0.3	1.2
Total (all species)	47.8	10.3	14.1	9.3	2.8	6.4
Dead weeds	-	16.6	-	-	1.3	-
Moss	5.0	10.3	2.1	3.0	4.7	0.2

Estimates of snail populations on 15 May 1967 were as follows:

Common snail (*Helix aspersa*)	3311/ac
Banded snail (*Cepaea* sp)	1252/ac
Wrinkled snail (*Helicella caperata*)	5968/ac
Strawberry snail (*Hygromia striolata*)	980/ac

Both *C. nemoralis* and *C. hortensis* were present but because identification between them can be difficult they were counted together as *Cepaea* sp. A few specimens of Hairy snail (*Hygromia hispida*) and Copse snail (*Arianta arbustorum*) were also collected. Although snails are gregarious and appear to 'home' to a communal resting place, occasionally as many as 40 to an individual bush, when conditions were dry the population as a whole were reasonably well spread over the plantation.

The results of the copper spray trials on the snail population are given in Table 2. The high population of the Wrinkled snail fell from about 6000/ac in 1967 to 110/ac in April 1968, and so the counts in the trial were restricted to the Common snail and Banded snails, the most numerous species.

Table 2

Snail populations - effect of copper sprays

Treatment	No. *Helix aspersa*/ac on 14 April	29 July	No. *Cepaea* spp/ac 14 April	29 July
Sequestrene 0.6 lb/ac	1416	4846	762	1851
Sequestrene 2.4 lb/ac	4247	9583	2831	3267
Oxychloride 0.6 lb/ac	4519	4683	3213	3920
Oxychloride 2.4 lb/ac	3321	2614	3376	2940
Oxychloride 2.4 lb/ac* (bush-line)	2886	5009	1960	4247
Oxychloride 2.4 lb/ac* (alley)	4465	9583	2723	5881
Unsprayed control	6697	6534	5227	6752

Spray dates 17 April 1968 and 29 May 1968

* Bush-line or alley sprayed only

The molluscicide trial results are given in Table 3. The mortality was assessed by counting the total number dead on each plot and the survivals by making counts on 12 bushes on each plot. The period of irrigation had caused a disintegration of the pellets, but they were readily discernible at 4 days, found with difficulty at 7 days and no trace after 14 days. The molluscicide did not cause rapid death and the affected snails remained immobilised for 4 or 5 days in the treated area often in exposed situations and without the excessive induced slime secretion associated with metaldehyde.

Table 3

The effect of methiocarb on snail population

Bait in lb/ac	Mortality after 4 day	7 day	14 day	Alive after 17 day
	No. of *Helix aspersa*/ac			
5	-	1519	2096	2635
10	1231	2096	3115	1819
20	1615	2577	3577	1634
Control	0	0	19	3452
"	0	0	19	3267
	No. of *Cepaea* spp			
5	-	519	1000	6991
10	846	1577	2865	5717
20	2077	3154	4577	8625
Control	0	0	38	10007
"	0	0	58	7078

DISCUSSION

Snails are not a pest of the blackcurrant crop in the normal sense of the word and none of the species collected was observed feeding on blackcurrant foliage either in the field or laboratory. It was thought at the beginning of the investigation, on information from NAAS entomologists, that there were never more than small numbers of snails on the bushes in plantings when snails had contaminated harvested fruit. The counts at Highnam (approx 12000/ac combined Common and Banded snails for 1968 and

1969) indicated that relatively large populations could be present in infested plantations. Although the examination of each bush was carried out carefully it is almost certain that the numbers obtained considerably underestimated the true population.

Control by herbicides did not result in complete eradication of all weeds but gave sufficient control for good management. It was concluded that under such conditions the amount of weed cover, although varying from about 2 to 50% cover, was sufficient to support a high population of snails. It is appreciated that complete eradication may be impractical especially if there is a possibility of soil erosion but poor weed control would most certainly lead to even higher populations.

From the limited data available on the occurrence of snails in blackcurrant plantations it appeared that the build-up of populations in the last few years may be associated with the introduction of organic fungicides with a corresponding decrease in the use of post-picking copper fungicide sprays. The use of copper sulphate for the control of slugs (MAFF, 1959) suggested that applications of copper might be toxic to snails. Applications of a soluble copper sequestrene and an insoluble copper oxychloride to the ground and butts of the bushes had no significant effect on the number of snails present. The bushes were not sprayed to avoid copper deposits on the developing fruit. Laboratory experiments confirmed that these compounds were non-toxic as deposits but affected feeding behaviour and either depressed or prevented hatching of eggs (Stringer, 1966). It would seem that the post-picking spray, which roughly coincides with the mid-period of egg laying (June to September), would be the most effective. The eggs are laid in batches of 20 to 50 in the top 2 in of soil. Depending upon the persistence of copper deposits, it would probably take several years for the fungicidal sprays to exert a pronounced effect.

It is appreciated that one snail in many tons of fruit can be a serious problem if it leads to one snail in a pot of jam, so that any reduction of populations by either orchard hygiene or chemical control is desirable. It would appear that the degree of control by metaldehyde was not sufficient to hold the population down to a satisfactory level. The first molluscicide discovered with a toxicity greater than metaldehyde was the carbamate methiocarb (Getzin & Cole, 1964). A 4% methiocarb bait was tested in the laboratory against the Common snail (Crowell, 1967) and gave a kill of 80% as against 20% for 4% metaldehyde bait. The results obtained from the preliminary trial with methiocarb were promising. The bait was taken in a hot dry period and resulted in a considerable progressive mortality over a period of 14 days. It was difficult to assess the true effectiveness of methiocarb because of several competing factors, such as the effect on persistence of the irrigation, the behaviour and mobility of the snails especially that affecting their descent from the bushes to the ground and immigration from surrounding areas. The problem of assessing the true populations presents one of the main difficulties in interpreting the data. It is evident from Table 3 that large numbers of snails were killed by the methiocarb. The kill increased with dosage from 40 to 70% for Common snails and from 12 to 30% for Banded snails, if one accepts the observed counts as the best estimate and no migration into or out of the treated plots. Also the kill for the Common snail was greater and this may have been due to its observed greater mobility from the bushes and on the ground during the experiment.

The above paper is an account of the preliminary investigations into the problem of snails in blackcurrants and it is hoped that it will provide a basis for further work.

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SOME PROBLEMS INVOLVED IN THE USE OF STUPEFYING BAITS TO CONTROL BIRDS

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Summary Research on the use of stupefying substances to control birds has resulted in the development of techniques for catching feral pigeons, house-sparrows and wood-pigeons employing alpha-chloralose as the stupefying agent. Although these techniques are basically straightforward, certain problems have arisen in practice. The main problem has been the possible risks to protected bird species which are negligible in urban areas but greater in rural situations unless adequate safeguards are adopted. Other problems have arisen due to difficulties in baiting technique and to the method of bait formulation; some of these problems have yet to be resolved. Alpha-chloralose has proved an efficient substance for use against house-sparrows but, owing to its slowness of action, it is less efficient when used against pigeons. In spite of its shortcomings, the method can serve as a useful tool for the local control of bird pests.

INTRODUCTION

The 1954 Protection of Birds Act made it possible to use poisoned or stupefying bait, under licence, to control the birds listed in Schedule II to the Act - the unprotected species. Since 1955 research has been conducted on developing techniques for catching birds by laying bait treated with a stupefying drug. Most work has been directed against three pest species - the feral pigeon (Columba livia var.), the house-sparrow (Passer domesticus) and the wood-pigeon (Columba palumbus). Alpha-chloralose ($C_8 H_{11} Cl_3 O_6$), a condensation product of chloral hydrate and glucose, has been used as the stupefying agent. At an early stage it was decided to concentrate on using stupefying drugs, rather than quick-acting poisons, as it was considered that their use would be more humane and would present fewer risks. A number of stupefacients were screened and of these alpha-chloralose gave the best results. Techniques for catching the three species have been established and the method has been in use commercially against feral pigeons since 1961, and against house-sparrows, infesting buildings, since 1963. This year licences have been issued to farmers permitting baits to be used against wood-pigeons.

Some publicity has been given to this method of bird control and has resulted in some sections of the community coming to regard stupefying baits as a kind of "magic formula" for removing bird pests - a method which will eliminate unwanted birds with little or no work involved and with a minimum of difficulty. The truth is far from this. In theory the technique is attractive and basically simple: a suitably attractive bait is treated with a stupefying drug and is laid where the target species will take it; once the birds become stupefied, they are picked up and humanely destroyed. Full details of the method have been adequately described elsewhere (Ridpath et al., 1961; Murton et al., 1963; Thearle, 1968; Murton et al., 1968) and will not be repeated here. Baits used have included whole wheat and maize for feral pigeons; breadcrumbs, small cereals and seeds for house-sparrows, and tic beans for wood-pigeons.

It was obvious from the outset that certain problems were involved and others have developed in practice. It is these problems that will now be considered and some attention must be paid to those arising in urban areas, where most of the developmental work on stupefying baits took place. The majority of these problems must be solved before the method can be applied successfully for crop protection.

THE RISK TO PROTECTED SPECIES

Perhaps the greatest problem to be overcome when using stupefying baits is the attitude of the general public. Adverse criticism of the technique falls into two main categories. First, the killing of any birds, whether they are considered harmful or not, is thought to be inhumane by many people - and to them the use of poisons is particularly objectionable; such an attitude can only be alleviated by good public relations and can probably never be overcome completely. Second, there are those who, whilst admitting that bird control may be necessary, are concerned about the risk to protected bird species when poisonous substances are used. One reason for employing a stupefying drug rather than a quick-acting killing agent is to give some measure of protection to species which accidentally take the bait - they can be allowed to recover and then be released. If it could be guaranteed that only the target species would feed on the bait then, in many ways, a direct poison would be preferable. But this guarantee cannot be given and although the risks are lessened by using a stupefying agent, it is not possible to prevent some birds from taking an overdose, so that accidental deaths do occur. Nevertheless, in urban situations the risks are negligible and this can be shown by considering the numbers of birds caught during operations in which alpha-chloralose has been used; these figures have been taken from the reports on treatments submitted to the Ministry of Agriculture, Fisheries and Food by local authorities and servicing companies since licences were first issued.

Table 1 shows the number of birds caught with stupefying baits in all operations

Table 1.

The use of stupefying baits against feral pigeons. Numbers of birds caught by servicing companies and local authorities from 1961 to 1968
(Figures in parentheses give percentages of the total catch)

No. of operations	No. of feral pigeons caught	No. of house-sparrows caught	No. of protected species			No. of other species caught
			Caught	Died	Recovered	
951	69007	10375	37 greenfinches 31 blackbirds 9 mallard 7 collared doves 5 robins 4 thrushes 4 "finches" 3 chaffinches 2 goldfinches 2 moorhens 2 "doves" 1 linnet	16 6 4 6 1 2 2 1 2 2 - - 1	21 25 5 1 4 2 2 2 - - 2 -	165 starlings 86 wood-pigeons 49 jackdaws 32 rooks 13 "crows" 3 "gulls"
	Totals:	(13%)	107 (0.1%)	43 (0.05%)	64	348 (0.4%)

against feral pigeons (which have been almost entirely confined to urban areas) up to the end of 1968. It is seen that the non-protected house-sparrow, which is frequently taken in feral pigeon treatments, forms 13% of the total catch and that protected birds form 0.1%. But the important figure is the number of protected birds that died from the effects of the stupefying drug which amounts to only 0.05% of the total catch; this figure can be used as an "index of risk".

Similarly, Table 2 shows the number of birds caught in operations against house-sparrows at urban and industrial sites. Here the number of protected birds killed represents about 0.1% of the total catch. Thus in urban operations against both feral pigeons and house-sparrows the risks are not great, but in rural areas the risks are greater as can be seen by reference to Table 3. This shows the number of

Table 2.

The use of stupefying baits against house-sparrows. Numbers of birds caught by servicing companies at urban and industrial sites from 1963 to 1968

(Figures in parentheses give percentages of the total catch)

No. of operations	No. of house-sparrows caught	No. of protected species			No. of other species caught
		Caught	Died	Recovered	
1907	85360	345 blackbirds	50	295	2194 starlings
		124 robins	34	90	532 feral pigeons
		55 thrushes	10	45	21 rooks
		25 dunnocks	5	20	9 "crows"
		15 blue tits	4	11	6 carrion crows
		14 wagtails	6	8	2 magpies
		9 chaffinches	2	7	1 wood-pigeon
		6 greenfinches	-	6	1 "seagull"
		6 black-headed gulls	2	4	
		6 "doves"	-	6	
		4 linnets	-	4	
		2 skylarks	-	2	
	Totals:	611 (0.7%)	113 (0.1%)	498	2766 (3.1%)

birds caught in operations against house-sparrows at rural sites since such operations were first licensed in 1965. Here the figure for protected birds accidentally killed rises to 1.8% of the total catch - a considerable increase on the urban figures. Experiments at farm buildings have produced similar results and, if the method is to be used for crop protection, this increased risk must be taken into consideration, although it can be reduced by taking suitable precautions, such as confining baiting for house-sparrows to the interior of buildings.

In operations against feral pigeons and house-sparrows, the bait is usually laid on hard surfaces so that it can be cleared completely at the end of a treatment. Feral pigeon treatments normally last for a few hours, whilst those for house-sparrows may continue for one or two days. For wood-pigeons, the bait has to be thinly scattered on open fields and cannot be cleared at the end of a treatment; sometimes it remains exposed for many days before it is cleared by the birds or becomes ineffective through weathering. It is obvious that the greatest dangers must arise when baiting for wood-pigeons where, in addition to small protected species, game birds are also at risk. Much of the research on wood-pigeons,

therefore, was directed towards developing a selective bait, and it was found that tic beans were reasonably satisfactory in this respect, being fairly attractive to

Table 3.

The use of stupefying baits against house-sparrows. Numbers of birds caught by servicing companies at non-agricultural rural sites from 1965 to 1968

(Figures in parentheses give percentages of total catch)

No. of operations	No. of house-sparrows caught	No. of protected species			No. of other species caught
		Caught	Died	Recovered	
83	4464	98 blackbirds	37	61	516 starlings
		37 robins	11	26	14 magpies
		22 dunnocks	8	14	11 feral pigeons
		21 chaffinches	21	-	11 jackdaws
		18 wagtails	7	11	6 rooks
		17 thrushes	7	10	3 "crows"
		6 blue tits	1	5	
		5 black-headed gulls	2	3	
		2 meadow pipits	-	2	
		1 yellow hammer	-	1	
		1 skylark	1	-	
	Totals:	228 (4.3%)	95 (1.8%)	133	561 (10.7%)

wood-pigeons but considerably less so to pheasants and partridges; tic beans are too large to be taken by most small protected species. In twenty-eight experimental operations against wood-pigeons from 1963 to 1967, in which tic beans treated with alpha-chloralose were used, the accidental deaths of game birds formed 0.4% of the total catch whilst those of protected species formed 0.3% (Murton et al., 1968). Thus the risks involved when baiting for wood-pigeons can be kept at an acceptable level.

BAITING

Although the problem of the dangers to non-target species can be largely overcome, certain other difficulties arise due to aspects of the baiting techniques and the method of bait formulation, and these are more difficult to control. Alpha-chloralose is a fine white powder which is applied to the bait base in the form of a coating, using technical white oil as a sticker. The drug is rather slow in action and a surface application on the bait has proved more effective, as regards speed of effect, than other formulation methods; if the chloralose is incorporated in the bait, there is greater delay before absorption occurs. Bait prepared by the coating method presents few problems when used against feral pigeons and house-sparrows, where treatments are short-term and often take place under cover. But against wood-pigeons, where treatments last for days rather than hours and the bait remains exposed on fields, complications can arise due to weathering effects as rain will quickly wash the chloralose off the bait so that it becomes only partially effective or even completely ineffective before being taken by the birds. A further complication has recently come to light due to the fact that, when used against wood-pigeons, bait has to be prepared in bulk. Against feral pigeons and house-sparrows small quantities of bait are normally used and these are prepared by the local authority or servicing company responsible for the treatment. For wood-pigeons

larger quantities are necessary and farmers are required to purchase ready-made bait. This has entailed the mixing of bait on a commercial scale and the method of formulation, although efficient when used on a small scale, has proved difficult to apply successfully in bulk. It is possible that this difficulty could be alleviated by using a different sticker, which would coat the drug more firmly on the bait, although the fact that at present the chloralose can be easily washed off the bait is, to some extent, a safeguard - preventing effective bait from remaining on fields for long periods when uneaten by the birds.

The present method of bait formulation, even when used on a small scale, restricts the concentration of bait that can be applied. With alpha-chloralose, which is used at concentrations of 1.5% or 2% by weight, this is not a problem; but above 2% some of the drug tends to fall off the bait so this is the maximum concentration that can effectively be used. This can be an important factor when considering an alternative substance to alpha-chloralose, the search for which has been prompted by the fact that, as mentioned above, chloralose is slow in action. The slowness is particularly noticeable in feral pigeons, where it can take between 20 and 50 minutes for a bird to become immobilised and such a delay can result in birds moving some distance from the baiting point after feeding. A wide scatter of affected birds is not only an embarrassment, but leads to inefficiency in pick-up and increases the time spent on an operation. It also means that care must be taken to ensure that birds are not attracted to the baiting point from flocks situated some distance away, as these are likely to leave the site as soon as feeding is finished and before the drug has taken effect. A chemical that is quicker in action than chloralose would obviously be an improvement, but most substances tested have not shown any significant improvement in this respect, although better results have been obtained using alpha-chloralose in combination with other drugs, notably barbiturates. This work is still in progress. To be effective a substance must not only act at a relatively low dosage, but must also have a wide margin between the hypnotic and lethal dose. Repellency must also be considered and some drugs have been rejected because of their unpalatability to the birds.

Certain other factors adversely affect treatments against feral pigeons and these are largely connected with the timing of operations. Because these operations are not popular with the general public and because it cannot be determined where affected birds are likely to land, it is necessary that treatments be carried out when people and traffic are largely absent; consequently they are normally done in the early morning - soon after dawn - and are completed by 0800 h. To condition the birds to feeding at this time, prebaiting is carried out for approximately 2 weeks before an operation. Nevertheless, it is difficult to ensure that the pigeons will feed at a particular place and at a particular time of day. Some of the population may well prefer to feed later in the day and, when breeding pairs are involved, the female is likely to be sitting on the nest throughout the baiting period so that the full population is not at risk. All these factors have contributed to the fact that operations against feral pigeons have been less successful and less popular than those against house-sparrows and this is reflected in Tables 1 and 2, from which it can be seen that considerably more treatments have been carried out against the latter species. Concentrated trapping is still often preferred as a control method for feral pigeons in spite of the labour and length of time involved and it must be admitted that, under certain conditions, trapping can achieve a satisfactory result. In the present climate of public opinion, it is difficult to see how the stupefying bait technique for feral pigeons can be improved other than by using a more efficient drug than alpha-chloralose. If such a drug were found, it would also increase the efficiency of treatments against wood-pigeons, where it is also desirable to restrict the scatter of affected birds.

Against house-sparrows problems of baiting technique are fewer and can more easily be overcome. Most of the treatments against these birds take place in buildings, particularly bakeries, granaries and food stores, and that the birds will

feed in these buildings and accept the bait can readily be determined by pre-baiting. Alpha-chloralose has proved to be quicker-acting and more effective against house-sparrows so that problems due to delay between feeding and hypnosis are not so apparent, and most birds are affected close to the baiting areas.

Perhaps the greatest baiting problems arise when operating against wood-pigeons. It is not possible to attract them to a particular site by pre-baiting and bait has to be laid on fields where the birds have already started to feed or are likely to feed in the near future. This means that some knowledge of the birds' normal feeding pattern is an advantage; it is useless to lay the bait on a field in the hope that the birds may come to feed on it - there has to be good reason to suppose that the birds will shortly visit it. Bait exposed for too long may become ineffective, so that the timing of bait laying is important. As bait has to be laid on fields of crops, it is essential that the bait is more attractive than the crop itself. Tic beans are, to some extent, a compromise bait; wheat is preferred by wood-pigeons but presents too great a risk to protected and game species. There is, therefore, the risk that by laying bait, one is attracting pigeons on to the very crop that it is intended to protect. These problems mitigate against the chances of success when baiting for wood-pigeons and consequently failures do occur.

POPULATION OR LOCAL CONTROL?

The term "control" is widely used when considering pest species, but it is necessary to elaborate on what is meant by this term when it is applied to birds. It is used to indicate a reduction in numbers of the species, and this reduction is normally achieved by killing. With birds many people think in terms of "population control" - a general reduction of the whole population of a species so that any damage it causes is eliminated or lessened. But with the majority of bird species, population control is virtually impossible using any of the existing control methods, including stupefying baits. Murton (1965) has shown that in the wood-pigeon, the annual adult mortality is about 36% and the average annual productivity is 2.1 young/pair. Thus fifty pairs of adults produce 105 young and of these only thirty-six are required to replace adult losses and keep the population constant, so that the annual juvenile mortality must be about 66%. The wood-pigeon population of Britain is more or less stable, so each year large numbers of juveniles must die through natural causes (largely by starvation). With such a large reservoir of young birds, it follows that a great proportion of the population must be killed to achieve a long-term reduction - a far greater proportion than is possible with any existing control method as at present applied, where great efforts are made to kill birds that would have died through natural causes. With house-sparrows, where the juvenile mortality is 70-80% (Summers-Smith, 1963), the position is similar.

From 1965 to 1968 a population study of the feral pigeon was carried out in the Manchester Docks. The results obtained in this study are in the process of analysis, but it is abundantly clear that the removal of large numbers of pigeons from the docks made no significant difference to the resident population - any birds removed were replaced within a matter of weeks. In sites such as dockyards there is a saturated population and it is unlikely that all the birds breed every year; nevertheless many are capable of breeding all the year round and the breeding potential of feral pigeons is very great. With all three species under consideration large-scale population control is a practical impossibility.

Small-scale local control, however, is possible. Small discrete populations of feral pigeons at sites such as factories and warehouses can be kept in check by means of stupefying baits, although repopulation is always likely to occur and regular treatments are usually necessary to keep the sites clear of birds. Similarly, regular treatments can keep buildings relatively free of house-sparrows. With this species repopulation is also a problem, but as house-sparrows are colonial, once a colony is eliminated repopulation will not occur except from juveniles which normally

try to establish themselves at a site during late autumn and winter. It follows that if a successful stupefying bait operation is carried out just at the start of the breeding season, a site can remain clear of house-sparrows for almost a year and an annual treatment should be sufficient to keep the site relatively sparrow-free.

This year licences have been issued to farmers and growers to allow the use of alpha-chloralose treated tic beans against wood-pigeons in order to protect pea and brassica sowings or seedlings. Bait is laid on these crops just as damage is about to occur and, provided it is taken, birds which would otherwise be causing damage are removed. As it may be several weeks before other birds move in to fill the gap, protection is given to the crop for a limited period at a time when it is particularly vulnerable. Thus local control is being used as a form of crop protection without attempting any long-term reduction in wood-pigeon numbers.

CONCLUSION

This paper has concentrated on the problems arising in using stupefying baits to control birds and has tended to paint a rather gloomy picture. Nevertheless the method can be of considerable benefit if properly applied and it must not be thought that most of the problems are insuperable. Against house-sparrows in buildings, particularly those in urban areas, stupefying baits have proved highly successful and useful, and even if their use against feral pigeons has been rather less effective, the method is still widely used against these birds. Agricultural damage by feral pigeons is comparatively rare, but it does occur close to urban populations and can be locally severe; under these circumstances, operations in an urban environment can benefit an agricultural district. House-sparrow treatments in farm buildings, such as granaries, can help to reduce damage to stored crops; it has not proved possible to bait successfully for sparrows when they are damaging cereal fields, prior to harvest, but operations in buildings, earlier in the year, may have some effect on the local population and thus incidentally lessen this type of damage. For wood-pigeons it is too early as yet to forecast what the future of the method will be. The results obtained in experimental operations were encouraging, but those obtained in this year's treatments appear rather less so, although they have yet to be fully analysed. Undoubtedly there are problems, but modifications may well ensure more success in the future. It must be emphasised, however, that regular treatments are necessary and that stupefying baits are not a once and for all answer to bird control. It is interesting to reflect that growers, who quite readily accept the need to use pesticides annually for the control of insect pests, are often reluctant to consider that annual treatments are required for controlling birds, and clamour for a method of control that will reduce the population permanently - in spite of all the arguments put forward showing that this is virtually impossible. Provided its limitations are recognised the use of stupefying baits can make a useful contribution to the local control of bird pests, to be carried out in conjunction with, and not necessarily replacing, more conventional methods of control.

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METABOLISM OF SOME ORGANOPHOSPHORUS INSECTICIDES BY STRAINS OF HOUSEFLY

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Summary Six strains of housefly were used in a study to relate the genetics of their resistance to organophosphorus compounds to their detoxification of these insecticides. Inconclusive results were obtained with whole flies, but subcellular fractions decomposed diazinon, diazoxon and ethyl chlorthion by several mechanisms. One mechanism, located in the microsomes, was common to all strains. Two other mechanisms, one soluble and another in the microsomes, were associated with resistance factors on chromosomes II and V, respectively. The effect of the synergists sesamex and S,S,S-tributylphosphorotrithionate on these mechanisms partly explains their effect on toxicity to the different strains.

Knowledge of how a substance affects a sensitive mechanism is not enough to predict its biological activity. Thus, organophosphorus insecticides kill by inhibiting cholinesterase but anti-cholinesterase activity is not a reliable guide to toxicity, because toxicity is much affected by other phenomena. These include entry of the poison into the insect (Farnham, A. W. et al., 1965) and metabolism of the poison (Matsumura & Hogendijk, 1964; Nakatsugawa, T. et al., 1969) to compounds of greater or lesser toxicity. When the dynamics of these processes follow the usual courses of biochemical reactions, these will depend on the concentrations of the insecticides and metabolites, which, in turn, are determined by the distribution of these compounds between the several phases of the insect, and some insecticides are strongly sorbed on insect solids, others are not (Gwiazda & Lord, 1967; Lord, 1968).

In addition, the size and shape (i.e. morphology) of the insect affects the dose required to kill, so toxicity can be described only in terms of numerous phenomena, each likely to influence, quantitatively if not qualitatively, others. At present few if any of these phenomena can be described precisely. In an attempt to control and minimise the effects of gross differences in morphology and chemical composition, we restricted our work to houseflies. Using strains of known genetic composition, which differ in their susceptibility to insecticides, we sought and examined differences of metabolism of poisons in attempts to relate the differences to susceptibility.

We are fortunate to have strains of houseflies in which resistance mechanisms have been genetically separated by Sawicki & Farnham (1967, 1969), who also supplied information about the toxicity of compounds and the effects of synergists on toxicity to these strains (Table 1). Diazinon has been used as the standard insecticide, because our first strains of flies were resistant to it. Ethyl chlorthion was used because some strains of flies resisted both it and diazinon, and others only diazinon (Sawicki & Farnham, 1969), suggesting different mechanisms of resistance, one effective against both and one against diazinon alone. This offered the possibility of identifying the mechanisms, in addition to separating them by genetic selection. We made preliminary studies of poisons applied to living flies, but because penetration is slow, only a small proportion

of the insecticide is metabolised. As penetration differences contribute to resistance, an uncertain proportion of the dose of insecticide can be metabolised, making it difficult to evaluate and compare metabolism in different strains. To study the metabolism of standardised amounts of insecticides, we therefore macerated houseflies and used subcellular fractions.

Table 1

The toxicity† of diazinon and ethyl chlorthion to strains of houseflies and the effects of synergists

Strain	LD50 µg/fly					
	Ethyl chlorthion			Diazinon		
	Alone	+TBTP	+sesamex	Alone	+TBTP	+sesamex
ocra SRS	0.067	0.023	0.16	0.029	0.014	0.012
ac;ar;bwb;ocra SRS	0.095	0.070	0.22	0.040	0.025	0.024
29	-	-	-	0.62	0.36	0.14
393	4.6	0.28	*	0.50	0.023	0.96
466.500	0.081	0.049	0.20	0.39	0.27	0.025
SKA	42.0	15.5	*	13.0	10.0	6.2

* excessive antagonism - LD50 not determined

† from Farnham & Sawicki (1967, 1969)

METHOD AND MATERIALS

Diazinon, diazoxon, and [^{14}C-ethoxy] diazinon were kindly given by Messrs J. R. Geigy, and [^{32}P] diazoxon was bought from the Radiochemical Centre, Amersham, Bucks.

Desethyl diazinon and desethyl diazoxon were synthesised by methods described by Hollingworth, R. M. et al., (1967) for the preparation of analogous derivatives of other insecticides, and the identity confirmed by NMR spectroscopy.

Ethyl chlorthion was given by Farbenfabriken Bayer. [^{14}C-ethoxy] ethyl chlorthion was synthesised by a method adapted from Hilton & O'Brien (1965) using ^{14}C ethanol purchased from the Radiochemical Centre, Amersham, Bucks. Its identity was confirmed by NMR spectroscopy and co-chromatography with authentic un-labelled material.

Desethyl ethyl chlorthion was prepared by a method analogous to desethyl diazinon, also by reacting one mole of ethanol with one mole of O-3-chloro-4-nitrophenyl dichlorophosphorothionate under conditions analogous to those used for the preparation of labelled ethyl chlorthion. The products co-chromatographed in system III and the results of NMR spectroscopy were consistent with the desired product. Monoethyl phosphorothioic acid (MEPTA) was prepared by condensing ethanol with thiophosphoryl chloride and separating the products on paper using system I. Diethyl phosphorothioic acid (DEPTA) was prepared in the same way as MEPTA, also by alkaline hydrolysis of parathion, and separated by chromatography. Diethyl phosphoric acid (DEPA) was bought (Eastman Kodak).

Sawicki & Farnham (1967, 1969) described the detailed genetics of the strains of houseflies studied (Table 2). The flies were kept at 20°C and fed on ad lib water, sugar and milk until used 4 to 7 days after emergence.

Table 2

Factors of diazinon-resistance in strains of housefly

Strain*	Type of resistance	Located on chromosome	Detoxification mechanism
ocra SRS	None (Susceptible)	-	C+D
ac;ar;bwb;ocra SRS	None (Susceptible)	-	C+D
29	gene a (low aliesterase)	II	A+C+D
393	gene a (low aliesterase)	II	A+C+D
466.500	sesamex-inhibited	V	B+C+D
SKA	gene a + penetration factor + sesamex-inhibted	II III V	A+B+C+D

* strains 29, 393 and 466.500 were derived from the SKA strain (Sawicki & Farnham, 1967, 1969)

1 µl of a solution of insecticide in acetone was applied to the ventral side of the thorax of individual flies, which were then kept at room temperature (20°C) for 1 or 2 hours as necessary and examined for decomposition products of the insecticides.

Batches of flies were ground in an all-glass macerator with hexane (10 flies/ml), to extract the bulk of unchanged insecticide. The solids were then reground with N HCl (4 flies/ml), centrifuged (1,000 g for 10 mins), washed with more N HCl (20 flies/ml) and re-centrifuged. The combined aqueous layers were extracted with 3 portions of 2 ml ether. The aqueous and ether layers were chromatographed and autoradiographed to separate and find the metabolites formed.

A second extraction procedure was also used for comparison. After extraction with hexane, the solids were reground with 80% aqueous acetone (4 flies/ml), centrifuged, washed with acetone (20 flies/ml) and re-centrifuged. The acetone extractions were combined and examined for metabolites.

Subcellular fractions were prepared by differential centrifugation of whole-fly homogenates in phosphate buffer (0.15 M, pH 7.3) containing bovine albumin (2% w/v; British Drug Houses). The supernatant fluid from centrifuging at 10,000 g for 10 mins was recentrifuged at 100,000 g for 60 mins to give a soluble fraction (fluid) and a microsomal fraction (precipitate). Typical reaction mixtures contained material from 10 flies, 2 mg NADPH (Boehringer Corp, London) and 4 mg reduced glutathione (Boehringer Corp, London) in 2 ml buffer and were shaken in air or nitrogen at 30°C. Sesamex (20 µgm), a methylene dioxyphenoxy type of synergist, and S,S,S-tributylphosphorotrithionate (TBTP) (10 µgm), a synergist that inhibits aliesterase, were added in 10 µl acetone. The reaction was ended by adding cold acetone (3.0 ml), and an aliquot of the supernatant fluid was chromatographed.

Chromatography

Four systems of chromatography were used to separate the insecticides and their metabolites. Two used Whatman No 1 paper with (I) acetonitrile:water: ammonia (40:9:1) and (II) propan2-ol:ammonia (75:25) as solvent. The third (III) used silica impregnated paper (Whatman SG81) and hexane:acetone (4:1) as solvent. The fourth (IV) used Whatman No 1 paper impregnated by dipping in 10% liquid paraffin with acetonitrile:water (3:7) as solvent.

Rf values of materials with solvent I were the same whether Whatman No 1 or SG81 paper was used, and solvent I was sometimes used after solvent III on SG81 paper in 2-way chromatography. Table 3 gives the Rf values of materials in these systems.

Table 3

Chromatography of diazinon, ethyl chlorthion and some possible metabolites

Compound	Rf values in solvent systems			
	I	II	III	IV
diazinon	1.0	1.0	-	0.05
diazoxon	1.0	1.0	-	0.8
desethyl diazinon	0.85	0.95	-	-
desethyl diazoxon	0.60	0.80	-	-
DEPTA	0.65	0.75	0	-
DEPA	0.40	0.70	0	-
ethyl chlorthion	1.0	1.0	0.87	-
ethyl chloroxon	1.0	1.0	0.30	-
desethyl ethyl chlorthion	0.95	0.95	0	-
MEPTA	0.06	-	0	-

A fifth system using Whatman No 1 paper with n-butanol:acetic acid: water (11:4:5) was used to try and separate amino acids and some radioactive products.

Radioactivity present in chromatograms was detected by autoradiography (Ilford Industrial "G" X-ray film) and assessed by solid scintillation counting (Naton 136) of dissected areas of chromatograms.

Phosphorus compounds were detected by exposing the chromatograms to bromine vapour and then spraying with 0.5% ferric chloride in 0.5% HCl in 80% ethanol. Phosphorus compounds showed as white areas against a "pink" background.

Insecticides were detected by spraying with 2% 4-(p-nitrobenzyl) pyridine in acetone, heating to 110°C for 10 mins and then spraying with 10% tetraethyl pentamine in acetone (Watts, 1965).

Metabolism by whole fly (in vivo)

Diazinon is metabolised to diethyl phosphorothioic acid (DEPTA) and diethyl phosphoric acid (DEPA). These metabolites are detected within 10 mins of dosing and their quantity increases with time of incubation.

Significant interstrain differences were not detected, and variation within the same strain was as great as between strains. Flies used more than 9 days after emergence metabolise diazinon less rapidly than younger flies and produce less DEPA.

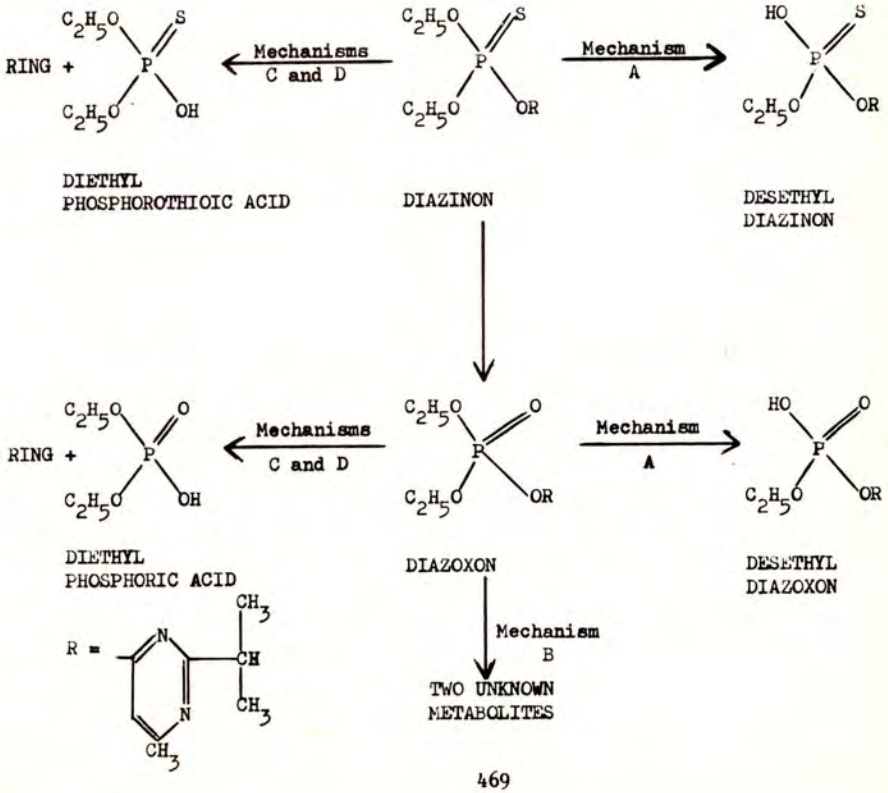
Ethyl chlorthion is metabolised by the SKA strain to several substances that resemble chromatographically those formed when ethyl chlorthion is incubated with insect subcellular fractions.

Metabolism by subcellular fractions (in vitro)

Three mechanisms concerned with detoxification of diazinon and diazoxon were described by Lewis (1969) (Table 2).

Figure 1

Metabolism of diazinon



Mechanism A, desethylation of diazinon and diazoxon, occurs entirely in the soluble fraction and requires reduced glutathione (GSH) as cofactor, but not NADPH or oxygen. This mechanism occurs only in strains with gene a (strains 29, 393 and SKA).

Mechanism B, degradation of diazoxon but not diazinon to two unknown metabolites, is microsomal and requires NADPH and oxygen. It was found only in strains with the sesamex-inhibited factor on chromosome V (strains 466.500 and SKA).

Mechanism C, the cleavage of diazinon and diazoxon to DEPTA and DEPA respectively, also occurs in the microsomes and requires NADPH and oxygen. It occurs in all strains and seems to be a common mode of detoxification. Significant interstrain differences (except for the SKA strain which metabolised slightly faster) were not found in this mechanism, in contrast to the results of El Basheir and Oppenoorth (1969).

A fourth mechanism (D), cleavage of diazinon and diazoxon to DEPTA and DEPA respectively, by a soluble, GSH-requiring enzyme, was found. It also occurs in all strains, but is distinct from, and much less powerful than, mechanism C.

As mechanisms C and D occur in all strains, only A and B can be considered resistance mechanisms. Diazinon is activated to diazoxon by the microsomes, and this reaction requires NADPH and oxygen, in all strains.

GSH, in addition to being essential for mechanisms A and D, increases the activity of all the microsomal mechanisms (B, C and diazoxon formation). Two other reduced sulphur compounds, dithiothreitol and cysteine, also increase microsomal metabolism, but desethylation does not occur in their presence. Iodoacetate (3 mM) and p-chloromercuribenzoate (0.05 mM) inhibit microsomal metabolism, thus confirming a role for sulphur.

Microsomal metabolites were formed rapidly initially, but did not increase after 60 mins incubation, whereas the products from the soluble, GSH-requiring mechanisms increased steadily for at least 2 hours, confirming that these two pathways are distinct.

Sesamex inhibits all the microsomal mechanisms (B, C and diazoxon formation), whereas TBTP only inhibits the soluble mechanisms (A and D), which are unaffected by sesamex.

The metabolism of ethyl chlorthion is less clear. Mechanism C, the microsomal cleavage to DEPTA, takes place in quantities similar to that for diazinon.

However, desethylation (mechanism A) is less obvious than with diazinon, although toxicity studies with different strains (Sawicki & Farnham, 1969) suggest that this should be a major pathway of detoxification of ethyl chlorthion in the strains with gene a. Four metabolites are produced by the soluble fraction of gene a strains (29, 393 and SKA) only in the presence of GSH, which suggests a similarity to mechanism A (desethylation of diazinon). One of these metabolites (Rf = 0.1 in solvent I) is also found in diazinon metabolism and is probably S-ethyl glutathione. The other three (Rf = 0.15, 0.2 and 0.35 in solvent I) have not been identified. The metabolite with Rf = 0.35, produced in considerable quantity, is not DEPA, which also occurs. TBTP inhibits the formation of these four metabolites, but sesamex does not.

Preliminary tests suggest that the compound with Rf = 0.35 may be an amino acid derivative, because in chromatograms run in system I it is invariably associated with ninhydrin staining material. Two-way chromatograms, run first in system I then system V, did not disprove this hypothesis. It was thought that the compound might be a glutathione derivative analogous to that found with BHC (Clark, A. G. et al., 1969) but when the substance was dissected from a chromatogram and

hydrolysed overnight in 6N HCl at 110° in a sealed tube, chromatograms run in system V had mostly yellow ninhydrin-reacting substances, not corresponding to the constituent amino acids of glutathione.

DISCUSSION

Although our results are incomplete, they suffice to explain in terms of metabolism of poisons some of the toxic effects of diazinon and ethyl chlorthion observed by Sawicki & Farnham (1969). Consider first the toxicity and metabolism of diazinon and diazoxon in relation to the strains of housefly. The detoxication mechanism C is inhibited by sesamex, which increases toxicity not only to the susceptible strains in which this mechanism predominates, but also to the strain that has the sesamex-sensitive mechanism B. Sesamex does not increase the toxicity of diazinon and diazoxon to the strains 29 and 393 which have gene a as sole resistance factor and use desethylation (mechanism A) as a major degradative pathway. TBTP inhibits mechanism A and increases diazinon toxicity to the strains 29 and 393. For reasons not understood, neither synergist greatly affects the toxicity of diazinon to the polyfactorial strain SKA.

There is less information about the metabolism of ethyl chlorthion, but a soluble, glutathione-requiring mechanism (A) is probably common to both diazinon and ethyl chlorthion, although the products of decomposition are not strictly analogous, as relatively less desethyl chlorthion is formed and more of the unidentified metabolite (Rf = 0.35 in system II) suspected of being an amino acid derivative. The simplicity of this supposition is attractive, because TBTP increases the toxicity of both ethyl chlorthion and diazinon to the gene a strains (393 and 29).

The diminished toxicity of diazinon in gene a strains, and ethyl chlorthion in all strains, caused by sesamex possibly results from a partial inhibition of the microsomal oxidation to the analogous phosphates, rather than from a modification of detoxification mechanisms.

There remains one major anomaly in the toxicological results; it is the lack of synergism of both diazinon and ethyl chlorthion by either TBTP or sesamex in the polyfactorial SKA strain. This cannot be satisfactorily explained.

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INVESTIGATIONS WITH SOME BIO-DEGRADABLE DIELDRIN ANALOGUES

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Summary The examination of potential metabolic pathways available for two cyclodiene epoxides (dieldrin analogues) in insects and other animals revealed species differences that are interesting from the point of view of selective toxicity. Investigations indicate that one compound in particular is detoxified in mammals and some birds by an additional metabolic pathway (epoxide ring hydration) that is insignificant in, for example, houseflies, which possess only oxidative detoxication mechanisms. The results indicate that combinations of this compound with suitable synergists might be effective against insects such as the housefly, while retaining low toxicity to other animals possessing the additional metabolic route.

INTRODUCTION

Popular demand for toxicants that combine high insecticidal efficiency with low or moderate persistence in the environment has left the organochlorine insecticides with few friends; properties for which they were hailed in the early post-war years as the final solution to the disease vector and other insect problems are now condemning them. Nevertheless, current predictions regarding the growth of world population and the associated problems of food supply make it questionable whether we can afford to dismiss these insecticides quite so readily.

The selective toxicity of many existing insecticides is largely accounted for by species differences in rates of metabolic toxication and detoxication, and once it is known that low toxicity toward a particular species is due to some form of detoxication mechanism, there is always the hope that this capability may be absent in another target species, or may be more easily inhibited in one species than in another, as for example, by a suitable synergist.

This requirement for high insecticidal activity combined with low mammalian toxicity and low persistence is not readily met in the cyclodiene series, although there are several commercial compounds of this type whose mammalian toxicity is much lower than that of dieldrin.

Investigations, with houseflies (Musca domestica), of structure-activity relationships among cyclodiene compounds (Brooks and Harrison 1963, 1964a and b) revealed the existence of detoxication mechanisms for several analogues of dieldrin and the inhibition of some of these mechanisms by suitable synergists was accompanied by enhancement of toxicity. This paper summarises comparative investigations on two of these analogues with insects, and with tissue preparations from several other animal species. One compound has metabolic patterns that illustrate particularly well the principles involved in selective toxic action.

METHOD AND MATERIALS

1,2,3,4,9,9-hexachloro-exo-5,6-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene (HCE; 1, Fig. 1) and 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene (HEOM; 5, Fig. 1) were prepared as previously described (Brooks and Harrison 1964a). Sesamex [2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane] was supplied by Shulton Chemicals Inc., and SKF 525A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) by Smith, Kline and French Laboratories Ltd.

Methods used for preparing microsomes and for the extraction of insecticides and their metabolites following incubation with microsomal preparations were similar to those reported previously (Ray 1967, Lewis et al. 1967, Brooks and Harrison 1969). In experiments with avian livers, microsomes and 11,000g supernatants from liver homogenates (fortified with NADP and glucose-6-phosphate when appropriate) were incubated with the epoxides for 90 minutes at 42° (El Zorgani et al. 1969).

Figure 1

Metabolic products of HCE and HEOM
1, HCE; 2, one of the possible HCE oxidation products; 3, dieldrin; 4, HCE-trans-diol; 5, HEOM; 6, HEOM-trans-diol.

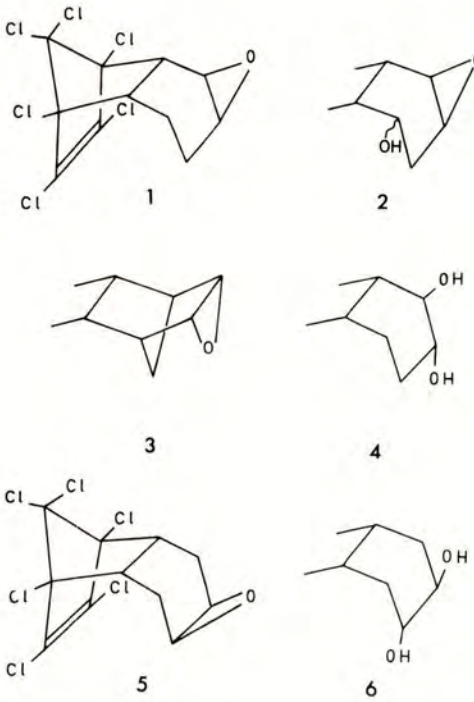


Table 1

Toxicity (LD50; mg/kg) of HCE to various species

	HCE (alone)	HCE (with sesamex)	Dieldrin
Housefly	100	2	1
Blowfly	>250	42	2
Mouse (oral)	200-400	-	75
Rat (oral)	>400	-	45

RESULTS

Toxicity tests with female houseflies showed that the dieldrin analogue HEOM (5, Fig. 1), which lacks the 5,8-methylene bridge of dieldrin (partial structure 3, Fig. 1), is virtually without toxicity to this insect, while the related epoxide HCE is about 100x less toxic than dieldrin (Table 1) on topical application. However, in the presence of the pyrethrum synergist sesamex, an established inhibitor of oxidative microsomal detoxication, HCE was strongly synergised, while the toxicity of HEOM was scarcely affected. Thus, in the presence of sesamex, HCE became nearly as toxic as dieldrin (Table 1) and similar effects were found with blowflies (Calliphora erythrocephala).

Houseflies hydrated the epoxide ring of HEOM rapidly and completely to give the corresponding innocuous trans-diol (6, Fig. 1) and, in vitro, housefly microsomes were found to be particularly active in effecting this conversion, which was somewhat inhibited by SKF 525A in vitro but not sufficiently strongly in vivo to stabilise HEOM, or to synergise its low toxicity. HCE was also metabolised fairly rapidly by houseflies and the synergistic effect with sesamex was associated with blocking of this metabolism and consequent accumulation of HCE in the tissues. Moreover, in contrast with their action on HEOM, housefly microsomes hydrated the epoxide ring of HCE extremely slowly, but extensive oxidative metabolism occurred (Table 2) which required the presence of $NADPH_2$ and oxygen and was inhibited by sesamex.

Information regarding the fate of these compounds in vivo in mammals is scanty but liver microsomal preparations from rabbits and pigs readily hydrated HEOM and converted HCE into both hydration and oxidation products. Rat liver microsomes converted HEOM into a mixture of oxidation and hydration products and HCE into mainly oxidation products. For all three species formation of the oxidation products resulted from mixed function oxidase action and was blocked by sesamex, which did not inhibit the hydration reactions. Chromatographic evidence indicated that the mammalian and insect microsomal preparations each produced two main oxidation products from HCE, and these appeared qualitatively similar for all these species. There is chemical evidence (Brooks and Harrison, unpublished results) for their formulation as hydroxylation products of HCE retaining the epoxide ring (such as 2, Fig. 1).

Partial hydration of racemic HCE by pig liver microsomes to give the trans-diol (4, Fig. 1) was rapid but the enantiomeric forms of the epoxide were converted at greatly different rates, so that one optically active form could be recovered from the microsomal system when hydration of the racemate had ceased. The recovered enantiomer was also hydrated very slowly by rabbit liver and rook liver microsomes.

Table 2

Comparative metabolism of HCE by microsomes from various species (at pH 7.4)

(results with avians after El Zorgani et al. 1969)

Species[a]	Hydration[b]	Oxidation[b]
Housefly	0	40
Rat	1	40
Pig	30	30
Rabbit	10	60
Quail[c]	2	60
Pigeon	0	90
Rook	12	50
Jackdaw	3	80
Fulmar	2	70

(a) First four species, incubations 30 min at 37°; last five, 90 min at 42°.
(b) Percent conversion of epoxide added.
(c) 11,000g supernatant used.

Approximate metabolic capabilities toward HCE of microsomal preparations from various species are compared in Table 2.

In some circumstances houseflies were observed to recover from poisoning by HCE, which otherwise produced toxic effects exactly similar to the irreversible ones observed with dieldrin. The toxicity to houseflies of the optically active form of HCE recovered from pig liver microsomes was not significantly different from that of the racemate when topically applied in acetone (Brooks et al. 1968).

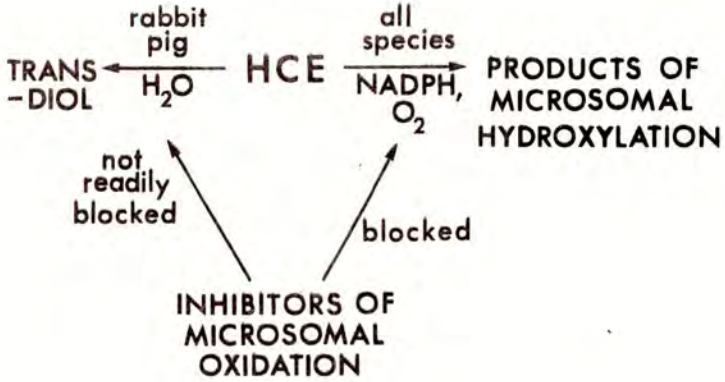
DISCUSSION

By analogy with the structures of related cyclodiene epoxides such as dieldrin and HCE, there is reason to suppose that HEOM is, intrinsically, a highly toxic compound. Its very low toxicity toward houseflies appears to be adequately explained by rapid inactivation through epoxide ring hydration. The ease of hydration of this compound is in complete contrast with dieldrin, which is highly stable in the housefly. Although compounds such as triphenyl phosphate, tri-o-cresyl phosphate and SKF 525A showed some inhibitory effect on this process *in vitro*, only SKF 525A had a slight stabilising effect on HEOM *in vivo* in houseflies and this appeared inadequate to potentiate the toxicity, so that at present no adequate synergist is available for this compound. It might, however, be highly effective against insects which lacked the hydrative capability and tests against more insect species would be of interest in this connection.

While fulmar and pigeon liver microsomes, and quail and pigeon liver

Figure 2

Summary of metabolic pathways for HCE, and the effect of inhibitors



homogenates, are relatively inactive toward HEOM in the absence of $NADPH_2$ and oxygen, rook and jackdaw liver microsomes readily hydrate the epoxide ring (El Zorgani et al. 1969) and thus resemble more closely the mammalian preparations so far examined. On this basis HEOM might be rather toxic to pigeon, fulmar and quail, but additional oxidative pathways are probably available and would doubtless modify the situation in vivo.

HCE is more interesting because of the apparent absence of the hydration route in some species, the stereoselective nature of the process in species that do hydrate, and the availability of inhibitors for the oxidative processes, which appear to be well developed in all species so far examined (Table 2). Its intrinsic toxicity toward the housefly appears to be close to that of dieldrin (Table 1), and in the absence of natural tolerance mechanisms other than metabolism, it should be highly toxic to insects lacking both hydrative and oxidative detoxication mechanisms, and synergisable against insects, such as the housefly (Tables 1 and 2), possessing only the oxidative capability. Preliminary tests with rats and mice (Table 1) indicate favourable oral toxicities relative to dieldrin but no experiments have been conducted with synergists.

Synergists such as sesamex, inhibiting microsomal detoxication, differ markedly in structure from HCE and it may be hoped that differential absorption and elimination of the two compounds would minimise the possibility of toxic effects in accidentally exposed non-target species. This situation is apparently realised with the carbaryl/2,3-methylenedioxynaphthalene combination, which is highly toxic to houseflies but of low toxicity to mice because of the faster elimination of the synergist from the mammal (Sacher et al. 1969). Moreover, present indications are that some non-target species (Table 2) possess the hydrative capability that is not blocked by commonly used synergists, and are thus afforded an additional protective detoxication route, at least against the hydration-labile enantiomer (Fig. 2). All species so far examined apparently hydrate the same HCE enantiomer and oxidise the other, hydrolytically stable, enantiomer normally.

The foregoing remarks regarding toxicity require a correlation between the processes *in vitro* and events *in vivo*. This exists in houseflies, and probably also in blowflies. For the other species there appears to be qualitative similarity between the products formed by microsomes and by the more complete system represented by homogenates. Some also appear to excrete products similar to those found *in vitro* and conjugation products are undoubtedly formed, but no detailed information is available at present.

HCE is the first compound related to dieldrin shown to exhibit reversible poisoning of insects (Brooks and Harrison 1964a). Houseflies were found to recover from the poisoning effects after extended periods of apparent death, although they appeared to suffer permanent wing damage. This phenomenon is clearly related to the availability of detoxication mechanisms operating for HCE but not for dieldrin and, with the reasonable assumption that the mode of action of these compounds is similar, indicates that the prostrate stage of dieldrin poisoning is a narcosis that is normally irreversible because this particular epoxide persists in the tissues indefinitely.

The conclusion that the enantiomeric forms of HCE (and other asymmetric cyclodiene epoxides) do not differ greatly in their toxicity to the housefly supports indications from earlier studies of structure-activity relationships in this series (Brooks 1966) that the positioning of the epoxide moiety in the terminal ring is not too critical for toxicity of these molecules, provided that the latter do not become too large.

Acknowledgement

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THE PROTECTIVE AND VAPOUR-PHASE ACTIVITIES OF SOME DINITROPHENOL FUNGICIDES

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Summary The importance of vapour-phase phenomena on the anti-mildew activities of formulations of 4-(1-cyclopentylalkyl)-2,6-dinitrophenol fungicides is discussed. High protective activity is shown by those phenols which have high vapour-phase activity, although such compounds will not necessarily have high vapour pressures. However, the importance of vapour-phase activity is not easily interpreted since crotonates with low vapour-phase activity are good protectants when applied to give good coverage.

INTRODUCTION

Despite the introduction of novel mildew fungicides, formulations based on dinocap have the advantage of controlling many species of these fungi and are therefore still widely used. The separation of dinocap into its six constituent isomers (Clifford et al. 1965) and subsequent biological testing of these compounds indicated the superior anti-mildew activities of the 4-(1-substituted alkyl) 2,6-dinitrophenols over their 2,4-dinitrophenol analogues coupled with a virtually total lack of phytotoxicity (Byrde et al., 1966., Pianka and Sweet, 1968).

Thus there was an obvious need to synthesise further 4-substituted-2,6-dinitrophenols and to study the mode of action of these potential anti-mildew fungicides. Woodcock and Byrde (1967) indicated the high anti-mildew activity of the 4-(1-cycloalkylalkyl)-2,6-dinitrophenols and these form the basis of a British Patent 23,712/67 assigned to N.R.D.C. This report describes some investigations on the anti-mildew activities of one homologous series of such compounds - the 4-(1-cyclopentylalkyl)-2,6-dinitrophenols.

MATERIALS AND METHODS

The synthesis and chemical properties of the 4-(1-cyclopentylalkyl)-2,6-dinitrophenols have already been published (Fieldgate & Woodcock, 1968).

Biological assays of these materials were carried out using cucumber powdery mildew caused by Sphaerotheca fuliginea. Cultures of this fungus were maintained on vegetable marrow plants (var. Green Bush), and similar plants, trimmed to leave one true leaf, were used for in vivo assays, the leaves being inoculated with conidia in a dusting tower.

Vapour-phase activity tests in vitro

Spots of fungicide applied to glass slides with a microsyringe were incubated beneath clean slides uniformly dusted with mildew conidia for 20hr at 23°C in a water-saturated atmosphere, the slides being kept apart by glass spacers of 1-2mm thickness. In all cases, examination of the control slides indicated that at least 50% of the conidia had produced germ tubes. In some cases, a marked zone of inhibition of germination occurred under conditions which precluded direct contact with the spores. The sizes of the zones were measured using a microscope and calibrated mechanical stage.

Vapour-phase activity tests in vivo

Materials were formulated as aqueous suspensions or solutions using up to 5% acetone with 0.025% (w/v) Ethylan C.P. as dispersing agent. Discrete drops of the formulations (0.001 ml) containing 10^{-8} mole (ca. 3×10^{-6} gm) of active ingredient were placed on the leaves using a micrometer syringe and allowed to dry. After inoculation and incubation for 10 days in a well-ventilated glasshouse, control leaves were uniformly covered with mildew lesions, whilst treated leaves showed circular zones of protection around the applied deposits. The diameters of the zones of protection were measured with calipers. This technique does, of course, exaggerate any inherent phytotoxicity shown by the applied material.

Protective activity tests

Formulations of fungicides were applied to plants with a de Vilbis spray gun to give uniform deposits of a low-volume type, the deposits allowed to dry and the plants inoculated as before. The area of leaf infected was assessed using a scoring system and estimated ED50 values were obtained from plots of percentage of infection against spray concentration on logarithmic probability paper.

RESULTS

Table 1 shows the *in vitro* and *in vivo* vapour activities of the 4-(1-cyclopentylalkyl)-2,6-dinitrophenols and of their crotonic esters. The *in vivo* and *in vitro* vapour activities of the phenols rise to a maximum with increasing side chain (R_2) length and then fall rapidly. The crotonates, however, show no vapour activity *in vivo* but high initial activity *in vitro* decreasing rapidly to zero.

The figure shows plots of the *in vivo* vapour and protective activities against the number of carbon atoms in the side chain R_2. It is clear that optimal activity of each type is shown by the butyl homologue.

DISCUSSION

Bent (1967) and Hislop (1967) drew attention to the fact that the vapour-phase activities of foliar fungicides may be more important than had previously been realised. This is particularly true of fungicides used for the control of powdery mildews since the conidia of these fungi most usually germinate and infect host plants in the absence of water, and this precludes the uptake of fungicides from solution. Laboratory and glasshouse tests have shown that very good protection can be achieved with poor covers of fungicides which are active in the vapour phase. Unpublished work (Clifford and Hislop, 1969) carried out at Long Ashton indicates that the vapour-phase activity of a fungicide is not necessarily related to its saturated vapour pressure. The *in vitro* vapour-phase activities of the dinitrophenylcrotonates employed in this study decrease with decreasing saturated vapour pressure whereas those of the corresponding phenols *increase* with decreasing vapour pressure. Furthermore, the crotonates show no real vapour-phase activity *in vivo* whilst that of the phenols *in vivo* parallels that indicated *in vitro*. The latter observation suggests that partition of a dinitrophenol through the conidial membrane in the vapour-phase may be a very important factor.

The physical characteristics of the crotonates may be unfavourable for partition into the spore but suitable for partition into the surface waxes of the leaf resulting in "encapsulation" (Winchester, 1967; Clifford *et al.*, 1969) and consequent non-availability of active material. This would explain the poor *in vivo* vapour-phase activity of those crotonates which show such activity *in vitro*. However, protective activity tests which involve good coverage of the leaf surface, showed no significant difference between the anti-mildew activities of the dinitrophenols and their corresponding crotonates (Clifford and Hislop, 1969). The vapour-phase activity reported by Bent (1967) for "Crotothane", which is essentially a mixture of alkyldinitrophenylcrotonates, is almost certainly due to the presence of free phenols in the mixture (Kurtz and Baum, 1969).

The fact that the practice of formulating dinitrophenol pesticides in the form of their esters reduces phytotoxicity but may reduce any potential vapour activity, emphasises the importance of efficient application techniques. Nevertheless, it may be inferred that an uneven coverage of the leaf surface by a free dinitrophenol might well give adequate protection as a result of vapour-phase activity.

This discussion covers part of an extensive study of the anti-mildew activities of a wide range of alkyldinitrophenols and it is hoped to publish a fuller account of this work in the near future.

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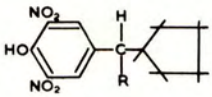
Table 1.

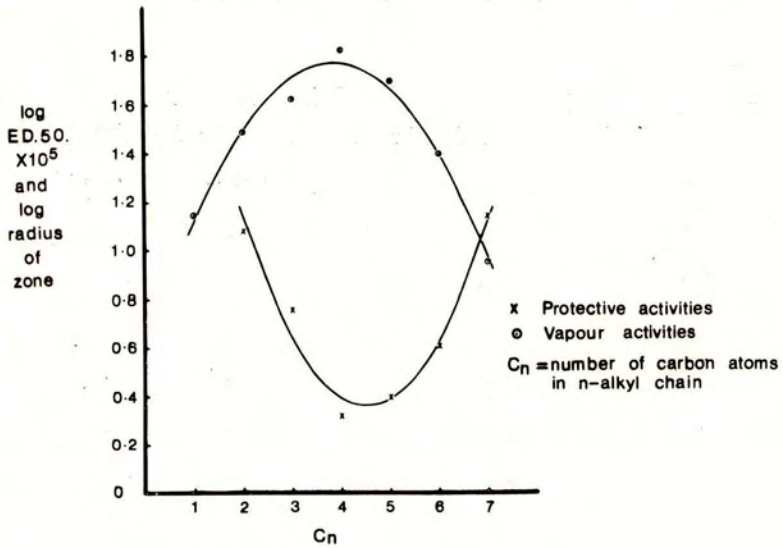
Vapour activities of 4-(1-cyclopentylalkyl)-2,6-dinitrophenols and their crotonates against Sphaerotheca fuliginea



	Mean radius of zone (mm)			
	in vitro inhibition		in vivo protection	
Homologue	phenol	crotonate	phenol	crotonate
Methyl	2·9	10·3	1·4	1·4
Ethyl	3·2	13·4	3·1	1·4
Propyl	2·4	7·9	4·2	1·4
Butyl	3·4	0	6·7	1·8
Pentyl	6·0	0	5·0	1·4
Hexyl	6·8	0	2·5	1·4
Heptyl	0	0	0·9	1·4

Fig. 1

Comparison of Protective and Vapour phase activities of 4-(1-cyclopentylalkyl)-2,6-dinitrophenols



Proc. 5th Br. Insectic. Fungic. Conf. (1969)

THE MODE OF ACTION OF A NEW GROUP OF SPECIES SPECIFIC PYRIMIDINE FUNGICIDES

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Summary

There is evidence that a new group of pyrimidine fungicides are non-competitive inhibitors of enzymes related to C-1 metabolism. The fungicides do not however inhibit dihydrofolic reductase, N^5N^{10} methylene tetrahydrofolate reductase or the enzymes of de novo purine biosynthesis in enzyme preparations of cucumber powdery mildew.

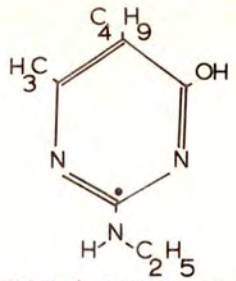
A new group of pyrimidine fungicides (Elias et al, 1968) Fig. 1 give a species specific control of powdery mildew, an obligate parasite on a wide range of plants. Systemic activity has been obtained in herbaceous plants with both dimethirimol (Fig. 2) and ethirimol. A single soil drench gives complete protection both to pre-existing and to newly formed leaves for at least eight weeks. The compounds are poorly translocated in woody tissue (Fig. 3).

As both ethirimol and dimethirimol are fungicidal within the cucumber leaf at an internal concentration equivalent to less than 10^{-7}M, it is assumed that the mode of action is that of a non-competitive enzyme inhibitor. To locate the site of this inhibition of fungal metabolism, a number of compounds have been supplied to the fungus via the host plant in an attempt to reverse the fungicidal activity of ethirimol on cucumber powdery mildew. In these experiments the fungicide (0.75 or 1.0 µg) was supplied to a cotyledon stage plant in water culture. Following uptake of the fungicide reversing agents were supplied at concentrations of 10^{-6} - 10^{-4}M and the plants infected and maintained in a spore filled atmosphere. Good reversals have been obtained with purines, folic acid and to a much lesser extent with thymine. Orotic acid and cytosine gave very much poorer reversals. This would suggest an inhibition of tetrahydrofolate (THFA) directed C-1 metabolism. In their biosynthesis purines derive carbons 2 and 8 via a THFA dependent reaction, as does the CH_3 group of thymidine. In their degradation, purines may donate a C-1 unit to THFA via formiminoglycine and pyrimidines via β-alanine.

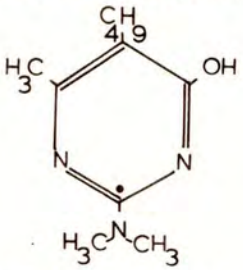
We have therefore examined the effect of dimethirimol upon enzymes of THFA metabolism.

No inhibition of either dihydrofolate reductase (Fig 4) or N^5N^{10} methylene tetrahydrofolate dehydrogenase has been obtained (Fig 5) using an enzyme preparation from cucumber powdery mildew. In addition, no dimethirimol inhibition has been found in the incorporation of $H^{14}COOH$ into purines or into formyl glycinamide ribotide. The ability of purines to reverse the fungicidal effect contrasted with the absence of any inhibition in the enzyme systems required for their biosynthesis may result from the nature of the label used, $H^{14}COOH$. The apparently anomalous result could arise from the difference between the reactions in which HCOOH, HCHO and CH_3 donate a 1-carbon unit to THFA and that in which either the β-carbon of serine or the α carbon of glycine is donated. In the normal course of metabolic events, C-1 units arise either from serine (serine hydroxymethylase) or from glycine via the amino levulinic synthetase reaction (Shemin et al 1955).

Fig. 1



PP149 (ETHIRIMOL)



PP675 (DIMETHIRIMOL)

5-butyl-2-ethylamino-4-hydroxy-6-methyl-pyrimidine (ethirimol) and
5-butyl-2-dimethylamino-4-hydroxy-6-methyl pyrimidine (dimethirimol).

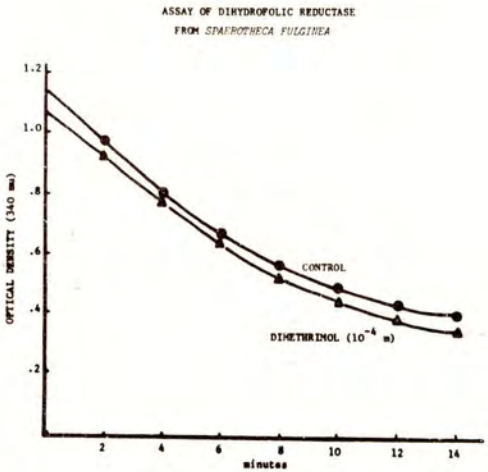
Fig. 2

Autoradiograph of freeze-dried cucumber plants grown in John Innes No. 2 compost and transferred to water culture before being supplied with 2-^{14}C dimethirimol for 6 hours. The isotope solution was then removed though some continues to adhere to soil particles on the roots. Plants fixed at time of isotope removal (0 hours) and intervals to 112 hours. An accumulation into the leaf margin, not seen at 0 hours has become obvious by the 24th hour.

Fig. 3

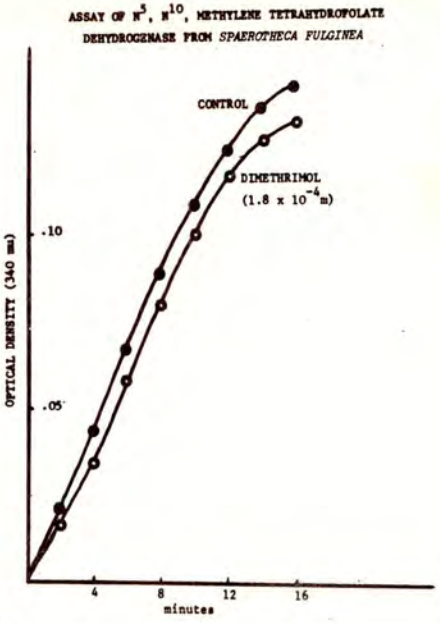
Autoradiograph of a freeze dried grape plant four days after a soil watering of 2-^{14}C-dimethirimol at 500 ppm. The radioactivity is largely confined to vein and stem.

Fig. 4



Assay of dihydrofolate reductase prepared and assayed after the procedure of Baker, Ho and Neilson (1964).

Fig. 5



Assay of N^5N^{10} methylene tetrahydrofolate dehydrogenase from an unpurified extract of cucumber powdery mildew by the method of Scrimgeour and Huennekens (1963).

These reactions, unlike others in which a C-1 unit may be donated to THFA, are dependent upon the co-enzyme pyridoxal (vitamin B_6).

That the blockage in metabolism is related to pyridoxal catalysed reactions is further evidenced by the ability of γ-amino levulinic acid, serine, glycine and pyridoxal to reverse the fungicidal action of ethirimol. In these experiments the fungicide is again supplied before introduction of the reversing agent. This prevents any chemical neutralization of the fungicide taking place outside of the plant. A photochemical reaction of this type has been found between dimethirimol and riboflavin. The possibility that such chemical reactions may occur within the leaf tissue is being investigated. The presence of the pyridoxal dependent enzymes serine hydroxymethylase and the decarboxylases of leucine and glutamic acid in cucumber powdery mildew has been demonstrated. It has not been possible to obtain any preparation requiring an addition of pyridoxal for full activity, so the failure of ethirimol to inhibit is probably not significant. Attempts to obtain pyridoxal requiring enzyme preparations are being made.

Although the powdery mildews are obligate parasites we have found that fungal respiratory activity continues for up to one hour following the removal by suction of parasite from host. We have therefore supplied 1:4-^{14}C succinic acid to isolated cucumber powdery mildew both in the presence and in the absence of ethirimol. Up to 13% of the supplied succinate is converted to malate and this is unaffected by ethirimol or by the further addition of non-radioactive acetate.

Of the radioactivity appearing in malate, only a fraction of 1% is released as $^{14}CO_2$ and this is malonate sensitive. With chromatographed extracts no radioactive compounds other than succinic and malic acid were detectable. We were therefore, unable to determine whether aminolevulinate synthesis was susceptible to the fungicide or even whether the fungus was capable of synthesizing this compound.

DISCUSSION

A biochemical investigation of an obligate parasite presents unusual difficulties. No assumptions may be made as to what enzyme systems must be present. When using the intact organism it must either be taking metabolites from the host or be in the process of dying. Similarly, if compounds are to be supplied to a healthy powdery mildew growth, then they must be supplied via the host plant. An unequivocal demonstration of the mode of action will therefore depend upon isolating enzyme systems that are non-competitively inhibited. Should it be confirmed that the fungicides act as pyridoxal antagonists it will be of considerable biochemical interest. Through C-1 metabolism, pyridoxal is involved in the biosynthesis of purines, thymidine and amino acids. In addition it is involved in thirteen different classes of enzymic reaction. Thus it would be possible to determine the species specificity for an enzyme and also the variation in specificity for different pyridoxal dependent enzymes of the same species. This may throw some light on the extent to which universal co-enzymes place constraints upon protein evolution.

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