STUDIES ON HALO BLIGHT OF BEANS

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Summary During studies on halo blight disease of French and Runner bean, caused by the bacterium Pseudomonas phaseolicola, a seed test was developed which involved the grinding of the seed to a flour and the assessment of its basic bacteriological content. Rapid and accurate identification of the pathogen in this flour was achieved by the use of specific phages or antisera. By these methods at least 1 infected seed in 1000 clean seeds could be detected. The methods have also been used for testing the effectiveness of seed treatments in the laboratory.

INTRODUCTION

Halo blight of French and Runner bean is caused by the internally seed-borne bacterial pathogen Pseudomonas phaseolicola. Disease free French bean crops can only be obtained by the use of seed grown in semi-arid, disease free regions of the world. Even here, in recent years, some crop infection has occurred. Runner bean seed, which is produced in this country, is always likely to be infected and may act as a source of infection for nearby French bean crops. Wharton (1967) has shown that runner beans are a source of race 1 of the disease in this country, whereas race 2 occurs mainly on imported French beans. Chemical control of the disease has so far been ineffective since normal seed dressings are unable to penetrate the seed. Even streptomycin seed treatment was found by Hagedorn (1967) to have no significant effect on the disease.

Whatever method of control is to be used, a primary requirement is a good seed test. Much work has been done in this respect. Wilson (1938) soaked seed in a selective medium to extract the bacteria, then inoculated pods to obtain positive confirmation of infection. Katznelson and Sutton (1951) used species specific phages to detect the pathogen in ground bean samples and Guthrie et al (1965) used antisera to detect the pathogen from seed. However little information was given regarding the exact levels of detection obtained by any of these methods. More recently Wharton (in press) has shown that infected white-seeded beans fluoresce under ultra-violet light, and using this method he was able to determine infection levels.

As a necessary preliminary to the development of a seed test, methods for the accurate identification of Ps. phaseolicola were studied. In this paper these methods are described and evaluated in relation to a dilution plate technique which has been developed for the detection of bacterial levels in seed.

METHOD AND MATERIALS

Cultures

Both pathogens and saprophytes were isolated from bean plant material showing typical halo blight symptoms, and from seed. Isolations were also made of various other plant pathogenic bacteria, and a range of cultures was obtained from the National Collection of Plant Pathogenic Bacteria and other sources. These isolates were used to investigate the specificity of various identification techniques.

Biochemical tests

All isolates were tested for their reactions on Kings medium B, 5% sucrose nutrient agar and by Kovacs oxidase test. Most pathogenic pseudomonads produce a green fluorescent pigment on Kings medium B, which fluoresces blue under ultra violet light. Saprophyes which produce fluorescent pigments tend to fluoresce green

under UV light. Certain isolates were also examined for their reaction on gelatin and aesculin. These tests are described by Lelliot et al (1966).

Lesion tests

Detached bean pods were inoculated either by stabbing or by infiltrating the pods with a suspension of bacteria using a hypodermic needle. Pods were stored in humid containers at room temperature where lesion development usually occurred within 3 to 4 days.

Phage tests

Phages were isolated from soil and plant material taken from crops infected with halo blight. The methods used for the isolation, purification and production of high titre phage stocks were basically those of Adams (1959) and Billing (1963). Three phages, 11P, 12P and 48P, to Ps. phaseolicola and 2 phages, 22P and 23P, to the Xanthomonas blights caused by X. phaseoli and X. phaseoli var fuscans, were isolated. Phage 12S isolated by Billing (1963) and of general specificity to a wider range of phytopathogenic pseudomonads, was also used. Phage preparations were stored over chloroform at 4°C.

Isolates were tested for phage sensitivity by spotting concentrated phage preparations onto glycerol agar plates previously seeded with the isolate under test. Plates were incubated overnight, the production of a lysis zone being regarded as a positive response. Using this method, isolates were tested for sensitivity to several phages on the same plate.

Serological tests

Rabbits were used for the preparations of antisera to halo blight. Three antisera were produced, 2 to race 1 and 1 to race 2. Isolates were tested using either gel-diffusion or tube agglutination tests. As gel-diffusion tests took 24 to 48 hr to give results the quicker tube agglutination method has been used in most experiments. In this method equal volumes of a turbid suspension of the bacteria in 0.85% saline and dilute antiserum were incubated in a water bath at 50°C. Agglutination of the bacteria (usually within 5 min.) was taken as a positive response.

Three methods were used in the preparation of bacteria (antigen) for testing. Bacteria were sonicated (disrupted in an ultra-sonic disintegrator) to release more antigens, tested whole, or heat treated (autoclaved) before testing.

IDENTIFICATION

A prime requirement of any tests of seed, either to determine their basic bacterial content, or the effect of seed treatments, is that the organisms shall be identified with certainty. The work so far has shown that this can be done either, in the case of seed with unknown bacterial content, by using several tests in conjunction, or, if the original infecting organism is known and only the level of infection of the organism is required, by the use of single selective tests.

The types of organism encountered and the methods of identifying them are as follows.

(a) Xanthomonas species. These (X. phaseoli or X. phaseoli var fuscans) may occur on imported seed. They can be detected by their yellow colony colour and by the use of suitable phages.

(b) Ps. syringae. An occasional parasite of beans in Britain. This can be distinguished from Ps. phaseolicola by biochemical, phage, serological or lesion tests.

(c) Ps. viridiflava. This weak pathogen occurs frequently on bean seed, and is identified using the same tests as for Ps. syringae.

(d) Pseudomonas saprophytes. These are common on bean seed and are distinguished by the type of fluorescent pigment production on Kings medium B.

(e) Ps. phaseolicola. The primary pathogen. It can occur in two pathogenic forms (rough and smooth) and as 2 races. The smooth forms react with specific phages but the rough forms do not. The latter react positively however in inoculation tests and against specific antisera. Race identification is normally by inoculation of the bean variety Mexican red (resistant to race 1 and susceptible to race 2) but recent work suggests that laboratory identification may also be possible.

SEED TESTING

As already noted, Wharton (in press) has developed a method for the detection of infected white bean seeds, by examining them under UV light, when the infected seeds show a blue fluorescence. The method will not work, however, on pigmented seeds. In the present study many types of seed were encountered and it was therefore necessary to develop a technique which, although it might be less rapid than Wharton's method, was capable of universal application. It was also necessary to obtain some estimate of the numbers of bacteria present in the seeds.

The rapid phage plaque count method of Katznelson and Sutton (1951) and the selective medium method of Wilson (1938) were first tested but both had disadvantages. The former was effective but there were variations in bacterium phage reaction and naturally occurring phages sometimes confused the results. It was also found that runner bean seed contained a phage inhibitor which necessitated soaking the seed before testing and lengthened the test period. The selective medium method inhibited growth of the pathogen as well as saprophytes although its restriction of the latter made it of use for some purposes.

Dilution method

Because of the disadvantages of the other methods a new technique was devised which is as follows.

The seed was ground to a flour in a laboratory hammer mill. The flour was suspended in sterile water and the basic bacteriological content of the seed assessed by plating out samples direct and after serial dilution. Samples were plated out in molten Kings medium B, the plates incubated for 3 days and examined under UV light for blue fluorescent colonies. Colony counts indicated the bacterial level in the seed, and colonies were picked for confirmatory tests against antisera or phage. These were necessary because Ps. viridiflava and Ps. syringae can both occur in bean seed.

The test depended on the number of viable cells of the halo blight bacterium in an infected seed. No enrichment culture was used since under such conditions rapidly growing saprophytes, especially green fluorescent saprophytes, tended to obscure the plates.

Tests on single seeds from a heavily infected seed stock showed that the bacterial level in individual seeds varied considerably, from 1,000 to 32,000,000 bacteria per infected seed. However, 7 out of 8 seeds infected with halo blight had a bacterial concentration of 100,000 cells/seed or more.

The level of detection of this method was tested by adding 5 seeds from a heavily infected seed stock (5 seeds containing at least 1 infected seed) to clean seed bulks of 100, 500 and 1,000 seeds, and in all cases positive identifications were made.

Seed stocks of both French and Runner bean varying in content of infected seeds from 0.2% to 20% have been tested successfully by this method. The limit of the technique will be examined in further tests.

Old seed stocks have been found to contain low bacterial concentrations even when a high proportion of the seed was infected, suggesting that the number of viable bacteria per infected seed may decline with age. Because the method requires no incubation or enrichment culture for the extraction of bacteria from the seed it may be used to screen control measures without the chemical having any residual effect.

Control Measures

So far only preliminary investigations have been undertaken. It is apparent that since the pathogen is internal, chemicals must be able to penetrate the seed and for this reason the tests have so far been confined to the use of chemicals in solution. Two methods have been examined viz. (i) 24 hr soak (ii) vacuum treatment.

Using these two methods, seeds have been treated with streptomycin sulphate and hexachlorophene. Control of infection was assessed by a seed test for viable pathogen, and by a seed box test for phytotoxicity.

The vacuum treatment was ineffective using both chemicals. Streptomycin soaking gave control only at a concentration phytotoxic to bean seed. Hexachlorophene has given variable results and is still being investigated. The problem of control will require intensive study of all possible methods, both physical and chemical.

DISCUSSION

French beans are an extremely important crop to the processing industry and the recent increase in halo blight infection has caused very serious losses. So far, no satisfactory field control of the disease has been developed and the success of the crop depends, therefore, especially in a wet season, on the initial health of the seed. For this reason the present investigation, which is still in a preliminary stage, has concentrated largely on the determination of seed infection, both as a guide to the basic disease content of the seed, and as an essential preliminary to the study of methods of seed disinfection. The work has so far indicated some of the problems likely to be encountered and has indicated ways of overcoming them. It has also given preliminary information on the best ways of testing seed treatments which will form the next stage of the investigation.

Acknowledgments

I am greatly indebted to Mr. R.B. Maude and Dr. W.G. Keyworth for their guidance and advice and to Dr. D.G.A. Walkey for preparation of the antisera.

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THIRAM SOAK TREATMENT FOR THE CONTROL OF FUNGAL SEED-BORNE DISEASES

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Summary Sixteen seed-borne fungal pathogens of vegetables, cereals and flowers have been eliminated from seed without loss of germination, by soaking the seed in a 0.2% aqueous thiram suspension for 24 hr at 30°C. Two other pathogens have been almost completely eradicated but one (Ascochyta in tick bean) was not adequately controlled.

Thiram has been shown to dissolve from the suspension to concentrations of up to 10 p.p.m. and the compound was taken up by the seed during imbibition. Methods for the commercial application of the treatment have been developed.

INTRODUCTION

The thiram soak method was first developed for the treatment of pea seed infected with Ascochyta pisi or Mycosphaerella pinodes. Heat treatment, either in water, carbon tetrachloride or steam/air mixtures had been found to be ineffective, but 24 hr soaks in aqueous suspensions of either captan or thiram gave marked control at 25°C and complete control if the temperature was raised to 30°C (Maude 1966). A 24 hr treatment in 0.2% thiram suspension at 30°C was eventually selected as giving complete kill of the pathogens without loss of laboratory or field germination.

Because of the practical problems involved in soaking and drying large quantities of pea seed, the treatment was considered to be of economic value only for nuclear stocks of such seed. However the method was thought to have potential value for the commercial treatment of high value seeds e.g. celery, where the problems of soaking and drying were not limiting. Tests were therefore begun on the thiram soak method on celery seed internally infected with Septoria apii-graveolentis (Maude 1964). These have now been concluded and have demonstrated 100 percent disease control at all stages of crop growth. The treatment has therefore been tested against seed-borne diseases of other crops and in the majority of cases complete disease control has been obtained.

This paper describes (i) the soak treatment and its mode of action (ii) the range of effect of thiram soaking on seed-borne diseases (iii) the effect of soaking on seed germination and crop yield (iv) the commercial application of the method.

METHOD AND MATERIALS

A 80% w.p. formulation of thiram was used at the rate of 0.2% a.i. (0.25 g in 100 ml) in most of the tests. Temperature was maintained at 30°C (±0.2°C) by means of thermostatically controlled water baths or by heater circulators which attached to tanks of different capacities.

In laboratory tests of thiram and other fungicides, treated seeds were washed prior to drying on a bench seed drier - usually overnight at 20 - 25°C in air circulated through them at 70 cu ft/min.

Laboratory assessments for M. pinodes and A. pisi on pea seed were carried out by identification of pycnidia after 9 days incubation at 21°C on moist cotton wool. Assessment of most of the fungi listed in table 3 was made after 7 days incubation on prune lactose yeast agar containing erythromycin and streptomycin (Maude 1963). Septoria spore viability was checked after 48 hours at 21°C using the agar streak method (Maude 1964).

Glasshouse experiments were conducted over a period of 6 weeks in either compost or sterile grit. Tests on celery were conducted under conditions of high humidity to obtain maximum expression of disease.

Many crops were also grown in the field and disease assessment conducted throughout the life of the crop.

Estimations of thiram in solution or in seeds were made by U.V. spectrophotometry or colorimetrically using modifications of methods devised by Kress (1951), Schäfer (1948) and Scheele and Gensch (1953).

THE SOAK TREATMENT AND ITS MODE OF ACTION

Tests on many pathogens have shown that complete control was obtained only when the soak temperature was maintained at 30°C. This is illustrated in table 1.

Table 1.

24 hr soaks of pea seed in captan and celery seed in thiram suspensions

Seed	Temp. of soak °C	% disease in seeds soaked in fungicide	water
Peas	30	0	73
	25	6	93
Celery	30	0	9.7
	25	3.4	59.4

In other tests at 30°C, soaking for a total of 24 hours has been demonstrated to be necessary for complete disease control. This is illustrated for Helminthosporium avenae on oats in table 2.

Table 2.

The effect of different lengths of soak in 0.2% thiram at 30°C on H. avenae on oats

Soak time (hr)	% infection
24	0
18	5.0
12	19.2
6	22.2
2	56.6
1	57.5
Water for 24 hr at 30°C	35.5
Untreated infected seeds	65.8

The reasons for the need for a 24 hr soak at 30°C are not fully understood. As shown in table 1 the increase in water temperature itself caused some reduction in infection. It was also noted in other tests that the temperature increase also caused more rapid imbibition of water by the seed. The fact that such imbibition is important is suggested by the need for a full 24 hr soak with the implication that during this period the seed absorbs, not only water, but also fungicide in solution.

Analyses demonstrated that static aqueous suspensions of 0.2% thiram kept for 24 hr at 30°C contained 10 p.p.m. thiram in solution, a concentration which was toxic to the fungus. In other experiments celery seeds have been shown to absorb a mean of 50 μg/g (dry wt.) thiram from the suspension after 24 hr even though they were contained in a dialysis bag into which the thiram must presumably have moved in aqueous solution.

It is now considered, therefore, that the internal therapeutant effect of the treatment is caused by the absorption by the seed of thiram in concentrations which have no adverse effect on the tissues of the seed (but see below for exceptions) and which selectively kill the fungi within these tissues.

In practice the treatment has a further effect if the thiram is circulated in suspension because solid thiram is deposited on the seed surface. In analyses of thiram on the surface of treated celery seed up to 4000 μg/g was recovered which represents three times the amount that would have been applied using a 50% thiram seed dressing at the rate of 1 oz to 28 lb of seed.

It is apparent therefore that with this treatment the seed is both internally disinfected and externally protected.

THE RANGE OF EFFECT OF THIRAM SOAKING

The thiram soak method has been tested in comparison with current control measures on the crops and seed-borne pathogens given in table 3.

Table 3.

Seed-borne diseases treated by the thiram soak method

Host	Pathogen	Percentage disease control
Peas	Ascochyta, Mycosphaerella	100
Celery	Septoria	100
Carrot	Alternaria, Stemphylium	100
Brassicas	Phoma	100
	Alternaria	99
Beet	Phoma, Colletotrichum, Fusarium	100
Trefoil	Ascochyta	100
Flax	Botrytis	100
Oats	Helminthosporium	100

Table 3. (continued)

Host	Pathogen	Percentage disease control
Wheat	Septoria) Tilletia)	100
Barley	Ustilago -	99
	Helminthosporium -	100
Tick beans	Ascochyta -	70-80
Lobelia	Alternaria -	100

In 16 out of 19 diseases 100 percent control was achieved demonstrating the extreme versatility of the method. This level of control was superior to any currently recommended seed treatment. An example is illustrated in table 4 for Septoria on celery seed.

Table 4.

A comparison of thiram soaking and hot water treatment of infected celery seed

Seed treated in	% Septoria infection on seeds	seedlings	mature plants
0.2% thiram for 24 hr at 30°C	0	0	0
Water at 50°C for 25 min.	0.19	0.3	0.875
Untreated infected seed	63.0	16.0	100

Very many tests of this nature have been made and although hot water treatment often gave 100 percent control it sometimes failed to do so whereas thiram soak treatment has invariably eliminated seed borne infection.

In two diseases 100 percent control was not achieved using thiram soaking, but the method was at least as effective as hot water treatment.

Table 5.

Comparison of methods where thiram did not achieve 100% control

Seed treated in	Percentage infection due to Alternaria on cabbage	Ustilago on barley
0.2% thiram for 24 hr at 30°C	1.0	0.008
Water at 50°C for 25 min.	4.5	-
Water at 42.7°C for 2 hr	-	0.095
Untreated infected seed	96.0	0.95

One disease (Ascochyta infection of tick bean) did not respond satisfactorily to thiram soak treatment, but no commercial seed treatment was available for comparison.

THE EFFECT OF THIRAM SOAKING ON SEED GERMINATION AND CROP YIELD

Thiram soaking has proved to be non-phytotoxic on all vegetable varieties so far tested (except for 1 lettuce variety). In laboratory tests of 26 celery cultivars, hot water (25 min. at 50°C) depressed germination in 5 varieties whereas thiram soaking depressed it in one variety only which was already of low viability. In field tests of celery, final crop yields from thiram treated seed were at least as good as those from hot water treated seed. In field emergence tests of 114 cultivars of the genus Brassica, thiram treated seed gave as good as or better emergence than untreated seed. In trials of brussels sprouts and cabbage, thiram soaked seed gave significantly better emergence than hot water treated seed. In laboratory, glasshouse and field tests of red beet thiram soaking has stimulated emergence as is shown in table 6.

Table 6.

Laboratory and field germination of thiram treated and untreated red beet

		Percentage germination			
		Laboratory		Field	
Red beet	% Phoma	untreated	thiram treated	untreated	thiram treated
Avonearly 1	63	54	89	32	96
Avonearly 2	72	78	90	9	73
Avonearly 3	82	75	91	19	69

The field sowing in this experiment was carried out in early March. While in general this stimulation is due to the leaching out of natural germination inhibitor from the seed during thiram soaking, in the field the effect of elimination of seed-borne pathogens and the effect of the protectant fungicide on the seed must be taken into account.

Some flower seeds are known to be adversely affected by thiram and were classified by Allen (1963) on this basis. It has been found that the thiram soak treatment similarly suppresses germination of these flower seeds but not others.

THE COMMERCIAL APPLICATION OF THE TREATMENT

The thiram soak treatment offers, for the first time, a commercially acceptable method of producing certain seeds completely free of fungal infection. This has considerable advantages from the point of view both of the grower and of the seedsman, several of whom have been quick to appreciate the potentialities of the method. Thus one seedsman treated 1½ tons of red beet seed in 1967 with highly satisfactory results, both in respect of lack of disease and of increased field emergence. Others are installing treatment tanks and drying facilities for use on a range of small seeds. The practical approach to the method has already been described (Maude and Keyworth 1967).

The use of the method on seeds such as sugar beet and cereals seems at the moment to present considerable practical problems, largely associated with the need for a 24 hr soak and subsequent large scale drying. It is therefore less likely to be commercially acceptable even though it is advantageous from the point of view of disease control. These matters are, however, still under investigation.

DISCUSSION

As already indicated, the thiram soak treatment provides a means of ridding many

seeds of internal infection with fungal pathogens. The diseases caused by many of these pathogens are of concern to growers and processors of vegetables. Some of these diseases, such as Septoria blight of celery, can cause very severe reductions or spoilage of crop but the effects of others have sometimes not been fully appreciated. Thus Phoma on red beet can cause severe seedling losses and is of special concern on precision sown crops, but it can also cause a root rot which may prove troublesome on small beet for bottling. Perhaps, only as really disease free seed comes into more general use, will its value become apparent.

The effectiveness of thiram in this treatment has led to a search for other compounds of equal or greater effect. This has shown that many compounds of copper, mercury, quinones and dithiocarbamates were ineffective and so far only thiram, captan and drazoxolon have given positive results against a range of fungi.

Further tests of compounds are in progress to determine whether any can be used at lower concentrations or temperatures or for shorter times. An obvious ideal to aim at is the use of a dry seed dust material which would dissolve in the soil water and be taken up by the seed but such compounds which would be effective against a wide range of pathogens do not seem to be currently available. The recent introduction of "Vitavax" is of great interest in this connection as it has systemic activity when applied as a dry dressing although it is only effective against fungi of the Basidiomycete group.

Acknowledgments

I am extremely grateful for the help and encouragement given to me by Dr. W.G. Keyworth during the course of this work.

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THE PREVALENCE OF PATHOGENIC FUNGI ON SEED TUBERS

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Summary Some fungi that infect potato seed tubers occur in almost all stocks, with incidence ranging widely from a few to most of the tubers infected; others occur less widely and only a few stocks contain many infected tubers.

Visible symptoms do not always correctly indicate the prevalence of pathogens; for example, the Phoma spp that cause gangrene are often present in soil adhering to apparently healthy tubers, and many potato eyes may be infected by Oospora pustulans without the tubers showing skin spots.

Careful storage and husbandry, or fungicidal dipping, may sometimes decrease damage by pathogens but are seldom effective enough to eliminate infections or to ensure healthy progeny tubers. Experiments in progress offer hope of decreasing the amounts of some pathogens by planting healthier seed, particularly on farms where foundation stocks are multiplied.

INTRODUCTION

Legislation on the health of seed tubers was first introduced for potato wart disease and has developed to ensure purity of variety, freedom from virus diseases and potato root eelworm. Recently complaints have chiefly concerned fungus diseases but there is little quantitative information about the extent of these diseases and their seasonal, varietal and regional occurrence. Such information is essential in assessing their practical importance, and to get it a survey was undertaken in collaboration with the Potato Marketing Board. Tubers were collected from seed stocks to be grown in England and Wales soon after they arrived at farms; the stocks sampled were taken from areas in proportion to the acreage grown and from certificate categories in proportion to their use. Sub-samples were examined (a) for macroscopic symptoms on tubers both at receipt and again at planting time, after storage on chitting trays under artificial light; and (b) for the presence of pathogenic fungi visible on excised potato eyes after 5 days incubation in a damp atmosphere at 15°C. The survey, covering the 5 years from 1962, was designed to determine disease incidence on a representative sample of the King Edward and Majestic seed received by farmers in England and Wales and to provide some information on the varieties Pentland Dell, Arran Pilot and Record. It may not accurately estimate the diseased seed actually planted, because we hope that farmers rejected at planting some of what we saw.

RESULTS

Probably the most important disease of potatoes is potato blight (Phytophthora infestans) which, besides causing loss of yield, can rot tubers and, when diseased tubers are planted, provide a source of infection in the crop. In four years out of five more than 2% of King Edward seed tubers were affected (Table 1) and allowing for one half of these to carry dead infections or be rejected at planting and less than 1% of these to produce invaded stems, this could result in almost one infected plant per acre to initiate the epidemic (Hirst and Stedman, 1960). Up to 15% of Pentland Dell and Arran Pilot seed was affected with powdery scab, a disease common after wet seasons but thought to be of minor importance because it causes only a surface blemish. However, the causal fungus Spongospora subterranea is now known to transmit potato mop top virus (Calvert and Harrison, 1966), so more attention must now be given to it. In contrast to powdery scab, common scab (Streptomyces scabies) is often severe following a dry summer and in some soils the disease can be severe and greatly decrease the value of ware tubers. Up to one half of Majestic seed tubers were affected, 8% severely; in other varieties the disease was less prevalent. Black scurf (Rhizoctonia

solani) occurred on about one fifth of the tubers, but the most common disease was skin spot which affected almost all stocks and up to three quarters of King Edward tubers.

Table 1.

Occurrence of diseases in King Edward seed 1962 to 1966

Disease	% tubers affected				
	1962*	1963	1964	1965	1966
Skin Spot	78	31	40	59	39
Gangrene	6	4	7	10	9
Blight	2	3	1	3	3
Black Scurf	9	22	19	17	20
Common Scab	19	13	32	16	24
Powdery Scab	8	10	5	10	12
Dry Rot	5	2	2	1	1

* year when produced

During the winter, buds may be killed, especially when tubers are stored in bulk, and in the survey the causal fungus Oospora pustulans was found on almost all dead buds. Gangrene (Phoma spp) infection generally takes place through mechanical injury done at lifting, grading or during transport. Up to 10% of the seed tubers showed the characteristic rots, but experimentally damaging sub-samples of each stock increased the incidence fourfold. Soil removed from seed tubers also induced lesions on test tubers, even from stocks which had no gangrene. These results suggest that many more tubers bear inoculum than at present show disease, and although present handling methods are not ideal, not all the potential disease is expressed. Dry rot also develops on damaged tubers but, excepting Arran Pilot in which 10% of tubers were affected, the incidence of the disease was small.

Table 2.

Occurrence of pathogenic fungi on King Edward seed 1962 to 1966

Fungus	% eyes affected				
	1962	1963	1964	1965	1966
Oospora pustulans	51	29	33	44	40
Rhizoctonia solani	26	39	26	21	27
Helminthosporium atrovirens	36	27	34	19	33
Verticillium spp	29	28	28	31	21

Conidiophores of Verticillium spp produced on exposed tuber tissue after incubation were found on about one third of the eyes of King Edward (Table 2). Most of the isolates obtained were identified as V. tricorpus, a species that, as far as is known, does not induce disease symptoms in the potato plant. A small proportion were identified as the weak parasites V. nubilum and V. nigrescens (MacGarvie and Hide, 1966). Early death of crops in this country can be caused by V. dahliae but we have not found that this fungus is tuber-borne.

Average disease incidence of the seed crop as a whole gives no account of the differences between stocks, and generally the diseases follow two patterns. First are those that occur in half or more of the stocks but with only a few stocks severely affected. These include blight, gangrene and dry rot. Secondly, diseases such as skin spot, black scurf and silver scurf (Helminthosporium atrovirens), which occur in almost all stocks, but the proportion of affected tubers ranges very widely. The first group of diseases should be the easier to control by concentrating on the few bad stocks but the second group presents the greater challenge for many more stocks and tubers are initially at risk.

Crop diseases vary in severity from year to year largely because of differences in the weather, but it is unprofitable to investigate the effects of weather without quantitative information about incidence and with only imperfect knowledge of the requirements of particular pathogens. Our results do allow a start to be made and at least show interesting contrasts between diseases. Dry soils, especially when tubers are forming, favour common scab (Lapwood, 1966), and this was more prevalent in 1964 crops than in other wetter years (Table 1). Silver scurf showed similar annual variations to common scab (Table 2) whereas blight and powdery scab were less prevalent in 1964 and their incidence suggests that their requirements are the opposite of those for common scab and silver scurf. Skin spot and gangrene had similar annual variations and were the opposite of those for black scurf, but skin spot was most common in 1962 whilst gangrene was most severe in 1965, a year when tubers suffered an abnormal amount of mechanical injury.

The incidence of common scab differs in different parts of the United Kingdom (Large and Honey, 1955) and our survey suggests that other diseases are also more common in some regions than others (Tables 3 and 4); for example, seed produced in England had more silver scurf and common scab but less powdery scab than seed produced in Scotland and Ireland.

Table 3.

Occurrence of diseases in King Edward seed by country of origin
(mean of 5 years)

Disease	% tubers affected		
	Scotland	Ireland	England
Skin Spot	58	43	34
Gangrene	9	3	6
Blight	2	1	3
Black Scurf	15	23	20
Common Scab	19	11	25
Powdery Scab	10	20	2
Dry Rot	2	3	1

This may reflect a smaller rainfall, because most of the English seed was produced in East Anglia, but surprisingly it also had most blighted tubers. This probably resulted from later haulm destruction and lifting than in the traditional seed producing areas and perhaps less rigorous inspection of tubers. The practice of planting once-grown seed for ware crops is apparently increasing and much more attention needs to be given to blight control and to inspection of tubers to reject infected ones from seed and so prevent early introduction of the disease into ware crops. Irish seed, had the fewest tubers affected with blight, but most with powdery scab and black scurf. Scottish seed had most gangrene and mechanical injury to tubers, also most skin spot and buds killed by O. pustulans. The incidence of this fungus on eyes, however, was similar in seed from all countries (Table 4), which suggests that post-lifting conditions in Scotland must favour development of skin spot.

Table 4.

Occurrence of pathogenic fungi on King Edward seed by country of origin
(mean of 5 years)

Fungus	% eyes affected		
	Scotland	Ireland	England
Oospora pustulans	45	36	39
Rhizoctonia solani	27	42	26
Helminthosporium atrovirens	24	33	41
Verticillium spp	26	20	32

Of the five varieties included in the survey in 1966, Record was most severely affected by skin spot, although King Edward had the more buds killed by O. pustulans than any other variety. Black scurf was most common in Pentland Dell and Arran Pilot, and blight and gangrene in King Edward. Although relative varietal susceptibility can sometimes be assessed by laboratory tests, only surveys of commercial seed measure diseases as they actually occur. Surveys do not necessarily indicate only inherent varietal susceptibility, but also the effects of agricultural factors including date of lifting, storage, handling and chitting, which may differ with different varieties.

The importance of seed tuber diseases lies not only in the damage done to the seed and the growing crop, but also in the transmission of infection to the next generation of tubers. Planting seed with skin spot, black scurf and gangrene may mean not only a loss of yield and quality in ware potatoes, but will infect progeny tubers and perpetuate infection of seed stocks. At present, all commercial stocks are infected with at least the skin spot fungus; if stocks free from this and other fungi are produced, more study will have to be given to contamination from soil, but meanwhile there is enough of the fungi on seed to account for most of the infection of plants and young tubers.

The damage caused by several of these diseases can be ameliorated by husbandry methods; early lifting and boxing will prevent buds being killed by skin spot. Avoiding mechanical damage and cold will decrease gangrene. But such measures do not eliminate the source of infection. Treating seed with a fungicide provides some degree of control; the most widely used are of the organo-mercury type, and treatment soon after lifting decreases skin spot in badly diseased stocks so that only a few eyes are still infected at planting time. Such seed will produce crops that emerge quickly and uniformly but infection is not eliminated and the progeny tubers are infected, so treatment has to be repeated annually. Successive treatment of stocks over several years may sometimes help to decrease the inoculum and we have had most success by selecting only lightly affected stocks and dipping them annually. These organo-mercury compounds can be beneficial when correctly used, but they are toxic to mammals and must be used with great care. There is an urgent need for less noxious but equally effective substances, so that treatment of seed tubers can become more common.

This survey provides information on the occurrence of diseases in seed stocks including their annual, regional and varietal distribution and it also indicates the proportion of seed likely to be unsuitable for planting. But information about disease incidence gives no practical measure of yield losses likely to occur, so complementary trials are needed to measure the effects of these diseases in field crops. Such experiments now in progress at Rothamsted are showing the importance of tuber-borne inoculum on growth of the crop, on yield and size of tubers and on infection of the young tubers.

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THE DEVELOPMENT OF SKIN SPOT IN RELATION TO LIFTING AND STORAGE FACTORS

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Summary In studies on factors determining the development of skin spot, storage temperature and humidity conditions were found to exert a major effect on level of infection. Time of lifting had no marked effect on surface infection where storage conditions were constant, but the lowest levels of eye infection were found with earlier lifting, when tubers were stored under warm, dry conditions. Haulm removal was found, in general, to have no effect on disease development. High temperature in the initial holding period only proved effective in controlling disease development when coupled with dry conditions. At high humidities, prolonged periods of high temperature were necessary to check the disease. Transferring tubers from cold, damp conditions to intermediate temperatures and low humidities afforded control of infection when carried out in November but not in January. In testing fungicides for skin spot control none of the compounds used proved as effective as the organo-mercury compound.

INTRODUCTION

Skin spot is generally recognized as an important problem in seed tubers of certain varieties. The development of infection by the causal fungus, Oospora pustulans, in storage may cause the death of some or all of the bud tissues of the eyes of the tuber and the planting of infected tubers of susceptible varieties, such as King Edward, may be related to delay or failure in plant emergence to an extent depending upon the degree of eye infection (Boyd and Lennard, 1961). Effective control of skin spot can be achieved by dipping the seed tubers in an organo-mercury solution and boxing immediately after lifting (Greeves and Muskett, 1939; Foister, 1943; Boyd, 1957, 1960). However, health hazards are introduced with the use of mercury compounds and the measure itself is difficult to apply in practice at the recommended time. In testing fungicides, other than mercury compounds in solution, Greeves and Muskett (1939) found formalin and a proprietary dusting compound to be non-effective, while delay in applying the treatment after lifting reduces its effectiveness (Greeves and Muskett, 1939; Boyd, 1960). Edie and Boyd (1966) indicated that the efficiency of delayed treatment depends on the date of lifting and concluded that the earlier lifting occurred the greater were the chances of effective control. It was suggested by these workers that the effectiveness of control was associated with the temperature of subsequent box storage.

Boxing seed tubers at lifting without disinfection has also been shown to reduce the incidence of skin spot, but not so effectively as the disinfection treatment (Boyd, 1957, 1960). In studies by Edie and Boyd (1966) boxing alone was found to be most effective when carried out before normal harvest and it was suggested that box storage would only seem useful as a control measure if the subsequent store temperature was relatively high. According to Boyd (1957), early lifting was also of benefit in reducing skin spot development where tubers were clamped. However, McGee (1967) found that early lifting reduced the disease incidence only when tubers were boxed, and in clamp storage time of lifting had no significant effect on level of infection which tended always to be relatively high. Boyd (1957) found that early haulm removal reduced skin spot infection despite continued proximity of the tubers to the inoculum in the soil, but McGee (1967) failed to show any significant trend in disease development whether the tubers were boxed or clamped.

The higher level of disease incidence under clamp storage in comparison with

box storage may be associated with the conditions of high humidity and low temperature which tend to develop in clamps. Boyd and Lennard (1962) indicated that above average rainfall over the lifting period and below average temperature in early storage showed a close relationship with a high level of infection, while high humidity, both before and after infection (Allen, 1957), and low temperature and high humidity during storage (Edie, 1964) have been found to increase disease incidence. Wilson (1962) has suggested that forced draught ventilation of seed tubers in bulk storage in the initial holding period during the day, when temperatures are relatively high and the humidity low, would discourage the spread of skin spot.

In some of the recent work at Edinburgh attention has been given to factors at lifting and in storage in relation to skin spot development with special reference to the effects of haulm destruction, time of lifting, and storage temperature and humidity conditions. In addition tests have been made with a range of fungicides in an attempt to find less hazardous alternatives to mercury compounds in skin spot control.

METHOD AND MATERIALS

During the season 1966-67 small scale studies were carried out on the effects of various factors at lifting and in storage on skin spot development.

In an investigation of the effects of time of lifting, haulm destruction and storage conditions, tubers of the variety King Edward were lifted at five different times, at intervals of 2 weeks from 23 August, 1966, and stored at about 39°F under conditions of high humidity or at about 49°F at low humidity (60 to 75 per cent R.H.). With the exception of the first and last lifting dates, comparable samples of tubers were lifted from plots where haulm destruction with sulphuric acid had been carried out 2 weeks before lifting. In a second, parallel experiment, where storage treatments were the same, tubers of the variety Redskin were lifted at three different dates at intervals of 3 weeks from 25 August, 1966 and comparable tubers were lifted at the second 2 lifting times from plots where haulm removal by cutting had been carried out 3 weeks before lifting.

In an investigation of the effects of high temperature treatment for varying periods after lifting, prior to storage at low temperature (39°F) and high humidity, tubers of the variety Kerr's Pink, lifted on 12 October, 1966, were stored at 58°F for 2, 6, 10 or 14 weeks under conditions of high humidity or at atmospheric humidity. Further samples of tubers were held continuously at high and low temperatures under the respective humidity conditions.

A study was also made of the effect of transferring tubers of the variety Kerr's Pink from storage at low temperature (39°F) and high humidity to warm temperature (49°F) and dry storage conditions at 2 different dates, 16 November, 1966, and 3 January, 1967. Other comparable lots of tubers were held constantly at the 2 respective storage conditions from lifting on 12 October, 1966. In a final study further samples of tubers were held constantly under 3 temperature conditions approximating 39°F, 43°F and 49°F under conditions of high humidity or at low humidity.

In all the studies tubers were placed in lots of 10 in small cardboard boxes replicated 7 or 8 times for each treatment and the condition of high humidity (almost 100 per cent R.H.) was maintained by wetting the boxes regularly. Skin spot assessments were made in March, 1967, using the method described by Boyd (1957) for the estimation of surface infection (S.I.I.) and the method of Nagdy and Boyd (1965) for the estimation of eye infection (E.I.I.).

Preliminary attempts were also made to find less hazardous alternatives to mercurial compounds in the control of skin spot. Tubers of the variety King Edward lifted on 6 October, 1965, and 17 October, 1966, were placed in standard chitting trays immediately after lifting and various disinfectants tested, using 2 boxes per

treatment. The trays were held in an insulated shed and skin spot assessments were carried out in the following March of each year.

RESULTS

It is seen from the results in Table 1 that storage conditions had a marked effect on disease incidence but there were no significant differences in levels of surface infection for the different times of lifting. With eye infection, however, there was a trend for levels of infection to increase as lifting was delayed, with the exception of the last lifting date for King Edward. This trend was more evident for tubers stored at the higher temperature under dry conditions.

Table 1.

Skin spot infection in relation to time of lifting and storage treatment

Time of lifting	Surface infection index Storage conditions 39°F High humidity	49°F Low humidity	Eye infection index Storage conditions 39°F High humidity	49°F Low humidity
Variety: King Edward				
	± 0.6	± 0.6	± 3.7	± 3.7
23 Aug.	7.1	0.6	45.0	10.0
5 Sept.	8.8	1.5	50.0	26.4
21 Sept.	6.7	1.3	43.8	24.4
3 Oct.	7.6	2.0	52.5	38.5
17 Oct.	6.4	1.1	36.9	22.5
Mean	7.3	1.3	45.6	24.3
	± 0.3	± 0.3	± 1.6	± 1.6
Variety: Redskin				
	± 1.7	± 1.7	± 3.2	± 3.2
25 Aug.	8.8	2.0	46.9	22.5
14 Sept.	15.3	2.4	53.1	30.6
5 Oct.	13.9	2.8	50.0	36.3
Mean	12.7	2.4	50.0	29.8
	± 1.0	± 1.0	± 1.9	± 1.9

Haulm destruction, either 2 weeks before lifting for King Edward or 3 weeks for Redskin, was found, in general, to have no effect on level of subsequent infection. There was, however, an unexpectedly high level of eye infection in Redskin tubers, stored at the higher temperature and low humidity, with the haulm removal treatment, (Table 2).

In studying the effect of storing tubers at high temperature (about 58°F) for varying lengths of time after lifting, prior to storage at low temperature and high humidity (Table 3), high temperature coupled with dry conditions for 2 weeks was found to reduce appreciably the disease incidence compared with continuous storage at low temperature and high humidity. Prolonging the period at high temperature and low humidity progressively increased the effectiveness of the treatment and for periods greater than 6 to 10 weeks a high level of control was achieved. Where a high humidity was maintained during the length of time at high temperature, relatively long periods were required to bring about any measure of disease reduction and it was only with continuous storage at high temperature that very low levels of infection were obtained. Continuous storage at low temperature in dry conditions also reduced disease incidence.

Table 2.

Skin spot infection in relation to time of lifting, haulm destruction and storage treatment

Time of lifting	Surface infection index Storage conditions 39°F High humidity		49°F Low humidity		Eye infection index Storage conditions 39°F High humidity		49°F Low humidity	
	Haulm removed	Untreated	Haulm removed	Untreated	Haulm removed	Untreated	Haulm removed	Untreated
Variety: King Edward								
	± 0.7	± 0.7	± 0.7	± 0.7	± 3.6	± 3.6	± 3.6	± 3.6
5 Sept.	6.7	8.8	1.0	1.5	50.6	50.0	17.3	26.4
21 Sept.	6.5	6.7	1.4	1.3	45.0	43.8	30.0	24.4
3 Oct.	8.9	7.6	1.7	2.0	53.8	52.5	36.9	38.5
Mean	7.4	7.7	1.4	1.6	49.8	48.8	28.1	29.8
	± 0.3	± 0.3	± 0.3	± 0.3	± 1.5	± 1.5	± 1.5	± 1.5
Variety: Redskin								
	± 1.9	± 1.9	± 1.9	± 1.9	± 2.8	± 2.8	± 2.8	± 2.8
14 Sept.	11.9	15.3	3.0	2.4	51.3	53.1	39.8	30.6
5 Oct.	11.5	13.9	3.7	2.8	49.4	50.0	46.8	36.3
Mean	11.7	14.6	3.4	2.6	50.4	51.6	43.3	33.5
	± 1.0	± 1.0	± 1.0	± 1.0	± 1.4	± 1.4	± 1.4	± 1.4

Table 3.

Skin spot infection in relation to high temperature treatment of tubers under conditions of high or low humidity for varying periods after lifting

Variety: Kerr's Pink

Period of high temperature treatment (weeks)	Surface infection index High humidity	Low humidity	Eye infection index High humidity	Low humidity
	± 1.6	± 1.6	± 2.7	± 2.7
0	21.7	7.6	54.3	43.6
2	18.7	7.8	53.0	40.7
6	19.0	3.9	48.9	29.3
10	12.6	2.1	45.0	24.7
14	7.7	1.5	42.6	12.3
24	0.7	0.6	11.7	10.7
Mean	13.4	3.9	42.6	26.9
	± 0.7	± 0.7	± 1.1	± 1.1

Where tubers were transferred from storage at low temperature and high humidity to warmer temperatures (about 49°F) and low humidity in November some measure of disease control was achieved. When the treatment was delayed until January the resulting level of infection was high (Table 4).

Table 4.

Effect of transferring tubers from low temperature and high humidity to warm temperature and low humidity at different times on skin spot development

Variety: Kerr's Pink

Storage treatment and date of transfer	Infection index Surface	Eye
	± 1.8	± 2.8
Continuous warm (49°F), dry	4.1	30.0
16 Nov.	7.6	37.5
3 Jan.	15.3	49.4
Continuous cold (39°F), humid	14.0	47.5

The results in Table 5 indicate that very high levels of infection are associated with temperatures below 40°F and conditions of high humidity. A moderate disease incidence may still occur at low temperatures under dry conditions or at intermediate temperatures between about 40 and 50°F where the humidity is high.

Table 5.

Skin spot infection in relation to storage temperature and humidity

Variety: Kerr's Pink

Mean storage temperature and range	Surface infection index High humidity	Low humidity	Eye infection index High humidity	Low humidity
	± 0.8	± 0.8	± 2.4	± 2.4
39°F (32 to 48°F)	16.8	7.7	50.0	43.7
43°F (38 to 53°F)	8.8	5.5	50.0	34.3
49°F (43 to 54°F)	8.4	6.2	46.8	41.8
Mean	11.3 ± 0.5	6.5 ± 0.5	48.9 ± 1.4	39.9 ± 1.4

From the results of tests of various fungicides for skin spot control, applied at the recommended rates, none proved as effective as the mercurial compound (Table 6). Maneb afforded some control in 1965 but not in 1966. Copper sulphate reduced the disease incidence but caused injury to the tubers.

Table 6.

The effect of disinfection treatment with various fungicides at lifting on skin spot development

Variety: King Edward

Treatment	Infection index Surface	Eye
1965-66 (Lifted 6 Oct.):		
Ethoxy-ethyl mercuric chloride	0.7	13.6
Maneb	1.7	25.9
Captan	3.4	51.5
Thiram	3.8	44.4
Boxed	3.8	40.5
1966-67 (Lifted 17 Oct.):		
Ethoxy-ethyl mercuric chloride	0.7	10.8
Copper sulphate	2.6*	29.4*
Fentin acetate	4.1	31.4
Copper oxychloride	4.4	38.9
Maneb	4.9	33.2
Boxed	4.9	35.6

* Chemical injury to tubers

DISCUSSION

The reduction in skin spot by boxing tubers may be attributed to exposure of tubers to dry conditions which would check fungal activity. Under field conditions boxing tubers after lifting only reduces skin infection to any extent if lifting is carried out early (Edie and Boyd, 1966; McGee, 1967). This may be attributed to higher ambient temperatures at earlier times of lifting which render the boxing treatment more effective. On the other hand, the failure of boxing to control the disease effectively in late-lifted tubers may relate to disease establishment in the field having already reached a stage where it is no longer checked by changing humidity. From the present investigations on the effects of time of lifting the results indicate that, where a temperature of about 50°F is maintained, exposure of tubers to dry conditions after lifting can give effective control of skin spot at late harvest dates. Eye infection still tended to increase, with the exception of one anomolous result, as lifting was delayed, which might suggest a more rapid establishment of eye infection than surface infection in the field. Haulm destruction appeared to have no effect in reducing the level of infection. Transferring tubers in November to a temperature of 50°F and dry conditions from conditions of low temperature and high humidity still afforded some control of skin spot, but there was no disease reduction when the changeover was delayed until January, when presumably infection had reached too advanced a stage.

The exposure of tubers to high temperatures (of about 60°F) for short times during the initial holding period only appears to be effective in controlling skin spot if the storage atmosphere is dry. Such low humidities would probably be difficult to achieve in bulk storage. At high humidities, high temperatures over a long period were necessary to exert any control and such treatment would inevitably lead to excessive sprouting. Intermediate temperatures within the range 40°F to 50°F may be associated with moderate levels of infection where the humidity is high. The results suggest that under normal conditions of continuous bulk storage it seems unlikely that measures taken to control skin spot would prove consistently effective in practice.

The results of tests on various fungicides for the control of skin spot showed that no satisfactory alternatives to the organo-mercury compound could be recommended.

Acknowledgments

The work on the testing of fungicides was carried out in collaboration with Mr. D.C. McGee and thanks are due to Dr. A.E.W. Boyd for his interest in the work, to the staff of the Plant Pathology Department and Botany Department, The Edinburgh School of Agriculture, for technical assistance and to the Potato Marketing Board for their financial assistance.

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POTATO GANGRENE: SOME INTERCONNECTED SOURCES AND FACTORS

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Summary

The cause of gangrene, Phoma foveata seems to be more restricted to the potato plant than the other associated species, P.solanicola and P.eupyrena. It can invade soils but does not appear to be a persistent soil inhabitant which these two are; nor is it known to affect other plants, as they do. Infection on the seed tuber is transmitted to the stems and there is evidence of some spread to other stems during growth. Spores are produced on moribund and dead haulm and in some circumstances tubers of the new crop begin to carry a latent infection before harvest - acquired most likely from the affected stems. Early infection of the tubers can be prevented or killed by chemical disinfection. Latent infection of the tuber is mostly confined to the skin and there is a greater chance of a lesion starting if the skin is impacted.

INTRODUCTION

Gangrene is a demarcated rot of the potato tuber caused by a fungus, Phoma foveata (Sphaeropsidales). Lesions start as dark sharp-edged depressions in the skin, often from wounds, lenticels or eyes, and may enlarge irregularly to a width of two inches or more; depth is variable, the least being a superficial skin necrosis. Affected flesh is dark brown, grey or purplish, at first dry and compacted but later with cavities; exceptionally the rot is soft and wet through the action of pectolytic bacteria.

Gangrene is important commercially because it develops on what had appeared to be sound tubers. It has become prominent in the British Isles and in parts of southern Australia; but in Britain at least only after a period of about 20 years following its first description (Alcock and Foister, 1936) during which it was scarcely known except from the coldest parts of Scotland. Table 1 shows how common it is now and also illustrates how it varies with cultivar and year.

Table 1.

Incidence of gangrene in some stocks officially-inspected in Scotland

Reason for inspection	Year	Tonnage inspected	Percentage of tonnage with any gangrene
Export (cv Majestic)	1963-64	680	14
" "	1964-65	1,310	20
" (cv Up-to-Date)	1963-64	2,170	3
" "	1964-65	5,900	9

Elsewhere, in North America, continental Europe and Asiatic Russia a similar disease has been reported occasionally, the main cause of which appears to be the widely distributed soil inhabitant P.solanicola. P.foveata closely resembles this species and has been regarded as a form of it (Malcolmson, 1958); but the two differ in some ways significantly and can easily be distinguished. Unqualified use of the older name, in place of P.foveata, obscures the distinctive nature of this pathogen.

Both P.solanicola and P.foveata, and a third species, P.eupyrena (sensu Wollenweber) which is not pathogenic to tubers, can be found also on potato stems and in the soil. We have isolated P.solanicola, or closely similar fungi capable of rotting potato tubers, from pycnidia on stems of Lavatera, Carduus lanceolatus, Centaurea nigra; on leaves of Iris; from a lesion on a rose stem; and from a dying root of raspberry (see also Dennis, 1946; and Maas, 1965, who equated P.solanicola with the soil-dwelling P.exigua). P.foveata has not been isolated from any plant other than potato.

Gangrene would be easier to control if one understood how infection on these substrates is interconnected. This paper describes our work on the subject.

THE DISTINCTIVE PATHOGENICITY OF P.FOVEATA

That P.foveata is the main cause of gangrene throughout Scotland has been shown by isolations and pathogenicity tests. From 80 cases involving 30 cultivars which were studied between 1955 and 1966, P.foveata was associated with 69, P.solanicola with five, and P.eupyrena with six. Isolates of P.eupyrena inoculated to tubers failed to cause gangrene but the fungus did invade moribund parts. P.solanicola isolates could cause large lesions when inoculated to tubers, but seldom when unwounded tubers were dipped in suspensions of macerated agar cultures and afterwards kept damp and cool. Table 2 summarises results from comparative tests with P.foveata in which the latter method was used.

Table 2.

The comparative extent (mean and range) of gangrene lesions/tuber induced in tests with 5 isolates of P.foveata and 5 of P.solanicola

10 to 12 tubers/isolate

Cultivar	P.foveata	P.solanicola
King Edward	48(23-81)	2(1-4)
Majestic	79(41-114)	10(4-19)

INFECTIVITY OF SOILS

P. eupyrena and P.solanicola can be isolated without difficulty in dilution plates from arable soil. Being also commonly isolable from apparently sound skin of potato tubers and from pycnidia on dead potato haulm they rank as microbial associates of the potato plant.

P.foveata has been obtained by Malcolmson (1958) from soils sampled after potato cropping and tested by tuber inoculation; and from land not lately used for potato (Anon. 1967). We have tested samples of soil from the following sources: (1) fields, before potato planting; (2) fields planted with affected seed and which had yielded an affected crop respectively 2, 14 and 26 months previously; and (3)

small field plots and glasshouse compost immediately after bearing crops grown from affected seed.

Soil crumbs were inoculated into tubers which also were given control stabs with made-up compost. Gangrene did not clearly result on any of a total of 486 so treated. On the other hand 41 of 45 tubers were infected from a compost to which some culture suspension had been added six months before and which had since borne a potato crop. Only from this soil was P.foveata isolated in dilution plates, the colony distribution suggesting that the fungus had been associated with the heavier soil particles.

This compost has remained infective under glasshouse conditions for at least two successive crops but much less so for the second. Sterilised compost has been made infective to potato plants somewhat more naturally by introducing to it fractions of dead haulm with pycnidia - but for how long is not yet known.

In some respects then, our results on soil infectivity differ from those obtained in Northern Ireland (Anon. 1967) either through technical defect or because massive infectivity was not a normal feature of our samples.

INFECTION ON POTATO STEMS

The nature of stem infection

Pycnidia of Phoma spp. are common on dead potato haulm in Britain. On supported stems of glasshouse plants, presumably little exposed to any mechanically-transmitted or wind-blown infection, pycnidia are most common on the first 20 cm of stem, though rare below soil level on stems, stolons or roots. They may be massed over the lowest part of a stem but elsewhere, as far as 60 cm from the base, they occur usually as spindle-shaped clusters up to about 10 cm long, very often around a leaf scar. Pycnidial groups may be associated topographically with a previous lesion but mostly they appear on undifferentiated parts of senescent stems. Pycnidia are at first green or yellowish, becoming dark brown or black; those formed latest may remain lighter and there are differences among strains in the apparent ability to form dark pycnidia. Each cluster is normally of one species or strain.

Working with P.foveata we have tried to discover to what extent stems are infected in a latent manner before pycnidia are developed on them.

In one test P.foveata was not isolated from any of 142 small pieces of green stem plated on malt agar from 15 plants of Majestic grown in infective compost. However, pycnidia of P.foveata developed on several of these pieces incubated for 2 to 3 weeks.

In another series of isolations, 20 green stems were sampled in early August from a crop of Redskin planted in May from seed tubers many of which had gangrene, and a comparable sample was taken every 10 to 12 days until late September. From the first sample, P.foveata was isolated on malt agar from pieces of two stems. Further isolations confirmed the presence of the fungus in the same parts; and pycnidia developed within 2 to 3 weeks on both stems and on four others kept in germinators. Later samples gave similar results except that it became easier to isolate P.foveata in September as the haulm matured. P.foveata was isolated from sections of the stem base and from small pieces of the outer part of either sound or abraded stem. Although what thus appeared to be latent stem infection was not hard to detect it did not occur generally throughout the stems. Even from those proved to be affected, P.foveata was isolated from just 37 of 205 pieces cultured in August.

Next, some stems of glasshouse-grown plants were infected, variously from a

a suspension of macerated agar culture, a spore suspension from agar culture, or from spores exuded in a drop of water from pycnidia on haulm; inocula were applied to leaf scars with a camel-hair brush. Eighteen plants of Dunbar Standard were each inoculated on two occasions: (1) prior to full growth when three leaves at 5 to 20 cm from soil level were broken off at the base of the petiole; and (2) two months later when three senescent leaves at 20 to 40 cm were removed. Comparable control plants were similarly treated and sterile water applied.

Sixteen of the 54 first-infected leaf scars and six of the second lot showed no sign of infection for some time. The others quickly developed a necrotic patch which mostly did not increase beyond 1 to 2 cm. Nearly three months after the first inoculations and three weeks after the second, pycnidia were present on the necrotic patches or leaf scars of 24 of the first set and 50 of the second, but hardly beyond these limits. One month later, when the stems were dying, almost all the inoculated sites had produced pycnidia, or further pycnidia in a zone surrounding those formed earlier.

Thus it seems that stems can be infected at any time but that pycnidia are formed only on moribund parts and predominantly on the ageing stem. As to whether latent infection in a stem is restricted or extensive our observations suggest that the fungus is usually localised while the stem is green but extends as the latter senesces. The results of a recent test in which the progress of infection in detached stem pieces was followed supported this view.

Once formed on the moribund or dead haulm, groups of pycnidia seem not to enlarge and, judged from a few clusters we have observed, no more pycnidia are developed. Spores can be produced almost forthwith but their release from a group is extendible at least over some weeks. We have observed pycnidial clusters of P.foveata on pieces of haulm put outside in mid-December, at a height of 3 ft, at soil level, and at 2 in below soil level. Within five weeks many of the pycnidia were almost spent, especially those set at 3 ft. After five months, pycnidia exposed above ground and at soil level were again examined. The former were all empty as were most of those set at soil level; however some of the latter were still full of viable spores. Pycnidial groups seem thus to be capable of releasing spores throughout the period of potato harvest; but another function of pycnidia may be to conserve the viability of spores for much longer or even to distribute the fungus.

Sources of stem infection

To demonstrate the incidence of the three common species of Phoma on haulm we have cultured extensively from spores exuded in sterile water mostly from single pycnidia representing groups at different parts of a stem (at an average rate of 10/stem). Results are summarised in Table 3.

It may be significant that, in two-thirds of the sources studied, P.foveata and P.solanicola appear to be almost mutually exclusive. Predominance of P.foveata was associated with gangrene on seed or crop, or with artificially-infected soil; otherwise P.solanicola predominated. In three of the five cases where both species occurred commonly together, P.foveata appeared to have gained a hold from the affected seed planted. The other two (d and m) seem to illustrate spread - because a distinctive strain of P.foveata included in the inoculum had been transmitted.

Apart from the artificially treated soils none tested in association with these sources has yet been proved infective. That gangrenous seed can provide infection for stems is, on the other hand, virtually certain, the best evidence being that a distinctive strain used to infect seed tubers reappeared on the stems. Logan (1967b) also has associated seed tuber and stem infection, and he has shown, further, that spores transmitted from haulm to crop can result in gangrene. Finally, some spread to the growing plant seems to have been demonstrated. This is in keeping with the results of our stem infection experiments but it is not known how it takes place in the field.

Table 3.

Incidence of Phoma spp. on haulm related to site, seed and soil

	Source of stems	No. of pycnidia	Percentage of pycnidia P.fov.	P.sol.	P.sup.
(a)	E. Craigs 1956 General	397	3	59	22
(b)	" 1965 "	53	0	70	11
(c)	" " Rows with artificially infected seed	142	29	45	15
(d)	" 1965 Alternate rows controlling (c)	178	16	38	36
(e)	Aberdeen 1958 1 crop with gangrene	67	9	0	78
(f)	Angus, Ayr, Lanark 1965 3 crops with gangrene	87	77	4	17
(g)	N. Ireland 1965 1 crop	25	52	4	36
(h)	Cambridge, Oxford 1956 2 crops	97	0	38	18
(i)	E. Craigs 1956, 1965 Glasshouse	69	0	93	7
(j)	" 1966 Glasshouse, latent-infected seed	36	44	56	0
(k)	" 1966 Glasshouse, affected seed	89	21	79	0
(l)	" 1966 Glasshouse, $\frac{1}{2}$ tubers in compost infected from cultures	440	91	8	0
(m)	" 1966 Glasshouse, $\frac{1}{2}$ tubers, controlling (l)	103	29	59	0
(n)	" 1967 Glasshouse, $\frac{1}{2}$ tubers in compost infected from haulm	98	93	4	0
(o)	" 1967 Glasshouse, $\frac{1}{2}$ tubers controlling (n)	55	2	53	0

INFECTION ON TUBERS

The nature of tuber infection

Alcock and Foister (1936) indicated that unwounded tubers were susceptible to gangrene; but many growers have felt that potatoes are infected through rough handling during the second half of the storage season. Our work has shown that the latter explanation of gangrene is over-simplified.

For more than a decade we have conducted official tests of varietal susceptibility, the procedure being to expose unwounded tubers to infection by dipping them in a suspension of macerated agar cultures of P.foveata, and to store them in damp cardboard boxes at 36°F. for a month and thereafter at about 45°F. Lesions develop usually in a few weeks from eyes, lenticels and apparently sound skin; the statistical record per tuber is the sum of the squared lesion diameters. Tubers are susceptible throughout the storage season but become a little more resistant as the season advances; on the other hand lesions develop faster in the later months.

Tubers exposed to infection in this manner appear to absorb it quickly. Our method of detecting such absorbed or latent infection is as follows. Washed tubers are dipped for 15 min in a fresh 0.1% solution of mercuric chloride containing non-ionic wetter, rinsed and allowed to dry. Part of one side of each tuber is scorched to kill any infection there and the skin removed. Skin or flesh on the opposite side is sampled by means of a sterilised cork-borer pushed through the tuber. The circular samples of skin (i.e. of periderm and outer cortex, $\frac{3}{8}$ in wide and about 1/16 in thick) are placed on malt agar slants. Similar pieces of flesh subtending the skin samples have also been tested as a routine but seldom with any suggestion that they carry infection.

Skin samples were taken from different surface-disinfected tubers one, three, seven and 21 days after exposure to infection. The numbers shown to be infected, aggregated from three tests were, respectively: 0/30; 0/30; 1/30; and 16/30. Thus it takes 7 to 21 days for infection to become sufficiently protected or absorbed to resist the sterilising effect of mercuric chloride demonstrable earlier.

The same method has revealed absorbed or latent infection in other interesting circumstances. Apparently sound tubers were taken in spring 1966 and 1967 from six stocks (three each of Majestic and Redskin) in which gangrene had begun to appear. Of 329 tubers tested, an average of 33% (ranging 3 to 54%) yielded P.foveata from skin pieces; P.eupyrena was isolated from 18% and P.solanicola from 3%. This must explain the commercially-embarrassing quality of such stocks! Again, latency is involved in varietal resistance to gangrene. For example, in two tests, Majestic tubers with an average of nine lesions each yielded P.foveata from 39 of 44 pieces (89%) of remaining sound skin; Arran Consul and two other resistant cultivars with a mean of 0.7 lesions per tuber yielded P.foveata from as many as 66 of 90 skin samples (73%). The suggestion is that a significant part of varietal resistance lies in an ability of the tuber to contain rather than to exclude the pathogen.

The nature of the association between gangrene and mechanical damage

Although unwounded tubers are susceptible, gangrene is closely associated with mechanical damage. In damaging tubers during the incubation period we have attempted to discover whether wounds incite lesions directly or, perhaps additionally, activate latent infection elsewhere on the tuber.

Five tests have been carried out variously with ten cultivars. Tubers were exposed to infection by dipping. After 3 to 6 weeks they were washed and either rubbed with spirit and flamed or, in one test, dipped in 0.1% aqueous mercuric chloride and dried; any lesion already formed was marked. Tubers were then injured in standardised ways: either pierced in two places to a depth of about $\frac{1}{2}$ in with a sterilised drill bit; or dropped through about 18 in on to a wooden block with partly driven nails on a 1 in square grid. This device was sterilised with spirit and flamed between replications. Fifteen tubers per cultivar were assigned to each treatment and there were 15 unwounded controls. The tubers were afterwards kept in dry paper bags each with a single tuber per cultivar, the treatments being separated.

The results summarised in Table 4 illustrate those obtained throughout the tests. Injury can add greatly to the number of lesions on a tuber and wounds may partly localise its potential to bear lesions. Some previously uninfected checks injured with the nail device contaminated with inoculum did not develop more lesions on the wounds than are shown in Table 4 for the latent-infected tubers. Evidently, however, wounds act only locally: elsewhere on wounded tubers no more lesions developed than on the uninjured controls.

Table 4.

Occurrence of gangrene lesions in relation to injuries inflicted during the latent period of infection: results averaged from several tests

Estimated no. gangrene lesions/10 tubers

Treatment	Arran Consul (2 tests)			Dunbar Standard (3 tests)			Gladstone (2 tests)			Majestic (3 tests)		
	1/	2	3	1	2	3	1	2	3	1	2	3
Uninjured	6	-	48	10	-	8	3	-	26	7	-	31
Injured (a)*	4	10	7	10	12	8	5	16	14	18	17	35
" (b)	3	24	3	9	23	6	6	35	5	19	33	15

* Injured (a) - pierced by drill bit; (b) - dropped on nails

/ Lesions formed, 1, before treatment; 2, on injury points; 3, after treatment but not on injury points

The incidence of lesions from injuries depended on the type of wound. Those caused by the nail device were of two kinds: either an impacted pit in the flesh or a shallow dent with a partial skin break. Wounds caused by the drill bit were, of course, ragged holes. Figures for the incidence of lesions developed from drill holes or nail holes were similar: means derived from all the tests were 69 and 71% respectively. From dents caused by nails the incidence was almost always lower than for pits, averaging 47%. Differences in the susceptibility or defences of tissues may explain these figures. But if latent infection is in some way absorbed or sealed off in the skin, impact may simply push this potential inoculum into the flesh when a new opportunity is then created for its extension.

Time and possible sources of tuber infection

The observations already given suggest that tubers can be infected or become very closely associated with P.foveata long before lesions appear, if they ever do. Injury can undoubtedly introduce infection from outside but it can also activate what is already there. Considering the possibility that tubers may become infected in the ground we have examined newly-lifted tubers from several environments for latent infection. None was found in the following lots:

(a) 40 tubers of Majestic and an un-named cultivar produced in the field from affected seed; pycnidia of P.foveata had been formed on the haulm. (Of 209 tubers from the same source stored dry over winter, 4 developed gangrene lesions laterally and 3 from the stolon end; and of 212 tubers from healthy control plants 3 developed lateral lesions)

(b) 51 tubers of Majestic grown in infective compost in the glasshouse; pycnidia of P.foveata had been developed freely on the haulm.

However, P.foveata was obtained from a field crop of Redskin planted in May, 1967 with inferior seed tubers many of which were affected with gangrene. Fifty sound tubers were dug at 10 to 12 day intervals and skin samples tested as usual except that the tubers harvested from 24 Aug. to 14 Sept. inclusive were not disinfected with mercuric chloride. This was precautionary as the tubers were still somewhat immature and tended to scuff. These tubers were well washed before being tested, and all those that yielded P.foveata were surface-disinfected and retested at the end of September. P.foveata was isolated from the 50-tuber samples

as follows: 2 Aug., 0/50; 14 Aug., 0/50; 24 Aug., 1/50; 4 Sept., 6/50; 14 Sept., 10/50. From the 17 apparently affected tubers that had merely been washed P.foveata was re-isolated after the later treatment only from one.

Infection on the green stems of this crop was demonstrable throughout the tests. Pycnidia, and hence spore release, were not discerned on stem samples in the field. However, they quickly formed on cut stems kept in germinators and so there may have been some early sporulation and transmission of infection to the tubers. Other possibilities, soil infectivity or underground spread from affected setts, were not explored.

Early tuber infection is thus at least an occasional feature of gangrene, but some of our results suggest that it does not invariably follow soil infectivity or the presence of the fungus on seed tuber or haulm. Rosser and Jones (1956) also report a case in which gangrene did not develop in the crop from affected seed. However, the results of several experiments with various farm stocks carried out at East Craigs (Graham and Todd, unpublished; Graham, 1964) and elsewhere (Boyd, 1960; Logan, 1967a) have shown that gangrene can be controlled by disinfection of tubers with organo-mercury immediately after lifting, and the most obvious interpretation is that early infection is significant. We have investigated some of these tubers for latent infection. P.foveata was isolated in March from 6 of 21 sound tubers that had been washed at lifting - a treatment not good enough to control gangrene; whereas no latent infection was demonstrated in 18 tubers of the same crop which had been washed and disinfected at lifting.

DISCUSSION

P.solanicola can cause rotting of potato tubers; it is closely associated with the potato plant and is also widely distributed in the soil and on other plants. That it is not commonly a cause of gangrene in Britain highlights the position of the morphologically similar P.foveata as a parasite adapted to potato. This species is more distinctively pathogenic to tubers than P.solanicola, has not been found on other plant species in the field and, in our experience, is not an easily demonstrable soil inhabitant. (Soil can be invaded, however, and the fungus may persist for some months at least after potato cropping). The resemblance between P.solanicola and P.foveata together with the complementary features of their ecology supports the view that the two fungi are closely related.

Seed tubers with gangrene produce stems with localised latent infections. The methods of transmission from tuber to stem have not been studied. Possibly, some parts of a shoot are infected as the bud grows but there may also be indirect infection through the soil: certainly stems can be infected from contaminated soil. Stem infections extend and produce pycnidia as the haulm matures. Pycnidial groups are capable of producing spores throughout the autumn and it seems likely, as Logan (1967b) had indicated, that these spores infect the crop, either directly or indirectly by growth in the soil. Different experiences of the incidence or control of gangrene in suspect crops may be attributable to the uncertainties of transmission between stem and tuber. This would be less likely if the haulm were destroyed early, artificially or by blight (Gray and Malcolmson, 1966) or if the crop were lifted early or, as Foister (1952) indicated, in a dry autumn. Because pycnidial groups sporulate over an extended period, the chance of incurring gangrene would seem to increase steadily as autumn advances, especially in a wet season. Admittedly, all these effects might be attributable to soil-inhabiting infection, but our evidence does not clearly support this view.

Tuber infection, or at least absorption of inoculum by the tuber may begin before lifting and can be accomplished quickly. This fits in with the practical finding that gangrene is controllable by chemical disinfection of newly-lifted tubers. The slightly penetrative property of organo-mercury may help to kill inoculum that has just been absorbed; and in searching for less toxic disinfectants one should keep this feature in mind.

The latent-infected tuber is literally a most delicate object to handle. Even uninjured, such a tuber will develop gangrene in a damp environment. If it is roughly treated, lesions will form around cuts and impacted skin. It is possible also that secondary infection from contaminated machinery is important but we have not studied this in practice.

Acknowledgements

We wish to thank Miss P. M. Smith and Miss M. F. Tripney for their technical assistance; and the Seed Potatoes Branch of the Department for the data we have summarised in Table 1.

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SOME PROBLEMS IN CHEMICAL CONTROL OF DISEASES OF SEED POTATO TUBERS

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Summary

In north-east Scotland, gangrene in potato tubers was reduced by organo-mercury compounds applied at lifting but not at grading. Effective control was not obtained by dipping in formaldehyde, oligomycin or rimocidin or by dusting with α- chloronaphthalene, dibrometetrachlorethane, diphenyl, parachlorophenol, pentachlorethane or tecnazene.

Germination of black scurf sclerotia was controlled by wet treatment of tubers with organo-mercury and chlorophenol preparations and by trioxymethylene fumigation of clean tubers.

Washing tubers increased incidence of gangrene. Chemical treatment of washed tubers was on occasion associated with bacterial rotting of treated tubers, increase in number of plants showing blackleg in the resulting crop and reduction in yield.

It is considered that partial failure of some chemical treatments to control gangrene was due to establishment of infection before harvest and that effect of treatment was influenced by the state of maturity of the crop.

INTRODUCTION

Of the diseases which may be controlled by the treatment of washing and dipping in organo-mercury preparations (Boyd, 1960), gangrene caused by Phoma solanicola and black scurf caused by Corticium solani are of particular interest to seed producers in northern Scotland. Gangrene is a source of complaints from buyers in Britain and Europe. Consignments for South Africa must be disinfected before packing or after delivery in order to prevent damage to sprouts by the black scurf fungus.

Treatment has not always proved satisfactory and in the course of advisory work examples have been seen of the increased incidence in blackleg noted by Graham and Volcani (1961) in their trials concerned with seed for export to warmer countries, and of soft rot and failure of sprout growth in stocks treated commercially.

In a study of the factors affecting gangrene in north-east Scotland, it was found that dipping unwashed, newly-lifted tubers for 30 seconds in 0.5% solution of a proprietary formulation of ethoxyethylmercuric chloride (E.E.M.C.) containing 3% mercury considerably reduced the incidence of gangrene; treatment at grading did not give effective control. The incidence of gangrene after treatment indicated that some infection takes place before harvest and that haulm need not be an important source of this. The rate of haulm destruction and incidence of gangrene were related, both in dipped and undipped tubers, less gangrene occurring where haulm was removed or killed rapidly by treatment with an efficient chemical. It was considered that there is a connection between the maturity of the tubers and the incidence of the disease.

Some indication that treatment at harvest does not eradicate established infection was obtained by dipping tubers in E.E.M.C. after

inoculation of the skins by sprinkling with soil from a field which in the previous year produced potatoes affected by gangrene. Gangrene developed on 11.8% of the inoculated tubers, on 4.8% of tubers dipped in E.E.M.C. after being inoculated and on 7.2% of the uninoculated, untreated controls.

In several seasons severe gangrene was recorded in commercial stocks treated with tecnazene to control Fusarium dry rot; however it was reported (Mills, 1958) that gangrene was apparently no longer a problem in potatoes from north-east Scotland since the practice of treating the tubers with tecnazene at lifting-time had been adopted.

Further trials were made with tecnazene and organo-mercury preparations and, as wet treatments with the latter create practical difficulties, the possibility of using other chemicals was explored. For all trials, tubers were from commercial seed crops harvested by elevator digger.

RESULTS

In 1953, newly-lifted tubers of the varieties Arran Pilot and Up-to-Date were dipped in wire baskets in E.E.M.C., then stored until spring in lots of 20 lb in closed containers under warm (8 to 18°C.) and cool (2°C.) conditions, and in chitting trays under straw in a frost-free shed. Newly-lifted tubers of the variety Arran Banner were stored similarly after treatment at 5 lb/ton with dusts containing α- chloronaphthalene (2%), parachlorophenol (2%), or pentachlorethane (2%). No gangrene developed in the Arran Pilot and Arran Banner tubers kept in warm storage and the incidence of the disease was reduced in them by all the chemical treatments (Table 1). Under cool storage conditions the incidence of gangrene was considerably higher when ventilation was reduced.

Table 1.

Effect of chemical treatment in relation to storage conditions, 1953 - 54

Variety	Treatment: At lifting in	Chemical	No. (%) tubers with gangrene in March: in closed containers at 8-18°C	in closed containers at 2°C	under straw in frost-free shed
Arran Pilot	October	None	0	54.2	25
	"	E.E.M.C.	0	36.6	13
Up-to-Date	November	None	0	4	1.52
	"	E.E.M.C.	1.3	16.4	0.14
Arran Banner	"	None	0	29.2	5.3
	"	α-chloronaphthalene	0	25.6	1.9
	"	parachlorophenol	0	7.4	4.6
	"	pentachlorethane	0	10.4	1.91

In 1954 trials were made with dusts containing α- chloronaphthalene (2%), parachlorophenol (2%), pentachlorethane (2%), diphenyl (3%) and dibromotetrachlorethane (1 and 5%). Treatments were applied at lifting in November to lots of 5 cwt of two stocks of Majestic and of 1 cwt of one stock of Arran Pilot; one stock of Majestic was also treated at grading in December. The incidence of gangrene recorded in April 1955 was extremely high in all the treatments (Table 2) and, contrary to the previous results, none of the chemicals gave effective control of the disease.

Table 2.

Effect of fungicidal dusts, 1954 - 55

Tuber treatment		No. (%) tubers with gangrene in April					
		Majestic					Arran Pilot (+)
Chemical	rate (lb/ton)	Stock 1* treated at lifting Nov. 3	Stock 1* treated at riddling Dec. 22	Stock 1 + treated at lifting Nov. 3	Stock 1 + treated at riddling Dec. 22	Stock 2 ø treated at lifting Nov. 17	treated at lifting Nov. 3
untreated	-	72	100	100	100	34	23
3% diphenyl	10	55	100	100	100	60	38
2% α-chloro naphthalene	5	67	100	100	100	51	34
2% parachloro-phenol	5	100	100	100	100	62	43
2% pentachlor-ethane	5	64	100	100	100	61	28
1% dibrometatra-chlorethane	6	78	100	100	100	35	31
5% dibrometetra-chlorethane	6	54	100	100	100	68	31

* stored in sections of 5 cwt under straw in unheated store
\+ stored in trays of 28 lb " " " " "
ø stored in crates of 100 lb " " " " "
(+) stored in trays of 28 lb uncovered in heated store

In Aberdeenshire and Kincardineshire, between 1948 and 1954, severe attacks of gangrene were observed in commercial stocks of Arran Pilot, Craigs Royal and Ulster Chieftain treated at lifting with tecnazene and stored in bulk throughout the winter or graded subsequently into chitting trays. Since these observations conflicted with those of Mills (1958), the effect of treatment and storage conditions was examined. In October 1958, 400 lb newly-lifted tubers of the variety Majestic were treated with tecnazene (3% tetrachloronitrobenzene) at the rate of 10 lb/ton and stored under straw in perforated cartons beside the commercial crop. The same quantity of untreated tubers was stored in the same way alongside, separated by sacking. In mid-February, 280 lb of both the treated and untreated tubers were riddled on a mechanical grader and replaced in the cartons and of these, half were replaced under straw in the potato shed where the temperature remained mostly below 5°C; the remainder were stored under sacking at about 12°C. The percentage of tubers with gangrene in April, although small, were less where tecnazene was used and this was not related to storage temperature (Table 3). In 1959-62 the incidence of gangrene was negligible and satisfactory assessment of treatments with tecnazene could not be made.

Table 3.

Trials with tecnazene, 1958 - 62

Season	Treatment	Storage temperature (°C)	No. (%) tubers with gangrene (March - April) treatment at lifting	
			none	tecnazene
1958-59	not riddled	< 4.5	5.7	2.8
	riddled 14th Feb.	< 4.5	9.9	1.9
	riddled 14th Feb.	12.0	6.9	2.2
1959-60	not riddled	< 4.5	0.9	0.2
1960-61	not riddled	< 4.5	0.7	0.3
1961-62	not riddled	< 4.5	3.2	0.1

The antiobiotics oligomycin and rimocidin sulphate, obtained from Messrs. Pfizer, were tested in 1956 on the variety Majestic at a farm in Aberdeenshire. Newly-lifted potatoes, in wire baskets, were dipped in solutions of 1 gm antibiotic/gal water. The amount of gangrene was recorded in March 1957 (Table 4). Treatment with oligomycin at this dosage caused skin necrosis.

Table 4.

Effect of treatment with antibiotics

Treatment	No. (%) tubers with gangrene
untreated	1.4
oligomycin	1.1
rimocidin	2.2
E.E.M.C.	0.8

Formaldehyde has proved effective in surface sterilising small quantities of tubers for experimental purposes. It was compared with E.E.M.C. and methoxyethylmercuric chloride (M.E.M.C.), both at 150 p.p.m. mercury, in treating newly-lifted tubers from a commercial seed crop of the variety Majestic. Half were washed mechanically, and any excess moisture was allowed to drain from them before further treatment was applied. Tubers from washed and unwashed lots were placed in wire baskets, then dipped in a solution of formaldehyde (2%), E.E.M.C. or M.E.M.C. For each treatment 2 cwt tubers were used. After being dipped, they were placed in trays and left uncovered for 24 hours in an airy shed with open sides to dry. The trays were then stacked in a store, alongside the normal commercial crop, covered lightly with straw, and subsequently covered to the same depth as the commercial crop. In April the presence of disease was recorded (Table 5). Possibly because of a serious outbreak of late blight in 1958, dry rot was severe among the washed tubers. This is reflected in the smaller numbers of tubers which remained sound. The incidence of gangrene was increased by the treatment with formaldehyde and reduced by the organo-mercury preparations. In each case the incidence of gangrene was higher among the washed tubers.

Table 5.

Effect of washing and dipping at harvest

Treatment	No. (%) tubers with gangrene		No. (%) sound tubers	
	Unwashed	Washed	Unwashed	Washed
untreated	2.2	4.3	96.8	87.8
formaldehyde	3.0	7.9	93.9	68.0
E.E.M.C.	0.9	2.6	96.9	87.7
M.E.M.C.	0.7	3.8	97.1	76.0

Similar treatments with formaldehyde and E.E.M.C. were applied in January, 1958 to graded tubers of Arran Pilot from two farms and Majestic from a third farm in Aberdeenshire. Half of the tubers from each stock were washed mechanically before chemical treatments were applied. From each stock one chitting tray of washed and one tray of unwashed tubers were dipped in solutions of 2% formalin or 0.5% E.E.M.C. Trays of washed and unwashed tubers from the respective stocks served as controls. The trays were placed under a fan at 10°C. to allow the dipped tubers to dry before they were placed under straw in a normal commercial potato store for two months.

The amount of gangrene among the tubers treated with formaldehyde (Table 6) was greater than in the controls and with one exception, less gangrene was recorded in the tubers treated with E.E.M.C. Again, the incidence of gangrene was in general higher among the washed tubers.

Table 6.

Effect of washing and dipping after grading

Tubers graded Nov. 1957	No. (%) tubers with gangrene, April 1958					
	Majestic		Arran Pilot			
Treatment Jan. 1958	Farm 1		Farm 2		Farm 3	
	unwashed	washed	unwashed	washed	unwashed	washed
untreated	9.9	12.7	10.8	25.0	30.4	27.6
formaldehyde	11.0	40.7	19.9	29.0	52.0	67.0
E.E.M.C.	6.0	17.4	9.7	17.0	20.5	20.8

Although treatment with organo-mercury preparations can give some degree of control of gangrene when applied at lifting, increased wet rot in storage and blackleg in the resulting crop have been noted following such treatment. In the 1960-61 seasons, the incidence of wet rot and blackleg following treatment with organo-mercury preparations and other chemicals for control of black scurf was recorded. Within 18 hours of lifting, tubers of the variety Up-to-Date were washed and dipped in either of two proprietary organo-mercury compounds or a chlorinated phenol preparation. In addition one lot of the washed tubers and one of the unwashed tubers were fumigated for 24 hours with a trioxymethylene preparation. For each treatment 4 cwt tubers were used. After the wet treatment, the tubers were divided into lots of 56 lb, placed in open crates and dried off at a temperature of about 15°C. in an airy shed ventilated by fans. Throughout the winter the tubers were stored uncovered in a frost-free shed. No

gangrene was evident in any of the tubers but the incidence of wet rot was recorded in April. There was little rotting of any other type during storage. Dipping in the organo-mercury and chlorinated phenol preparations prevented germination of sclerotia of the black scurf fungus. Fumigation with trioxymethylene was moderately effective with washed tubers but not with unwashed tubers.

To determine the effect of the treatments on the subsequent crop, sound chitted tubers from each treatment were planted in a randomised block design of six blocks each of seven plots. The plots, each of four drills 11 yd long, were planted with 132 tubers of size range $1\frac{1}{4}$ to $1\frac{3}{4}$ in, and the two middle rows of each plot were harvested. The treatments had little effect on yield, except for organo-mercury compound (3) which caused a slight reduction. Blackleg, confirmed by Dr. A. M. Paton as being caused by Pectobacterium carotovorum var. atrosepticum occurred in all the plots and its incidence was increased by chemical treatment of washed tubers (Table 7).

Table 7.

Effect of chemical treatments on black scurf, wet rot, blackleg and yield

Treatment (October 1960)	% sclerotia germinating (March 1961)	No. (%) tubers with wet rot (Apr. 1961)	No. (%) plants with blackleg (Sept. 1961)	Yield tons/acre (Oct. 1961)
Tubers washed mechanically				
untreated	65.6	0	3.36	12.5
fumigated 24 hrs. with trioxymethylene	3.9	25	9.85	13.9
dipped 1 min. in 0.5% Aardisan (organo mercurial)	0	12	11.49	11.3
dipped 5 min. in 0.5% E.E.M.C. + wetter*	1.0	6	9.85	12.7
dipped 1 min. in 2% chlorophenol	0	81	8.90	14.0
Tubers not washed				
untreated	57.3	0	0.97	13.2
fumigated 24 hrs. with trioxymethylene	32.1	0	1.52	13.3
			± 1.39	± 0.66

*octylphenolpolyethylene

In the winter of 1961-62, tubers of the varieties Arran Pilot, Kerr's Pink and Ulster Prince from dry consignments, brushed free of soil, were fumigated with trioxymethylene. Germination of sclerotia was reduced to 12%, 8% and 3% respectively, compared with 100% from untreated tubers. The treated tubers sprouted normally and did not produce plants with blackleg.

The effect of treating the parent crop with a proprietary foliar nutrient dust containing 2,4-D, designed to improve periderm formation, combined with washing and dipping in E.E.M.C., was tested in 1961 on tubers from dusted and untreated portions of two crops of Up-to-Date.

Washing and dipping treatments were carried out immediately after lifting and 6 and 12 weeks thereafter as indicated in Table 8. The treated tubers were dried by means of an electric fan and all tubers were stored in open crates in a commercial potato store until spring. No other rots developed. The incidence of gangrene recorded in March was low (Table 8). There was no clear difference between treatments in length or number of sprouts produced during storage. From each treatment sound chitted tubers of size range $1\frac{1}{4}$ to $1\frac{3}{4}$ in were planted in a randomised block design of two blocks of twenty-eight plots. The plots consisted of four drills, 7 yd long planted with tubers 1 ft apart, and the records were taken from the middle two rows of each plot. The incidence of blackleg was negligible. Yields from seed from crop 2 were not affected but there were significant effects due to treatment of crop 1. Higher yields were obtained from untreated seed and yields were better from tubers washed but not treated than from any of the chemical treatments. Averaged over both stocks, the highest mean yield was from untreated seed, from untreated parent crop (Table 9).

Table 8.

Effect on gangrene of treatment of foliage and tubers (1961-62)

Treatment of tubers	No. (%) tubers with gangrene Treatment			
	Crop 1		Crop 2	
	none	2,4-D	none	2,4-D
Untreated	0	6	1	0.5
Washed in Oct.	2	3	0	0
Washed and dipped E.E.M.C. in Oct.	5	5	1	0
Washed in Oct. dipped E.E.M.C. in Dec.	1	6	1	0
Washed in Oct. dipped E.E.M.C. in Jan.	3	4	3	2
Washed and dipped E.E.M.C. in Dec.	0.5	2	2	0.5
Washed and dipped E.E.M.C. in Jan.	2	3	5	2

Table 9.

Effect on yield of treatment of parent crop foliage and of seed tubers

Treatment of tubers	Yield of tubers in tons per acre Treatment					
	Crop 1			Crop 2		
	none	2,4-D	mean	none	2,4-D	mean
Untreated	16.6	16.3	16.5	14.2	13.8	14.0
Washed in Oct.	16.0	15.5	15.7	14.0	15.1	14.6
Washed and dipped E.E.M.C. in Oct.	15.6	13.7	14.6	15.1	16.5	15.8
Washed in Oct. dipped E.E.M.C. in Dec.	14.5	12.5	13.5	14.9	13.9	14.4
Washed in Oct. dipped E.E.M.C. in Jan.	15.1	14.5	14.8	13.5	14.4	13.9
Washed and dipped E.E.M.C. in Dec.	12.8	14.2	13.5	15.6	13.9	14.8
Washed and dipped E.E.M.C. in Jan.	14.0	15.3	14.6	14.1	13.5	13.8
Mean	15.0	14.6		14.5	14.4	

Standard errors of means (13 d.f.)

Overall seed treatment	± 0.44	± 0.70
Overall crop treatment	± 0.23	± 0.38
Within seed x crop treatment	± 0.62	± 0.99

DISCUSSION

The various trials reported were carried out with relatively small quantities of tubers, using improvised equipment, and not in a commercial plant. It can be claimed that the tubers had firm skins and were not injured excessively, that treatments were made within the recommended limits of temperature, that drying was speedy and adequate and storage thereafter was in airy, frost-free buildings. From earlier experience of washing and of flotation in brine and soil suspensions, using tubers from the same districts, it was known that wet treatment could be beneficial or damaging, depending on removal of surface moisture from skins after treatment and on state of tubers when treated.

It may be significant that phytotoxic effects and failure to control gangrene are reported from north-east Scotland. There the state of soil moisture during the growing season is believed to favour pre-harvest infection with the gangrene fungus and crops mature less rapidly than in the areas where the bulk of the tubers treated successfully on a commercial scale are produced. Incomplete control of gangrene presumably results from treatment after infection is established. It is known that extent of damage and reaction to damage are influenced by state of maturity of the tuber at the time of harvest (Mackenzie, 1967). Degree of maturity is related to growing conditions, including length and uniformity of the growing season (Burton, 1966).

Better control of gangrene by chemical disinfection could no doubt be achieved provided harm were destroyed before the tubers became infected, and rate of fertilizer adjusted so as to reduce susceptibility of tubers to damage. At lower rates of fertilizer, the number of plants showing blackleg in the growing crop increases and may exceed the level permitted for certification, an important consideration for the seed producer. There would appear also to be an association between increased incidence of blackleg in the growing crop and relative earliness of development of the crop resulting from climate, time of planting and the use of chitted and of damaged and rotting seed tubers. Thus acceleration of plant growth by the disturbance by disinfection processes may be a factor influencing manifestation of blackleg.

In northern Scotland, soft rots under lenticels, associated with bacterial infection though not necessarily with blackleg bacteria, have been most noticeable in tubers treated after the wet growing seasons and wet harvests of 1960 and 1965. It has been reported in discussions with growers and officials from South Africa that wet treatment there of imported Scotch seed prior to planting does not cause rotting and does not increase blackleg. This may be due to the method which dispenses with washing and mechanical drying and consists of immersing unopened perforated cartons of tubers in the organo-mercury preparation, draining off excess liquid and planting soon afterwards.

Treatment with thiram (Nielsen, 1965) has not so far given satisfactory results in Aberdeenshire but some other dust formulations now being tested on newly brushed tubers have proved superior to dipping in E.E.M.C. in preventing growth from black scurf sclerotia. An objection to the use of dusts and fumigants is the difficulty of ensuring good coverage. For seed

for export, a treatment which can be applied by the producer is to be preferred and it should remove soil and soil-borne organisms, especially nematodes, in addition to controlling black scurf. Wet treatment has obvious merits but there is a need to define the state of tuber for which treatment is suitable.

Acknowledgments

From 1956 - 60, investigations on gangrene at the North of Scotland College of Agriculture were financed in part by a grant from the Agricultural Research Council. We are indebted to the farmers and merchants who allowed us the use of land, crops and stores, and to N.V. Aagrunol Chemische Fabrieken, Bayer Agriculture Ltd., J. W. Chafer Ltd., Imperial Chemical Industries Ltd., Lunevale Products Ltd., Pfizer Ltd., and Sir Thomas Wedderspoon for chemicals, to colleagues in the former Agricultural Research Council Potato Storage Investigation for assistance in some of the trials and to the Agricultural Research Council Unit of Statistics, formerly at the University of Aberdeen, for statistical analyses.

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SEED TUBER DISINFECTION

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Summary

Experimental results are given of a continuous process of washing and disinfecting seed potato tubers using a solution of methyl ethoxy mercuric chloride (MEMC). A high degree of control of gangrene and dry rot was achieved in both the varieties used, Majestic and King Edward, and blight was also reduced when this disease was present in the stocks. The level of skin spot in these stocks was not high but adequate control was effected. The reduction of bacterial hard rot symptoms was assumed to be associated with reduction of damage by removing pintle rollers in the washing process. Damage sustained by mechanical grading either with or without previous tuber washing increased the incidence of blackleg in the growing crop in comparison with that resulting from hand-riddling. Seed treatment generally stimulated sprout growth and this was reflected in earlier emergence after planting. In one series with the variety Majestic the number of stems was significantly increased. No significant effect was observed on total yield from apparently sound treated and untreated seed.

INTRODUCTION

Fungicidal treatment of seed potatoes has been used to control various tuber diseases since Bolley (1891) first advocated the use of mercuric chloride for this purpose. The introduction of organo-mercury compounds after the first World War stimulated work in Austria (Claus et al 1923), Germany (Appel 1923) and in the U.S.A. (Clayton 1929) where they have been employed to disinfect seed pieces before planting to give protection against seed piece decay caused by Erwinia spp and Fusarium spp and to reduce the spread of common scab (Streptomyces scabies). In the Netherlands tuber disinfection during storage and before boxing is undertaken to minimise subsequent Rhizoctonia attack on young sprouts (van Emden 1958).

Disinfection immediately after lifting was first carried out to reduce late infection of tubers by blight (Phytophthora infestans) by Small (1935) using formalin and later by Greeves (1937) using mercury compounds. Organo-mercury compounds were subsequently shown to provide a successful control of skin spot (Oospora pustulans)(Greeves and Muskett 1939, Foister 1943, Boyd 1957) and of dry rot (Fusarium caeruleum) (Foister 1940; Foister and Wilson 1943). All this experimental work in the U.K. had been carried out simply by immersing seed tubers in baskets or sprouting trays for between $\frac{1}{2}$ and 1 min in a solution of an organo-mercury compound, usually ethoxyethyl mercuric chloride (EEMC) at a concentration of approximately 150 ppm mercury.

After a number of years of experimentation, a continuous disinfection process was developed commercially in 1956 in Scotland by Sir Thomas Wedderspoon at Eassie, Angus, and this has been described briefly by Muskett (1960) and Boyd (1960). The seed is separated from the ware after being first washed to remove adhering soil and to ensure freedom from eelworm cysts, particularly those of Heterodera rostochiensis (Mabbott 1960), then passed through a dipping tank containing a solution of ethoxy- or methoxy-ethyl mercuric chloride (EEMC or MEMC) maintained at a mercury concentration of about 100 ppm. The tubers remain in this solution for

12 min and are then elevated out of the bath, over absorbent rollers to remove surface moisture and delivered into sprouting trays for final drying and storage.

Examination of these tubers on a conveyor belt before final despatch facilitates the removal of any diseased or blemished tubers previously overlooked. Boyd (1960) using the varieties Doon Star and King Edward found that dry rot, skin spot and also gangrene were controlled satisfactorily by this method when this was undertaken immediately after lifting which took place up to about the end of October. Satisfactory control was not achieved by treatment of potatoes taken from storage six weeks later. Normal treatment was found to stimulate sprouting and to induce earlier emergence after planting but generally gave no significant increase in total yield.

Jennings, Calvert and Morrison (1964) using the same method with MEMC in Northern Ireland also showed that storage losses with Arran Pilot (mainly associated with Fusarium caeruleum) were reduced to negligible proportions and that the incidence of Rhizoctonia solani on the stem bases was considerably lower. Sprouting was stimulated and a higher number of stems were produced per plant but again the total yield was unaffected. Reduction of germination of sclerotia of R.solani on the tuber surface by KEMC and MEMC has been shown by Graham (1960)

In work carried out to investigate the control of blackleg (Pectobacterium carotovorum var atrosepticum), Graham and Volcani (1961) noted that where washed but not disinfected seed of the variety Up-to-Date gave rise to severe blackleg after planting, the additional disinfection of the seed with KEMC provided a significant reduction. They also noted that in some commercial crops disinfected seed had given rise to an increased number of infected plants than untreated seed from the same stock. Jennings et al (1964) observed a high incidence of blackleg in Arran Pilot plants grown from washed-only seed but in plants from seed disinfected after washing this was considerably reduced although not quite to the level of that of the control plants.

Another facet of the problem of blackleg development subsequent to washing tubers appeared when Logan (1964) described bacterial hard rot, showing its association with the blackleg organism. It takes the form of a black, dry, gangrene-like lesion somewhat similar to the arrested type described by Rudd Jones and Dowson (1950). It is associated with bacterial invasion of lenticels and wounds during the washing process and subsequent storage in a dry environment. Disinfection immediately after washing tended to reduce but not entirely prevent its incidence and a high proportion of infected tubers produced plants with blackleg symptoms.

Many of the problems associated with the efficiency of a large scale disinfection process are related to damage to the potatoes both before and during the washing and to the rapid drying after treatment. In the present paper, some of the results are given of work in progress since 1965.

METHODS AND MATERIALS

Washing and treatment of the tubers was undertaken in the disinfection plant at Castleton, Eassie, Angus. Standard procedure was used with MEMC as the active ingredient of the disinfection fluid and commercial stocks of two varieties Majestic and King Edward treated just after lifting on 23 October 1965, and on 26 October 1966 (King Edward) and 7 November 1966 (Majestic). In all cases the crops had been burnt down not less than one month before lifting. In 1965, there were three experimental variants.

A. Washed, machine-riddled and disinfected: dried in sprouting trays.

B. Washed, machine-riddled and disinfected: dried in pallet boxes.

C. Untreated: hand-riddled into sprouting boxes.

In 1966 two further variants were added.

D. Washed, machine-riddled, not disinfected: dried in sprouting trays.

E. Untreated: machine-riddled into sprouting trays.

After 10 days storage, samples of about 800 tubers were taken from each of the treatments, retained in bags for one week to simulate time of transport and then taken to a potato store at Bush or at East Craigs, Edinburgh and kept in trays at temperatures thermostatically controlled to minimum temperatures of 42°F in 1965 and 37°F in 1966. Boxes were moved in the stacks periodically to maintain even lighting conditions.

RESULTS

Disease control

Periodic examinations were made for the presence of storage rots and final disease assessments were made on 31 March 1966 and 30 March 1967 of all tubers in all the boxes.

Table 1

The effect of various treatments upon the incidence of tuber rots, 1965-66

Treatment	Gangrene %	Dry rot %	Hard rot %	Other rots %	Total rots %
		Majestic			
A	3.0	0.1	6.3	0.8	10.2
B	3.2	0.2	6.4	0.8	10.6
C	65.2	5.2	0.0	0.5	70.9
		King Edward			
A	1.0	0.2	0.0	0.1	1.3
B	1.8	0.2	0.7	0.1	2.8
C	6.3	0.6	0.0	0.1	7.0

Table 2

The effect of various treatments upon the incidence of tuber rots, 1966-67

Treatment	Gangrene %	Dry rot %	Blight %	Other rots %	Total rots %
		Majestic			
A	1.7	1.2	0.6	0.6	4.1
B	1.9	1.1	0.3	0.2	3.5
C	17.2	8.5	3.9	0.4	30.0
D	9.4	6.9	1.2	0.3	17.8
E	23.2	13.2	2.8	0.1	39.3
		King Edward			
A	4.0	0.2	2.0	0.2	6.4
B	2.0	0.2	1.7	0.8	4.7
C	16.9	3.2	3.5	1.5	25.1
D	28.0	6.9	4.2	0.8	39.9

Tables 1 and 2 show that both disinfection treatments A and B are equally successful in reducing all tuber rots to a very low level.

Gangrene: Control of this disease was most marked, particularly with Majestic in 1965. The high incidence of 65.5% occurred in hand-riddled potatoes which normally receive less damage than those dressed by machine. In 1966, opportunity was taken to use a mechanical grading machine for the variety Majestic and this was shown to increase gangrene still further over the equivalent hand-riddled potatoes.

Dry rot: The incidence of this disease appears to be lower than it was some years ago, but it can still cause heavy losses. Tables 1 and 2 show again that successful control of dry rot is achieved by organo-mercury treatment of the seed, so that both of the major tuber diseases usually associated with wounds may be successfully treated by adequate disinfection at lifting time.

Blight: In the 1966 experiments, blight was present in the stocks of both varieties. All obviously affected tubers were removed before storage began and the reduction of this disease associated with disinfection may be an indication of an eradicant action by the mercury of late infection from the soil. Blight was also associated with some of the dry rot and gangrene lesions, but the positive identification of blight in some of these lesions was so difficult that they are all considered under the gangrene or dry rot categories. Dry rot often follows initial blight lesions but there is no record as far as is known of gangrene occurring in this way. However, even in these cases, disinfection provided a satisfactory control.

Hard rot: Logan (1964) showed that symptoms of this disease are caused by the entry of blackleg organism into lenticels and wounds and were related principally to the washing process. They were evident only in 1965 and almost entirely in the variety Majestic. Graham (1967) has evidence that much of the blackleg infection is associated with damage by pintle rollers in the washing and grading process. Such rollers were used in the 1965 experiments but not in the following year.

Other rots: These consisted mainly of various bacterial soft rots, including a few blackleg and, in 1966, pink rot. The combined total of these was negligible in every case.

The effect of washing: Treatment D was carried out in 1966 only but its effect on the two varieties was quite different compared with that of hand-riddling (c). It was associated with more tuber rots in King Edward and fewer in Majestic and the reason for this is not clear.

Table 3

The effect of various treatments on the incidence of skin spot, 1965-66

Treatment	Percentage Majestic tubers with		Percentage King Edward tubers with	
	Infection at some eyes	No obvious infection	Infection at some eyes	No obvious infection
A	3	81	2	80
B	1	83	2	88
C	9	69	22	51

Skin spot: This disease has been shown to be very amenable to control by organo-mercury disinfectants and although the results in Table 3 show some reduction none of the stocks used was highly infected. The fact that all the treatments spent almost the entire storage period in boxes is probably responsible for the low incidence of skin spot in the untreated control.

Blackleg: Samples of apparently sound tubers from each treatment were planted either in randomised block or Latin square design, 80 tubers per plot, and counts of blackleg-affected plants made on one occasion in the 1966 crop and periodically in 1967. Results are shown in Table 4 in which the statistical data are given only for those of 24 July and 4 September. It is clear that, as has been noticed by other workers, an increase in blackleg is associated with washed and disinfected seed compared with untreated and hand-riddled seed although, because of considerable plot variation, the large increase in 1966 is not statistically significant. Evidence that such treatment is less damaging to the tubers than mechanical grading is seen in its association with the development of less gangrene and dry rot. Consequently blackleg incidence after treatment should be compared with that after mechanical grading (E) which unfortunately was carried out only with Majestic in 1966. The effect of washing alone is to increase blackleg still further so that, as shown by Graham and Volcani (1961), Jennings et al (1964) and Logan (1964), the disinfection treatment tends to reduce infection initiated in the washing process. It is not known why there is a difference in the effect of the two forms of drying, i.e. in trays and in boxes, in each year. From these results it would appear that one of the principal causes of blackleg increase is damage to the tubers and even in spite of removal of pintle rollers, which, it is believed, served to reduce or eliminate hard rot, damage such as occurs on a mechanical grader can allow the entry of the blackleg bacterium.

Table 4

Percentage blackleg in plots of Majestic and King Edward plants grown in 1966 and 1967 from seed variously treated in 1965 and 1966.

Treatment	1966 8 Aug.	1967 14 June	25 June	24 July %	24 July Angles	4 Sept. %	4 Sept. Angles
			Majestic				
A	25.6	2.9	3.8	4.2	0.20	11.5	0.35
B	16.3	4.5	7.4	7.7	0.27	17.6	0.43
C	5.6	1.3	2.0	2.2	0.13	3.8	0.19
D	-	4.8	9.3	9.9	0.32	21.0	0.47
E	-	3.6	7.5	8.2	0.28	18.3	0.44
LSD 5%	NS				0.08		0.07
1%					0.10		0.10
			King Edward				
A	4.4	0.0	1.0	-	-	13.9	0.38
B	4.0	0.6	0.6	-	-	17.4	0.43
C	0.0	0.0	0.0	-	-	2.3	0.15
D	-	2.1	4.6	-	-	20.5	0.47
LSD 5%	NS						0.05
1%							0.08

This can occur even when mechanical riddling is followed simply by boxing but is greatly increased in the presence of water. The increase is smaller, however, when tubers are immediately disinfected.

The expression of blackleg symptoms in the field depends very much on environmental conditions and sometimes it is not easy to detect. Detailed examinations in these experiments revealed many plants with only slight symptoms which might well be missed in a more casual assessment. Hence the relatively high counts which are shown in Table 4. In 1966, blackleg reached epidemic proportions in many crops of untreated seed after very high rainfall in June and the incidence did not increase much towards the end of the season (Graham and Harper, 1967).

Another aspect shown in Table 4 is the gradual increase of blackleg symptoms during the course of the 1967 growing season and the sudden increase in early September when plants were beginning to lose vigour. Graham and Harper (1966) have shown that the rate of fertiliser application can alter the incidence of the expression of blackleg symptoms which are less obvious where high rates are used.

Sprouting and sprout emergence.

Periodic measurement of sprout numbers and length were made on random samples of 100 tubers and, after planting, the rates of emergence recorded as the average number of days for all plants to emerge. The results for 1966-67 are shown in Table 5.

Table 5

Effect of various treatments on sprout length, rate of emergence and blanking, 1966-67

Treatment	Percentage tubers with no sprouts 10 Jan.	10 Feb.	Percentage tubers with sprouts $\frac{1}{8}$ in or more 10 Jan.	10 Feb.	Av. no. of days for complete emergence	Percentage blanking
			Majestic			
A	100	47	0	13	45	0
B	100	56	0	7	43	0
C	100	93	0	1	48	0
D	100	75	0	5	48	2
E	100	94	0	0	54	2
			King Edward			
A	74	0	9	84	39	0
B	72	1	8	74	43	1
C	82	12	8	65	45	2
D	82	6	2	79	46	1

Table 5 provides further evidence that sprout development is stimulated by the disinfection treatment. This was clear with Majestic in each year but in 1965-66, there was a slight initial retardation with King Edward but only in tray-dried tubers. In both varieties in each year, however, seed treatment tended to give a quicker emergence. There was also a tendency for slight sprout stimulation in washed-only tubers particularly with Majestic. This has been found to occur previously but not consistently (Boyd 1960).

Stem numbers: Jennings et al (1964) recorded an increase in the number of stems per plant arising from disinfected Arran Pilot tubers, while washing without disinfection tended to cause a decrease in stem number. This was confirmed in our experiments in 1967 with Majestic but not with King Edward where there were no significant differences. The results in the previous year were also not significantly different.

Yield: In field assessments from treated and untreated seed, Boyd (1960) found no significant differences except in one trial where treatment resulted in an increase. Jennings et al (1964) also found no significant differences in total yield but noted a tendency for high number of seed-size tubers. Yield differences to date in the present experiments have not been significant.

Table 6

The effect of various treatments on number of stems per plant in Majestic and King Edward 1966-67

Treatment		Majestic	King Edward
A		4.56	5.95
B		5.08	5.34
C		3.70	4.87
D		2.83	5.05
E		3.30	-
LSD	5%	0.78	NS
	1%	1.07	
	0.1%	1.24	

DISCUSSION

The problem of so-called latent diseases is one which is of paramount importance in the production of seed potatoes. The development of the disinfection process represents a serious attempt to overcome this problem and to control some of these diseases the high incidence of which has been for some time the target of much criticism.

The method has been shown to provide adequate control for dry rot and skin spot. The other notorious cause of storage loss, gangrene, was shown by Boyd (1960), Graham (1964) and Logan (1967) to be satisfactorily controlled in this way although Gray and Malcolmson (1966) did not obtain a constant reduction, the level of gangrene being sometimes higher in the treated tubers. The results presented here again show a very high degree of control of this disease, which, being largely associated with tuber damage, is very difficult to control by other means.

Increase in the rate of emergence brought about by treatment cannot be claimed to have a clear and consistent effect on yield but the fact that seed tubers are stored in sprouting trays until planting and not immediately taken from bulk store before planting is in itself a beneficial factor. In these experiments only sound and sprouted tubers were planted and yield increases because of seed treatment per se, are not the main objective of the process. It is in the prevention of storage losses that the chief benefit lies and after planting if blanking because of skin spot or dry rot is controlled then yield could well be affected. Direct loss of yield, on the other hand, might be related to extensive blackleg infection.

One of the chief drawbacks is that sometimes washing and disinfection is followed by an increase in blackleg in the field. Damage at lifting or in grading or according to Graham (1967) by the pintle rollers in the washing process is sufficient to allow the entry of the bacterium. Immediate disinfection can reduce this considerably but Graham (1963) found the use of disinfectants in the washing water to be unsatisfactory. The removal of pintle rollers appears to have eliminated the incidence of bacterial hard rot in the 1966-67 experiments. It may be that disinfection without prior washing, which would probably require a higher mercury concentration to counteract soil adsorption, could minimise the blackleg problem.

Naturally the incidence of blackleg and other diseases fluctuates according to season, the handling of the seed and the growing of the subsequent crop. More benefit will probably accrue in treatment of varieties particularly susceptible to

the various diseases which can be controlled and treatment must be accomplished at the earliest opportunity consistent with the maturity of the potatoes after haulm destruction. Treatment immediately after lifting is the general rule but, as Edie and Boyd (1966) have pointed out, the lifting operation may be extended over almost a two month period. The operative factor of this is believed to be the environmental temperature at that period. The earlier that lifting is carried out, at least with respect to skin spot control, the more satisfactory will be the result. It is possible that gangrene, and dry rot may be influenced in the same way.

The use of mercury compounds always presents a toxic hazard not so much for the grower but for the operator and other less toxic but equally effective compounds are urgently required. In this connection, Graham (1964) investigated the properties of organo-tin compounds but concluded that even although tributyl tin acetate provided an excellent control of gangrene, dry rot and reduction of viability of the sclerotia of Rhizoctonia solani, it was also phytotoxic to the tubers and could not then be recommended.

Acknowledgements

This work was made possible by a Research Grant from the Potato Marketing Board for the drying of washed and disinfected potato tubers under the supervision of Dr. I. M. Robertson of the National Institute of Agricultural Engineering, Scottish Station, Dr. D. C. Graham, Department of Agriculture and Fisheries for Scotland, Agricultural Scientific Services and the senior author. Our sincere thanks are due to Scottish Treated Seeds Ltd. for their willing co-operation in the part of the work recorded in this paper.

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THE EFFECT OF SULPHUR APPLICATION TO THE SOIL IN THE CONTROL OF SOME TUBER DISEASES

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Summary In field trials from 1962-66, sulphur application to the soil consistently controlled the potato tuber diseases common scab, powdery scab, black scurf and blight provided that the quantity applied was sufficient to attain a soil acidity of approximately pH 5.2. Application of other substances controlled common scab and black scurf in some trials while lime increased the incidences of all four diseases. There are numerous references in the literature to the control of common scab at low pH values but this effect has not previously been recorded for the other three diseases. Good results have been obtained in large scale trials on farms where the rates of sulphur application were based on the original soil acidity and on the soil type; the necessity to use rates calculated for each site rather than standard rates is stressed.

INTRODUCTION

Investigations on the control of common scab were resumed in Northern Ireland in the 1950's following reports of increasing numbers of crops affected. The increase in incidence of common scab was concurrent with and thought to be related to the increase in liming resulting from grassland development and a rapidly expanding acreage under barley. The existence of a valuable export trade in seed potatoes accentuates problems of disease control owing to the stringent health regulations imposed by importing countries and because some of the large number of varieties necessarily grown which are very susceptible to common scab are in great demand and thus cannot be replaced by less susceptible varieties. In consequence methods of control investigated in preliminary experiments were tuber disinfection and soil treatment. Tuber disinfection was found to give inconsistent results and thus regarded as unsuitable for general use. Both quintozene and sulphur were effective in controlling common scab and it was of special interest that, in one experiment, sulphur also reduced incidence of powdery scab, black scurf and tuber blight for by this time incidence of tuber blight as well as common scab appeared to be on the increase. A series of plot and field experiments was, therefore, instituted to determine the reliability of the sulphur treatment and its suitability for commercial use.

PLOT AND FARM INVESTIGATIONS

Plot Experiment

The plot experiment was laid down in 1963 in a light medium loam with a clay subsoil. The treatments compared were:- sulphur: lime: quintozene: untreated control. The sulphur and lime were applied a few weeks before planting. Ground sulphur was applied at the low rate of 5 cwt/acre and hydrated lime at the high rate of 140 cwt/acre as the existing soil acidity was close to the critical level for the development of common scab. Quintozene was applied at planting at the rate of 60 lb a.i./acre. The treatments were replicated and the plots suitably randomized. The variety planted was Up-to-Date and normal cultivation was carried out. Lifting took place 3 weeks after the foliage was burned down. Marginal drills and rows were discarded and the entire produce of the remaining 30 plants in each plot was bagged. The tubers from each plot were divided into the commercial grades of ware, seed and chats, using $1\frac{1}{4}$ and $2\frac{1}{4}$ inch riddles and the tubers in each grade were weighed and counted.

In the disease examination 50 tubers from the seed grade of each plot were

washed and the incidence of common scab (Streptomyces scabies), powdery scab (Spongospora subterranea), black scurf (Corticium solani), and tuber blight (Phytophthora infestans) assessed in each sample. Tuber blight was assessed by counting the number of blighted tubers. The other diseases were assessed by scoring tubers from 0 to 4 on an arbitrary scale according to the severity of attack as indicated by the extent and distribution of scabs or sclerotia. The totals obtained per sample for each disease were recorded and are herein quoted as percentages of the maximum count, i.e., that corresponding to all tubers heavily infected. The scores adopted were, 0 where the disease was absent, 1 for the range from the smallest recognisable amount up to about 5% cover of the surface and 4 for surface coverage over about 20-25% with 2 and 3 for intermediate areas.

The experiment was repeated in 1964 and 1965 on the original layout without further soil treatment except fertilisers.

Table 1

Field Station plot experiment 1964

Incidence of disease expressed as percentage of maximum

Treatment	Common scab	Powdery scab	Black scurf	Tuber blight
Control	19	5	8	11
Sulphur	3	1	4	4
Lime	74	8	13	50
Quintozene	3	3	3	21

The pattern of disease incidence was similar in all three years of this experiment, disease levels being moderate to low in 1963 and higher in 1964 and 1965. The 1964 results have been selected for consideration and are shown in Table 1. The incidence of the four diseases, common scab, powdery scab, black scurf and tuber blight was increased by the lime treatment and reduced by the sulphur treatment. The figures show marked differences between treatments particularly with regard to the control of common scab and blight. The overall effect may be indicated by describing the crop from the lime treatment as unsaleable, and that from untreated plots as average and that from sulphur treated plots as excellent. It will be seen that in quintozene-treated plots considerably more blighted tubers occurred than in untreated or sulphur treated plots but that the incidence of other diseases was less than in untreated plots.

Samples of soil were taken on several occasions during the course of the experiment. Representative figures for soil pH from untreated, sulphur treated and lime treated soils were, respectively, 5.1, 4.8 and 7.2

Farm Trials

In 1965, field trials were laid down to examine the effect of sulphur on the incidence of tuber diseases under farming conditions. Sulphur was applied at the rate of 10 cwt/acre to a $\frac{1}{4}$ acre plot in a field on each of 11 farms which included a number of different types of soil. The whole of each field was planted with the farmer's stock of seed and cultural operations were carried out by each farmer according to his normal practice. Five varieties of potato were represented in all. Disease assessments were made as described previously on samples drawn from small bulks of tubers lifted from 8 sites in the treated area and from 8 sites in the untreated area at each trial, and are shown in Table 2.

Table 2.

Farm trials with sulphur 1965

Incidence of disease expressed as percentage of maximum

	Sulphur cwt/acre	Common scab	Powdery scab	Black scurf	Tuber blight	pH
Up to Date (1)	0	0	9	21	30	5.6
	10	0	0	0	1	4.9
Up to Date (2)	0	28	25	15	7	5.9
	10	21	8	7	2	5.5
A. Banner	0	32	8	11	7	5.9
	10	8	0	0	1	4.5
Home Guard	0	7	0	11	2	6.2
	10	12	0	7	2	5.8

In 1966 in a similar series of trials 2 plots were treated at each field, one at 5 cwt/acre and one at 10 cwt/acre and the sampling rate was reduced from 8 to 6.

For simplicity the results of only 4 of the 11 trials are given in Table 2 where 0% indicates that infection was absent or negligible. With few exceptions the sulphur treatment resulted in the reduction in incidence of each disease at all trials sometimes to a striking extent. Thus common scab was reduced from 32% to 8% in the Arran Banner trial and tuber blight from 30% to 1% in the Up-to-Date trial. In other trials with this variety crop savings recorded in regard to blight were 24%, 23% and 11%. Disease reductions in some trials were small but the consistency with which reductions occurred must be emphasised. An exception where no control was achieved can be seen in the figures for the Home Guard trial where the soil acidity at pH 5.8 was less than that necessary for the control of common scab, probably due to the trial being on a basalt soil.

The application of sulphur at 10 cwt/acre increased soil acidity to different extents in the 3 main soil types namely (approximately) 1.0, 0.75 and 0.5 units of pH for granite, mica schist and basalt soils respectively.

Table 3.

Farm trials with sulphur 1966

Incidence of disease expressed as percentage of maximum

	Sulphur cwt/acre	Common scab	Powdery scab	Black scurf	Tuber blight	pH
King Edward	0	13	25	19	6	6.2
	5	0	0	13	0	4.9
	10	0	0	0	0	4.5
Arran Banner	0	27	12	6	5	6.5
	5	12	8	6	7	5.3
	10	11	6	0	1	4.9
Up to Date	0	12	0	0	13	6.3
	5	10	0	0	11	6.0
	10	6	0	0	4	5.3

The 1966 results (some shown in Table 3) in general confirmed previous findings although the consistency of results was not so high as in 1965. This can be attributed, at least in part, to soil variation within the selected sites and to uneven distribution of sulphur rather than to inadequate application so far as the whole plots were concerned. This suggestion derives from observations at trials and from the figures for individual samples; for example, the 11% common scab recorded in the Arran Banner trial was composed of diseased tubers practically confined to 2 of the 6 replicates. In a few cases, however, the 5 cwt application was inadequate, for example the Up-to-Date trial recorded in Table 3.

In addition in 1966 trials were laid down on areas sufficiently large as to permit bulks of tubers to be kept separate at lifting and in storage in order to obtain information on the effects of sulphur in preventing disease development as assessed by commercial standards rather than by examination of washed tubers. It had been found impracticable to ensure adequate separation in plots trials where the $\frac{1}{4}$ acre plot was centred in a field. The area treated at each of 5 farms was 2/3 acre and rates of application, estimated from the soil type and pH, were $7\frac{1}{2}$ cwt/acre at 1 field and 15 cwt/acre at 4 fields. After storage bulks of tubers were prepared as for sale by each farmer and weights recorded of tubers discarded due to disease, tubers otherwise unsuitable for sale and of healthy tubers in the commercial size grades. The quantities so prepared at each farm were the same from treated and untreated areas but from farm to farm ranged from 10 cwt to 35 cwt.

Tuber blight and common scab were prevalent in this season and as estimated rates of application of sulphur rather than arbitrary rates had been used good disease control was anticipated. From the bulks of tubers prepared in the manner described the percentages of saleable tubers obtained from treated areas at the 5 trials were 92, 92, 95, 96, 98 and the corresponding percentages from the untreated areas 35, 57, 65, 76 and 70. The differences in the percentages, at a nominal yield of 10 tons/acre represent savings due to treatment of from 2 tons to over 5 tons/acre. In 4 trials with the variety Up-to-Date tubers were discarded almost entirely because of tuber blight and in 1 trial, variety Majestic, common scab was mainly responsible. As the percentage of saleable tubers other than in the seed grade were almost the same from treated and untreated areas crop savings can be related to seed tubers thereby simplifying estimates of crop value which in this season was £28/ton for the variety Up-to-Date. On the ton/acre basis as shown, the value of 2 tons less the cost of sulphur was £40 and similarly the over 5 tons saving was worth £120. Furthermore it is estimated that other costs associated with the treatment are considerably less than labour costs incurred in removing large quantities of diseased tubers in preparing stocks for sale.

Other observations

No evidence has been found from weighings of tubers from plots in the plot experiment or from short lengths of drills at farm trials that yield is affected by the sulphur treatment.

Tubers from sulphur-treated plots have, in general, a brighter and more attractive appearance than those from control plots. However deleterious effects have been noticed on a few occasions. In 1966, weather conditions were conducive to second growth and deep cracks which developed in tubers of the variety Ulster Prince in 2 trials were found on more tubers from treated areas than from untreated areas. Cracking occurred to a lesser extent on other varieties and was not always more prevalent on tubers from treated soil. In some seasons tubers of certain varieties may be prone to develop cracks in acid soil and it may be noted that conditions at the Ulster Prince trials were very acid (pH 4.5). Another form of cracking of a much finer and netted nature has frequently been seen on tubers of several varieties when produced in soil of pH 4.5 or lower. Such tubers are completely free of disease and the cracking appears harmless. The effects of sulphur persist for several years, as shown in the plot experiments where pH values were still low 3 years after application, and subsequent crops may be affected in soil of low pH values. However on trial fields where no readjustment of soil acidity has been made normal crops of grass and oats have, in general, been observed while growth of barley has been normal on some sites but impaired on others.

DISCUSSION

The use of sulphur applied to soil in controlling common scab has been investigated in several countries and the abundant literature is reviewed, amongst others, by Emilsson and Gustafsson (1954). It is generally, if not unanimously, accepted that control can be achieved when soil acidity is brought to pH 5.2. Soil acidity however is not static and different evaluations can be obtained on different days and because of soil variation different figures can result from separate samplings on the same day.

From the few figures for soil pH given in the present work control, if not elimination, of common scab would appear to be achieved between pH 4.9 and pH 5.3. Taking into account the above reservations the results tend to confirm that common scab can be controlled at pH 5.2.

The control of powdery scab by application of sulphur to soil has received much less attention and would appear to have been investigated only by Pethybridge (1913) in Ireland, Melhus et al. (1916) in the U.S.A. and Millard reported by Cotton (1922) in England. All obtained reductions in incidence of powdery scab on tubers from sulphur treated soil and Pethybridge and Melhus found that liming increased the incidence of the disease. No records have been found in the literature on the effects of sulphur applied to soil on the incidence of black scurf or tuber blight. From the results of the present investigations there are grounds for suggesting that control of these 3 diseases is also exercised through soil acidification and that the level of acidity is close to that required for control of common scab. Taking into account the erratic distribution of black scurf and what has been said about soil pH determinations, the few figures shown in Tables 2 and 3 suggest that control is obtained within the range pH 4.9 - pH 5.3. Whether or not soil acidity is the main or only factor involved in control may be in doubt but at least acidity can be measured and the measurements used to estimate the quantities of sulphur to be applied to produce crops relatively free from these diseases.

In practice it is necessary to have the rate of application estimated for each field by a soil chemist on the basis of the pH of a correctly taken soil sample, the soil type and other factors which may influence the development of acidity. Rates of application estimated in this way have ranged from 3 cwt/acre to 20 cwt/acre for the many fields sampled during the years of investigation. There is little experience of the effects of application of over 15 cwt/acre but from this rate excellent results have been obtained and no deleterious effects noticed. As tubers may grow in any part of a drill it is desirable that the whole of the soil forming a drill is acidified uniformly. It is preferable therefore that application should be on to a flat harrowed surface followed by rotovation or further harrowing so that when drills are formed and split after planting a reasonable admixture of soil and sulphur will have been obtained. When applied to a ploughed surface the powder tends to gather in hollows resulting in uneven distribution in the drill. Fertilizer spinners have been used to apply sulphur and are regarded as satisfactory provided only 1 cwt is put in the hopper at a time owing to the tendency of the powder to go into lumps.

Dates of application have varied according to circumstances on each farm and to weather conditions since even distribution is impracticable except in fairly calm weather, but in all cases application was at least a few days before planting. In most trials the requisite acidity appears to have been attained by the time tuberization started and infection became possible. Further investigation of the more efficient use of sulphur by earlier application is being considered and may involve a close examination of soil pH changes before and during the growing season. Figures given for pH of treated areas were obtained from samples taken after lifting and not for the period of tuberization.

The use of a common rate of application of sulphur resulted in different levels of soil acidity being produced from field to field which may account for the differences in growth observed in subsequent crops intolerant of acid conditions. In practice liming may be necessary where the adjustment of soil

acidity for potatoes has made conditions unsuitable for other crops.

The sulphur treatment is expensive: the cost per acre during the period covered by the investigations at the different rates used varied from £7½ to £30. It is obviously a treatment more suited to the production of seed potatoes than to ware as the value of the crop is more and the standards of health are higher. It is most useful where blight susceptible varieties are grown but it also ensures against attacks of powdery scab in the cooler wetter seasons and against common scab in the warmer drier seasons.

In deciding whether or not to use sulphur it is necessary to know the cost of the treatment for a particular field and to balance this against the risk of tuber diseases based on past experience and susceptibility of the variety grown.

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THE VALUE OF GRANULAR PARATHION FOR THE CONTROL OF WIREWORMS IN POTATOES

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Summary Results of trials carried out in the Midlands during 1966 and 1967, to evaluate granular parathion for the control of wireworms in potatoes, indicate that method of application is of paramount importance. Band-placed furrow treatments were more effective than broadcast treatments at equivalent rates of application. The optimum rate for band-placement was 32-40 oz a.i./acre. This rate effectively lessened tuber damage and compared favourably with the standard commercial treatment of aldrin. Dual applications of parathion and phorate produced only an insignificant improvement in control.

INTRODUCTION

The Advisory Committee on Poisonous Substances used in Agriculture and Food Storage recommended continued commercial usage of aldrin for the control of wireworms in potatoes because no effective economic alternatives existed at that time (Cook, 1964). Strickland (Cook, 1964, Appendix E) stated that the only possible alternatives to aldrin are organophosphates which are still under investigation and their relative efficiency is not yet known.

In 1965, Selleck and Evans reported the development of a stabilised granular formulation of parathion containing 10% a.i. which showed promise for the control of wireworm damage. Granular parathion was already recommended for wireworm control on potatoes and tobacco in the U.S.A. The main advantages of the granular formulation over liquid parathion were its increased persistence and lower dermal toxicity.

Bevan (1967), Brock (1967) and Rayner (1967) have since reported trials which showed that parathion was the most effective organophosphate so far examined, but its effectiveness varied according to the method of application.

Trials were initiated in 1966 to examine more closely the value of 10% parathion granules for control of wireworms in potatoes and the results form the contents of this paper.

METHOD AND MATERIALS

The trials were laid down in randomised block design with four replicates for each treatment and plots 160 yd^2 in area. Cultivar Majestic was used in all trials, planted in 28 inch rows.

The granular materials were applied by use of a Horstine-Farmery "Airflow" Microband Applicator fitted with 9 in spout end spreaders. For broadcast treatments, the spout ends were raised so that an overall treatment could be applied. The broadcast treatments were also incorporated by harrowing before ridging and planting. Band-placed treatments were applied to the furrow with the seed, the band width being about 6 in before covering the potatoes. Where phorate and disulfoton were involved in the trials, the commercial recommended rates for aphid control were used, similarly applied to the furrow. In the case of dual treatments of parathion and phorate or disulfoton, the materials were applied separately.

Aldrin was used as the standard commercial treatment for comparison and was applied as a spray, incorporated by light harrowing before ridging and planting.

RESULTS

1966 Trials

The trials carried out in 1966 were primarily designed to evaluate parathion granules for wireworm control but also to determine the relative efficiency of band-placed treatments against incorporated broadcast applications. The potatoes were planted rather later than normal for a maincrop but some interesting results emerged (Tables 1 and 2).

Table 1.

Site: Tythby, Nottinghamshire
Planting date: 13 May, 1966
Field history: 5 years grass
Soil: Sandy loam
Harvest date: 10-11 October, 1966
Wireworm population: 500,000/acre

Material	Type of application and a.i. oz/acre	Number of wireworm holes/100 tubers	Undamaged tubers %	
			%	Angles
Control	-	593	13	21.28
Parathion	10 G broadcast 24	207	48	43.71
Parathion	10 G broadcast 48	97	61	51.62
Parathion	10 G band-placed 24	70	66	54.60
Parathion	10 G band-placed 36	46	77	61.60
Aldrin	30 EC spray 48	79	72	58.01
Significant difference	(P = 0.01)	134		9.64
	(P = 0.05)	97		7.00

Table 2.

Site: Ryton-on-Dunsmore, Warwickshire
Planting date: 18 May, 1966
Field history: 12 years grass
Soil: Medium loam
Harvest date: 17-18 October, 1966
Wireworm population: 300,000/acre

Material	Type of application and a.i. oz/acre	Number of wireworm holes/100 tubers	Undamaged tubers %	
			%	Angles
Control	-	228	38	39.28
Parathion	10 G broadcast 24	104	57	49.20
Parathion	10 G broadcast 48	114	59	49.91
Parathion	10 G band-placed 36	66	66	54.29
Parathion	10 G band-placed 48	61	68	55.58
Aldrin	30 EC spray 48	78	65	53.47
Significant difference	(P = 0.01)	96		11.64
	(P = 0.05)	70		8.42

1967 Trials

On the basis of this preliminary information, the 1967 trials were designed with two objectives - a) to confirm the effective rate of parathion granules for wireworm control when applied to the furrow, and b) to examine the effect of treatments combined with granular aphicides now in wide usage on potatoes. Two trials were laid down of which details and results are given in Tables 3 and 4.

Table 3.

Site: Clifton, Nottinghamshire
Planting date: 30 March, 1967
Field history: Permanent pasture
Soil: Medium loam
Harvest date: 11 September, 1967
Wireworm population: 100,000/acre

Material	Type of application and a.i. oz/acre		Number of wireworm holes/100 tubers	Undamaged tubers %	
				%	Angles
Control		-	144	47	43.18
Parathion	10 G	32	33	80	63.28
Parathion	10 G	40	23	85	67.97
Parathion)	10 G	(32	14	90	71.48
Phorate)	10 G	(24			
Parathion)	10 G	(32	38	79	62.64
Disulfoton)	7.5 G	(16.8			
Phorate	10 G	24	54	70	56.54
Disulfoton	10 G	16.8	77	62	52.01
Aldrin	30 EC spray	36	34	80	63.31
Significant difference	(P = 0.01)		69		15.84
	(P = 0.05)		50		11.64

Table 4.

Site: Ockbrook, Derbyshire
Planting date: 12 April, 1967
Field history: Permanent pasture
Soil: Heavy loam
Harvest date: 18 September, 1967
Wireworm population: 250,000/acre

Material	Type of application and a.i. oz/acre		Number of wireworm holes/100 tubers	Undamaged tubers %	
				%	Angles
Control		-	128	52	46.15
Parathion	10 G	32	39	76	60.86
Parathion	10 G	40	24	83	65.83
Parathion)	10 G	(32	18	90	71.46
Phorate)	10 G	(24			
Parathion)	10 G	(32	16	88	69.63
Disulfoton)	7.5 G	(16.8			
Phorate	10 G	24	70	67	54.71
Disulfoton	10 G	16.8	126	51	45.44
Aldrin	30 EC spray	36	84	62	51.78
Significant difference	(P = 0.01)		70		13.39
	(P = 0.05)		52		11.07

DISCUSSION AND CONCLUSIONS

1966 Trials

All treatments in both trials produced a significant reduction of tuber damage over the untreated control, but differences between treatments were less pronounced. At equivalent rates, band-placed treatments were superior to incorporated broadcast treatments. This superiority, however, was not statistically significant at Ryton. The band-placed treatments of parathion compared very favourably with aldrin application in reducing wireworm damage, with an optimum rate in the region of 36 oz a.i./acre.

1967 Trials

Assessment of these trials was made earlier than in 1966, but a severe attack was recorded at both sites. It is interesting to note that damage was greatest at Clifton, although this site had the lower estimate of wireworms per acre.

With two exceptions, all treatments again significantly reduced tuber damage compared with the untreated controls. Similarly a high level of control was obtained with the parathion treatments compared with aldrin (in these trials at 36 oz a.i./acre). The higher rate of 40 oz a.i. parathion was only marginally better than 32 oz.

The combined parathion/phorate treatment produced an inconsiderable additive effect on wireworm control. The same can be said for parathion/disulfoton at Ockbrook, but this improvement was not significant, particularly in view of the results obtained for disulfoton alone.

A consistent feature in all these trials was the very promising control given by band-placed furrow treatments of parathion compared with aldrin application. This is in contrast to the results of Bevan (1967), and Brock (1967), where aldrin was always superior to parathion. The discrepancy between the results appears due, in part, to the higher level of control produced by parathion but also, and perhaps to a greater extent, due to poor performance of aldrin. In the 1967 trials, the poor showing of aldrin can be explained on the basis of low rate since only 36 oz a.i./acre were used, which was certainly inadequate at Ockbrook. Such an explanation, however, will not hold for the 1966 trials, where the reasons for poor control remain obscure. On the other hand, Selleck and Evans (1965) reported from Italy that with maize, both parathion granules and 10% aldrin granules provided equivalent wireworm control.

It is concluded from these trials that a band-placed furrow treatment of granular parathion at 32-40 oz a.i./acre will effectively reduce wireworm damage in potatoes. Above 40 oz a.i., the law of diminishing returns comes rapidly into effect, since higher rates only produced marginal improvements in control. Granular parathion should therefore prove a useful addition to the very limited number of chemicals known to be effective against wireworms, particularly for the control of damage to the potato crop.

Acknowledgements

Thanks are expressed to Mr. D. J. Higgons for helpful advice throughout this work, the staff of the Zoology Section, Lenton Research Station for technical assistance, the farmers who co-operated in the trials and the Directors of Boots Pure Drug Co. Ltd. for permission to publish this paper.

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THE USE OF PHORATE FOR THE CONTROL OF WIREWORM IN POTATOES

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Summary Experimental work is described with the organophosphorous insecticide phorate for the control of wireworm in potato crops. Field experiments carried out in 1966 and 1967 showed that phorate granules applied at 3.0 lb a.i./acre into the planting furrow gave a reduction of damage due to wireworm comparable to that obtained when aldrin was sprayed broadcast at 2.25 lb a.i./acre immediately before planting. Phorate at 3.0 lb a.i./acre in the furrow gave significantly better results than 1.5 lb a.i. applied in the same way and marginally better results than 3.0 lb a.i. applied broadcast.

INTRODUCTION

Field trials were started in 1963 by Cyanamid of Great Britain Limited to investigate the activity against wireworms (Agriotes spp.) of two organophosphorus insecticides, phorate (0,0-diethyl S-(ethyl thiomethyl) phosphorodithioate) and thionazin (0,0-diethyl 0-2-pyrazinyl phosphorothioate). The activity of both these materials against wireworms was first demonstrated by Cuthbert and Reid (1959) in screening tests using the southern potato wireworm (Conoderus falli). Further evidence of the activity of phorate and thionazin (E.N. 18,133) against the southern potato wireworm was furnished by Cuthbert et al (1959), Day et al (1964), Wolfenbarger (1965) and Workman (1966). In laboratory tests on the larvae of Agriotes spp. Griffiths and Bardner (1964) found thionazin to be more toxic to the larvae than phorate. In four out of five field trials in the years 1963-1965 Caldicott and Lindley (1965) showed that phorate 10% granules were more effective than thionazin 10% granules for reducing damage by wireworms at rates of 1 to 2 lb a.i./acre. It was also shown that thionazin did not give as good control of aphids as phorate. Phorate applied as a band of granules into the planting furrow was more effective against both aphids and wireworms than broadcast treatments followed by cultivations. At the rates used, phorate did not give as good a reduction of wireworm damage as aldrin at 2.25 lb a.i./acre. Bryden (1965) also found that phorate at 1.6 lb a.i./acre was not as effective as aldrin at 3.0 lb a.i./acre.

In trials in 1966 (Caldicott and Isherwood, 1967) phorate 10% granules were used at 2.0 lb and 3.0 lb a.i./acre in the hope that higher rates would prove comparable to aldrin when used at 2.25 lb a.i./acre. Phorate granules were applied into the planting furrow at 2.0 lb and 3.0 lb a.i./acre and at 3.0 lb a.i./acre broadcast. In addition a "part furrow" treatment was employed in which a 6-inch wide band of phorate granules at 3.0 lb a.i./acre was applied onto a partly filled furrow so that the granules lay in a band two inches above the newly planted tubers. This method and rate of phorate was found to be highly effective against the Pacific Coast wireworm (Limonius canus) by Onsager et al (1966) in trials in which this treatment as well as phorate and parathion, the latter two as side dressings of 3.0 lb a.i./acre, gave the best control of wireworm damage of nine chemical treatments tested. In only one of Caldicott and Isherwood's trials was there a heavy wireworm attack. All treatments reduced wireworm damage; the aldrin and 3.0 lb phorate furrow treatments gave the greatest effects. There was no significant difference between the effects of these two treatments. Aphid counts made at this site showed that whilst the phorate furrow treatments gave good aphid control for at least 10 weeks after planting, the broadcast and part-furrow treatments gave aphid control for only approximately seven weeks. In the two 1966 trials there were no significant effects of treatment on yield when the trials were considered separately, although there was an apparent beneficial effect due to phorate. When both trials were considered together, however, there was an increase of yield significant at the 5% level on the phorate 3.0 lb broadcast treatment.

Further evidence of the effectiveness of phorate in reducing damage to potatoes by wireworm has been furnished by other workers. Gair (1966) showed that phorate granules at 3.0 lb a.i./acre and also three other experimental materials gave a degree of protection not significantly different from that obtained with aldrin. Bevan (1967) showed that phorate granules at 2.0 lb a.i./acre gave the same degree of protection as parathion granules at the same rate but that neither material was quite as effective as aldrin. Wells and Guyer (1967) at Michigan State University used phorate granules broadcast at 3.0 lb a.i./acre plus a furrow treatment of 3.0 lb a.i./acre. Following these applications the protection from damage by the wheat wireworm (Agriotes mancus) was only slightly less than that obtained with aldrin at 3.0 lb a.i./acre. A phorate broadcast treatment alone or a furrow treatment alone gave good results, but either was less effective than the combined treatment.

Phorate applied at 1.5 lb a.i./acre into the planting furrow for main crop potatoes, has been established for several years as a standard aphicidal treatment for potatoes. This treatment has been shown to give protection from aphid attack for 12 to 14 weeks (Lindley 1963). This treatment has also been shown to reduce damage due to wireworm, but not to give a level of control equal to that of aldrin at standard rates. It was considered highly desirable that any treatment of phorate for the control of wireworm should also control aphids to the same level as was already achieved by the 1.5 lb a.i./acre furrow treatment. Thus in the 1967 trials it was decided to include the following: phorate at 1.5 lb a.i./acre in the furrow, as used in standard aphicidal practice; the same treatment doubled to 3.0 lb a.i./acre, which had already shown good control of wireworm; the 1.5 lb a.i./acre furrow treatment supplemented by 3.0 lb a.i./acre broadcast; and, a 3.0 lb broadcast treatment alone for comparison.

METHOD AND MATERIALS

The granular formulation used in this work contained 10% by weight of phorate on fuller's earth (22/44 mesh). The different levels of phorate tested were compared with aldrin 30% e.c. as the standard material.

Three trials were laid down in the spring of 1967. Each trial was in the form of a randomised block experiment with four replicates of six treatments each. Plots were either two or three rows wide and fifteen yards long. Single guard rows between the plots were treated with phorate at 3.0 lb a.i./acre in the furrow. Rows were 30 inches apart. The treatments were as follows:

1. Untreated control
2. Aldrin 30% e.c. at 2.25 lb a.i./50 gal/acre, sprayed immediately before planting and harrowed in.
3. Phorate 10% granules at 1.5 lb a.i./acre in furrow.
4. Phorate 10% granules at 3.0 lb a.i./acre in furrow.
5. Phorate 10% granules at 3.0 lb a.i./acre broadcast.
6. Phorate 10% granules at 3.0 lb a.i./acre broadcast + 1.5 lb a.i./acre in furrow

The aldrin spray treatment was applied with a knapsack sprayer. The broadcast phorate treatment was applied by hand. Both treatments were lightly harrowed in before ridging.

The phorate furrow treatments were applied before planting with a Horstine Farmery wheelbarrow-mounted applicator as a continuous narrow band into the open furrow. Granules were placed in the bottom of the furrows and then the potatoes planted by hand above the granules.

One site (132/1) was planted on 15th March on a clay loam soil in Huntingdonshire with a population of 100,000 wireworm per acre. The second site (132/2) was planted on 28th March on a sandy soil in Herefordshire with a population

of 100,000 wireworm per acre. A third site was planted on 7th April on a silt soil in Lincolnshire with a population of 425,000 wireworm per acre. Unfortunately this trial was prematurely harvested by the farmer and its information thus lost.

At harvesting, one yard of each row was dug out by hand from each end of each plot and discarded. Yields were taken from the remaining 13 yards of each row from each plot and converted into tons per acre. The total yield of tubers irrespective of size was recorded.

One hundred tubers from each plot were examined for wireworm damage. The number of deep wireworm holes (those not removed by peeling) and the percentage of tubers with deep wireworm holes were recorded.

RESULTS

The harvesting of trial 132/2 was carried out on 18th September and of trial 132/1 on 25th September. The total yield of tubers per plot was recorded and these are given in Table 1 expressed as tons per acre. There were no significant differences between the treatments in their effect on yield.

Table 1

Comparison of four treatments of phorate granules with aldrin broadcast spray for their effects on potato yield

Treatment	lb a.i./acre	Yield in tons/acre Trial 132/1	Trial 132/2
Control		17.7	18.4
Aldrin spray	2.25	18.7	18.3
Phorate in furrow	1.5	16.7	18.7
Phorate in furrow	3.0	16.9	18.7
Phorate, broadcast	3.0	17.3	18.6
Phorate in furrow 1.5 + broadcast	3.0	15.7	18.2
L.S.D. 5%		NS	NS

The mean numbers of deep wireworm holes per hundred tubers and the percentage of tubers with deep wireworm holes are given in Table 2.

At trial 132/1 all treatments except phorate at 1.5 lb a.i./acre in the furrow gave significantly fewer deep holes per hundred tubers than on the control. Significantly lower percentages of tubers with deep holes than on the control were recorded on plots treated with phorate at 3.0 lb a.i./acre in the furrow, with phorate at 1.5 lb a.i./acre in the furrow plus 3.0 lb a.i./acre broadcast, and with aldrin. In the observations on numbers of wireworm holes and on the percentage of tubers with deep holes, there were no significant differences between the treatments. However, the difference in the numbers of tubers with deep holes approached significance between phorate at 1.5 lb a.i./acre in the furrow and both the 3.0 lb a.i. furrow treatment and the 1.5 lb a.i. furrow plus 3.0 lb a.i. broadcast treatment.

Table 2

Comparison of four treatments of phorate granules with aldrin broadcast spray for potato wireworm control

Treatment	lb a.i./acre	No. deep wireworm holes/100 tubers		Percent tubers with deep wireworm holes (angles)	
		Trial 132/1	Trial 132/2	Trial 132/1	Trial 132/2
Control		28.8	33.0	24.4	25.2
Aldrin spray	2.25	11.3	6.2	17.3	12.4
Phorate in furrow	1.5	17.8	16.2	21.8	18.5
Phorate in furrow	3.0	7.8	4.0	15.9	9.3
Phorate, broadcast	3.0	14.3	10.5	18.7	15.1
Phorate in furrow 1.5 + broadcast 3.0		9.8	13.7	16.0	18.4
L.S.D. 5%		11.7	10.7	6.0	7.8
1%		16.2	14.3	8.4	10.7
0.1%		22.4	19.9	11.3	14.9

At trial 132/2 all treatments gave significantly fewer deep wireworm holes per hundred tubers than the control. Aldrin and phorate 3.0 lb a.i./acre both in the furrow and broadcast gave significantly lower percentages of tubers with deep wireworm holes than on the control plots. Phorate at 3.0 lb a.i./acre in the furrow was significantly better (at P = 0.05) than the 1.5 lb a.i./acre furrow rate for both assessments and significantly better than the 1.5 lb a.i./acre furrow plus 3.0 lb a.i./acre broadcast treatment in respect to percentage of tubers with deep holes.

DISCUSSION

The 1967 trials showed that phorate granules at 3.0 lb a.i./acre into the planting furrow gave a greater protection from wireworm damage than the same type of treatment at 1.5 lb a.i./acre. The differences were significant in one trial. The results also suggest that 3.0 lb a.i. phorate/acre in the furrow gives marginally better protection than the same rate broadcast. This confirms the same suggestion from previous trials in 1966 by Caldicott and Isherwood (1967) but conflicts with the results of Day et al (1964) who found broadcast treatments more effective. Both the 1966 trials and the trials described here have satisfactorily demonstrated that phorate granules at 3.0 lb a.i./acre in the planting furrow have given a control of wireworm damage in potatoes comparable to that obtained with aldrin when sprayed broadcast at 2.25 lb a.i./acre immediately before planting.

This work has not differentiated between actual kill of wireworms by phorate and the protection of tubers from damage. It was demonstrated in laboratory tests that phorate killed wireworms at rates of 2.0 to 20.0 lb a.i./acre by Griffiths and Bardner (1964) and at 0.25 to 1.0 lb a.i./acre by Workman (1966). Cuthbert et al (1959) obtained a 93% kill of southern potato wireworm larvae with 0.94 lb a.i. phorate/acre in a field plot experiment. Parathion at 1.0 lb a.i./acre gave the same initial mortality but phorate was shown to be more persistent. In field trials at Rothamsted Experimental Station, Edwards and Arnold (1967) showed that phorate at 4.0 lb a.i./acre broadcast reduced wireworm populations to less than one tenth of those on untreated soil. Wells and Guyer (1967) also found that phorate significantly reduced wireworm populations when used at 3.0 lb a.i./acre applied either

broadcast or as a furrow treatment.

It is concluded that in the current quest for materials to replace aldrin for the control of wireworm in potatoes, as recommended in the "Review of the Persistent Organochlorine Pesticides" (1964), phorate 10% granules applied at 3.0 lb a.i./acre into the planting furrow offers a satisfactory replacement.

Acknowledgements

The writers wish to thank Mr. F. Maskell, N.A.A.S., Cambridge, Mr. R. Gair, N.A.A.S., Bristol and Mr. H. Gould, N.A.A.S., Reading for carrying out the 1966 and 1967 wireworm counts.

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FURTHER TRIALS WITH BARK-APPLIED SYSTEMIC APHICIDES ON PLUM AND CHERRY

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The advantages of applying systemic aphicides in a more or less concentrated form to the trunks of trees were summarised at the First British Insecticide and Fungicide Conference (Pietri-Tonelli, Barontini and Biondi, 1962). With the increasing demand to avoid contamination of the environment with toxic chemicals, these advantages of bark application are even more attractive. In a recent review of the behaviour of systemic insecticides in trees, Norris (1967) emphasizes the advantages of bark over soil application. Damage, apparently due to excessively fast uptake of chemical, reported for citrus especially (Pietri-Tonelli et al., 1962) and woodland trees has been avoided on white pine by applying the active ingredient in 'diluent-adhesives' (Coppel and Norris, 1966). Trials with several systemic aphicides applied via the bark to two varieties of plum have been reported (Kirby, Bennett, Insley, Tew and Sillibourne, 1964); very successful control on young trees was achieved with demeton-methyl or dimethoate, and no sign of damage, either local or peripheral, was detected. These trials have been continued and extended to two varieties of cherry.

MATERIALS AND METHODS

Details of the treatments applied in each trial are given in the tables and figures, or in the appropriate part of the text.

Chemicals: Dimethoate and its N-formyl derivative, formothion, were used in most trials as their mammalian toxicities are more favourable than those of other systemic aphicides then available. Demeton-methyl (mixed isomers) and vamidothion were also used in one trial. All four were used as commercial emulsifiable concentrates, except in one trial for which technical grade dimethoate (93% dimethoate) was formulated with an equal weight of either the tridecanol/ethylene oxide condensate, Texofor T95, or tributyl phosphate, and sufficient diacetone alcohol to give solutions containing 30%, w/v, of pure dimethoate.

The trials on plum were conducted on the cultivars, Early Laxton and Victoria, on Common Plum rootstock, planted in 1955. Two plots of cherry trees on F12/1 rootstock were used; one, of Napoleon only, was 12 years old, and the other, of Napoleon and Roundel, was six years old. All trees were closer together than in normal commercial plantations.

Application techniques: In previous work, chemicals had been applied in cotton-wool bands tied round the trunk and covered with black polythene, or by pouring suitable dilutions down the entire trunk. These methods were used in the trials reported here, but in addition painting of neat commercial formulations (as pioneered by Jeppson, 1952, and Pielou, 1961) was included. High-volume spraying was included for comparison in one year, but adequate screening was not achieved and no further use of this technique was made. In the final

year of this series, pouring was replaced by squirting from an open-ended (nozzle without swirlplate) hand sprayer fitted with a glass container in place of the rubber tube that normally connects to a separate container; this approximated to the large-scale procedure in use on other stone-fruit plantations on this Station.

Dosage rates: As in the earlier trials, dosages were calculated as p.p.m. of estimated tree weight, using the graph relating tree weight and girth prepared by Miss Bennett from data obtained when trees were removed from this plot in 1959. It had to be assumed that this relationship remained unchanged and amounts of insecticide applied were increased as the estimated tree weights increased. Half the trees were removed in November 1966, enabling a further girth/weight distribution curve to be plotted; this showed that tree weights had increased faster than girths, so that rates of aphicide application had been increasingly overestimated. No data were available for cherry, and the amounts applied were related to girths using the first relationship established for plum. However, ten-year-old trees of the cultivar, Hedelfingen, were removed elsewhere on the Station in the autumn, 1966; the girths and weights of those that had been on the same rootstock (F12/1) were used to suggest the probable weight of Napoleon trees of comparable age that were used in the 1967 trial.

The annual girth measurements also served to assess the effects of treatments upon tree growth; in no year was evidence obtained that growth had been restricted by any treatment.

Analytical methods: Weighed samples of tip foliage (usually 10 g) were macerated with successive quantities of acetone. Aliquots of these extracts were diluted with water and extracted with chloroform. In 1965, volatile organically bound phosphorus was determined by the method of Chilwell and Beecham (1960) involving microdistillation at low pressure, oxidation with perchloric acid and the use of the molybdenum blue reaction for phosphorus. In 1966-7, dimethoate was determined by thin-layer chromatography following preliminary clean-up on alumina-charcoal columns.

RESULTS

Influence of method of application

A. Trials with plum

The performances of demeton-methyl and dimethoate were compared, together with that of vamidothion, in 1964; each chemical was applied at petal-fall (11th and 12th May) either by pouring at an estimated rate of 250 p.p.m. or by painting ($\frac{1}{2}$ inch brush) at half, and in the case of dimethoate, also at a quarter, the dosage. For pouring, the E.C. formulations were diluted from 1 to 64 to 1 to 96, according to the amount of active ingredient present. The percentages of shoots showing leaf-curl at three dates on Early Laxton are shown in relation to treatment applied in Table 1. All three aphicides largely prevented leaf-curl up to 16 days after application by pouring; dimethoate was very effective even up to the end of June, by which time this aphid species has usually migrated. Re-infestation apparently occurred on trees receiving demeton-methyl or vamidothion by pouring. None of these aphicides was so effective initially when painted on, but demeton-methyl appears to have had much more prolonged action. However, the lower rate used for

painting may account for the lesser effectiveness from this procedure. Vamidothion was the least effective by either procedure. Leaf-curl was slight on Victoria, and mealy plum aphid virtually absent from either cultivar.

Table 1

Incidence of leaf-curling aphid on Early Laxton plum in 1964 trial

Aphicide	Treatment Concn. p.p.m.	Applied (11.v) by	Percentage of shoots* with leaf-curl on: 28.v	3.vi	30.vi
None	-	-	48.6	58.4	86.4
Dimethoate	(250	Pouring	Nil	0.2	4.0
	(125	Painting	7.4	3.0	8.8
	(62.5	Painting	13.4	7.6	36.8
Demeton-methyl (mixed isomers)	(250	Pouring	Nil	Nil	34.8
	(125	Painting	28.6	27.0	10.0
Vamidothion	(250	Pouring	5.6	8.0	60.0
	(125	Painting	11.4	20.4	60.0

* obtained from 50-shoot sample

In 1965, all four methods of application were compared, using dimethoate. Spraying was done with the recommended rate, 16 fl oz of E.C. per 100 gallons, to run-off. The three bark treatments (painting, cotton-wool bands, and pouring) were applied at a rate intended to be 125 p.p.m., diluting 0, 7.2, and 96 times, respectively. Formothion was also included as a painting treatment. Applications were made on 29th and 30th April, when petal-fall on both cultivars had exceeded 80%. Mealy plum aphid was again scarce, but heavy attacks of leaf-curling aphid appeared on both cultivars (Table 2). The three bark treatments gave very similar control, both

Table 2

Incidence of leaf-curling aphid on 27th May on two plum cultivars in 1965 trial

Aphicide (applied 29.iv)	Treatment Method of application	Percentage of shoots with leaf-curl Early Laxton	Victoria
None	-	88	74
Dimethoate	(Sprayed*	4	20
	(Bands+	30	16
	(Poured+	21	16
	(Painted+	22	22
Formothion	Painted+	34	47

* 16 fl oz 32% product/100 gal + <125 p.p.m.

in relation to each other and to variety. Spraying seems to have been more effective on Early Laxton, where it reduced attack more than the bark treatments, than on Victoria where it did not. Analyses of foliage by the Chilwell and Beecham method showed the expected disappearance with time for sprayed dimethoate (Table 3).

Table 3

Level of 'dimethoate'* reached in young foliage of two plum cultivars in 1965 trial

Treatment			'Dimethoate'* present (p.p.m.) in young foliage on						
Aphicide applied 29.iv	Method of application	Cultivar	29.iv	30.iv	3.v	7.v	11.v	17.v	23.v
None	-	(E.L.	8	-	-	-	-	-	-
		(V.	2	-	-	-	-	-	-
Dimethoate	(Sprayed	(E.L.	70	55	21	-	2.7	1.5	0.9
		(V.	61	50	16	-	3.3	2.4	2.7
	(Bands	(E.L.	-	-	2.1	-	2.8	3.6	4.9
		(V.	-	-	1.9	-	1.6	3.0	2.8
	(Poured	(E.L.	-	-	0.6	-	-	3.6	-
		(V.	-	-	0.4	-	-	2.2	3.0
	(Painted	(E.L.	-	(2.2)	(1.9)	(1.3)	1.0	4.0	3.4
		(V.	-	(2.5)	(1.6)	(1.6)	0.7	1.9	-
Formothion	Painted	(E.L.	-	(9.6)	(3.7)	(2.2)	1.8	1.8	1.2
		(V.	-	(3.5)	(2.0)	(1.0)	0.3	1.0	Nil

* measured as volatile, organically bound phosphorus
E.L. = Early Laxton
V. = Victoria

Maxima were reached in tip foliage on bark-treated trees about three weeks after treatment, about 4 p.p.m. on Early Laxton and 3 p.p.m. on Victoria. The difficulties met in preventing spray reaching trees not intended to receive it are reflected in the amounts of dimethoate present initially on untreated trees and in the first week on bark-treated trees, and the data obtained in this period for the latter are therefore in parentheses (also in Table 8). The lesser control of formothion, especially on Victoria, seems to reflect the lower levels reached between two and four weeks after application.

B. Trials with cherry

Dimethoate was applied at petal-fall in two seasons by all four methods, and formothion by painting only, to the two cherry cultivars in the younger block. The average weight assumed for each variety was 15 lb in 1965 and 26 lb in 1966. Bark applications of both chemicals would then have been sufficient to provide 290 p.p.m. in 1965 and 150 p.p.m. in 1966; as the weights were probably underestimated, the rates would in fact have been lower.

Heavy attacks of blackfly appeared on both varieties in 1965, records taken at the end of July showing infection of almost all

shoots (Table 4). At this date, sprayed trees were showing 20 to 30% infested shoots. All the bark treatments had reduced infestation still further, especially on Roundel where aphids were found only on a few of the trees treated by the pouring technique. Formothion was completely effective on both cultivars.

Table 4

Incidence of blackfly on two cherry cultivars on 28th July in 1965 trial

Treatment		Percentage of shoots infested on	
Aphicide (applied 6.v)	Method of application	Napoleon	Roundel
None	-	97	100
Dimethoate	(Sprayed*	30	23
	(Bands+	17	Nil
	(Poured+	20	3
	(Painted+	3	Nil
Formothion	Painted+	Nil	Nil

* 16 fl oz 32% product/100 gal + < 290 p.p.m.

In 1966, blackfly remained at a very low level, so the effectiveness of the lower rate of the insecticides could not be assessed. However, the thin-layer chromatographic method was used to assess levels of dimethoate in foliage for a month after the treatments were applied (Table 5). Sprayed foliage had 25 p.p.m. present four days

Table 5

Levels of dimethoate reached in young foliage of two cherry cultivars in 1966 trial

Treatment		Dimethoate present in young foliage on:					
Dimethoate applied on 9.v by	Cultivar	10.v	11.v	13.v	16.v	27.v	7.vi
Spraying	(Napoleon	56.2	25.2	13.4	4.2	0.2	N.D.
	(Roundel	36.2	28.8	10.9	8.8	0.7	Trace
Bands	(Napoleon	-	N.D.	-	N.D.	0.7	1.1
	(Roundel	-	N.D.	-	0.2	1.1	-
Pouring	(Napoleon	-	N.D.	-	0.4	3.4	3.2
	(Roundel	-	0.2	-	0.6	3.0	1.9
Painting	(Napoleon	-	N.D.	-	N.D.	3.0	3.0
	(Roundel	-	N.D.	-	0.3	3.1	-

N.D. = none detected

after spraying, but almost none was found in young foliage picked on 27th May. Only traces were present in foliage from any of the bark-

treated trees a week after application, but after 18 days levels in young foliage reached about 3 p.p.m. on trees when painting or pouring had been used, and remained at this level for at least ten days longer. Uptake from bands was slower and had only reached 1 p.p.m. on 7th June; blackfly control in the previous year had been as effective from bands as from pouring.

As the cherry trees used in 1965-7 were very young, a trial was carried out in 1967 on somewhat older, 12 years, trees of the cultivar, Napoleon. These trees varied from 16 inches to 22 inches girth; if this cultivar has a girth to weight ratio similar to that for Hedelfingen of comparable age, the average weight would have been about 180 lb. Dimethoate, as the commercial E.C., was applied at two rates, 8 ml and 16 ml/tree; formothion, also as the commercial E.C., was applied at 14 ml/tree. The dose for each tree was diluted with water to one pint and applied by squirting on 10th May. If the weight estimate is reasonably correct, the average doses of dimethoate were 40 and 80 p.p.m., respectively, and of formothion 40 p.p.m. Lower levels of dimethoate were detected (about 0.5 p.p.m. from the lower rate, and about 2 p.p.m. from the higher) than in shoots from the younger trees thought to be receiving much the same dosage, but the errors in estimating weights of trees are potentially large enough to render comparison of these figures fruitless. A record of blackfly attack was taken on 21st July, when migration appeared to be complete; shoots were counted in three categories: 0, no sign of leaf-curl; 1, leaf-curl present but new growth resumed; 2, leaf-curl severe and no further shoot growth. The mean percentage attack on untreated trees was 85% (43 to 100%), on trees receiving the lower and higher rates of dimethoate was 18 and 11%, respectively, and on trees receiving formothion 22%; even with 12 replicates, the tree to tree variation was so great that differences between the three aphicide treatments were not significant at the 5% level.

Influence of time of application

In all trials up to and including 1965, applications were made at petal-fall. Before this stage of development, especially on plum, there is little foliage to provide transpiration and, hence, translocation from the lower parts of the tree. Moreover, application at petal-fall reduced the risk of contaminating nectaries to the minimum. However, the plum leaf-curling aphid hatches very early in the year and begins feeding on the buds even before they open (Anon, 1965). Earlier application could therefore be advantageous and, indeed, is advised for spraying (viz. between bud burst and white bud), although dimethoate applied via the bark had not failed to give substantial control in any year when this aphid was present.

In 1966, dimethoate and formothion were applied at two rates on both plum varieties at white bud or at petal-fall, i.e. at 31st March or at 25th April. The youngest foliage available was sampled and analysed at intervals up to 17th May, and leaf-curl was recorded at the end of the month. In spite of adequate water in the soil during early and mid-April, no dimethoate was found in foliage up to petal-fall (Fig. 1). At this time, soil temperatures began to rise, reaching 60°F at 8 inches deep in cultivated soil on the 2nd May. Dimethoate was then detected in foliage. The higher rates of application both led to leaf levels of at least 6 to 7 p.p.m. and the lower rates to between 3 and 4 p.p.m.; the maxima reached may well have been higher. However, the striking feature is the sharp rise from the white bud applications even though no dimethoate was

detected in young shoots at 24 days after bark application. Dimethoate appeared later from the petal-fall applications, though at least as soon as in the 1965 trial, but increased at a much lower rate from the lower level of application. The degree of control (about 90% from white bud applications and 20-30% from petal-fall) appeared to be more closely related to the time of application, i.e. to the earlier appearance of dimethoate in young, growing points, and not to the concentration applied to the bark, but the variation of the attack among untreated trees was too large to enable firm conclusions to be drawn. Similarly, a record of the incidence of mealy plum aphid, on 1st June, showed that a heavy attack had been eliminated by the white bud applications but not entirely by the petal-fall applications. The incidence of both species on the other cultivar, Victoria, was very erratic. Control by formothion was inferior, regardless of rate or timing, but the mealy plum aphid record suggested that the earlier application was more successful than the later.

As the number of trees had been halved in 1966, only four treatments were possible on the plum plantation in 1967. Formothion was therefore discarded, and three timings for dimethoate were tried. Applications were made by the squirting technique at white bud (23rd March), full bloom (4th April) and petal-fall (17th April), using 10 ml for each Early Laxton tree (now known to have averaged about 60 lb) and 20 ml for each Victoria tree (averaging about 110 lb); the rate of application was therefore about 80 p.p.m./tree. Analyses of foliage showed that no dimethoate could be detected for at least 35 days on Early Laxton, and 45 days on Victoria, after white bud application. Ample rain fell in the blossom period, but chemical appeared in the foliage on all trees only when temperatures at 8 inches below soil level had exceeded 55°F for a short period (Fig. 2). Although foliage levels rose first from the earliest application on Early Laxton, the highest level reached, 3.8 p.p.m., was associated with petal-fall application on both cultivars. It is difficult to understand why the lowest levels were associated, especially on Victoria, with full bloom application. Curling due to leaf-curling aphid, sparse on Victoria, was recorded on 30th May on Early Laxton; least curling was found on trees that had received dimethoate at full bloom, but the levels for the other two treatments probably did not differ significantly as there was much variation in attack.

Influence of formulation

As the amount of aphicide available for translocation in the sap stream must depend on the amount moving from the outside of the bark to the vascular system, the addition of substances aiding entry of the aphicide could be important. No basic studies of such action seem to have been made, but Pietri-Tonelli et al. chose tributyl phosphate as the vehicle for dimethoate in most of their experiments, and this was used as co-solvent in one treatment in the 1965 plum trial. At the 5th International Pesticides Congress, held in London in July 1963, Dr. A.E. Dimond stated that the best solvent found (at Connecticut Agricultural Experiment Station) for introducing fungitoxicants into trees for control of vascular wilt diseases was tridecanol etherified with a chain of some nine or ten molecules of ethylene oxide; such a product, Texofor T95, was also used as co-solvent in another treatment. Dimethoate was used at the same rate in both solvent systems and in the commercial E.C. On Early Laxton, substantially the same percentage control of leaf-curling aphid was

obtained with all three formulations, although the level of dimethoate reached in the young shoots was higher from the E.C. and the tributyl phosphate solution (Table 6); on Victoria, the Texofor T95 solution led to a higher level than that obtained with the ester solution, and this seems to be reflected in the lesser degree of leaf-curl on the T95 trees. However, there is insufficient evidence to suggest that such modifications of formulation would be any benefit.

Table 6

Influence of formulation on percentage infestation with leaf-curling aphid, and on levels of dimethoate reached in young leaves of two plum cultivars in 1965 trial

Treatment Dimethoate (<125 p.p.m.) painted on 29.iv	Cultivar	Dimethoate (p.p.m.) reached on: 3.v	7.v	11.v	17.v	24.v	% leaf-curl 27.v
Untreated	(E.L.	-	-	-	-	-	88
	(V.	-	-	-	-	-	64
Commercial E.C. (32% dimethoate)	(E.L.	(1.9)	(1.3)	1.0	4.0	3.4	22
	(V.	(1.6)	(1.6)	0.7	1.9	-	22
With equal wts Texofor T95 and diacetone alcohol	(E.L.	(1.2)	Nil	1.0	2.5	1.9	27
	(V.	(1.8)	(1.6)	1.6	2.2	3.8	6
With equal wts tributyl phosphate and diacetone alcohol	(E.L.	(1.8)	(0.9)	0.9	4.6	3.3	24
	(V.	(0.7)	(1.0)	1.6	1.8	2.5	25

E.L. = Early Laxton V. = Victoria

Claims have been made in recent years that dimethyl sulphoxide can aid in the entry of chemicals into plants (Keil, Smale and Wilson, 1965; Bean, 1965; Hocking, 1967); less satisfactory results have been reported by other workers (Newhall and Diaz, 1966; Helton and Kochan, 1967). An experiment was carried out in 1967 with this adjuvant on the two cultivars of cherry. Commercial E.C. (5 ml i.e. 2 g dimethoate) was diluted and applied to each tree by squirting on 10th May. If the Napoleon trees are assumed to have weighed 100 lb, the rate of dimethoate applied would have been 43 p.p.m. The Roundel trees would have been lighter, but the corresponding rate could have been about 50 p.p.m. The level of DMSO added was similar to that of the dimethoate, i.e. 2 ml (2.2 g)/tree. Two other treatments consisted of the same rate of formothion, with and without DMSO. Besides untreated control trees, DMSO alone was also used as a treatment. No dimethoate was detected up to the end of the month (Table 7), i.e. for at least three weeks after treatments were applied; maximal levels seem to have been reached in mid-June on both varieties. Very substantial control of blackfly was achieved, especially by dimethoate alone, but there was no evidence that DMSO improved the degree of control; indeed it is surprising that so little blackfly appeared where DMSO was added, as the levels of dimethoate found after two weeks in the tips on these trees was considerably lower than on trees receiving dimethoate without DMSO.

Formothion showed no advantage over dimethoate on either cultivar.

Table 7

Influence of dimethyl sulphoxide (DMSO) on the incidence of leaf-curling aphid, and on levels of dimethoate reached in young leaves of two cherry cultivars in 1967 trial

Treatment*	Cultivar	Dimethoate (p.p.m.) reached on: 31.v	13.vi	23.vi	% shoots with leaf-curl on 14.vi
None	(Napoleon	-	-	-	98
	(Roundel	-	-	-	100
DMSO, 2 g/tree	(Napoleon	-	-	-	83
	(Roundel	-	-	-	98
Dimethoate, 2 g+/tree	(Napoleon	N.D.	3.0	N.D.	1
	(Roundel	0.7	2.4	N.D.	4
Dimethoate, 2 g+) DMSO, 2 g)/tree	(Napoleon	N.D.	0.9	N.D.	14
	(Roundel	0.4	1.4	N.D.	8
Formothion, 2 g°/tree	(Napoleon	-	-	-	11
	(Roundel	-	-	-	12
Formothion, 2 g°) DMSO, 2 g)/tree	(Napoleon	-	-	-	7
	(Roundel	-	-	-	16

* all treatments applied by squirting in final volume of 250 ml
\+ 5 ml 40% product
° 8 ml 25% product

DISCUSSION

Of the systemic aphicides so far used, dimethoate remains the most effective when applied to the bark of plum or cherry trees. Even on 12-year-old trees, reasonable rates of use provide commercial control of aphid species. Experience is lacking with older trees, but may prove less favourable.

For the control of mealy plum aphid on plum and of blackfly on cherry, petal-fall may well be a suitable time for application. There is comparatively little delay in uptake and translocation at this stage in the season and adequate levels of aphicide are soon reached in the young growing points.

The situation for control of leaf-curling aphid on plum is perhaps less favourable. This pest is attacking buds and young leaves when the flow of sap is still very slow, and our experience with application at white bud or even in full bloom indicates that translocation of aphicide may well not occur until petal-fall. This appears to be closely related to the temperature of the soil which will govern the rate of uptake of water by the roots. Translocation is then more rapid, possibly indicating that the chemical is nearer to the vessels, than from petal-fall application, and the levels of dimethoate attained in the growing points are very similar. There is

thus remarkably little decomposition of dimethoate, wherever it is remaining, in the three weeks or more that may elapse between application and translocation. However, even petal-fall application has led to very effective control of leaf-curl in several years.

Attempts to assist entry of dimethoate by the simultaneous application of chemicals claimed to help with the entry of other pesticides into plants have led to no improvement. The use of dimethyl sulphoxide, which improves penetration of many chemicals through mammalian skin, may well be undesirable as well as expensive.

No sign of damage to any part of either species used in these trials has been found, but influences on cropping of stone fruit trees, especially of young cherries, are very difficult to assess.

Acknowledgements

The authors acknowledge with pleasure the assistance given in the trials reported here, by Miss M. Bennett and members of the Pathology Recording Team of this Station. Mrs. J.M. Lewis (née Sillibourne) was responsible for sampling and chemical analyses.

They are also grateful to Glovers (Chemicals) Ltd. for a gift of Texofor T95, and to Dr. E.F. Edson, Fisons Pest Control Ltd. for a gift of technical grade dimethoate.

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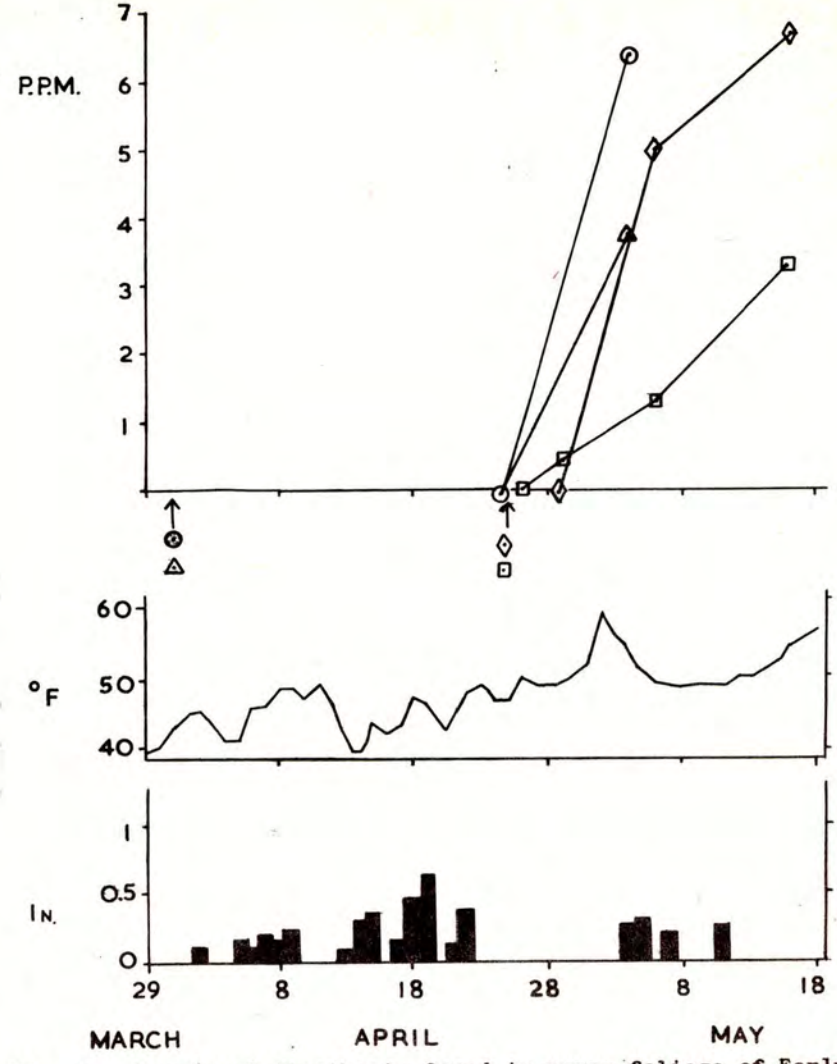

Fig. 1. Levels of dimethoate found in young foliage of Early Laxton plum in 1966 trial, with daily rainfall and mean daily temperature at 8 inches below cultivated soil surface.

⊙ 200)
△ 100) p.p.m. (approx) dimethoate applied at white bud

◇ 200)
⊡ 100) p.p.m. (approx) dimethoate applied at petal-fall

↑ Dates of application

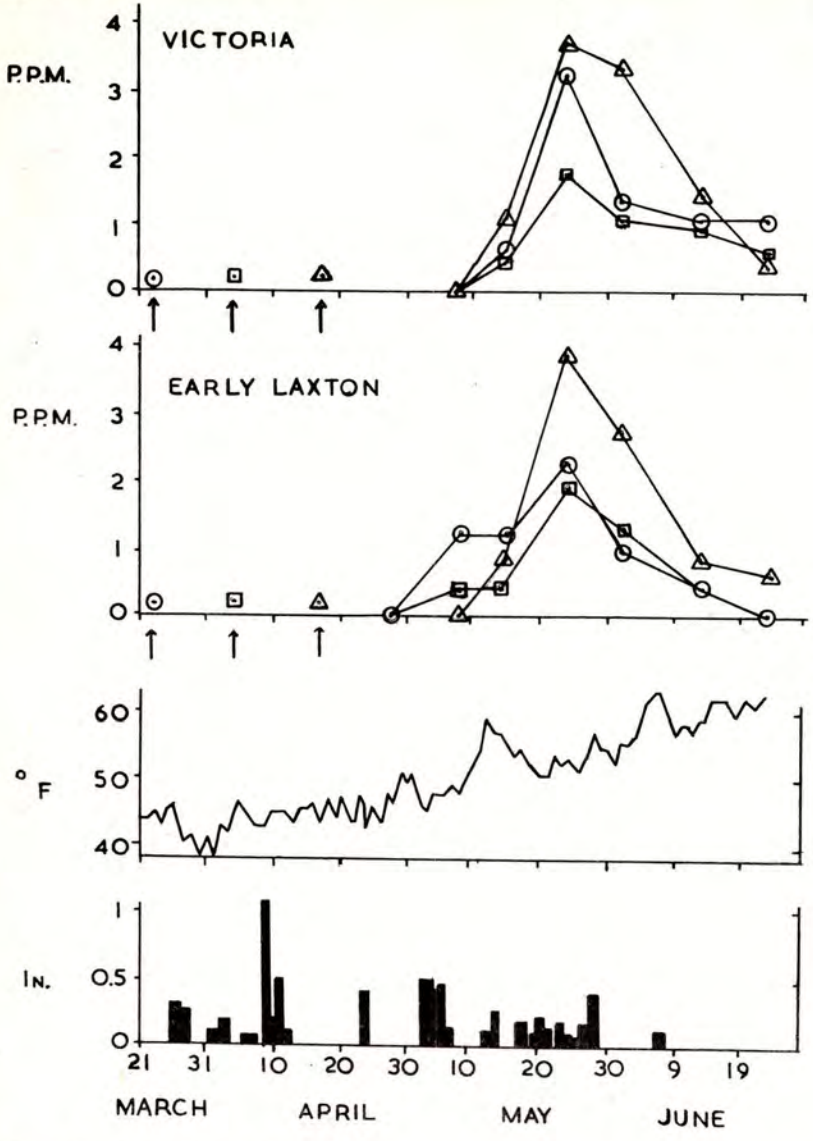

Fig. 2. Levels of dimethoate reached in young foliage (p.p.m. fresh wt) of two plum cultivars receiving about 80 p.p.m. dimethoate via the bark at white bud (23.iii.67), full bloom (4.iv.67), or petal-fall (17.iv.67), together with data for daily rainfall and mean daily temperatures at 8 inches below cultivated soil level.

⊙ white bud; ⊡ full bloom; △ petal-fall.

↑ Dates of application.

PENETRATION OF MALATHION INTO WHEAT GRAINS

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Summary

The penetration of malathion in the first few days after application to wheat grains has been examined. After a few days most of the intact malathion is in the endosperm. Moisture content does not seem to affect movement between pericarp and endosperm although insecticide only penetrates into the germ at moisture contents of 14% or lower.

Comparison of three organic solvents revealed no major effect on penetration.

INTRODUCTION

The use of organo-phosphorus insecticides for control of insects on stored food grains has increased rapidly during the past few years. In some countries exported grain is treated as a routine procedure, the most commonly used insecticide being malathion. Several others might be used in the future, notably fenitrothion; bromophos and DDVP.

The use of insecticides on food materials poses many problems, which can be divided into two main groups. The first concerns the application, whether it is effective, what are the best formulations etc. The second is essentially the problem of residues, both of the original insecticide and of its breakdown products, particularly in respect to the possible toxic hazard to the consumer. In both cases the distribution of the insecticide through the grain is important.

A certain amount of work has been done on the effectiveness of applications of organo-phosphorus insecticides to stored grains (Schesser, J.H. et al., 1958; Rowlands, 1967). The results have been valuable in elucidating optimum conditions for application. However very little has been done on the detailed picture of what happens to an insecticide when applied to grain. In particular the contribution of the different tissues to the overall picture. Wheat grains can be fairly easily dissected, in the dry state, into pericarp, endosperm and germ (Fig. 1) roughly corresponding to the bran, flour and germ of the milling process (Peterson 1965).

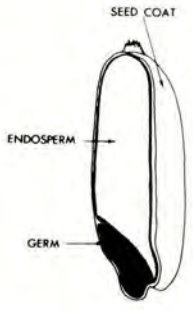

Fig. 1 DIAGRAM OF WHEAT GRAIN

Endosperm in the form of flour is the fraction mostly used for human consumption. Bran and germ are also present in most breakfast cereals and in whole wheat flour. In the present work we have examined the distribution of malathion between the three tissues both from the point of view of the effectiveness of application and of possible residue problems.

METHOD AND MATERIALS

Wheat grains, judged visually to be undamaged and healthy, were separated from freshly harvested English wheat. Five lots were equilibrated at 25°C with various humidities, obtained by using solutions of sulphuric acid (Solomon 1951) to give 96, 82, 57, 35 and 16% relative humidities.

A sample of radioactive malathion, labelled with C^{14} in the 2-C position of the thio-malate moiety, was diluted with inactive malathion to give the required specific activity. The insecticide, normally dissolved in n-hexane, was applied topically to individual grains in volumes of 0.1 µl, using a special capillary applicator (Hewlett and Lloyd 1960). The total activity applied was standardized at 640 c.p.m.

The grains were dissected by hand into pericarp, endosperm and germ and total radioactivity in each fraction determined by immersion in a liquid phosphor solution and measurement in a Beckman scintillation counter. To measure the breakdown products separately they were partitioned into buffer solution at pH 7, the intact malathion (and malaoxon) remaining in n-hexane. All counts extended over 5 min, giving a 2σ of about 4% for the 100% figures and 12% for the 10%.

RESULTS

Table 1 shows the moisture contents obtained at the different humidities. The grain was dried to constant weight at 105°C.

Table 1

% RH at 25° nominal	Moisture content of wheat %
96	22
82	17
57	14
35	10
16	7

Tables 2 and 3 give the C^{14} content of each fraction at different times after application of C^{14} labelled malathion, in n-hexane, to the attachment region and the back respectively. The results are expressed as percentages of the total C^{14} recovered, the mean total recovery figure for the thirty determinations was 84.2% of added C^{14} with a standard deviation of 4.7%.

Table 2

% moisture	% of C^{14} recovered											
	Pericarp				Germ				Endosperm			
	1hr	4hr	24hr	48hr	1hr	4hr	24hr	48hr	1hr	4hr	24hr	48hr
22	20	23	23	25	4	4	4	3	76	72	72	71
17	19	26	25		4	4	4		76	69	70	
14	7	12	25		31	30	28		62	57	61	
10	6	11	22		31	29	24		64	60	54	
7	5	10	21	25	22	22	19	12	72	68	60	63

Topically applied to attachment region

Table 3

% moisture	% of C^{14} recovered											
	Pericarp				Germ				Endosperm			
	1hr	4hr	24hr	48hr	1hr	4hr	24hr	48hr	1hr	4hr	24hr	48hr
22	100	93	73	45	0	0	2	4	0	7	25	50
17	100	97	76		0	0	1		0	6	23	
14	100	97	88		0	0	0		0	3	12	
10	100	96	78		0	0	1		0	4	20	
7	100	94	77	50	0	0	3	2	0	6	20	47

Topically applied to the back

Tables 4 and 5 give the results of experiments where the C^{14} contents of each tissue were separated into hexane solubles (intact malathion) and water soluble (breakdown products) fractions.

Table 4

% moisture		Tissue	% of C^{14} recovered 1hr	4hr	24hr	48hr
22	Hexane	Pericarp	17	22	11	5
		Germ	4	3	5	3
		Endosperm	78	63	53	48
	Aqu.	Pericarp	0	1	13	20
		Germ	0	1	1	0
		Endosperm	0	10	19	23
7		Pericarp	3	9	19	23
		Germ	22	20	19	12
		Endosperm	75	70	56	59
		Pericarp	0	0	2	2
		Germ	0	0	0	0
		Endosperm	0	0	4	4

Applied topically to attachment region

Table 5

% moisture		Tissue	% of C^{14} recovered 1hr	24hr	48hr	7 days
22	Hexane	Pericarp	100	64	42	11
		Germ	0	1	2	0
		Endosperm	0	26	35	40
	Aqu.	Pericarp	0	0	3	2
		Germ	0	1	2	2
		Endosperm	0	8	15	45
7	Hexane	Pericarp	100	80	49	22
		Germ	0	3	2	0
		Endosperm	0	17	33	44
	Aqu.	Pericarp	0	0	1	0
		Germ	0	0	1	1
		Endosperm	0	0	14	32

Applied topically to back region

Table 6 shows the distribution of C^{14} 24 hr after application of C^{14} labelled malathion in different solvents to the attachment region of 14% moisture content grain.

Table 6

Tissue	% of total C^{14} recovered at 24 hr after application in different solvents		
	n-hexane	carbon tetrachloride	dioxane
Pericarp	23	26	22
Germ	25	31	27
Endosperm	51	43	51

DISCUSSION

In this paper we have examined in some detail the penetration of malathion, applied in n-hexane to the back and the attachment region of wheat grains. Previous work had indicated that these two areas were the least and most rapid points of uptake respectively (Rowlands 1967).

The results given in tables 2 and 3 suggest two main effects. Firstly, moisture content has very little effect on the rate of penetration from the back region. Secondly, when applied to the attachment region, there is a marked difference in the amount of malathion reaching the germ, depending on the overall moisture content of the grain. Above 14%, very little insecticide enters the germ but below this moisture level approximately 30% is found to have reached the tissue.

As anticipated the rate of uptake from the back region is slower than from the attachment region but despite this difference the overall picture of distribution is similar if we except the increased amount of insecticide present in the germ at lower moisture contents. For example table 7 shows the distribution pattern 48 hr after application to the attachment and 7 days after application to the back region.

Table 7

Moisture content %	Tissue	Malathion content % Attachment 48hr from appl.	Back 7 days from appl.
22	Pericarp	5	11
	Germ	3	0
	Endosperm	48	40
7	Pericarp	23	22
	Germ	12	0
	Endosperm	59	44

Since the insecticide distributes itself similarly, after a few days, from both positions of application, it would seem that, after the first week or so, the distribution of malathion within the grain is independent of the original site of application. This suggests that even in grain of moisture content as low as 7% there is an effective transport system moving the insecticide from one tissue to another.

Table 6 shows the distribution of C^{14} 24 hr after adding the malathion in three different organic solvents. There is no significant difference between the three. It seems likely that the more volatile solvents evaporate very rapidly after application and would therefore be unlikely to have any major effect. Dioxane, which is less volatile than the others, also seems not to affect the distribution. Since the volumes used in this work are far in excess of the amounts of solvent that would be encountered in practice it is unlikely that any organic solvent would affect the distribution of insecticide.

It is hoped to extend this work to cover more insecticides and particularly to see if application as aqueous emulsion shows any radical difference. Preliminary work with bromophos, fenitrothion and dimethoate indicate similar qualitative results to malathion but considerable quantitative differences.

Acknowledgement

The authors wish to thank Cyanamid of America Inc. for the generous gift of a sample of C^{14} labelled malathion.

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EFFECT OF SOIL MOISTURE CONDITIONS ON UPTAKE OF SYSTEMIC INSECTICIDES FROM SOIL BY PLANTS

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Summary The influence of soil moisture conditions on the effectiveness of the soil applied systemic insecticides disulfoton, phorate, dimethoate and menazon against aphids was studied by experiments with wheat in pots and by irrigating potatoes in the field. Soil moisture was controlled in the pots osmotically. Results are interpreted in relation to other experiments on adsorption and diffusion in soil. In the pots effects of moisture were small, but generally effectiveness of dimethoate decreased and that of disulfoton increased with increasing moisture. Uptake by the wheat was probably not limited by movement to the roots, but by absorption by the roots which depends on the concentration in the soil solution. In the field irrigation increased effectiveness of dimethoate and menazon but not disulfoton and phorate. This is not simply because of different relationships between moisture content and diffusion rates for these insecticides in soil resulting from different physical properties as was previously suggested. Other possible explanations are considered.

INTRODUCTION

The uptake of different systemic insecticides from soil by plants may be affected in different ways by soil factors so that their relative effectiveness against insects may depend on soil conditions. Moisture conditions vary widely from soil to soil and season to season and their influence on the behaviour of systemic insecticides is therefore of interest. In the field, differences in rainfall or irrigation can affect soil-applied insecticides in two ways: first through differences in the soil moisture content and second through differences in the extent of transport with the water downflow, i.e. leaching. In experiments where soil moisture is controlled in pots, the soil volume is smaller, plant roots are confined, and water movement is less than in field soil so leaching should be less important and the static factor, moisture content, is more likely to cause any effects of moisture conditions.

Several workers have studied effects of soil moisture conditions. Specht and Chisholm (1965) reported, from experiments in the glasshouse, that soil moisture content had no effect on the uptake of disulfoton after application in the furrows with pea seeds or on pea aphid control. In the field, by contrast, Reynolds and Metcalf (1962) found that several insecticides including disulfoton, phorate and dimethoate applied as bands in furrows beside cabbage became effective much more quickly with irrigation than without. Jefferson, R. N. *et al.* (1964) also reported that frequent watering increased the effectiveness of surface applied granules of dimethoate, phorate, disulfoton and phosphamidon against aphids on carnations. These three reports are consistent with the view that dispersion of the insecticide into the root zone by leaching is important but moisture content is not, because effects were observed in the field and not in the glasshouse. However, Burt, P. E. *et al.* (1965) postulated that moisture content is important in the field also. They found that the relative effectiveness of different soil-applied granular systemic insecticides over several years depended on season and the physical properties of the insecticide. Phorate and disulfoton were more effective than menazon and dimethoate in dry years but all were equally effective in wet years. Because

aphid control was complete in all cases with disulfoton and nearly so with phorate, conclusions about the relative amounts of these insecticides absorbed by the plants in different seasons are difficult, but Burt, P.E. et al. (1965) postulated that whereas all four insecticides can move through the soil to roots in wet soil, only phorate and disulfoton are volatile enough to diffuse in sufficient quantities as vapour through the air-filled pores in dry soil. In support of the hypothesis, it was shown that when roots of wheat plants were in contact with only the vapours of the insecticides, phorate and disulfoton were much more effective than menazon and dimethoate against aphids, whereas all four were almost equally effective when absorbed from aqueous solution.

The objects of our investigation were to determine whether the seasonal differences observed by Burt, P.E. et al. (1965) were in fact due to differences in moisture conditions by making these the only variable, and to learn more about how these conditions exert their influence, if any. The effects of soil moisture on the effectiveness of different systemic insecticides were studied in the laboratory by pot experiments with wheat grown in soil under different moisture regimes which did not involve leaching and in the field by irrigating potatoes. The pot experiments were designed to test if the amounts of insecticide accumulating in the plant were governed by the ability to maintain a supply to the roots and to examine the effects of moisture content on the maintenance of supply. To give a reproducible and well-defined system which would allow theoretical calculations to be made, the insecticide was initially uniformly mixed with soil in the pots. Although this system cannot test the ability to establish an effective concentration at the root by transport from a distant source, continuing uptake depends on insecticide reaching the roots through the soil. Differences in ability to maintain supply would therefore result in differences in uptake which would increase as the experiment continued.

Only the results of bioassays from these experiments will be given here; radiochemical measurements were also made in the pot experiments but results have not yet been fully calculated. Bioassay results may be approximately related to concentrations available at the roots because the four insecticides considered were of similar toxicity to aphids when taken up by wheat from aqueous solution (Burt, P.E. et al., 1965).

METHOD AND MATERIALS

Pot Experiments For pot experiments sandy clay loam soil, derived from Lower Greensand and taken from Woburn Experimental Farm, was air dried and sieved (<2 mm) before use. Different moisture regimes were established by an osmotic method (Graham-Bryce 1967a). Pots made from Visking dialysis membrane tubing supported inside perforated polythene pipe were packed with soil mixed with the desired amount of water containing emulsified insecticide, and placed in tanks of polyethylene glycol (PEG) solution which controlled the potential at which water entered the soil through the membrane. PEG solutions were made up to give osmotic pressures appropriate for the soil water content (Table 1). With this method there is an initial adjustment of the soil moisture content over approximately one week when the rate of change becomes negligible and the soil moisture content below a drier surface layer varies little. The pots cannot be used for longer than four weeks because the membrane decomposes. Table 1 shows soil moisture contents on setting up and on dismantling after 28 days for the three regimes studied.

24 hours after packing the pots with soil, 12 small wheat plants, as similar as possible and having approximately $\frac{1}{2}$ to 1 inch shoots were planted $\frac{1}{2}$ to 1 inch deep at uniform spacing. Pots were kept in controlled environment rooms at the following conditions: temperature 18°C day, 15°C night; relative humidity 70% day and night; day length 18 hours. The toxicity of the foliage was tested at intervals of 1, 3, 7, 14 and 28 days after planting by confining ten late instar or adult apterous aphids (Rhopalosiphum padi) in small cages on the foliage of three plants in each pot as described by Burt, P.E. et al. (1965). Live aphids were

Table 1

Moisture regimes in pot experiments

Moisture condition	Initial water content % w/w	Final water content % w/w	PEG conc g/100 ml	Osmotic pressure of PEG soln atms.
Wet	20	15	2	0.06
Intermediate	15	9	5	0.39
Dry	10	6	10	1.8

counted after 24 hours. The ranges 0.05 to 6 mg disulfoton/kg soil and 0.05 to 3 mg dimethoate/kg soil were investigated by testing four different insecticide levels at each moisture content, and repeating the experiment three times using slightly different concentrations. Soil concentrations producing weighted mean LC50s in the wheat were calculated from results of the three experiments.

Field Experiments Field experiments were at Woburn Experimental Farm. Disulfoton, phorate, dimethoate and menazon granules were applied at the equivalent of 3 lb a.i./acre as bands along the furrows below potato tubers (Maris Piper) at the time of planting (27/4/67). Four different moisture conditions were established on plots (28 ft by 10 ft) as follows:-

1) Plots protected from rainfall or irrigation by polythene covers on wooden frames, placed in position one week after planting.
2) Plots receiving no irrigation, but open to the rain.
3) Plots irrigated during the first half of the experiment, until 23rd June.
4) Plots irrigated throughout the season.

Irrigation was applied at approximately one inch per week and moisture contents were determined gravimetrically in soil cores taken from the top 6 inches at several positions in each different plot at frequent intervals during the course of the experiment. The plots were split for insecticide and control treatments, sub-plots being single rows separated from neighbouring insecticide treatments by untreated rows. The toxicity of the plants to aphids was tested at approximately weekly intervals by caging ten late instar or adult apterous aphids (Myzus persicae) on the foliage of three plants in each sub-plot. Live aphids were counted after three days.

RESULTS AND DISCUSSION

Pot Experiments Fig. 1 shows weighted mean LC50s (mg insecticide/kg soil) during the 28 day experiment. Effects of moisture conditions are small. In the first four tests, disulfoton was somewhat more effective in wet soil than in dry whereas the opposite was true for dimethoate. This was reversed for both insecticides at 28 days. Thus the relative potency of dimethoate to disulfoton was greater in dry soil than in wet except at 28 days. However, very few differences were significant and these do not seem to be distributed according to any clear pattern. Analysis of trends over the course of the experiment shows that for the results as a whole, the effectiveness of disulfoton increased significantly with moisture content and that the opposite was true for dimethoate.

If uptake of insecticide by the plant is limited by diffusion in the soil, and diffusion of dimethoate relative to disulfoton decreases as the soil becomes drier as suggested by Burt, P.E. et al. (1965) then the effectiveness of dimethoate

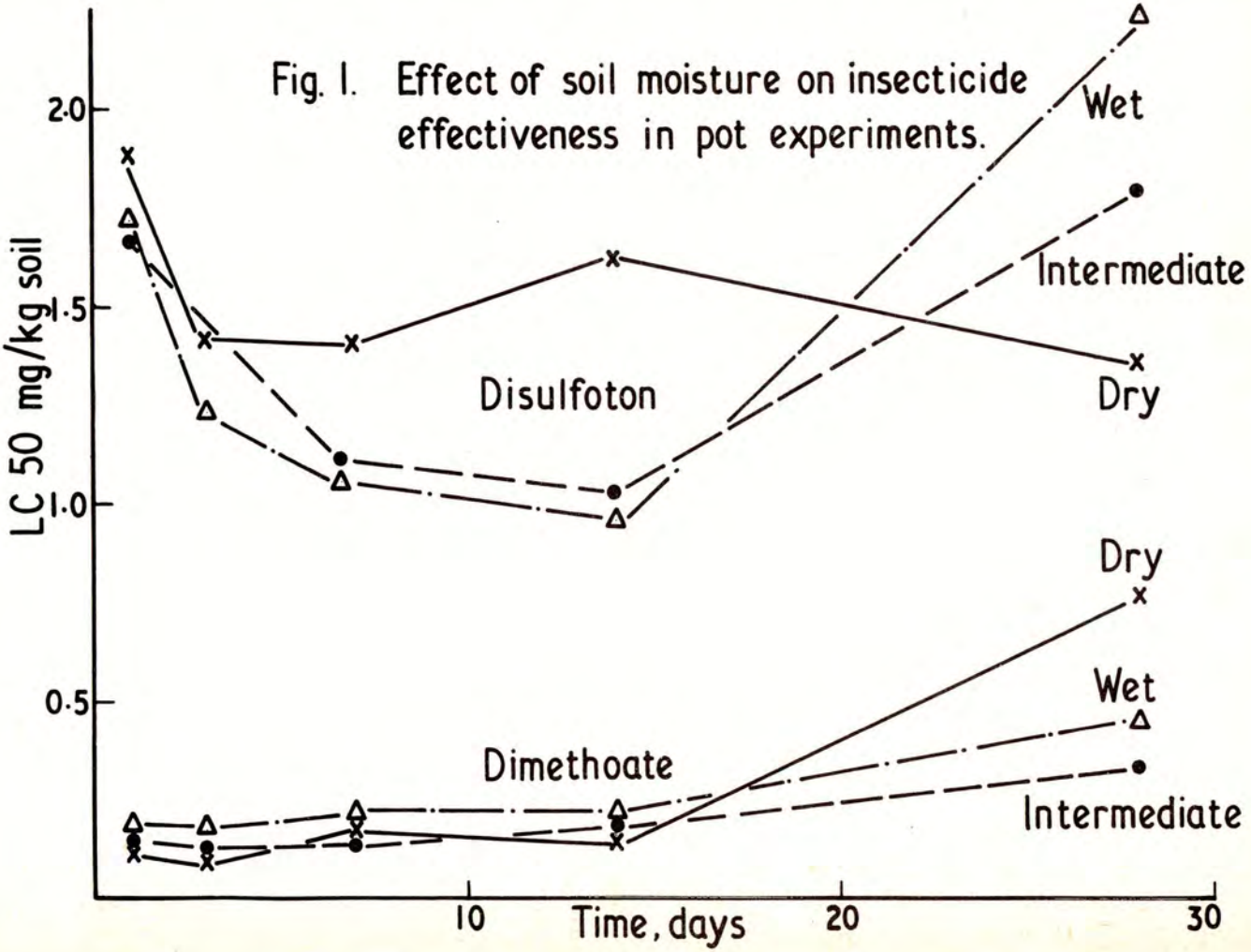
Fig. I. Effect of soil moisture on insecticide effectiveness in pot experiments.
LC 50 mg/kg soil
2·0
1·5
1·0
0·5
Wet
Intermediate
Disulfoton
Dry
Dry
Wet
Dimethoate
Intermediate
10
Time, days
20
30

relative to disulfoton, reflecting the amounts taken up, should also decrease as the soil becomes drier. Differences in our experiments were not clear cut and interpretation is difficult, but the general trend of the results is contrary to this hypothesis, except at 28 days. Other considerations also suggest that the hypothesis is not valid for these conditions without modification. Detailed calculations (Graham-Bryce 1967b) indicate that, at the intermediate moisture content, uptake was not limited by transport in the soil but by processes in the plant. It is highly probable that this applies to the other moisture conditions also. In this case the results can be partly accounted for by the different concentrations at the roots. Dimethoate is only very slightly bound to soil and would be almost all in the soil solution. The solution concentration would therefore be almost inversely proportional to the water content. Uptake by plants probably increases with the concentration at the roots at these concentrations and would thus be greater in dry soils. In contrast disulfoton is strongly bound to soil (Graham-Bryce 1967c) so that differences in water content have little effect on the concentration in the soil solution. Some other factor, possibly an effect of moisture content on adsorption, must account for the opposite trend.

In this system the insecticide is initially uniformly mixed with soil and only the maintenance of a supply to the roots depends on movement in the soil. It is possible that with a non-uniform distribution in the field, the establishment of a concentration at the root surface large enough to produce toxic amounts in plants depends on diffusion, influenced by moisture content as suggested by Burt et al. (1965). However, comparison of apparent diffusion coefficients suggests that this is also unlikely. The values of these coefficients are determined by the combined effects of all factors such as tortuosity of the pores, adsorption, and vapour movement which affect diffusion in soil. The apparent diffusion coefficient for dimethoate in this soil (3.14×10^{-7} cm^2/s) is much greater than that for disulfoton (2.62×10^{-8} cm^2/s) at the intermediate moisture content, because of differences in adsorption (Graham-Bryce 1967b). Although the relative values may change somewhat with moisture content, this almost certainly applies at the other moisture contents also, so that diffusion alone would result in a greater concentration of dimethoate at the root than of disulfoton for the same amounts present in the soil. Because the two insecticides are equally effective in solution, diffusion alone should therefore make dimethoate more effective than disulfoton at all moisture conditions. Further, mass flow with the transpiration stream is probably a more significant mechanism than diffusion for supplying dimethoate to roots than diffusion. The values for diffusion coefficients emphasise that insecticides cannot diffuse far in soil; the root mean square displacement of dimethoate calculated from the diffusion coefficient is only approximately 1 cm in a month.

The maintenance of a toxic concentration in the plant depends on its ability to absorb and translocate enough insecticide from the concentration presented at the roots, to compensate for dilution by its growth and for detoxication. Even when there is no leaching, significant differences in relative effectiveness caused by moisture content are likely to require complex explanations involving the interactions of such factors as transport in the soil, uptake at the root and translocation in the plant, transpiration, rates of growth of root and shoot and decompositon of insecticide in the soil and the plant. Analysis of the radiochemical results from this experiment should give some further help with this problem.

Apart from the effects of moisture content two features of the results are of interest. First dimethoate was more effective than disulfoton throughout the experiment at all moisture contents, probably because it was more concentrated in the soil solution for the same amounts added to the soil, as a result of the differences in adsorption. Second, the effectiveness of disulfoton was similar throughout the experiment, whereas after 3 days dimethoate became progressively less effective. Statistical analysis confirmed that there was a significant effect of time on the potency of dimethoate, but not disulfoton. This could be explained if dimethoate decomposes in soil or plant to non-toxic products faster than disulfoton.

Field Experiments The moisture regimes established by the various irrigation treat-

ments differed considerably. Total water between planting (27th April 1967) and the final bioassay (14th August 1967) was: covered plots, nil; uncovered, unirrigated plots, 7.82 inches; early irrigation, 10.9 inches; full irrigation, 17.33 inches. Average values for moisture contents of soil samples taken throughout the experiment were: covered plots, 5.3% w/w; uncovered plots, 7.9% w/w; fully irrigated plots, 9.5% w/w.

The bioassays showed that disulfoton was very effective and persisted well throughout the season at all moisture conditions. Phorate which is chemically very similar to disulfoton was almost as effective, but dimethoate and menazon, although initially as potent as disulfoton, soon became less so. In the final test menazon and dimethoate plots were not significantly more toxic (5% level) than control. Fig. 2 shows mean values from all irrigation treatments for the aphids surviving at each test, expressed as % of control numbers.

As with the pot experiments, the influence of moisture conditions on effectiveness was small. There was no significant effect of treatment on the potency of phorate relative to the control plots in any of the tests. Disulfoton was similar until the final test 3½ months after planting when plants on the irrigated plots were significantly more toxic than on the unirrigated. For dimethoate, differences between treatments were found in the 4th and 5th tests (June 30th and July 7th) when plants on both the irrigated and uncovered plots were significantly more toxic (5% or 1% levels) than on the covered plots. The maximum natural aphid infestation was in mid-July so that differences during this period could be particularly important in relation to aphid control. Irrigation also made menazon more effective. In each of the 1st, 2nd, 4th, 5th and 6th tests, it was significantly more effective on wetter plots than on drier ones in at least one of the possible treatment comparisons. Such differences as were observed, therefore, all confirm the observations of Burt, P.E. et al. (1965).

Results in the field differ in two ways from those in the pot experiments. First disulfoton was considerably more effective than dimethoate and second, although effects were small, the activity of dimethoate increased with increasing moisture content. Conditions in the field differed from those in the pots in several respects. The insecticides were not uniformly distributed, leaching was possible, the roots were not confined and a different plant was tested. Also tests were not possible until the potatoes emerged, 5 weeks after planting whereas pot experiments were restricted to 4 weeks. This difference in the periods studied probably accounts for the difference in relative performance of disulfoton and dimethoate in field and pot experiments. Fig. 2 shows that the activity of dimethoate decreased much faster than that of disulfoton. This could be because dimethoate decomposes faster in soil or plants than disulfoton, or because, being less strongly bound to soil, it is more easily leached out of the root zone. Decomposition is probably more important because persistence in irrigated plots was broadly similar to that in covered plots where there was no leaching. Also, during the pot experiments dimethoate, although more effective than disulfoton, became progressively less active, unlike disulfoton. Extrapolation suggests that if these experiments had continued, the order of effectiveness would have been reversed by the time tests were made in the field.

Although the considerations given earlier in this paper indicate that different relationships between water content and diffusion rates of insecticides in soil do not alone explain why dimethoate and menazon are likely to be less effective than disulfoton and phorate in dry soil, interaction of decomposition with effects of irrigation on transport to roots by mass flow and diffusion could account for our results. Also dimethoate and probably menazon are less strongly bound to soil than disulfoton and phorate, so that they would be dispersed more in the root zone by leaching. This could increase uptake of dimethoate and menazon on irrigated plots by making them available to more roots. However as with the pot experiments many factors could be involved and, without further experiments, any interpretation can only be highly speculative.

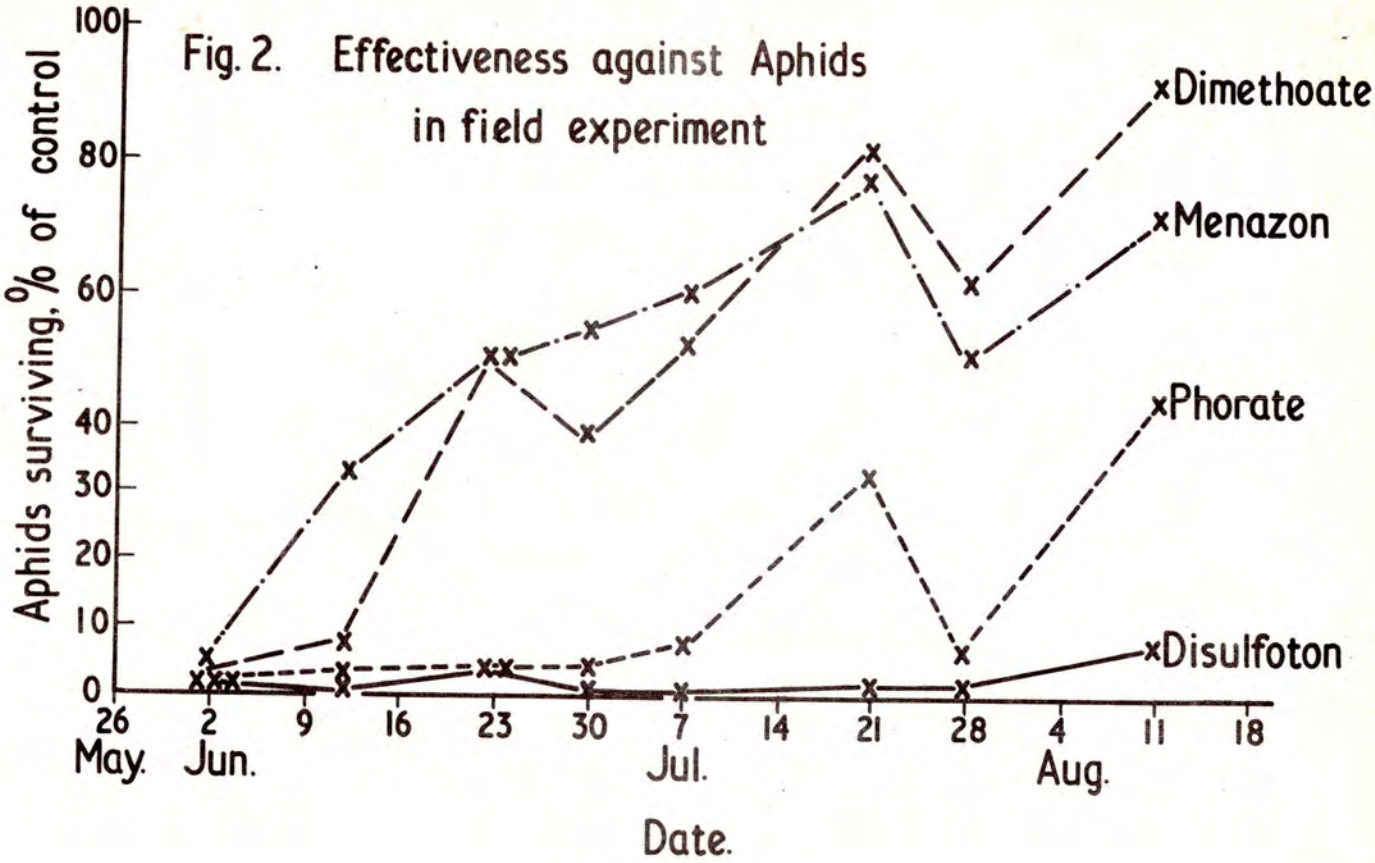
Fig. 2. Effectiveness against Aphids in field experiment
Aphids surviving, % of control
100
80
60
40
30
20
10
0
Dimethoate
Menazon
Phorate
Disulfoton
26 May.
2 Jun.
9
16
23
30
7 Jul.
14
21
28
4 Aug.
11
18
Date.

These experiments tested only those conditions considered by Burt, P.E. et al. (1965) and the conclusions apply only for granules placed along the furrows under potato tubers at the time of planting. Larger differences in the effects of moisture conditions might be found with plants whose roots are less well ramified, where the insecticide was initially further from the roots, or if tests were done sooner after application.

Acknowledgements

We thank Mr. G. J. S. Ross and Mr. J. H. A. Dunwoody of the Statistics Department, Rothamsted Experimental Station for help with the statistical treatment of the results. Insecticides were kindly supplied by Messrs. BASF United Kingdom Ltd., Baywood Chemicals Ltd., Cyanamid of Great Britain Ltd., Fisons Pest Control Ltd. and Plant Protection Ltd.

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SOME ASPECTS OF THE SPECIFICITY OF INSECTICIDE SYNERGISTS

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Summary

Strain and species differences may determine whether triphenyl phosphate synergizes malathion. A preliminary examination of the effects of other candidate synergists on the malathion metabolites produced by flour beetles, showed that both effective and ineffective synergists affected metabolism in a variety of ways. Several pyrethrin synergists which antagonized malathion inhibited malaoxon formation. Some compounds appeared to inhibit detoxication by phosphatases and synergized malaoxon. These failed to synergize malathion, probably because they also inhibited malaoxon formation, or the penetration of malathion.

INTRODUCTION

Insecticide synergists are compounds which, though not toxic in themselves strongly enhance the toxicity of the insecticides with which they are combined. Most appear to act by inhibiting detoxication mechanisms within the insect.

A compound may enhance the toxicity of a particular insecticide against one species of insect and fail to do so in another. In other words synergists may be species-specific. Moreover they may be effective only with certain strains of given species. In particular, compounds which are highly effective against insecticide-resistant strains may be ineffective against susceptible strains of the same species. For example, triphenyl phosphate is a very effective synergist for malathion against larvae of Dermestes maculatus but against Dermestes lardarius another species of the same genus it is ineffective. Similarly against malathion-resistant flour beetles (Tribolium castaneum) we found pronounced synergism, whereas against a susceptible strain of the same flour beetle no synergism was detected (Table 1).

We have reported elsewhere our studies of the metabolism of malathion in the two strains of this flour beetle (Dyte and Rowlands 1967). Besides oxidation to malaoxon, which is the toxic cholinesterase inhibitor, malathion was detoxified by the formation of carboxyesterase products and phosphatase products in both strains (Fig. 1). However, in resistant beetles carboxyesterase products were produced much more rapidly than in the susceptible strain. It seemed likely that the synergist, triphenyl phosphate, which overcame the resistance, was inhibiting this more rapid production of carboxyesterase products, and studies of the metabolism of malathion applied with triphenyl phosphate showed this to be the case.

We have now made further studies of malathion metabolism in these flour beetles, in which the emphasis has changed from the nature of malathion resistance to the specificity of some candidate synergists.

METHOD AND MATERIALS

Full details of the origin of the two strains of Tribolium castaneum, and the methods used are given by Dyte and Rowlands (1967). The resistant strain originated in Nigeria, and after selection in the laboratory had a resistance factor of x 450

Table 1

Strain and species differences in the synergism of malathion by triphenyl phosphate (TPP)

Topical application in 2-ethoxy-ethanol on larvae of Dermestes, or in cyclohexanone on adults of Tribolium. Ratio of malathion : synergist 1 : 20, data from Dyte et al. (1966) and Dyte and Rowlands (1967).

Insect and Insecticide	LD50 (µg/insect)	Factor of synergism
Dermestes maculatus		
malathion	19.08	x 12
malathion + TPP	1.57	
Dermestes lardarius		
malathion	1.92	< x 1
malathion + TPP	2.93	
Resistant T. castaneum		
malathion	12.3	x 189
malathion + TPP	0.065	
Susceptible T. castaneum		
malathion	0.027	< x 1
malathion + TPP	0.071	

(Table 1). Insecticide solutions in cyclohexanone, with or without synergist, were applied topically to adult beetles, 3-5 weeks old with a microcapillary tube of 0.05 µl. The treated beetles were kept at 25°C and mortalities assessed after 9 days. 75 beetles were used at each dose level and the ratio of malathion to synergist was 10:1. Beetles destined for homogenization were treated likewise with 0.05 µg malathion and 0.5 µg synergist/beetle, in batches of 30, and subsequently transferred to about -10°C after 20 or 30 hours. Homogenates were prepared within 36 hours of the beetles being frozen. Malathion, and the metabolites shown in Fig. 1, were separated from homogenates and measured separately by gas-liquid chromatography. The malathion and candidate synergists used were all pure, or high grade technical samples. 'Diphopip', 0,0-dimethyl O-(3,4-methylenedioxyphenyl) phosphate was prepared by reacting dimethyl phosphorochloridate with the sodium derivative of piperonyl alcohol. S421, and SKF 525A are code designations for bis-(2,3,3,3-tetrachloropropyl) ether and 2-diethylaminoethyl 2,2-diphenyl-n-pentanoate respectively. The latter compound was used as the free base.

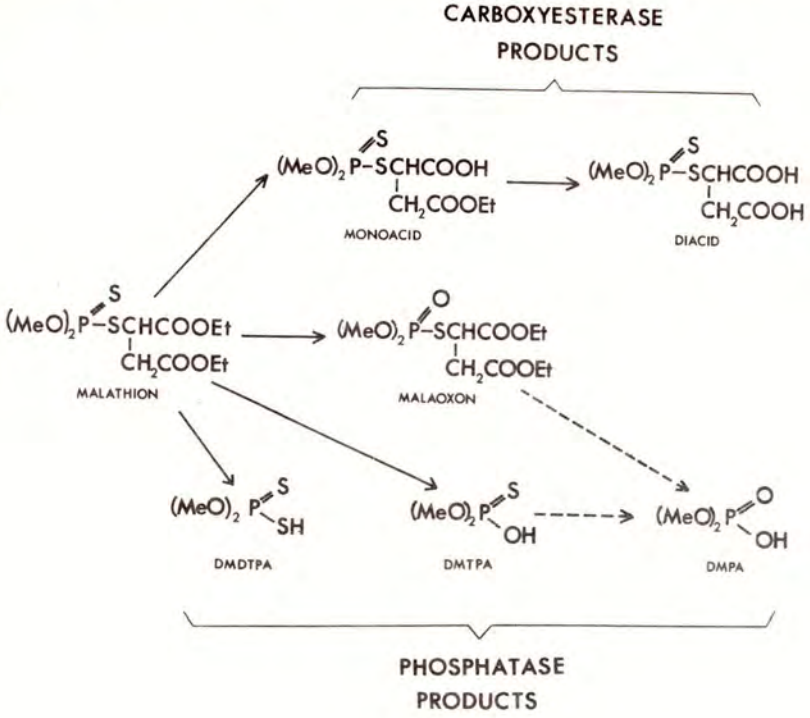
RESULTS

Of the five phosphate esters (listed in addition to triphenyl phosphate in Fig. 3), only diphenyl phosphate and triphenyl phosphate synergized malathion against the resistant strain, though neither was as effective as triphenyl phosphate. None of the five compounds synergized malathion against the susceptible strain, and diphenyl phosphate and tri-o-cresyl phosphate were slightly antagonistic.

Most of the other candidate synergists (listed in Fig. 9) antagonized the toxicity of malathion against both strains. However S421 and 'Diphopip' were neither synergistic nor antagonistic with either strain, and piperonyl butoxide did not affect the toxicity of malathion to the resistant beetles. This last result was unexpected but was confirmed in repeated tests. Tri-n-butyl phosphorothionate

Figure 1

Metabolism of malathion showing metabolites studied in this investigation



and SKF 525A synergized malaoxon against the susceptible strain.

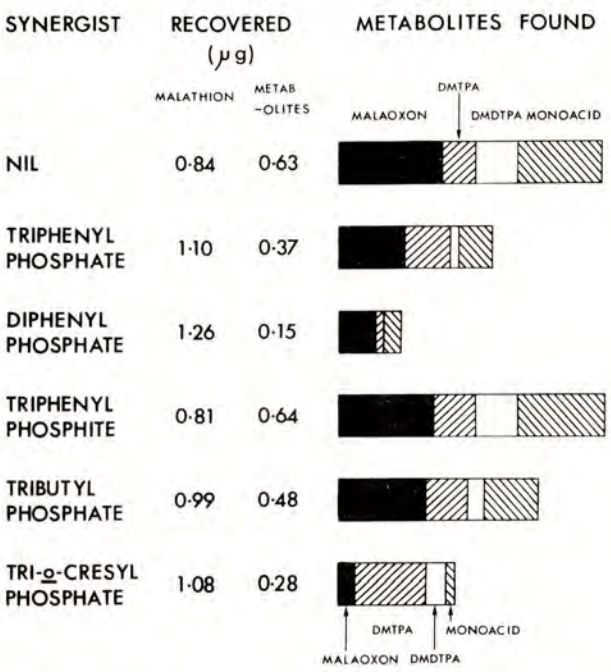
The metabolites recovered when malathion was applied with the candidate synergists are shown in figs 2 to 9; those obtained with malathion alone, and with malathion synergized with triphenyl phosphate, which have been reported more extensively elsewhere (Dyte and Rowlands 1967), being included for comparison.

DISCUSSION

In considering the possible effects of the candidate synergists upon malathion metabolism it is important to emphasize the limitations of the present study. As discussed elsewhere (Dyte and Rowlands 1967) our techniques did not distinguish internal malathion which had been absorbed from external malathion which was not absorbed or which may possibly have been excreted unchanged. Since pyrethrum synergists and other phosphorus esters are known to inhibit the cuticular penetration or organophosphorus insecticides (Abdallah 1963; Menzer and Casida, 1965), high malathion levels, and low levels of metabolites, could result from either reduced penetration or reduced metabolism or both. Moreover, our previous work has shown that whereas the total level of carboxyesterase products on phosphatase products in homogenates tends to increase steadily with time after the beetles are

Figure 2

Metabolites recovered from batches of 30 susceptible flour beetles 20 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle.



treated, the malaoxon level first rises and then falls in both strains. Obviously studies of malaoxon levels at 20 and 30 hours after treatment cannot reflect such changes with time, particularly since they may be affected by a reduced rate of malathion penetration.

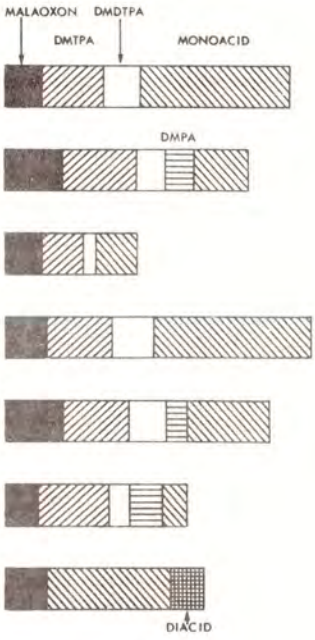
In view of these limitations, and because this preliminary study was intended to explore the complexities of synergist selectivity rather than explain them, it will suffice at this stage to draw attention to those effects on the relative proportions of the different detoxication products which seem of sufficient interest to merit further study.

With the phosphate synergists (Figs 2-5), the results obtained on the two strains and at the two observation times were similar, when allowance is made for the more rapid production of carboxyesterase products in the resistant strain, and the higher overall level of metabolism to be expected at 30 hours compared to 20 hours after treatment. They can therefore be considered together. The effects of the different candidate synergists varied. The beetles treated with malathion and the two compounds, diphenyl phosphate and tri-o-cresyl phosphate, which antagonized malathion against the susceptible strain, produced the lowest levels of malathion metabolites. This antagonism appears to be the result of reduced cuticular penetration, though despite this, diphenyl phosphate synergized malathion against the resistant beetles. Tri-n-butyl phosphorothionate completely inhibited the production of phosphatase products, whereas normal levels of carboxyesterase

Figure 3

Metabolites recovered from batches of 30 susceptible flour beetles 30 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle

SYNERGIST	RECOVERED (µg) MALATHION	METAB-OLITES
NIL	0·75	0·69
TRIPHENYL PHOSPHATE	0·86	0·59
DIPHENYL PHOSPHATE	1·17	0·32
TRIPHENYL PHOSPHITE	0·74	0·74
TRIBUTYL PHOSPHATE	0·80	0·64
TRI-o-CRESYL PHOSPHATE	0·99	0·44
TRIBUTYL PHOSPHORO THIONATE	0·87	0·48



products were formed (Figs 3 and 5). The failure of this compound to synergize malathion may well be due to an inhibitory effect on malathion penetration. It synergized malaoxon, and might well prove to be a selective synergist for organophosphate-resistant strains in which resistance is due to enhanced phosphatase activity, if these should evolve in Tribolium as they have in houseflies.

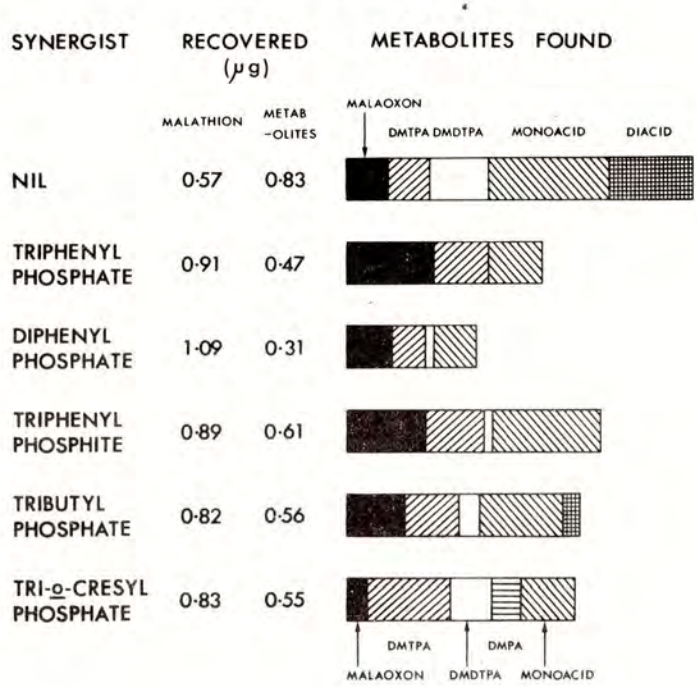
With the pyrethrin synergists (Figs 6-9) again the effects on the different strains and at the different times are comparable, except that piperonyl butoxide appeared to have different effects in the two strains.

The absence of malaoxon in the tests with piperonyl butoxide on the susceptible strain, and sesamex and SKF 525A on both strains, is probably the result of the inhibition of the enzymatic conversion of malathion to malaoxon. This effect of methylenedioxyphenyl synergists on phosphorothionate insecticides has been reported previously in other insects (Nakatsugawa and Dahm 1965) and probably accounts for the antagonism of malathion by these compounds.

In contrast to the methylenedioxyphenyl synergists, SKF 525A completely inhibited the production of phosphatase products, though the yield of carboxyesterase products appeared to be unaffected. This is of interest because both SKF 525A and the methylenedioxyphenyl synergists are inhibitors of microsomal oxidases, and their effects on insecticide metabolism are usually very similar. When the effects of this compound on malaoxon formation were avoided by treating

Figure 4

Metabolites recovered from batches of 30 resistant flour beetles 20 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle



susceptible beetles with malaoxon, synergism resulted.

Whereas with beetles treated with malathion more dimethylthiophosphoric acid (DMTPA) than dimethyl dithiophosphoric acid (DMDTPA) was produced, the reverse was the case with beetles treated with malathion and one of the methylenedioxyphenyl synergists (except with piperonyl butoxide on the resistant strain). This suggested that the oxidation of the dithiophosphoric acid to the thiophosphoric acid was inhibited by these compounds. A small test comparing susceptible beetles treated with the dithiophosphoric acid, and this acid together with piperonyl butoxide or 'Diphopip', tended to confirm this suggestion (Table 2).

Whereas piperonyl butoxide inhibited malaoxon formation and enhanced the proportion of dimethyldithiophosphoric acid formed in beetles of the susceptible strain (Fig. 7), neither of these effects were apparent in the resistant beetles (Fig. 9). This lack of effect of piperonyl butoxide on the metabolism of malathion in the resistant beetles was confirmed in additional tests. It is in accord with the failure of piperonyl butoxide to antagonize malathion in the resistant strain.

These preliminary studies have involved examples of two types of synergist. The phosphate synergists can be regarded as analogues of malathion, comparable perhaps to the use of DMC as a synergist for DDT. It appears that analogue synergists can be specific in that they are effective with only one type of insecticide and may affect only one type of detoxication pathway. They can be contrasted

Figure 5

Metabolites recovered from batches of 30 resistant flour beetles 30 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle

SYNERGIST	RECOVERED (µg) MALATHION	METAB-OLITES	METABOLITES FOUND
NIL	0·30	1·07	
TRIPHENYL PHOSPHATE	0·72	0·71	
DIPHENYL PHOSPHATE	0·98	0·45	
TRIPHENYL PHOSPHITE	0·78	0·96	
TRIBUTYL PHOSPHATE	0·59	0·79	
TRI-o-CRESYL PHOSPHATE	0·68	0·63	
TRIBUTYL PHOSPHORO THIONATE	0·77	0·69	

with the pyrethrum synergists which are structurally unrelated to the insecticides whose metabolism they affect. Methylenedioxyphenyl synergists areknown to affect the metabolism of different types of insecticide including carbamates, pyrethroids, some cyclodiene insecticides, and phosphorus compounds. They inhibit certain microsomal oxidases which affect the metabolism of a variety of drugs (Casida et al. 1966), and may affect the metabolism of a single insecticide in several different ways.

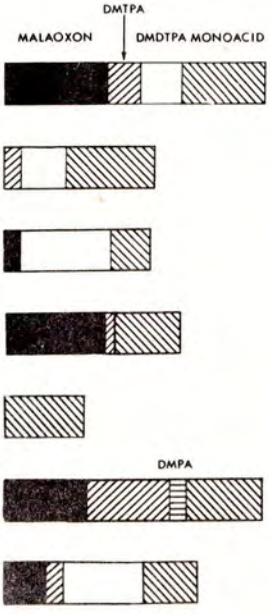
The synergism of organophosphates by non-insecticidal carbamates, and the synergism of carbamates by non-insecticidal phosphorus compounds reported by Plapp and Valega (1967) represents what may be regarded as a third type of synergism. These synergists are not analogues of the insecticide which they synergize, but analogues of other compounds which inhibit the same target enzyme. Some of them, such as s,s,s-tributy-phosphorotrithioate and butyl o-methylcarbanilate, are also effective analogue synergists.

Previous work on SKF 525A indicates it is a non-analogue synergist inhibiting the metabolism of a diversity of insecticides like the common pyrethrum synergists. Its ability to inhibit detoxication by phosphatases which are unaffected by the methylene dioxyphenyl synergists suggests it may possibly represent a fourth type of synergist. Since the organophosphorus insecticides must have affinity with both the esterases which detoxify them and with cholinesterase which they inhibit, it seems probable that alternative substrates for the detoxifying enzymes might be

Figure 6

Metabolites recovered from batches of 30 susceptible flour beetles 20 hours after treatment with 0.05 μg malathion and 0.5 μg candidate synergist/beetle

SYNERGIST	RECOVERED (μg) MALATHION	METAB-OLITES	METABOLITES FOUND
NIL	0·84	0·63	
SESAMEX	1·10	0·36	
PIPERONYL ALCOHOL	1·21	0·35	
S 421	1·0	0·42	
SKF 525A	1·23	0·19	
THANITE	0·73	0·62	
SAFROXON	0·91	0·47	



notably phosphate analogues, and analogues of other cholinesterase inhibitors such as carbamates, but also analogues of acetyl choline itself. SKF 525A might well be regarded as such a substance.

$MeCOO(CH_2)_2N(Me)_3$ — acetyl choline

$C_3H_7(Ph)_2COO(CH_2)_2N(Et)_2^+$ — SKF 525A

Figure 7

Metabolites recovered from batches of 30 susceptible flour beetles 30 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle

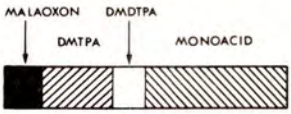
SYNERGIST	RECOVERED (µg) MALATHION	METAB-OLITES	METABOLITES FOUND
NIL	0·77	0·68	
PIPERONYL BUTOXIDE	1·07	0·30	
SESAMEX	0·90	0·63	
PIPERONYL ALCOHOL	0·84	0·50	
S 421	0·84	0·57	
SKF 525A	1·10	0·29	
THANITE	0·59	0·84	
'DIPHOPIP'	0·73	0·87	

Figure 8

Metabolites recovered from batches of 30 resistant flour beetles 20 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle

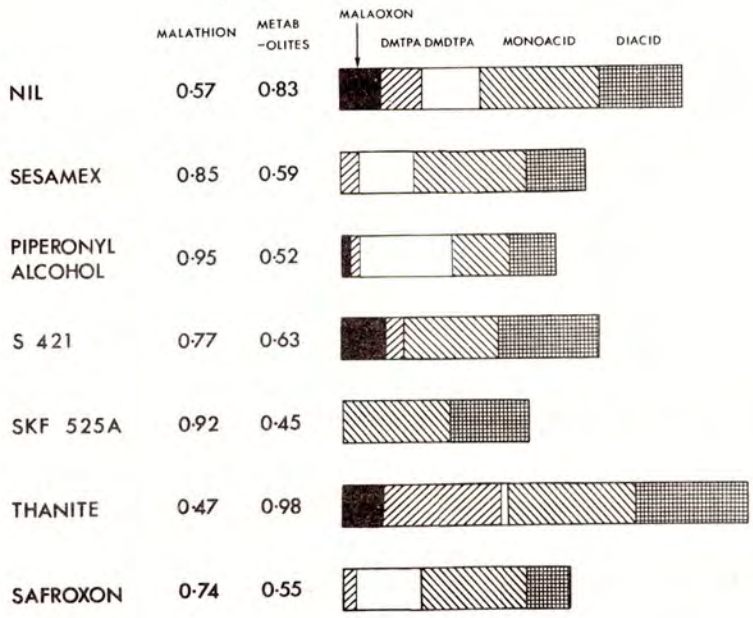


Table 2

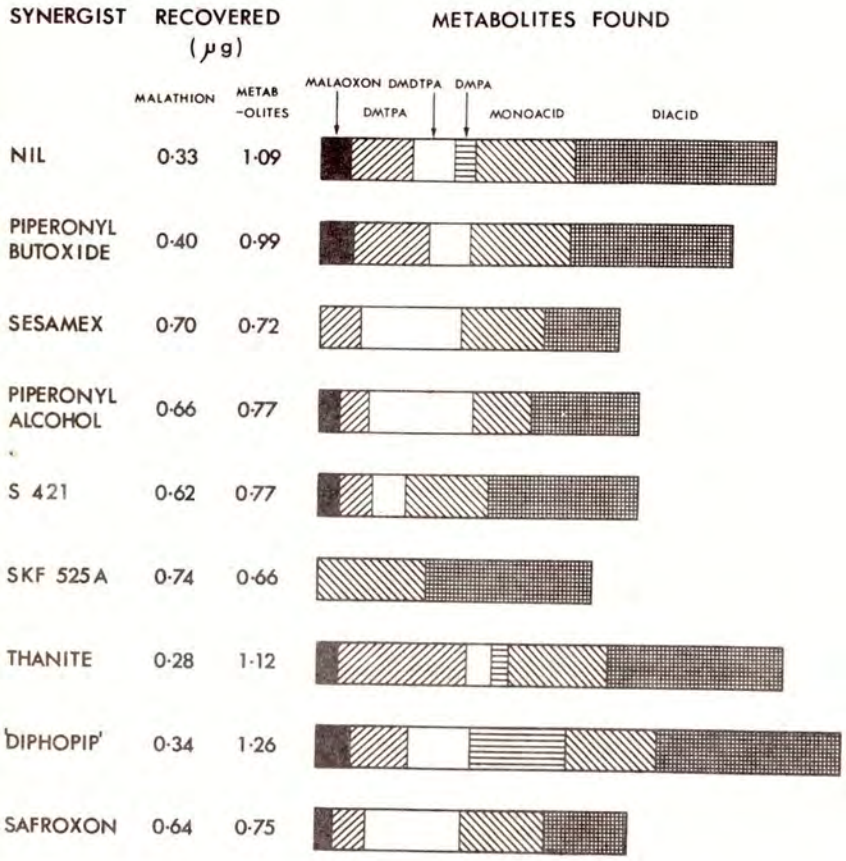
Metabolism of dimethyldithiophosphoric acid (DMDTPA) by susceptible Tribolium castaneum

Batches of 30 beetles were treated with 0.05 µg DMDTPA and 0.5 µg synergist/beetle. Homogenates were examined for metabolites after 30 hours.

Synergist	DMDTPA recovered (µg)	Dimethylthio-phosphoric acid recovered (µg)
None	1.02	0.23
'Diphopip'	1.22	0.09
Piperonyl butoxide	1.28	nil

Figure 9

Metabolites recovered from batches of 30 resistant flour beetles 30 hours after treatment with 0.05 µg malathion and 0.5 µg candidate synergist/beetle



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