STUDIES ON THE POTATO SPROUT SUPPRESSANT CHLORPROPHAM

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CONTENTS

SUMMARY

This thesis is based on studies of problems and consequences which develop from the use of chlorpropham as a potato sprout suppressant. Chapter 1 presents details of the the history of the chemical and its use, both as a herbicide and a sprout suppressant, and how it has become the main chemical in use in the potato processing industry today. A brief overview of the potato industry in the UK is given to enable the reader to understand how chlorpropham fits into sprout suppression in potato storage and also what problems applicable, in particular, to potato storage ensue from its use.

Chapter 2 describes available analytical methods for chlorpropham determination and also the modification of an existing method to suit equipment available in our laboratory. Chlorpropham was extracted from potato samples using hexane as the solvent in the presence of anhydrous sodium sulphate. A new clean-up procedure which allows low level (> 0.035 mg kg^{-1} fresh weight tuber) determination of the compound is also described.

Chapter 3 is a report of a storage and field experiment carried out to ascertain to what extent contamination of potato seed crops with chlorpropham affects subsequent growth. Potatoes of cv. Desiree and Pentland Crown, treated in February, 1977 with chlorpropham adsorbed on alumina to give rates of 0, 0.6, 1.25, 2.5, 5, 10 and 20 mg kg^{-1} potato tubers, were sampled for analysis immediately before planting at the beginning of May. Chlorpropham residues were

determined. Emergence and yield data showed that low levels (< 0.3 mg kg^{-1}) that had little effect on total emergence, increased the mean emergence time and reduced yield. High levels of chlorpropham drastically reduced total emergence and yield.

The results obtained above prompted an investigation into the influence of storage conditions of chlorpropham treated seed on residues and subsequent growth of the treated tubers (mean emergence time, % emergence and yield) and is reported in Chapter 4. This study involved the airing (for 9, 6, 3 and 0 weeks) of chlorpropham treated seed tubers and corresponding control tubers at two temperatures ($8^{o}C$ and $12^{o}C$). Chlorpropham treatment at a rate of 5 mg kg^{-1} was chosen for a number of reasons. Firstly, it produced a large enough effect in the field to be significantly different from controls in the previous experiment and secondly, the residues produced were large enough to enable reductions through airing to be quantified and were similar to chlorpropham residues found in contaminated seed received for analysis. When planted out, all chlorpropham treated seed emerged more slowly than controls; the total emergence in many cases was <100%. Even 9 weeks airing at $12^{o}C$ was unable to improve the performance to equal that of control seed. Once seed tubers have been contaminated with chlorpropham, it is unlikely that any changes in storage conditions will be able to completely overcome the effect of the chemical.

In addition to problems with its effect on seed tubers, chlorpropham is widely used in the potato processing industry and Chapter 5 describes a study of the distribution of chlorpropham in a large commercial box store. Chlorpropham was determined in tuber samples from the store and considerable variation in residues was

found (0.5 - 80 mg kg^{-1} unwashed whole tuber). It was not clear whether the distribution of chlorpropham was due to uneven application or a redistribution of the chemical after application. As it is difficult to remove tuber samples from box store without disruption, a further study, using filter papers placed at various positions in store during chlorpropham aerosol application, was carried out. Again, considerable variation (3.6 - 457 mg chlorpropham deposited on surface) was found, implying uneven application of the chemical.

In Chapter 6, some conclusions are drawn from the results in other chapters and in particular, the effect of new legislation providing statutory limits of residues currently being implemented is discussed. Suggestions for further work are also presented.

DECLARATION

Reports of much of the data in Chapters 3 and 5 have already been published - Boyd *et al.*, (1982) and Duncan *et al.*, (1986).

CHAPTER 1

AN INTRODUCTION TO CHLORPROPHAM AND THE POTATO CROP

This thesis is concerned with the study of chlorpropham and its use on the potato crop, particularly under UK conditions. Chapter 1 will describe chlorpropham, its history and current use as a herbicide as well as a sprout suppressant followed by a similar description of the potato crop to establish a background knowledge of subjects dealt with in the thesis.

Chlorpropham

Chlorpropham (isopropyl-N-(3-chlorophenyl)carbamate), also known as CIPC, is a member of the phenylcarbamate group of herbicides which includes propham (isopropyl-N-phenylcarbamate), swep (isopropyl-N-(3,4-dichlorophenyl) carbamate) and carbetamide (1'-(N'-ethyl-carbamoyl)ethyl-N-phenylcarbamate.

Propham was the first member of the group to be discovered (Templeman and Sexton, 1945) when its action on monocotyledons and not dicotyledons was noted. The chlorinated analogue, chlorpropham, was introduced in 1952 and quickly superseded propham in many uses such as the control of monocotyledons in sugar beet and peas. Chlorpropham is now the major member of the group of phenylcarbamates and is used

world wide. It is difficult to obtain figures for individual pesticides but in the USA alone in 1971, 8,300 tonnes of herbicidal carbamates were used (Green et al., 1977). World wide, the carbamate share of the herbicide market was estimated at 450 million dollars in 1979 (Anon, 1979). The main physical properties of propham and chlorpropham are summarised in Table 1.1. Chlorpropham can be synthesized by the reaction of 3-chloroaniline and isopropyl chloroformate or, alternatively, 3-chlorophenyl isocyanate with propan-2-ol. Both reactions are relatively easy to carry out and present the opportunity of synthesizing radiolabelled chlorpropham labelled either on the ring or the side chain. Neither propham nor chlorpropham is susceptible to alkaline or acid hydrolysis although the use of strongly alkaline or acid reagents can hydrolyse the compounds. Degradation by u.v. is not regarded as a serious problem with either compound; some degradation does occur (Guzik et al., 1978) but is not considered to be a major source of loss of activity under field conditions unlike herbicides such as trifluralin.

Mode of action of chlorpropham

There are two distinct ways of approaching mode of action studies,

1) Biochemical or physiological basis

Changes in e.g. rate of respiration, photosynthesis, RNA synthesis etc. In addition, changes in composition such as amino acids, sugars, proteins, RNA and DNA and hormone levels can all be followed. A major problem with this type of approach is that it is very difficult to decide between a primary cause and its subsequent effects.

2) Physical observation

It can be easier to monitor general growth responses visually and also responses at a cellular (cytological) level can be observed. As these are again complex systems the same problem of differentiating between cause and effect exists.

Ideally, the exact site of molecular interaction should be found but in practice this is very difficult. For many pesticides only approximate sites of action are known. The phenylcarbamates were discovered early on to be mitotic inhibitors but the mechanism of this effect took some time to explain. Initially, experiments to determine the site of action compared chlorpropham to colchicine in that the treatment effects were similar, e.g. arrested metaphases, distorted chromosomes and increased vacuolation etc. However, further study showed that although chlorpropham effects were superficially similar to that of colchicine, its action was not directly on microtubules but instead it acted in some way on the microtubule organising centres (MTOCs) in plants. Present knowledge of microtubules and their role in cell division, cell wall development etc. has been comprehensively reviewed (for example, Gunning and Hardham, 1982) and so only a brief account of their functions will be presented here.

Microtubules

All cells, plant and animal contain microtubules and tubulin sub-units from which the microtubules are composed. Microtubules can be split into three groups involved in

1. cell division
2. cell wall development
3. flagellar motility

Much of our knowledge of how microtubules function has been gained from studies using compounds such as colchicine which has been shown to disrupt the *in vitro* assembly of tubulin to microtubules. Most work with tubulin polymerisation has been with brain tubulin because it can be isolated and form microtubules under optimally defined conditions. Plant tubulin is notoriously difficult to isolate; this difference is generally attributed to variations in sugar side chain links (Fedtke, 1982). It is possible that some difference in action due to these variations may be found and it would be unwise to state that plant tubulin would behave like brain tubulin if it could be used in *in vitro* studies.

Higher plant cells do not possess a centriole but instead rely on a diffuse area known as the MTOC to assist in the parallel alignment of microtubules thus allowing the separation of chromosome pairs to opposite poles of the cell during mitosis. In some way, chlorpropham exerts an effect on the MTOCs causing changes in the alignment of microtubules and therefore affects cell division at metaphase. At present the interaction producing this effect is not known.

Chlorpropham metabolism in soil

Both chlorpropham and propham are soil applied and as they are volatile must be incorporated into the soil to minimise losses due to volatilisation. Many studies have been carried out using laboratory trials in attempts to quantify losses and are reviewed in Hance (1980). As soil acting herbicides, the effects of soil type (Harris and Sheets, 1965), weather (temperature and amount of precipitation), adsorption and pH have all been investigated. Adsorption onto soil components can result in less chemical being available to either the plant or micro-organisms and, in the case of chlorpropham, the soil organic matter content is the major influence on adsorption.

Microbial degradation of chlorpropham is well documented by Kaufman and co-workers (Kaufman and Kearney, 1965; Kaufman, 1967; Kaufman and Blake, 1973; Kaufman, 1977; Fletcher and Kaufman, 1979), and others (McClure, 1974; Marty et al., 1986). The phenylcarbamates are unusual in that other chemicals have been added to improve their use; the persistence of phenylcarbamates in the soil can be increased by the addition of a methylcarbamate - PCMC (4-chlorophenyl-N-methyl-carbamate) due to competitive enzyme inhibition (Dawson, 1969). It is rarely the case that one chemical alone is added to a crop or soil the possibility of effects of one chemical on another must be investigated. Using chlorpropham, both quintozene (Walker, 1970) and carbaryl (Kaufman et al., 1970) have affected persistence.

In soil, chlorpropham is readily degraded to 3-chloroaniline which can be further metabolised or bound, and propan-2-ol and CO_2.

Chlorinated anilines and their bioavailability have been discussed in some detail because a number of pesticides produce these compounds on degradation (Parris, 1980; Freitag et al., 1984). It is possible that condensation of these anilines could take place producing, in the case of chlorpropham, 3,3' dichloroazobenzene (Bartha and Pramer, 1967).

Chlorpropham metabolism in plants

Chlorpropham is effective on monocotyledons but not dicotyledons and many studies have been carried out to ascertain whether and to what extent metabolism, penetration or difference in plant development contributed to the difference in response. Toxicological and environmental implications of chlorpropham metabolism are another reason for examining metabolism in detail. Early on, it was noted that plant responses to chlorpropham treatment varied; resistant and susceptible species were observed. Ries (1953) hypothesized that the selectivity of chlorpropham was due to a substance or substances produced by plant species which lowered the effect of chlorpropham on the plant; the production of this substance varied with plant species.

The effect of chlorpropham on protein synthesis was the main effect found by Mann et al., (1965) when they reported the inhibition of incorporation of ^{14}C labelled methionine into polymeric material in susceptible plants treated with chlorpropham or propham. Resistant plants treated with these chemicals showed either no inhibition or a slight increase. Treatment with barban and swep also resulted in some inhibition in susceptible plants. Prendeville et al., (1968) examined movement and metabolism of ^{14}C labelled chlorpropham in three plant species; they found no differences in mobility of the compound either root or foliar applied and concluded that it was unlikely that transport of the herbicide accounted for differences in susceptibility. A variety of water soluble metabolites and rates of formation of these were noted and it seemed possible that this was more important in selectivity. As both ring and side chain labelled chlorpropham gave similar results, cleavage of the carbamate group was not observed.

Water soluble chlorpropham metabolites were found by James and Prendeville (1969) when they applied chlorpropham to leaf surfaces of several plant species. They found no evidence of cleavage of the carbamate group, no hydroxylation of the ring and identified B glucosides possibly linked through hydroxylation of the alkyl side-chain. In contrast, Still and Mansager (1971, 1972) found no alteration of the isopropyl side chain in soya beans root treated with ^{14}C chlorpropham.

They did find evidence of ring hydroxylation to produce a hydroxy-chlorpropham which was further metabolised to produce an O-glucoside. This was characterized by acetylation, B glucosidase hydrolysis and mass spectrometry (MS) and found to be isopropyl-5-chloro-2-hydroxycarbanilate (2-OH-chlorpropham). No cleavage of the carbamate group was observed. The use of a system consisting of chloroform: methanol: water in the ratio of 2:2:1.8 allowed extraction with a one phase solvent mixture that could easily be separated by the addition of methanol or water to produce a chloroform (organic) phase and a methanol/water (polar) phase (Bligh and Dyer, 1959). The radioactivity which remained unextracted at this point was termed insoluble and assayed by combustion. Differences in root and shoot metabolism were observed; in the root there was a large insoluble fraction and hydroxy-chlorpropham. In shoots, a metabolite or metabolites which may have been dechlorinated appeared. Further work was needed to characterise these metabolites.

Using a milder separation treatment after extraction resulted in the discovery of another ring hydroxylation product, 4-OH-chlorpropham (isopropyl 3-chloro-4-hydroxycarbanilate), (Still and Mansager,

1973a). The dechlorinated products described above were in fact artifacts produced from 4-OH-chlorpropham under the acidic conditions used to separate labelled components in earlier studies. The use of Sephadex columns allowed separation of metabolites without such degradation. After examining the metabolism of chlorpropham in the resistant soya bean and in the susceptible species, cucumber, they hypothesized that the formation of these two OH-chlorpropham metabolites and especially their further metabolism to glycosides and insoluble residues was associated with differences in resistant and susceptible species (Still and Mansager, 1973b). Aryl hydroxylation followed by glucosylation was the main method of detoxification of chlorpropham.

The effect of hydroxylated chlorpropham on cell division was investigated by Davis et al., (1977) and using model systems containing firefly luciferase, Rusness and Still (1974a; 1974b; 1977) concluded the rate of 4-OH-chlorpropham conjugation was the reason for susceptibility of cucumber to chlorpropham. In the studies described above on soya beans, no evidence of side chain hydroxylation was reported; Wiedmann et al. (1976) found the predominant metabolite in soya beans to be hydroxylated on the isopropyl side chain. They speculated that the differences in metabolites might be related to the fact that their plants were soil grown and in all other studies hydroponically grown. However, labelled propham applied to hydroponically grown alfalfa plants has been shown to produce almost equal amounts of 4-OH-chlorpropham, 2-OH-chlorpropham and 1-hydroxy-2-propyl 3-chlorocarbanilate (Zurqiyah et al., 1976) so this is unlikely to be the reason for the side chain hydroxylation differences.

As chlorpropham is applied to potatoes to suppress sprouting, its fate and behaviour in potato tubers has been studied by a number of workers (Jumar and Sieber, 1964; Steinbeiss et al., 1972; Coxon and Filmer, 1985; Heikes, 1985 Worobey and Sun, 1987 and Worobey et al., 1987).

The earlier studies working with radiolabelled chlorpropham applied to tubers could find no evidence of metabolites but a substantial portion of the label remained unextracted, indicating that the compound or metabolites were being bound, conjugated or incorporated into the plant material in some way. 'Bound' or non-extractable residues had been reported in most of the plant metabolism studies described above (Still et al., 1981) and an assessment of the environmental implications of these was carried out by Paulson et al., (1975).

Although the presence of hydroxylated chlorpropham was shown in a number of studies and hydroxylated propham in others working with propham, only the study by Heikes (1985) reported a methoxy group attached to the ring at the 4 position. The amount of 4-methoxy chlorpropham found was considerable 6 weeks after treatment with chlorpropham (4-methoxy-chlorpropham 0.17 mg kg^{-1} tuber).

Until recently, 4-methoxy-chlorpropham was the only metabolite identified in the potato but Worobey and Sun (1987) found trace levels (2 - 39 ug kg^{-1}) of 3,3'dichloroazobenzene and Worobey et al., (1987) found 3-chloroaniline in the peel of chlorpropham treated potatoes. The presence and implications of these metabolites will be more fully discussed in Chapter 6.

Chlorpropham metabolism in animals.

Ryan (1971) and Menzies (1978) reviewed the metabolism of pesticidal carbamates, including arylcarbamates. Since these reviews were published little has appeared in the literature on chlorpropham or propham in animal systems. Most reports that are available concentrated on feeding or dosing trial animals such as rats (Holder and Ryan, 1968; Grunow et al., 1970; Bobik et al., 1972; and Fang et al., 1974) goats (Paulson et al., 1973) sheep (Paulson et al., 1975) or chickens (Paulson et al., 1972; Paulson and Jacobsen, 1974) with single doses of ^{14}C labelled chlorpropham or propham and subsequent isolation and, if possible, identification of major metabolites.

It is possible to summarise the animal metabolism of these two compounds and state that generally, aryl hydroxylation, to form isopropyl-N-(3-chloro-4-hydroxyphenyl) carbamate in the case of chlorpropham, was the most common process (Grunow et al., 1970; Bobik et al., 1972) although side chain alkyl hydroxylation to form 1-hydroxy-2-propyl-N-(3-chlorophenyl) carbamate also occurred. Glucuronides or sulphate esters of the hydroxy-derivatives were the source of the majority of ^{14}C labelled metabolites found in the urine. Hydrolysis of the carbamate group occurred, producing metabolites derived from 3-chloroaniline, e.g. 2-amino-4-chlorophenol and 4-amino-2-chlorophenol (Grunow et al., 1970)

In the studies reported above, most (80%) of the ^{14}C label given to the animals was excreted within 96 hours of treatment, with the largest portion of this being excreted in the 24-48 hour period.

Some workers have voiced concern over results obtained from studies of chlorpropham in isolated cells (Timpson, 1970; Oliver *et al.*, 1978). Both studies showed that chlorpropham could exert a mitotic effect on animal cells *in vitro* but the more worrying aspect was that when chlorpropham was removed, the cells that survived developed multipolar spindles during mitosis and uneven partitioning of genetic material between daughter cells. These effects were time dependent, however, and it is possible that the rapid metabolism of chlorpropham in animals prevents sufficient build up of the chemical in cells *in vivo*.

The metabolism work reported above all describe the fate of chlorpropham or propham administered to animals; in practice, most animals will receive food which has been treated with chlorpropham or propham and therefore be exposed to the range of metabolites produced by plants.

Paulson *et al.*, (1975) fed alfalfa which had been treated with ^{14}C labelled propham to rats and sheep; alfalfa could convert propham to a variety of products and a substantial portion of the label remained unextractable. Of the label added to the roots, 26.4% of radioactivity found in the shoots was as insoluble metabolites and 77% found in roots was also insoluble. The treated alfalfa was split into two portions, one of which was extracted using the modified Bligh-Dyer method described earlier in the plant metabolism section to produce alfalfa containing mainly unextractable or insoluble radioactive label. These two types of alfalfa were fed to the test animals and urine and faeces samples collected. Measurements of the radioactivity showed that non-polar and soluble radioactivity was excreted in the urine; insoluble radioactive residues, on the other

hand, passed through the gut with apparently little uptake, suggesting that the insoluble metabolites produced by plants were not readily available to animal systems and were excreted in the faeces.

Bearing in mind the relatively large proportion of non-extractable radioactive label reported in the work of Coxon and Filmer, (1985) in studies on chlorpropham metabolism in potatoes, it seems that further investigation of this aspect is warranted.

The potato crop

World production of potatoes (*Solanum tuberosum*) is on average 300×10^6 tonnes per annum (Dalton, 1978). It is a mainly European crop with the U.S.S.R. also a major grower. Each year in the UK the potato crop harvest is expected to be approx. 6 - 7 million tonnes. The acreage grown is strictly controlled by the Potato Marketing Board (PMB) to avoid overproduction. Approximately 25% of this total will be processed in some way and over 10% goes to the crisping industry alone (Anon, 1986). As processors need a reliable source of material year round, many obtain their supplies by contract and then store the material themselves since storage requirements for the processing industry differ from that of ordinary ware storage.

In the UK maincrop seed potatoes are planted out at the end of April and are harvested late September onwards. Seed (32-52mm) and ware (45-85mm) tubers are separated at a grading stage where damaged, green or diseased tubers are removed. Depending on the scale of the operation, the tubers are then stored in small stores or transported to large centralised stores where because of damage caused by harvesting and grading procedures, they are allowed to wound heal for several weeks. This healing time is also known as curing. The wound healing process in a potato tuber results in a sealing off of wounded, diseased or cut tissue through the process of suberisation and cell division. Most sprout suppressant chemicals affect wound healing adversely and for this reason cannot be applied until after wound healing has occurred or applied in special slow release formulations to ensure adequate time for wound healing. Many diseases can attack potatoes in store and depending upon the investment in the stored

Table 1.1 Some physical properties of sprout suppressant chemicals.

	chlorpropham	propham	tecnazene	maleic hydrazide
molecular weight	213.70	179.22	260.96	112.10
vapour pressure at 25°C (Pa)	0.039	-[a]	0.06	not volatile
SVC[b] at 25°C ($mg\ m^{-3}$)	3.36	-	6.32	-
SVC[b] at 10°C ($mg\ m^{-3}$)	0.54	-	-	-
solubility in water at 25°C ($g.dm^{-3}$)	0.089	0.025-0.25	practically insoluble	6
melting point (°C)	41.4	87-88	99	296
boiling point (°C)	247 (with decomposition)	sublimes at R.T.	304 (with decomposition)	-

[a] not available

[b] saturated vapour concentration.

Most of the data gathered in this table can be found in the Agrochemical Handbook (Hartley and Kidd, 1983).

tubers, fungicides and bacteriacides may be applied at this stage in addition to a sprout suppressant. Examples of these include thiabendazole (2-(thiazol-4-yl)bezimidazole, TBZ) to reduce dry rot, gangrene, silver scurf and skin spot, dichlorophen (5,5'-dichloro-2,2'-dihydroxydiphenylmethane, RE49) which has broad spectrum bacteriacidal action and 2-aminobutane (2-AB) to reduce skin spot and gangrene.

When potatoes are harvested and put into store there is a natural lag phase where sprouting will not occur even if environmental conditions are favourable for growth. The length of this resting period as it was termed by Emilsson (1949) is cv. dependent. Once this phase has passed then sprout growth will commence unless controlled by some means such as temperature, chemical suppressants, irradiation or controlled atmosphere storage. Of these alternatives, at present only temperature control and chemical control are practised to any extent. At store temperatures of ca. $4^{o}C$, sprouting is inhibited. However, at this temperature biochemical changes in the tuber lead to low temperature sweetening which cannot be tolerated in potatoes stored for the processing industry and in particular the crisping industry. The processing of the tubers into crisps involves frying thin potato slices at ca. $180^{o}C$; at this temperature reducing sugars in the tubers react with free amino acids in the Maillard reaction and form dark brown coloured polymeric material which gives the crisp its characteristic golden colour. The reducing sugar concentration of the slices is the rate determining step for the reaction as the free amino acids are normally present in excess. Low temperature sweetening results in a large increase in the reducing sugar concentration and subsequently, unacceptable dark brown coloured crisps are produced.

The processing industry must, therefore, store potatoes at a temperature high enough to minimise low temperature sweetening and also low enough to minimise sprouting and water loss; in practice, a temperature of 7 - 8°C is considered optimal.

At this temperature, long term storage of tubers is practicable only through the use of sprout suppressant chemicals. It would be fair to say that if sprout suppressants were to be banned immediately, the processing industry and especially the crisping industry would be unable to produce an acceptable product for the major part of the year.

Types of store

Potato storage is normally confined to two main designs of stores and some less popular or older methods.

1. Bulk storage

In bulk storage as the name suggests, the potatoes are stored in bulk in large specially constructed or adapted stores with walls thickened to withstand the pressure generated by the potatoes when loaded into store. Temperature control in these stores is reasonable and fluctuations can be corrected by ventilation either internal recirculation or bringing in an amount of outside air dependent on the temperature wanted. To allow for the possibility of outside air being too warm for cooling some modern stores have refrigeration units built in, but these are generally confined to long term stores where the extra expense can be justified. Chlorpropham can be applied to bulk stores in a relatively straightforward manner; the fog produced by the

applicator can be passed down a main air duct and then along the ventilation ducts which are positioned lengthwise along the floor of the store at distances determined by the height of the stack. In these stores the chlorpropham fog has no other obvious route of travel and eventually seeps through the bulk of the potatoes. The main disadvantage of this type of storage system is that it is difficult to know what is going on further down the pile of potatoes. If rotting does occur it can be some time before it can be detected by smell and then a large section of the store must be emptied to find it. The sheer size of some bulk stores can also be a problem when the amount of time taken to fill the store and then allow adequate time for wound healing has resulted in the first tubers into the store being held at elevated temperatures long enough to break dormancy. This problem was one of the reasons behind the development of a first tecnazene sprout suppressant treatment.

2. Box storage

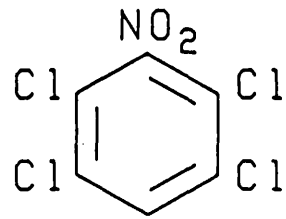
In this type of storage system the potatoes are loaded into 0.5 or 1 tonne wooden boxes which are stored in stacks up to 5 or 6 high. Despite the initial outlay on wooden boxes which is offset to some extent by no need to strengthen walls, this method of storage originally mainly for seed growers, has proved popular among processors. The control of temperature in this storage system is better than that of bulk stores and for the reasons already discussed, this is important in the processing industry. Boxes from individual growers or fields can be identified and readily removed if necessary so control of material is much greater. Disease and sprouting can be recognised more quickly by the store manager and steps taken to deal

with it. One major disadvantage is the problem of chemical application to the store during storage. Chlorpropham applied in a fog can be deposited throughout the store but problems with distribution of the chemical can occur (see Chapter 5 for more details). Also the quantity of tubers which can be stored is less than a similar bulk store.

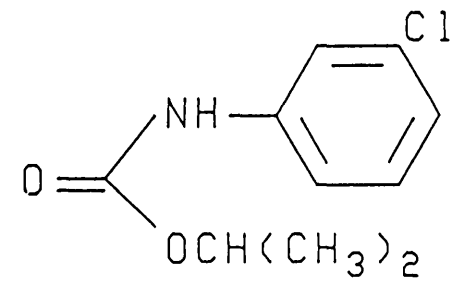
Other systems of storage include clamps, Dickie pie clamps and bin storage (Bishop and Maunder, 1980). These are not discussed here because chlorpropham use is not as common in these storage systems; tecnazene is preferred.

By the time the potato or its product is purchased by the consumer, it has been exposed to a number of chemical treatments designed to improve yield and quality. When the plant is growing, herbicides and fungicides are applied to the soil and foliage respectively. Once harvested however, any chemical treatment is carried out directly on the tubers which are destined for consumption.

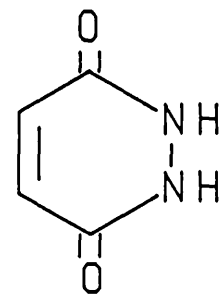
At present, there are only 3 chemicals which can be used as sprout suppressants on potatoes, tecnazene, chlorpropham and propham. A fourth chemical, maleic hydrazide has gained limited clearance at present. This chemical is unusual because it must be applied during the growing season to the foliage where it is translocated down to the tubers to inhibit sprouting in store. It is popular in North America and Australia but its use in the UK has been confined to onions because of toxicological worries over certain formulations. A summary of the main points of interest regarding the use of these chemicals is shown in Table 1.2.



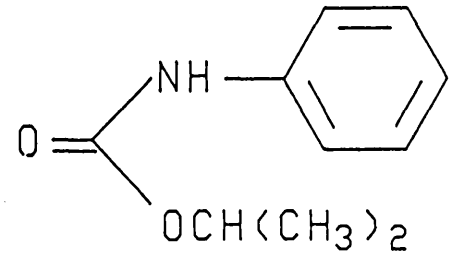
Tecnazene (TCNB)



Chlorpropham (CIPC)



Maleic Hydrazide (MH)



Propham (IPC)

Fig.1.1 Commercial potato sprout suppressant chemicals.

The chemicals shown in this table were not developed as sprout suppressant chemicals originally. After being accepted as agrochemicals for some other purpose, their use was then extended to that of sprout suppression of potatoes. Approximately £10 million was needed to introduce a new chemical onto the market in 1982 and large agrochemical firms involved in research will only consider developing new chemicals for markets such as cereals or soybean where returns can be made world-wide. Despite an improvement in our knowledge of pesticides and their effects, the possibility of tailoring specific pesticides to specific functions becomes less due to the costs involved in developing and marketing the product. This also means that most pesticides being marketed at present must have a wide range of activity or the chemical company would never have developed them that far.

The chemicals commonly used as sprout suppressants have been on the market for some time and although cleared for use on potatoes there is always the possibility of new research work revealing a problem.

Table 1.2 Comparison of main sprout suppressant chemicals and a possible new sprout suppressant.

Name	chlorpropham	tecnazene	maleic hydrazide	dimethylnaphthalene
Chemical name	isopropyl-N-(3-chloro-phenyl)-carbamate	2,3,5,6 tetra-chloronitrobenzene	1,2 dihydro-3,6 pyridazinedione	1,4 dimethyl naphthalene
Abbreviated name	CIPC	TCNB	MH	DMN
Mode of action	mitotic inhibitor	not known	mitotic inhibitor	not known
Uses	herbicide	fungicide	growth regulator	-
Formulations available	liquids BL500 (Wheatley) Mirvale 500 (Ciba-Geigy) MSS CIPC 50M (Mirfield) granules also available CIPC/IPC mixtures Atlas Indigo (Atlas Interlates) Pommetrol M (Fletcher)	dusts Arena 3 and 6 (Tripart) Fusarex (ICI) Bygran S (Wheatley) Hortag (Avon Packers) granules Fusarex (ICI) Hortag (Avon Packers) liquids (with thiabendazole) Hortag tecnazene plus (Avon Packers) Hytec Super (Agrichem) Storaid Storite SS (MSD Agvet) Fogging liquid Nebulin Wheatley	liquid	-
Amount needed for sprout suppression for 3-4 mo. at 10°C	10-30 g tonne^{-1}	135 g tonne^{-1}	-	100 g tonne^{-1}

Table 1.2 (cont.)

Approx. cost of one application	£0.40 tonne^{-1}	£2.00 tonne^{-1}	£1.40 tonne^{-1}	-
Ease of re-application	easy - call in contractors to re-apply	very difficult as powders or dusts fogable formulation available but low active ingredient	not possible	potentially easy to apply
Effect on wound healing	yes - curing period needed after harvest and before application	no - can apply as potatoes are loaded into store	may affect wound healing process but not possible to apply later	yes - some curing period needed
Is it used as a sprout suppressant in other countries?	yes - world wide use although some countries restricted use	not generally used in Europe or USA	very popular in USA not approved for use in U.K. - limited clearance only	Holland and U.K. looking at possible approval

Tecnazene

Tecnazene is the trivial name given to 2,3,5,6 tetrachloronitrobenzene. It was first introduced as a fungicide against dry rot (Fusarium spp.) and subsequently used as a sprout suppressant on ware and seed potatoes (Dalziel, 1978). The level needed to suppress sprouting is approximately 135 mg kg^{-1} although lower rates are employed in some formulations designed for short term control. The chemical has been formulated in many ways including foggable liquids, granules and powders.

Of the chemicals currently approved for use as sprout suppressants, tecnazene is unusual in that it is widely accepted that it does not inhibit wound healing of potatoes (Leonard et al., 1986; Burton, 1966). Consequently, it can be applied as potatoes are loaded into store after harvest and before wound healing has taken place. Because of the ease of application as potatoes are loaded into store, the chemical is very popular among farmers and small scale storers of potatoes.

For a summary of the main physical characteristics of tecnazene, see Table 1.1. The mode of action of tecnazene is, as yet, unknown and little has been reported on plant and soil metabolism studies. However, pentachloronitrobenzene (quintozene) is widely used as a soil sterilant and its metabolism in soil has been investigated to a greater extent. A knowledge of the degradation products of quintozene can be applied to tecnazene; the products produced by quintozene in soil include anisoles, thioanisoles, and anilines. Heikes et al., (1979) found tecnazene and related compounds in potatoes.

In the last 5-10 years tecnazene has become popular as a first sprout suppressant treatment when potatoes are put into store; subsequent control is obtained by chlorpropham applications. The use of tecnazene as the first treatment allows processors to fill stores and maintain good sprout control and as skin spot type blemish which appears late in the storage season has been linked with time of initial chlorpropham application, the appearance of the blemish as a problem is delayed (typically from Feb. to June) until later in the season (Leonard et al., 1986).

Substituted naphthalenes

1,4 dimethylnaphthalene (DMN) has been identified as a volatile component produced by potatoes and shown to have sprout suppressant properties (Meigh et al., 1973) in a simple bioassay. Studies carried out on a number of volatile chemicals which looked promising in the bioassay showed that although active, many were too volatile for use commercially because the effect wore off quickly as the chemicals dissipated. DMN was the only chemical tested which could maintain sprout control over a storage season and then allow tubers to be planted out and grow and looked promising from a toxicological point of view (Beveridge, 1981). Sprout control under commercial conditions in ware stores was also possible.

Since then, a number of workers have looked at DMN as a sprout suppressant but development of the chemical has been hampered by the fact that it is impossible to obtain a patent due to prior publication of its sprout suppressant properties.

Studies on a number of analogues or derivatives which could be patented have shown that many other substituted naphthalenes are

active as sprout suppressants. (Stephen and Duncan, 1984). Trimethylnaphthalene (TMN) was shown to be at least as active as DMN (Filmer and Rhodes, 1984) but unfortunately could not be patented for the same reasons. Attempts to explain the order of activity on a structural basis have failed; most substituted naphthalenes will suppress sprouting to some extent. Attempts to relate headspace concentrations of these chemicals with sprout suppressant activity have shown that the chemicals which are least successful as sprout suppressants also produce the smallest headspace concentration (O'Hagan et al., 1987).

The information available on the toxicology of DMN would suggest that further development of the chemical should proceed (Beveridge, 1979). The effect of DMN on some of the major quality factors in tubers has been studied and compared with both tecnazene and chlorpropham; no significant differences were found between the sprout suppressant treatments in terms of reducing sugars (glucose and fructose) and sucrose concentrations studied over a number of storage seasons and cvs. (Boyd and Duncan, 1984). Some evidence that DMN might inhibit wound healing, admittedly to a lesser degree than chlorpropham, suggests that until this aspect has been more fully investigated a short curing period after harvest should be observed with DMN.

Maleic hydrazide

Maleic hydrazide (MH) is the name given to 6-hydroxy-3-(2H)-pyridazinone. It has been used as a growth regulant for a number of years in the USA on tobacco, onions and as a potato sprout suppressant. Recently, MH was cleared for limited use in the UK on

potatoes after some years of use on onions. It is unique among sprout suppressants in that it is foliar applied and translocated to the tubers. One treatment at a rate of 1.7 kg ha^{-1} is sufficient to retard sprouting until the end of the storage season (Burton, 1966). The mode of action of MH appears to involve the ability of the compound to be incorporated into nucleic acid (Corbett, 1984). It is generally accepted that cell division but not cell enlargement is inhibited.

Although at first MH would appear to have many advantages over chlorpropham as a sprout suppressant a serious problem is the timing of application to the growing crop. MH has been used in the USA for a number of years and most of the information on application comes from American sources. For optimum performance the chemical must be applied as flowering is completed, and this results in a period when the potatoes can be sprayed of only, at maximum, two weeks. In the UK conditions for spraying can often be unsuitable for more than two weeks at a time especially in the north and west of the country. If spraying is carried out too early or too late then growth abnormalities can be observed in the crop (Poapst and Genier, 1970). One problem associated with the use of a translocated compound as a sprout suppressant is that residues in the tuber are inevitably higher than those resulting from sprout suppressants applied to the surface of he tuber after harvest. Residues of 6 mg kg^{-1} have been shown to retard sprouting for 6 months but residues of up to 40 mg kg^{-1} can be obtained with commercial formulations (Franklin and Longheed, 1964). It should be noted that these residues cannot be reduced by peeling as is the case with the alternative chemicals.

Propham

Propham (isopropyl-N-phenylcarbamate, IPC) is never used on its own as a sprout suppressant, it is always found in mixtures with chlorpropham. Propham is the more volatile of the two chemicals and much of its popularity stems from the belief that active sprout suppressant headspace levels can be achieved more quickly with this component in the formulation. Analysis of a PMB survey of chemicals on stored potatoes showed that propham/chlorpropham formulations constituted 20% of the amount of chlorpropham applied (Anon, 1981). As the compound is not used on its own, any comments regarding its effects on tubers or tuber quality are best left to the section on chlorpropham use on potatoes.

Chlorpropham

Chlorpropham was first introduced as a sprout suppressant by Marth and Schultz in 1950 and quickly became the most widely used sprout suppressant in North America and Europe. Only in the UK which has tecnazene as an alternative has its use been generally restricted to larger storage units. Its success can be attributed to a number of factors among which are its potency, cheapness, and ease of application to potatoes already in store. Problems with its use include its effect on wound healing in potatoes (Cunningham, 1953; Audia *et al.*, 1962; Reeve *et al.*, 1963; Leonard *et al.*, 1986) with resulting problems of increased disease and rotting if potatoes are not allowed to cure completely before application and some increase in internal sprouting under certain conditions (Hruschka *et al.*, 1965;

Ewing et al., 1968) although Sawyer and Dallyn (1964) maintained it was an environmental problem.

Chlorpropham effects on tuber components were observed by Ponnampalam and Mondy (1986) who showed a significant increase in the phenolic content of tubers; the ascorbic acid content generally decreased following chlorpropham treatment although this response was more cv. dependent.

The effect of chlorpropham on sugar, especially reducing sugar concentrations in potatoes has been studied by a number of workers with conflicting results (Zaehringer et al., 1966; Baijal and van Vliet, 1966; Burton, 1966; Moll, 1968; Dalziel, 1978; Boyd and Duncan, 1981). This is not surprising considering the complexity of the subject of carbohydrate metabolism in tubers. Generally, chlorpropham treatment decreased the reducing sugar concentration compared with controls over the bulk of the storage season. By April/May, senescent sweetening of the potatoes, which is to a large extent dependent on the physiological age of the tuber, can occur (Burton, 1978) and if this process does occur then sprout suppression will exacerbate the problem.

As this thesis is concerned with problems associated with the use of chlorpropham on potatoes, a method of quantifying the chlorpropham present in potatoes was required. This will be discussed in Chapter 2.

CHAPTER 2

THE DEVELOPMENT OF AN ANALYTICAL TECHNIQUE FOR CHLORPROPHAM

2.1 Introduction

Any analytical method used to determine chlorpropham must be extremely sensitive because chlorpropham can affect plant growth at very low levels. One of the main problems found with chlorpropham use in this country has been the occurrence of contamination of seed tubers which obviously affects their growth and development. The method chosen for routine analysis of tubers has to be capable of determining chlorpropham at < 0.2 mg kg^{-1} fr. wt. tubers.

Part of this chapter is concerned with a survey of methods available for chlorpropham determination under a wide range of conditions i.e. some developed for routine screening of freshly treated tubers and others for extremely sensitive but time consuming measurements. Before the available methods are described, a section on general principles and procedures in pesticide determination should be helpful. Analytical textbooks cover the basic principles involved in any analytical problem (see, for example, Fifield and Kealey, 1983 and Beyermann, 1984). Although the basic procedure involved in the determination of a substance will be discussed briefly below, the reader is referred to one of the textbooks mentioned above for more

information. All analytical procedures, including pesticide determination involve four basic steps,

Sampling

Extraction

Purification (clean-up)

Determination of the compound using one or a combination of techniques available to the analytical chemist.

Involved in these basic steps are a number of statistical procedures which should be carried out to ensure the accuracy and precision of the result is known. Checks of the method using blanks, spiked samples and replication are all included in deciding whether a particular technique would be suitable or not.

The first and most important step in any analytical procedure is sampling. No matter how accurate and precise a method is, it is of little use if the sample taken from the original material is unrepresentative or has changed. Sampling can be carried out in two stages. Firstly, a sub-sample often taken from field or store must contain an adequate number of tubers to allow for the inevitable variability in residues. The amount of sample to be taken at this stage is always difficult to determine and experience, recognised statistical procedures and even legal requirements can all be involved in the decision. There are procedures to minimise the possibility of unrepresentative samples being taken

1. Field collection of samples - always over sample if at all possible and if little is known of variability. Often, the laboratory chemist has no control over this stage.

2. Once samples reach the laboratory, they must be further sub-sampled to allow residue determination to take place. Procedures for this are already available and include:-

a) coring

b) quartering

c) mincing, macerating or homogenising

Generally, in studies reported in this thesis, samples were minced and then re-sampled after mixing to obtain representative samples of tuber material. The decision to wash or peel the tubers obviously must be taken before this stage.

Before the other stages of chlorpropham determination are discussed, it would be helpful to review the existing literature on chlorpropham determination. As many analytical methods as possible were included as examples but the survey cannot claim to be fully comprehensive.

Existing techniques for chlorpropham determination.

As a herbicide commonly used all over the world, many methods of analysis for chlorpropham have been developed and used over the years since its introduction in 1951. These techniques can be roughly classified according to the final technique used to determine chlorpropham and include

a) spectrophotometric methods of analysis.

b) direct techniques of measurement such as infra-red spectroscopy (IR), gas chromatography (GC) and high pressure liquid chromatography (HPLC).

Due to its widespread use, chlorpropham has often been included in multi-residue studies. Papers describing the analytical techniques used in these studies have not been included here because it was felt that as they were developed to include as many pesticides as possible, the efficiency of extraction and/or method of determination of any particular pesticide could be compromised. However, some examples of multi-residue studies which include chlorpropham are those of Friestad (1974), Johansson (1978), and Luke et al., (1981)

a) Spectrophotometric methods of analysis for chlorpropham.

The spectrophotometric methods of analysis for chlorpropham may be divided into 3 separate stages for easier discussion. These stages include

(1) The extraction of chlorpropham from plant materials using a convenient solvent.

(2) Hydrolysis (either alkaline or acidic) of chlorpropham to produce 3-chloroaniline, isopropanol and CO_2. As chlorpropham is resistant to hydrolysis, this step involves the use of strongly alkaline or acidic reagents.

(3) Quantitative determination of the 3-chloroaniline produced on hydrolysis, by the addition of a reagent causing the development of a colour.

Bissenger and Fredenburg (1951) described a method similar in outline to that listed above where the final colorimetric determination was of the blue coloured complex formed by the reaction of an amine (in this case, 3-chloroaniline) with calcium hypochlorite and phenol.

This same basic procedure was followed by others with some minor modifications (Gard and Rudd, 1953; Gard et al., 1954; Gard and Reynolds, 1957; Gard et al., 1959 and Gard , 1959).

In this earlier work, some of the references actually refer to propham with the assumption that chlorpropham determination could be carried out using exactly the same method.

Kroller (1962) discussed the problems of chlorpropham determination using a colorimetric method optimised for propham. He noted that 3-chloroaniline often produced a green or blue/green colour in the phenol/hypochlorite reaction whereas aniline (produced by the hydrolysis of propham) gave the expected blue colour. Also, the presence of heavy metals such as chromium, manganese and iron were found to have an effect on the blue colour of the aniline complex produced by the reaction of the hypochlorite and the phenol/ammonium hydroxide solution. He concluded that the use of an alternative colorimetric method, diazotising the 3-chloroaniline and subsequently coupling this intermediate product with naphthylethylenediamine to produce a red colour, could be less prone to these interferences.

In fact, this colorimetric method had previously been used by Montgomery and Freed (1959) for the estimation of propham in strawberries.

Following difficulties with the extraction of propham from strawberries, these authors employed direct hydrolysis of the plant material and steam distillation of the aniline produced on hydrochloric acid and the final determination by treatment with nitrous acid followed by coupling with 1-naphthylethylenediamine to produce a red coloured complex which was measured in a

spectrophotometer at 500nm.

Previously to this, Merz and Kammerer (1958) had reported the use of the coloured complex formed on the reaction of p-phenylenediamine with aniline to determine trace amounts of propham. Nultsch (1959) used a modified version of this procedure to determine levels of chlorpropham in potatoes; recovery of chlorpropham was low (33%) when spiked with less than the equivalent of 1 mg kg^{-1} tuber.

Other modifications of this method were reported by Gard and Ferguson (1963) and Ferguson and Gard (1969) in chlorpropham residue studies with recoveries of chlorpropham ranging from 80-120% in spiked samples (i.e. untreated plant material to which is added known quantities of chlorpropham). The minimum detectable amount (MDA) quoted by these authors was 0.05 mg chlorpropham per kg of fresh tuber.

Ercegovitch and Witkonten (1972) introduced a cellulose column clean-up of the final red coloured complex after encountering problems with variable blanks-untreated control samples. The use of this column resulted in a significant increase in the reliability and efficiency of analysis.

As can be noted from the review, methods of chlorpropham analysis based on colorimetric determination, have been changed and/or modified over the years in attempts to overcome problems that have appeared. Among the drawbacks of these colorimetric methods of analysis is the need for untreated control samples. These are of the utmost importance because they act as blanks for the analytical method. Obtaining untreated samples grown or stored under the same

conditions as the suspect plant material may prove difficult if not impossible.

In addition, the colorimetric methods of analysis are all based on the hydrolysis of chlorpropham to 3-chloroaniline and its subsequent quantification. Any other aniline based pesticide susceptible to hydrolysis may interfere. This interference is not confined to pesticides containing the 3-chloroaniline moiety because the extinction co-efficients of 2-chloroaniline, 4-chloroaniline and aniline itself are sufficiently close to that of 3-chloroaniline to cause problems.

In recognition of these problems, other methods have been devised in more recent years, based on the measurement of the intact chlorpropham molecule.

b) Direct measurement of chlorpropham.

Infra-red spectroscopy was used by Ferguson et al., (1963) to follow chlorpropham residues in potato tubers treated with the sprout suppressant. Although specificity towards chlorpropham was improved compared to colorimetric techniques, sensitivity was not (minimum detectable amount (MDA) 0.5 mg kg^{-1}).

Despite difficulties reported by Romagnoli and Bailey (1966) in the gas chromatographic determination of chlorpropham because of the instability of the compound at temperatures greater than 230^{o}C, this technique has since proved popular with other workers (e.g. Onley and Yip, 1971). However, problems with the GC of the intact molecule resulted in many workers attempting derivatisation of some sort. Gutenmann and Lisk (1964) reported a method involving electron

affinity gas chromatography of brominated anilines produced by hydrolysis of chlorpropham and subsequent derivatisation. The sensitivity of this method was quoted as 0.02 mg chlorpropham kg^{-1}. As hydrolysis of chlorpropham is involved, this method of analysis suffers from some of the drawbacks mentioned in connection with colorimetric analysis.

Caverly and Denney (1978) described a method for chlorpropham determination in soil which involved an acetone extraction followed by alkaline hydrolysis, steam distillation and concentration of the resultant 3-chloroaniline in toluene. Subsequently, the 3-chloroaniline was partitioned into hydrogen bromide and the brominated derivative determined by electron capture (EC) GC. A diazotisation step before derivatisation could remove any interfering anilines. This method has been used by Agricultural Development and Advisory Service (ADAS) analysts to determine chlorpropham levels in potatoes. The extraction of chlorpropham from peel or whole tuber samples was accomplished by maceration with acetone followed by extraction into chloroform and drying with anhydrous sodium sulphate. The solvent was removed and the chlorpropham either redissolved in acetone for determination by nitrogen specific (N) FID (MDA 0.05 mg kg^{-1}) or derivatised following the method of Caverly and Denney and determined by EC GC (MDA 0.01 mg kg^{-1}).

The above workers and others have taken advantage of the fact that most chlorpropham is concentrated in the peel of the tubers. Peelings from the potato samples would concentrate the chlorpropham by a factor of *ca.* 10, thus reducing the need for extremely sensitive techniques (Corsini *et al.*, 1978).

Lawrence and co-workers alkylated chlorpropham (both methylation and trifluoroacetylation) at the N-position and subsequently determined the product by GC (Lawrence and Laver, 1975; Lawrence, 1976a; Lawrence, 1976b).

Van Vliet and Hertog (1966) reviewed methods for chlorpropham determination and discussed the merits of direct steam distillation (not following hydrolysis) of chlorpropham from plant material in comparison with the extraction of the chemical using a suitable solvent, both followed by determination of the chlorpropham using GC.

Direct determination of chlorpropham by gas chromatography using flame ionisation detectors (FID) was described by Vogel and Deshusses (1965) although the sensitivity of the method was no greater than that of existing colorimetric methods. The analytical method consisted of a Soxhlet extraction of potato tubers using chloroform in the presence of sufficient anhydrous conditions.

Cerny and Blumenthal (1972) concluded that the lack of sensitivity in the above procedures was due to the fact that the solvent chloroform could extract many other components of the tubers. To improve sensitivity they suggested that the use of hexane in the presence of anhydrous sodium sulphate would result in extracts which could be more easily purified using an alumina column. Following this procedure they found that 0.02 mg chlorpropham kg^{-1} could be detected by GC using nitrogen specific detectors. A simplified method for determining chlorpropham residues in large numbers of samples was reported by Corsini et al., (1978). This involved the extraction of ground frozen potato peel for 20 hours using petroleum ether, concentration of the petroleum ether extract and subsequently

determination of chlorpropham using GC equipped with FID. The method was not particularly sensitive (MDA >1 mg kg^{-1} tubers) but was straightforward.

The use of high performance liquid chromatography (HPLC) in pesticide analysis has increased especially in the determination of compounds difficult to analyse by GC methods e.g. polar, non-volatile or thermally labile compounds. At the time of this project little had been reported on the use of HPLC for chlorpropham determination apart from a Waters Associates technical bulletin (Anon, 1976). Since then a number of studies have been published (e.g. Wilson et al., 1981; Pena and Sanchez, 1982; Krause, 1983). HPLC has been used in plant and animal metabolism studies because of its ability to separate and detect polar metabolites but at the time of this work no HPLC was available.

Development of an analytical method for chlorpropham.

For the studies reported in this thesis an accurate, sensitive, analytical method for chlorpropham quantification was needed. The methods described in the previous section either did not meet the above requirements or involved the use of laboratory equipment which was not readily available. The remainder of this chapter is devoted to finding a suitable method.

Firstly, the extraction of chlorpropham from potato tubers was necessary and suitable solvents were examined.

As GC equipped with FID were available in the laboratory, it was decided at an early stage that the final determination of chlorpropham

would be by GC. This meant that any tuber extracts would have to be cleaned to allow the quantification of low levels of chlorpropham by FID. Various column purification procedures were investigated.

2.2 Experimental.

2.2.1 Extraction of chlorpropham from potatoes.

2.2.1.1 Introduction.

The use of a selection of common solvents such as chlorinated hydrocarbons and lower alcohols in the extraction of chlorpropham from potato tubers was investigated. Three promising solvent extraction procedures were evaluated in turn. These consisted of

(a) polar organic solvents which could easily be reduced in volume using a rotary evaporator.

(b) ethanol extraction of chlorpropham followed by the partitioning of chlorpropham between ethanol and hexane.

(c) direct hexane extraction of chlorpropham from tubers in the presence of sufficient drying agent to maintain anhydrous conditions.

The efficiency of the extraction procedure was initially checked by determining the amount of chlorpropham recovered after the addition of the chemical to a potato sample which was known to contain no chlorpropham. GC was used to quantify the chlorpropham recovered. As 1 mg chlorpropham was used to spike the potatoes a clean-up procedure was not necessary at this stage.

2.2.1.2 Experimental.

Materials and methods.

All solvents and chemicals used were of Analar (AR) quality (Hopkin & Williams, England) unless stated otherwise.

Sample preparation.

A large quantity of tubers was washed and comminuted using a mincer (Model AL 2-1, Bauknecht, W. Germany). The minced material was stirred vigorously to ensure homogeniety and sub-samples were weighed out as needed. Each sub-sample was spiked with exactly 1 mg chlorpropham prior to extraction.

Extraction procedure.

a) polar organic solvents.

50 g sub-samples of spiked minced tubers were blended for 1 minute at high speed in an electric blender (Ato-mix, MSE, England) with 150 cm^3 of solvent. This procedure was carried out with ethyl acetate, dichloromethane and chloroform.

b) ethanol/hexane. (glass distilled grade, Rathburn Chem. Co., Scotland)

100 g spiked minced tubers were blended at high speed for 1 minute with 100 cm^3 ethanol. 200 cm^3 hexane were added to the blender and the resulting mixture blended for a further 1 minute, transferred (with washing) and filtered using a Buchner assembly. The residue in the Buchner funnel was washed with 2 x 50 cm^3 hexane. The filtrate was transferred, again with washing to a 1 dm^3 separating funnel where 100 cm^3 of saturated sodium chloride solution was added. The mixture was shaken and the aqueous layer discarded. Further washing of the hexane layer with 200 cm^3 10% sodium bicarbonate solution (w/v) followed by 200 cm^3 deionised water was carried out, the aqueous layer being discarded each time. The hexane solution was dried over

anhydrous sodium sulphate and reduced in volume in a rotary evaporator at less than 40°C, then made to 2 cm^3.

Suitable samples of the final solution were injected into a GC and the amount of chlorpropham recovered determined. The procedure was repeated several times.

c) hexane extract.

50 g spiked minced tubers were blended at high speed for 2 minutes with 150 cm^3 hexane and 80 g anhydrous sodium sulphate. The mixture was transferred with washing to an aluminium bottle, shaken on a wrist shaker for at least 30 minutes and filtered using a Buchner assembly. The residue was washed four times with 50 cm^3 hexane and the filtrate obtained was reduced in volume in a rotary evaporator and made to 2 cm^3. As before, suitable samples of the solution were injected into a GC and the whole procedure repeated several times.

Determination of chlorpropham by GC.

The optimum conditions for chlorpropham determination by GC were found by trial injections of chlorpropham standards dissolved in hexane. A Pye 104 (Pye Unicam, England) series gas chromatograph with flame ionisation detectors (FID) was used for the majority of analyses described in this thesis. 2 m x 4 mm i.d. columns of 5% OV17 on 100/120 mesh Gas Chrom Q (Phase Separations, England), held at a temperature of 195°C, were used. The injection ports were maintained at 220°C and the detector temperature was 275°C. The concentration of chlorpropham was determined by comparison with standard solutions. Triangulation was used to measure peak areas.

Occasionally it was necessary to use a Packard Becker 419 GC or Pye Unicam PU4500 equipped with FID. 5% OV17 columns under similar conditions to those listed above were used in these machines. Although not the most sensitive of detectors available for GC, FID does have the advantage of linearity of response over a wide range of concentrations.

2.2.1.3 Results and discussion.

Direct determination of chlorpropham by GC is possible providing stringent precautions are taken in the preparation of columns etc. for the GC. The OV series of phases are popular due to their thermal stability and low bleed characteristics. To enable chlorpropham determination by GC, glass columns which have been thoroughly acid and solvent washed must be silylated before packing. Once prepared, the filled columns should be conditioned for at least 48 hours at a temperature 20^{o}C below the maximum temperature (for OV 17, the maximum temperature is approximately 350^{o}C). On badly prepared columns, chlorpropham peaks can tail drastically resulting in difficulties measuring peak heights and areas. This problem can be countered to some extent by injecting a silylating solution directly onto the GC column, having first extinguished the flame to prevent a build up of silica in the detector. Commercial preparations such as Silyl 8 or Glass-Treet (Alltech Ass., England) are available for this purpose but NO-Bis(trimethylsilyl)acetamide (BSA) from BDH Chems., England was found to be as useful and is cheaper. Providing glass columns and an all-glass lining straight to the FID are used and column operating temperatures are kept below 230^{o}C then direct determination of chlorpropham should pose no problems.

The choice of solvents to be evaluated was limited by considerations of cost and purity and also safety aspects such as flammability and toxicity. For these reasons solvents such as diethyl ether, benzene and 1,4 dioxane were not included in this study.

(a) Although chlorpropham is readily soluble in ethyl acetate, dichloromethane and chloroform, the formulation of stable emulsions formed during blending gave rise to problems. Van Vliet and Hertog reviewed techniques used by other workers to overcome or avoid this problem i.e. the soaking of the plant material in the chosen solvent for a period of 3-10 days (Hardon et al., 1961). Centrifugation and careful removal of the organic layer was also suggested by Van Vliet and Hertog (1966).

Due to the amount of time, equipment use and probable increase in experimental error that would ensue, this procedure was abandoned.

(b) The formation of stable emulsions is avoided if the plant material is first blended with a solvent which is miscible with water such as ethanol, methanol or acetone. Further blending with organic solvent immiscible with water results in the partitioning of chlorpropham between the ethanol/water phase and the hexane phase. This two step procedure has the advantage of better penetration of the plant material by the initial water miscible solvent. Ethanol and hexane were the two solvents chosen for this extraction procedure because they were already routinely used in the laboratory for the extraction of another sprout suppressant, tecnazene, and some experience of hexane extracts of potatoes using this method was already available.

However, when the ethanol/hexane extraction procedure was repeated several times with spiked tuber samples, the recovery of

chlorpropham varied greatly. If the saturated sodium chloride step which increased the speed of partition of the phases was omitted, the recovery of chlorpropham dropped to 16.4% (mean of two replicates).

The obvious step to check was the partitioning of chlorpropham between aqueous and organic phases in the extraction procedure. It was discovered that only approximately 60% of the added chlorpropham was partitioned into the hexane phase under the conditions previously described (see Table 2.1). Increasing the water content of the aqueous phase coupled with re-extraction of this phase with fresh hexane improved the recovery of chlorpropham greatly but resulted in a cumbersome procedure that still lacked the reproducability required. Other solvents producing an improved partition co-efficient for chlorpropham could have been investigated but lack of time precluded this.

Cerny and Blumenthal (1972) claimed that a hexane extraction from potatoes was possible provided the extraction was carried out in the presence of sufficient anhydrous sodium sulphate to ensure anhydrous conditions. Their extraction procedure resulted in high and equally importantly, reproducible recovery of chlorpropham.

Cerny and Blumenthal (1972) had previously compared residue values obtained from this procedure to those obtained by a direct soxhlet extract of potato material in the presence of anhydrous sodium sulphate and had found good agreement between the methods. Re-extraction of the Buchner funnel contents showed that < 3% chlorpropham was removed indicating that recovery was almost complete. It was decided that this extraction procedure would be followed in future chlorpropham analysis.

Table 2.1 The effect of various ethanol/water mixtures on the partitioning of chlorpropham between aqueous and organic phases.

% ethanol in H_2O (in 50 cm^3)	% chlorpropham extracted into 50 cm^3 hexane	% chlorpropham extracted into 50 cm^3 hexane after the addition of 2 cm^3 saturated NaCl solution.
30	92.6[a]	98.5[a]
40	82.7	90.7
50	62.8	63.8
60	38.9	39.3
70	23.9	25.8

[a] each figure is the mean of two replicates

Recovery of chlorpropham from samples to which the chlorpropham is added prior to the extraction procedure can only indicate the efficiency of the procedure with respect to freely available chlorpropham. Metabolites with different characteristics to the parent compound would not be extracted and any bound or aged chlorpropham residues would be more difficult to extract. Exhaustive extraction where the procedure is repeated a number of times with the same sample can be employed to estimate how well chlorpropham is removed but comparisons of results with those by previous methods is still necessary. Extra care had to be taken at two stages in the procedure to maximise recovery of chlorpropham. Firstly, shaking the contents of the blender for 30 minutes to one hour is important as it permits sufficient time for the drying agent, anhydrous sodium sulphate, to absorb the water present in the plant material thus allowing chlorpropham to be extracted by this stage. A faster drying agent, magnesium sulphate, was considered but its dessicating action proved so rapid that blender blades frequently jammed. Alternative drying agents were either less efficient and/or as rapid in action as magnesium sulphate. Therefore, sodium sulphate was retained as the drying agent despite the extra time involved in its use.

Secondly, careful washing of the residue on the filter pad was essential for high recovery of chlorpropham. Four washings of 50 cm^3 hexane were used at this stage.

Although solvents, which had been dismissed in the first section because of emulsion problems in the presence of relatively large quantities of water, could now be reconsidered for use with a drying agent, hexane was preferred because fewer tuber components were co-

extracted using this solvent (Cerny and Blumenthal, 1972). This could lead to fewer problems during extract purification. Also it was envisaged that a GC equipped with electron capture detectors (ECD) could be used for the determination of chlorpropham and hexane is one of the preferred solvents for use with ECD due to its low electron capture properties.

2.2.2 Crude Extract Purification.

2.2.2.1 Introduction.

When spiked hexane extracts of potatoes were reduced in volume, made to 2 cm^3 and aliquots injected into a GC with FID, it was possible to measure chlorpropham by triangulation and comparison with standard chlorpropham injections. However, when the chlorpropham residue falls below a level of 5 mg kg^{-1} tuber, chromatograms such as that shown in Fig 2.1 were obtained and it is more difficult to quantify the chlorpropham present.

As much of the work in this thesis involved the quantification of low levels of chlorpropham in tubers, a suitable clean-up was necessary. Ideally, the clean-up should give complete recovery of chlorpropham and remove all the co-extracted material in the shortest possible time.

The most suitable method of clean-up was felt to be column chromatography rather than thin layer chromatography (TLC) or solvent washing. TLC was not used because of the relatively small quantity of concentrated extract that could be applied to a TLC plate - even a preparative plate. Solvent washing was considered but in this case, the degree of clean-up is usually much less than that of column chromatography and purified extracts were needed for the analysis of low levels of chlorpropham.

Several types of column could have been evaluated e.g. florisil, celite, silica and alumina have all been used to purify extracts of plant material, but the flexibility obtained by the addition of

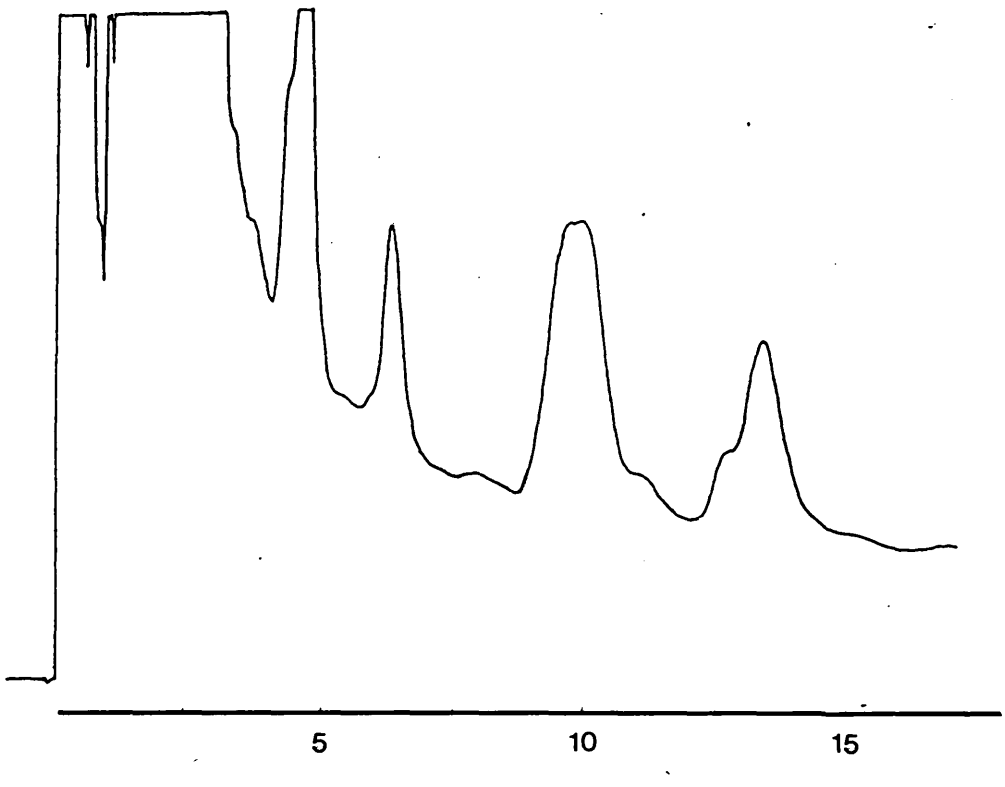


Fig. 2.1 GC chromatogram obtained from a 5 mm^3 injection of a concentrated (2 cm^3) hexane extract before clean-up. GC conditions as follows: detector temperature 275°C, injector temperature 220°C, oven temperature 195°C, column 5% OV17 on Gas Chrom Q, attenuation x200, N_2 flow rate 40 cm^3 min^{-1}.

varying amounts of water to alumina of different types made alumina columns the first choice.

Acidic and neutral aluminas of various grades (Woelm alumina for column chromatography) were evaluated.

2.2.2.2 Experimental.

Each column was prepared by pouring a hexane slurry of alumina of type and grade to be evaluated (ICN Pharmaceuticals, W. Germany) into glass column, 300 mm x 9 mm, to a depth of 150 mm. A 10 mm plug of anhydrous sodium sulphate was added to the top of the alumina to reduce the risk of accidental introduction of water which could drastically alter the reaction characteristics of the column.

To settle the column, approximately 20 cm^3 of solvent (hexane) was run through the column at a rate of 0.75 cm^3 min^{-1} before commencing the clean-up.

Using a Pasteur pipette, a concentrated hexane tuber extract spiked with 1 mg chlorpropham was applied to the top of the column as the level of solvent reached the anhydrous sodium sulphate plug. To ensure quantitative transfer, the Pasteur pipette and container were washed twice with hexane; these washings were then used to wash the spiked extract fully onto the column. A reservoir containing the developing solution was connected to the top of the glass column. The flow rate was maintained at approx 0.75 cm^3 min^{-1}.

The eluate from the column was collected in 25 cm^3 batches, concentrated by evaporation and samples of the concentrate made to volume and analysed by GC under the conditions previously stated.

When the volume needed to fully elute chlorpropham from the column had been ascertained, the procedure was repeated and the fraction containing chlorpropham was collected in one batch to determine recovery from the column.

2.2.2.3 Results and discussion.

Two approaches to extract purification using alumina chromatography were possible.

(1) The plant extracts could be applied to an activated alumina column i.e. one with little or no water added. Under these conditions, the chlorpropham and contaminants would probably be held strongly until the developing solution hexane was made more polar by the addition of solvents such as ethyl acetate, diethylether, acetone etc. Usually only small amounts of these solvents are sufficient to remove the compound of interest from the alumina column. However, a change in polarity of the developing solution inevitably removes some of the contaminants from the column.

(2) The plant extract could be applied to an alumina column which had been deactivated by the addition of a known amount of water to produce various grades of alumina. By deactivating the alumina column, chlorpropham could be eluted from the column using hexane as a developing solvent. In this way, less co-extracted material would be removed at the same time as chlorpropham.

The second procedure was followed using neutral alumina of various grades. It was found that the column had to be deactivated to grade 5 (15% water addition) before chlorpropham could be eluted in a reasonable amount of time. When the volume of hexane needed to fully

elute chlorpropham from the column had been ascertained, the procedure was repeated with the first 80 cm^3 of eluate being discarded and the next 120 cm^3 collected, concentrated to a small volume and made up to 2 cm^3. Samples of this purified extract were injected into a GC under the conditions previously described and the concentration of chlorpropham was determined by comparison with standard solutions. The recovery of chlorpropham from the column was found to be greater than 96%. The extraction and clean-up were carried out on a chlorpropham treated potato sample and the resulting GC chromatogram is shown in Fig 2.2. Each time a batch of alumina was prepared by addition of water, it was checked to ensure that the behaviour of chlorpropham on the column did not alter.

Since the work in this thesis was carried out, small disposable cartridges containing a variety of sorbents have been developed for rapid sample preparation. Sep-pak cartridges (Waters Associates, USA) and Bond-Elut (Analytichem International, USA) units have been investigated for the possibility of chlorpropham purification from potato hexane extracts. Sep-pak cartridges were originally available in two forms -C18 for aqueous extracts and silica for non-polar solvent extracts. Although the silica cartridges enabled separation of tecnazene and DMN from co-extractives in the hexane solution, the procedure was not successful in the case of chlorpropham. Ethyl acetate had to be added to the developing solvent (hexane) at a level of 20% v/v before chlorpropham was eluted from the cartridge. At this level of ethyl acetate addition, many other co-extractives were removed in the process.

Bond-Elut columns have the advantage of being available in a much greater range of sorbents. In addition to silica, diol columns

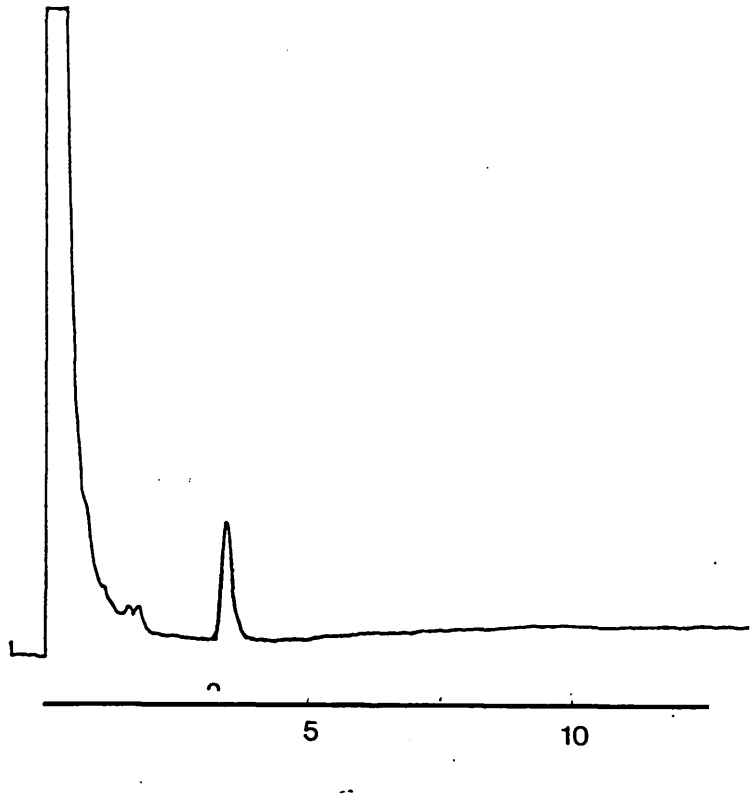


Fig. 2.2 GC chromatogram obtained from a 5 mm^3 injection of a concentrated (2 cm^3) hexane extract after alumina column clean-up. GC conditions as follows: detector temperature 275°C, injector temperature 220°C, oven temperature 195°C, column 5% OV17 on Gas Chrom Q, attenuation x200, N_2 flow rate 40 cm^3 min^{-1}.

are available and when these were evaluated it was found that the chlorpropham was separated from the co-extractives quickly and easily with 10 cm^3 of hexane. After concentration to 2 cm^3 an injection as previously described resulted in a chromatogram very similar to Figure 2.2. Thus, Bond-Elut columns are now used in the clean-up of chlorpropham extracts.

2.2.3 Derivatisation of chlorpropham for GC using an electron capture detector.

2.2.3.1 Introduction.

An electron capture detector (ECD) was available for the determination of low levels of chlorpropham. It had already proved useful in the determination of residues of tecnazene which contains 4 chlorine atoms as well as a nitro group.

The principles involved in electron capture (EC) detection of compounds can be found in many textbooks on gas chromatography and the authors agree that although some compounds appear in theory eminently suitable for EC detection, in practice, little or no response is observed. Chlorpropham, containing chlorine, nitrogen and an ester group is one of these compounds. Initially, it was not clear whether the lack of response found in EC was due to lack of sensitivity of the technique towards chlorpropham or the possibility of breakdown of chlorpropham at the high temperature of the detector. The nature and design of the ECD leads to the compound being exposed to hot metal surfaces at the time of detection. In an FID a glass lining straight

to the flame ensures no degradation of chlorpropham before the flame itself burns the compound in the detection process, but in EC detection where the stucture of the compound is important any breakdown could minimise the detector response. Methods using EC detection of intact chlorpropham could not be found in the literature although a personal communication from IBVL in Holland revealed that ECD is used routinely for chlorpropham determination. It was assumed thet as the compound can be successfully determined by EC, the detector used in our laboratory degraded chlorpropham. This was substantiated by the fact that injections of increasing concentrations of chlorpropham failed to give any response.

A method for more sensitive detection of chlorpropham would be useful as using FID, the MDA was 0.03 mg kg^{-1}. The possibility of derivatising chlorpropham to enable EC detection was investigated in this section. Although a number of derivatising agents have been used for chlorpropham analysis and described earlier, it was decided to investigate hydrolysis of chlorpropham to 3-chloroaniline followed by derivatisation with heptafluorobutyrylimidazole (HFBI) to produce a derivative which would be highly responsive to EC detection. Conditions for the small scale hydrolysis of chlorpropham and its subsequent derivatisation were investigated. Some of this work was originally carried out in a Final Year project by a student in Agricultural Chemistry and later expanded and checked.

2.2.3.2 Experimental.

The work described in this section was carried out in Reactivials (Pierce Chem. Co., USA).

Chlorpropham hydrolysis.

50 mm^3 samples of a 10 mg cm^{-3} stock solution of chlorpropham in acetone were placed in Reactivials. After allowing the acetone to evaporate, 1 cm^3 of 40% w/v potassium hydroxide (KOH) was added and the mixture refluxed for one hour with constant stirring (at 80-100°C). After cooling, the 3-chloroaniline and unreacted chlorpropham were extracted into 1 cm^3 of hexane or diethylether. Samples of these solutions were injected onto a GC and compared to standard 3-chloroaniline and chlorpropham.

Recovery of 3-chloroaniline from the aqueous KOH solution using hexane and diethylether was determined by the use of 3-chloroaniline and 1 cm^3 40% KOH mixtures and immediate extraction by shaking with hexane and diethylether. The possibility of 3-chloroaniline breakdown using strong alkali was checked in another series of Reactivials using 3-chloroaniline heated to 100°C for one hour with 10, 20 and 40% KOH.

The GC was as previously described and the column employed was 5% OV 17 on Gas Chrom Q. Column temperatures were 130°C and 200°C for 3-chloroaniline and chlorpropham respectively. The detector was maintained at 250°C.

Derivatisation reaction.

The method described in the Pierce General catalogue 1976-77 was used.

To 20-50 ug of 3-chloroaniline were added 20 mm^3 of HFBI. The mixture was heated at 85°C for one hour. 2 cm^3 toluene and 0.5 cm^3

water to stop the reaction were added and the mixture stirred. The aqueous layer was removed and the toluene layer washed three times in water to remove excess HFBI. The solution was centrifuged for 2 minutes and samples of the toluene layer injected into the GC fitted with ECD (^{63}Ni source). The column (5% OV 17) was maintained at 130^{o}C.

2.2.3.3 Results and discussion.

The extraction of 3-chloroaniline into diethylether was >85% but into hexane was only ca. 40%. Diethylether was, therefore, the preferred solvent.

It was found that 10% KOH gave the largest percentage hydrolysis (91%) of chlorpropham to 3-chloroaniline. When breakdown of 3-chloroaniline under the reaction conditions was investigated, stronger KOH lead to some breakdown. This presumably explained why percentage hydrolysis of chlorpropham was less with 20% and 40% KOH solutions since the hydrolysis was measured through 3-chloroaniline production.

The reaction to form the HFBI derivative of 3-chloroaniline resulted in a good peak on the chromatogram and seemed quantitative (see Fig. 2.3). Increasing the reaction time from 30 minutes to one hour decreased peak areas slightly so it would appear that shorter reaction times are better although further investigation is needed to optimise conditions.

It is, therefore, possible to hydrolyse chlorpropham obtained from a column clean-up using 1 cm^{3} of 10% KOH for one hour at 100^{o}C followed by extraction of the resulting 3-chloroaniline into diethylether. After evaporation of the diethylether, derivatisation

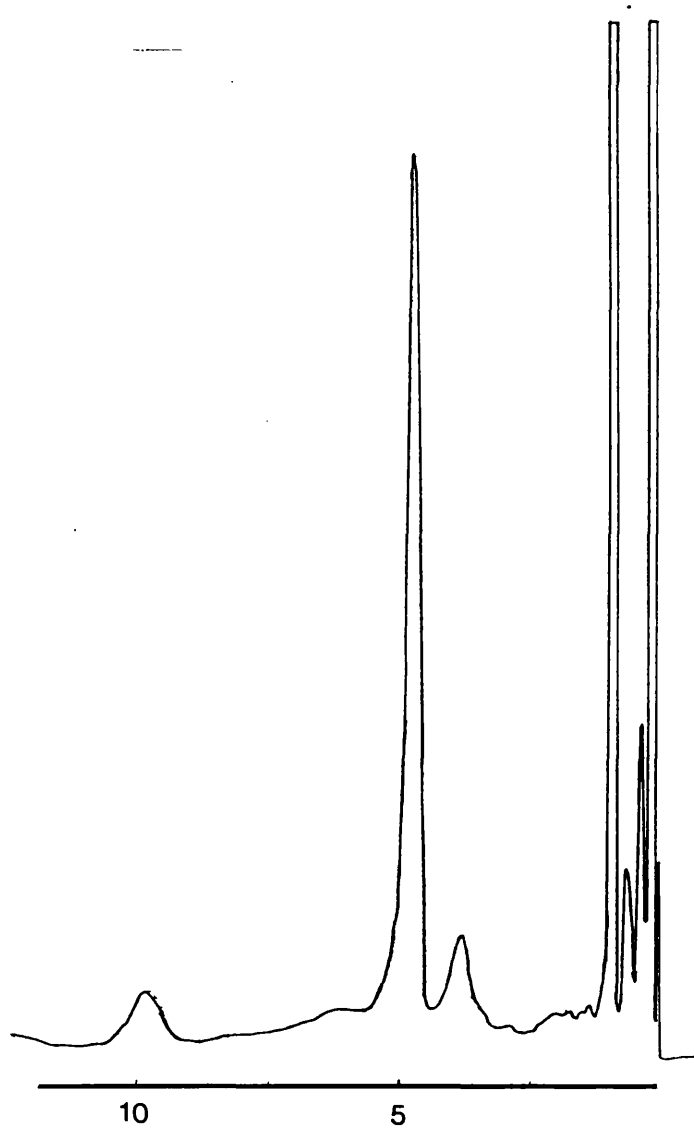


Fig. 2.3 GC chromatogram produced by a 0.4 mm^3 injection of an HFBI derivative of 3-chloroaniline by ECD. GC conditions as follows: detector temperature 250°C, injector temperature 140°C, oven temperature 130°C, GC column 5% OV17 on Gas Chrom Q, range x128, N_2 flow rate 45 cm^3 min^{-1}.

using HFBI at 85°C for <30 minutes followed by extraction of the derivative into toluene would produce extracts suitable for injection onto a GC fitted with ECD.

Although the procedure has not been fully quantified, it has been shown to be possible with high yields in different sections of the procedure. Time was not available for further studies on the method but it was available as an option if residue levels in field experiments described in subsequent chapters were found to be beyond the scope of the existing GC method.

Chlorpropham determination - method summary.

1-3 kg samples of potatoes were comminuted using a powerful mincer. The minced material was stirred vigorously to ensure its homogeneity and a 50 g sub-sample was extracted by blending for 1 min with 150 cm^3 hexane (glass-distilled grade, Rathburn Chem. Co., Scotland) in the presence of 80 g anhydrous sodium sulphate (A.R. Hopkin and Williams). The mixture was transferred with washings to an aluminium bottle, shaken on a wrist shaker for at least 30 minutes and filtered using a Buchner assembly. The residue was washed four times with 50 cm^3 hexane and the filtrate obtained was concentrated to ca. 1 cm^3 on a rotary evaporator keeping the temperature below 40°C to prevent chlorpropham loss.

A slurry of neutral alumina (activity grade 5, Woelm) in hexane was poured into a column, 300 x 9 mm to a depth of 150 mm. A 10 mm plug of anhydrous sodium sulphate was added to the top of the column to maintain anhydrous conditions and the column equilibrated by running 20 cm^3 hexane through it at a rate of 0.75 cm^3 min^{-1}. The

concentrated extract and washings were applied and eluted through the column with hexane at a maximum flow of 1 cm^3 min^{-1}. The first 80 cm^3 of eluate were discarded, the next 120 cm^3 collected, concentrated to a small volume and made up to 2 cm^3.

Samples of 0.5-5 mm^3 of purified extract were injected into a Pye Series 104 GC equipped with FID. 5% OV 17 on 100/120 mesh Gas Chrom Q columns (2 m long) with an inner diameter of 4 mm were used. These were held at a temperature of 195°C. The injection ports were maintained at 220°C and the detector temperature was 275°C. The nitrogen flow was 40 cm^3 min^{-1}. The concentration of chlorpropham was determined by comparison with standard solutions. Triangulation was used to measure peak areas.

Recovery.

1 mg amounts of chlorpropham were applied to 5 separate batches of diced potatoes prior to blending and by the procedure described above, recovery was found to be 91.2% $\pm$4.9 sd (standard deviation) All subsequent results were corrected for this factor.

CHAPTER 3

THE EFFECT OF CHLORPROPHAM ON THE PERFORMANCE OF SEED TUBERS

3.1 Introduction.

As stated in Chapter 1, chlorpropham, a mitotic poison should never be used on seed potatoes because it can permanently impair growth (Reeve et al., 1963). However, contamination of seed stocks with chlorpropham occurs and in most cases this contamination is not noticed until the seed is planted out and poor or no crop emergence is noted. This chapter will be concerned with the problem of seed tubers being accidently contaminated with chlorpropham at some stage in either their harvest or storage.

ADAS reports, especially, highlight this problem as year after year, instances of poor or failed emergence are investigated and after disease or poor husbandry are eliminated as likely causes, analysis of dug-up seed tubers often reveals the presence of either chlorpropham or tecnazene and in some unusual cases, both chemicals.

Tecnazene is interesting for the reason that, of the sprout suppressant chemicals available for use, it alone does not inhibit wound healing (McGee, 1984) i.e. its mode of action does not seem to be linked to inhibition of cell division but instead it maintains dormancy through some unknown mechanism (Dalziel, 1978). The fact that it is volatile, can suppress sprouting and the sprout suppressant effect can be removed by airing coupled with its use as a fungicide

for control of dry rot (Fusarium spp) in tubers make it attractive as a treatment for seed tubers.

In Scotland, until recently much of the seed crop was treated with tecnazene each year to maintain quality. Although the use of sprout suppressants on seed potatoes is officially banned, seed growers can use tecnazene as a fungicide for control of dry-rot, its sprout suppressant property being an added advantage but not the sole reason for its use. Although less active as a sprout suppressant than chlorpropham and at least as volatile, seed airing regimes to minimise tecnazene residues before planting can sometimes be inadequate, leading to cases of poor emergence in the field (Dalziel, 1978; Lindsay and Ruthven, 1986). ICI Plant Protection who market tecnazene under the trade name "Fusarex" recommend a 6-8 week airing period before treated tubers are planted out. Thus, residues of tecnazene in seed tubers can be expected and on some occasions, usually after inadequate airing, emergence problems encountered.

The presence of traces of chlorpropham in seed tubers is much more difficult to explain. It is clearly stated in the Approved Products Handbook for farmers and growers that chlorpropham should never be used on or in the vicinity of seed tubers. In addition, seed tubers should not be stored in buildings previously used to store chlorpropham treated ware potatoes. Despite this warning, chlorpropham contamination continues to be a problem each year (Anon, 1975-1980).

The main reasons for the success of chlorpropham as a potato sprout suppressant are

(1) Volatility - complete cover of tubers is not necessary for sprout control.

(2) Potency - 10-30 mg kg^{-1} is needed for sprout control. This is a factor of 10 less than tecnazene and newer sprout suppressants currently known to be being tested for approval.

These two factors are also responsible for the majority of contamination problems. Chlorpropham has been shown to be active in the vapour phase and as it is volatile it can drift from one section of a store to another, can be absorbed by store fitments and the store itself and slowly released into the air. Even small amounts released by this process could cause problems if seed potatoes were to be stored nearby because chlorpropham is active at low levels. Reeve et al., (1963) showed that a chlorpropham dose of 1 mg kg^{-1} could have an effect on potato cell mitosis. Danielson (1959) showed chlorpropham to be active as a vapour in bioassays using cucumber seedling growth. In his study, 0.5 ug chlorpropham applied to a Petri dish significantly affected cucumber seedling growth compared with controls. Work by a colleague in the Agricultural Chemistry section has resulted in measurements of the chlorpropham content of store air before and after chlorpropham treatment of ware potatoes (Boyd, 1984) and found that chlorpropham was present in the air of a commercial potato store even at the begining of the storage season when freshly harvested potatoes were placed in the store and before the initial chlorpropham application that season. The chlorpropham concentration was 0.17 mg m^{-3} store air while the potatoes were being loaded in and after chlorpropham application to the store as a thermal fog approximately 3 weeks later, the concentration had risen to 0.5 - 0.8 mg m^{-3} store air. As various workers have reported chlorpropham to be

present in dust, wood and concrete taken from treated stores, the presence of chlorpropham in the air at the start of the storage season could be expected. However, Filmer and Land (1978) failed to detect any chlorpropham in store air prior to the first application of that storage season despite the fact that their minimum detectable amount was reported as 10 ug m^{-3}.

In many cases, problems with chlorpropham contaminated seed can be traced back to situations where the grower or merchant had inadvertantly stored his seed close to or in a store previously used to treat or store chlorpropham treated ware despite the warnings given in the Approved Products Handbook (Anon., 1986)concerning this practice. In addition to seed potato growth problems resulting from exposure to chlorpropham, cereal seed germination and growth can also be badly affected. ADAS reported a case of stored grain being affected by chlorpropham although the building used to store the grain had not contained treated potatoes for at least three years. The residue of chlorpropham was still sufficient to inhibit germination. Although cereal contamination does occur it will not be investigated in this thesis although obviously any discussion of how to avoid or reduce chlorpropham residues would also be applicable to cereal contamination problems.

Little work on the effect of chlorpropham on seed potato performance has been published apart from the work of Kim et al., (1972) who noted that seed tubers sprouted more slowly than controls and that the delay was related to the concentration of chlorpropham applied.

ADAS workers have noted chlorpropham residues in seed tubers that failed to grow satisfactorily every season since sprout suppressant analysis began (Anon, 1975-82).

At the time of this study, no-one could predict the effect that a particular chlorpropham level in seed tuber would have on the seed performance. As chlorpropham contamination problems became more widely publicised, our laboratory regularly received samples for chlorpropham analysis a few weeks prior to planting if there was any possibility of chlorpropham contamination having occurred. In these cases, if chlorpropham was detected our advice to the grower had to be not to plant the seed as performance was likely to be affected but to what extent could not be predicted.

The study described in this Chapter involved treating tubers of two cultivars with various levels of chlorpropham, noting the level of chlorpropham in the tubers at planting and then following the performance of the seed through to harvest and grading.

3.2 Experimental.

3.2.1 Materials.

Chlorpropham, glass distilled hexane and anhydrous sodium sulphate were as previously described (Chapter 2). Alumina 'O' was purchased from Spence and Son, Scotland.

Seed of cvs. Desiree and Pentland Crown (AA1 grade) were purchased from J. and E. England and Sons, Abernethy, Perth. Grammmoxone (paraquat) and Sanspor (captafol) were purchased from ICI Plant Protection Division (England). Temik 10G (aldicarb) was purchased from Union Carbide U.K. Ltd. (England)

3.2.2 Methods.

Chemical treatments.

Chlorpropham was applied to the potatoes on a solid carrier. The application mixture was prepared by adding suitable quantities of chlorpropham into glass jars containing 25 g alumina 'O'. The jars were sealed, warmed gently for 2 hours to assist the distribution of chlorpropham throughout the carrier and shaken on an end-over-end shaker for 24 hours.

Batches, weighing 7.5 kg, of each of the two cultivars Desiree and Pentland Crown were dusted with the alumina to give treatment rates of 0 (control), 0.6, 1.2, 2.5, 5, 10 and 20 mg kg^{-1}. There were duplicates for each rate and cultivar and the treated tubers were stored at $10^{o}C \pm 0.5^{o}C$ in cardboard boxes with closely fitting lids from the treatment date on 21 February 1977 until the controls had commenced sprouting. At this time (6 April 1977), the degree of

sprouting in each box was quantified by measuring the length of the longest sprout on a 50 tuber sample. After measurement the tubers were desprouted because the controls at this time had already reached an average length of 45 - 50 mm and by the time of planting could have been very difficult to handle without damage. The desprouted tubers were transferred to wooden trays and stored under illuminated conditions to maintain sprouts at a manageable level until planting on 3 May, 1977.

The degree of sprouting was again quantified on 23 April 1977 as previously described. Immediately prior to the planting, 1 kg samples were removed from each replicate treatment, washed and stored at -18^{o}C until required for analysis.

Planting.

The experimental area was situated at Hattrick Farm, Bridge of Weir, Renfrewshire (NS 355673) and consisted of a sandy loam soil which had been uniformly treated with FYM. Immediately before planting, potato fertilizer (SA1) was applied at a rate of 1400 kg ha^{-1}. Also at planting time 10% aldicarb granules (Temik 10G) were applied at the recommended dose rate.

Experimental Design.

The site was planted in two independently randomised blocks protected by guard drills. The tubers were planted by hand on 3 May 1977 at 330 mm spacing. Each plot consisted of 3 drills (710 mm apart) 6 m long, containing 18 tubers/drill.

Husbandry.

A combination of paraquat (Grammoxone) spraying at emergence and hand weeding when necessary was practised. Chemical spraying was delayed until 5% emergence and as a knapsack sprayer was used, emerged plants were avoided during the spraying operation. Blight was controlled by captafol sprays (Sanspor) applied at fortnightly intervals from August onwards.

Emergence Counts.

Scores of emergence were taken at 2 day intervals from 28 May until 27 July. Each of the 3 drills in the plot was individually assessed. From these scores, the number of plants emerged since the previous count was calculated.

Harvesting and Grading.

The crop was mechanically defoliated on 29 September, 1977 and harvested on 5 October 1977. Each of the drills in the plots was harvested separately, stored in 12 kg nets, mechanically graded over 52 mm and 32 mm riddles and the yield of each drill recorded.

Residue determination.

The washed 1 kg samples were removed from storage and after thawing for 30 minutes, comminuted using a powerful mincer (Model AL 2-1, Bauknecht, W.Germany). The minced material was stirred vigorously to ensure homogeneity and chlorpropham was determined in a 50 g subsample as previously described.

3.2.3 Analysis of results.

Sprout lengths.

The results are expressed as a mean ±standard deviation. Significant differences were tested by analysis of variance (AOV). If significant, the least significant difference (LSD) was calculated as

$$LSD = t_{0.05}\sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_1}}$$

where t has N-k degrees of freedom

k - number of treatments

n_1, n_2 - number of replicates of each mean

s^2 - residual mean square from AOV

Emergence and yield data.

Analysis of variance (AOV) was used to check for any significant effects and the Tukey Honestly Significant Difference method for multiple comparisons was used to calculate the LSD (Dowdy and Weardon, 1983).

$$LSD = q_{(0.05,t,v)} \times \sqrt{\frac{s^2}{n}}$$

where t = number of variables

s^2 - residual mean square from AOV

n - number of replicates

v - residual degrees of freedom

3.3 Results

The effect of chlorpropham on sprout lengths of tubers is shown in Table 3.1. A visual record of the effect of chlorpropham on growth is given in Plates 3.1 - 3.3. Details of the emergence of seed tubers treated with chlorpropham are shown in Table 3.2 and histograms of interval emergence with time are shown in Figures 3.1 - 3.8. A summary of the main emergence and yield characteristics associated with chlorpropham residues from analysis at planting are given in Tables 3.2 and 3.3. The relationships between emergence and yield and residue at planting with emergence and yield are shown in Figs. 3.9 - 3.12 with associated linear regression model parameters detailed in Table 3.4.

Plate 3.1 Chlorpropham treated seed tubers immediately prior to planting.

cv. Pentland Crown

cv. Desiree

1 = control. No chlorpropham applied.
3 = chlorpropham applied at a rate of 1.2 mg kg^{-1}
5 = chlorpropham applied at a rate of 5 mg kg^{-1}
7 = chlorpropham applied at a rate of 20 mg kg^{-1}

Plate 3.2 Influence of chlorpropham on the emergence of seed tubers. cv. Pentland Crown (planted on 3 May 1977 and photographed on 13 July 1977).

a. control

b. chlorpropham applied at a rate of 1.2 mg kg^{-1}

Plate 3.2 cont.

c. chlorpropham applied at a rate of 5 mg kg^{-1}

d. chlorpropham applied at a rate of 20 mg kg^{-1}

Plate 3.3 Influence of chlorpropham on the emergence of seed tubers. cv. Desiree (planted on 3 May 1977 and photographed on 13 July 1977).

a. control

b. chlorpropham applied at a rate of 1.2 mg kg^{-1}

Plate 3.3 cont.

c. chlorpropham applied at a rate of 5 mg kg^{-1}

d. chlorpropham applied at a rate of 20 mg kg^{-1}

Table 3.1 Mean sprout length of tubers treated with chlorpropham and stored at 10°C

cultivar	chlorpropham applied (mg kg^{-1})	sprout length (mm)			
		stored at 10°C in 10 kg boxes and measured on 6/4/77		desprouted, aired on trays and measured on 23/4/77	
		mean[a]	sd[b]	mean	sd
Pentland Crown	0.0	48.6	19.2	9.7	5.4
	0.6	19.4	17.7	7.5	4.0
	1.2	16.1	16.5	5.3	4.3
	2.5	8.8	12.9	4.9	5.0
	5.0	3.7	5.7	3.3	3.2
	10.0	2.7	5.2	1.7	1.0
	20.0	1.8	3.8	1.5	1.0
Desiree	0.0	44.6	23.7	13.7	8.1
	0.6	13.6	16.3	9.7	6.4
	1.2	7.5	10.5	6.9	5.7
	2.5	4.9	7.7	4.2	4.3
	5.0	4.1	7.3	2.0	1.9
	10.0	2.4	5.1	1.7	1.5
	20.0	2.0	3.3	1.5	1.0
$LSD_{0.05}$[c]		6.33		2.46	

[a] mean of 100 replicate sprout lengths

[b] standard deviation

[c] Least significant difference which allows multiple comparisons between all pairs of treatments.

Fig. 3.1 Emergence profiles of chlorpropham treated seed tubers. cv. Pentland Crown. (1976-1977)

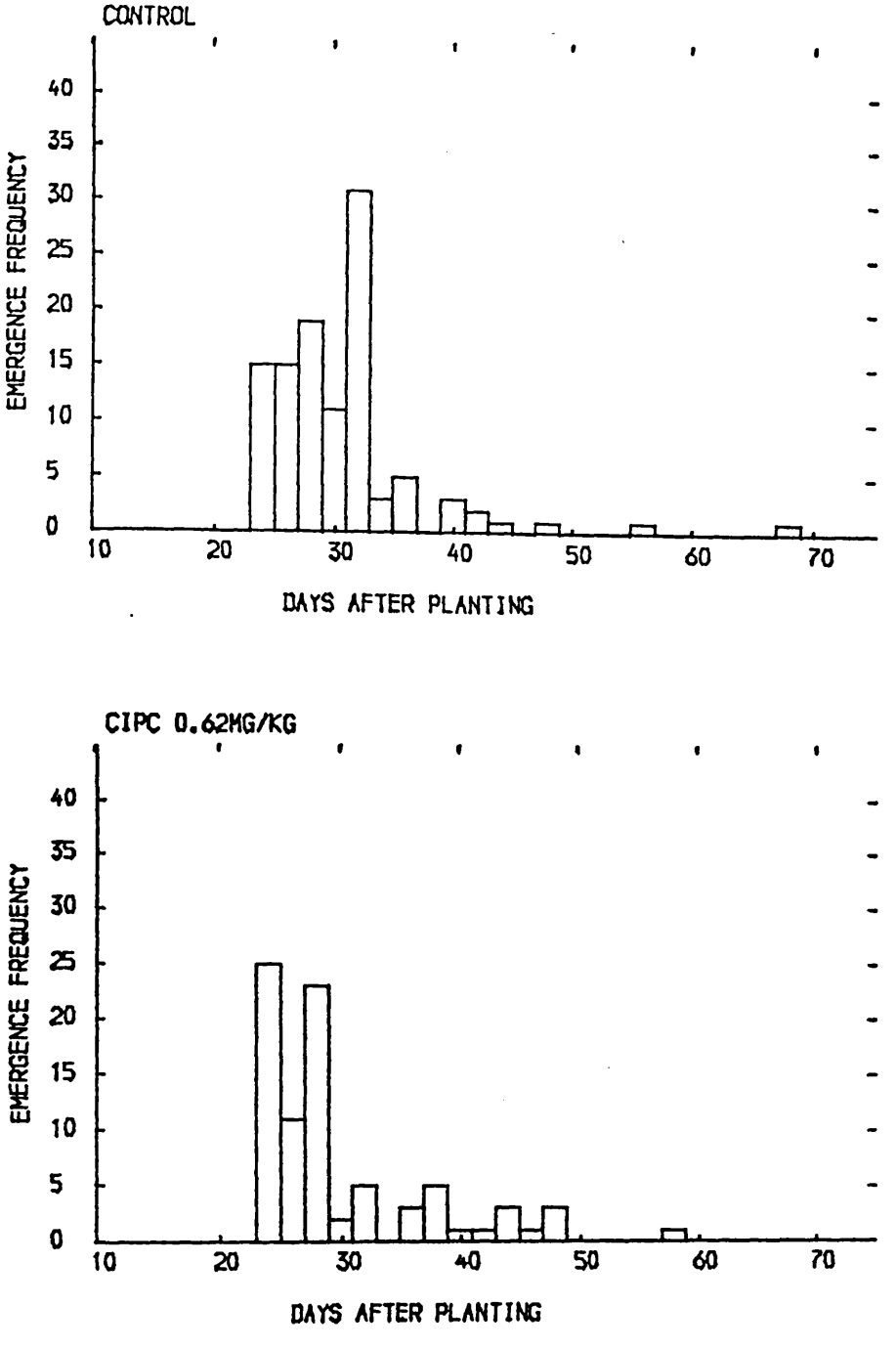


Fig. 3.2 Emergence profiles of chlorpropham treated seed tubers. cv. Pentland Crown. (1976-1977)

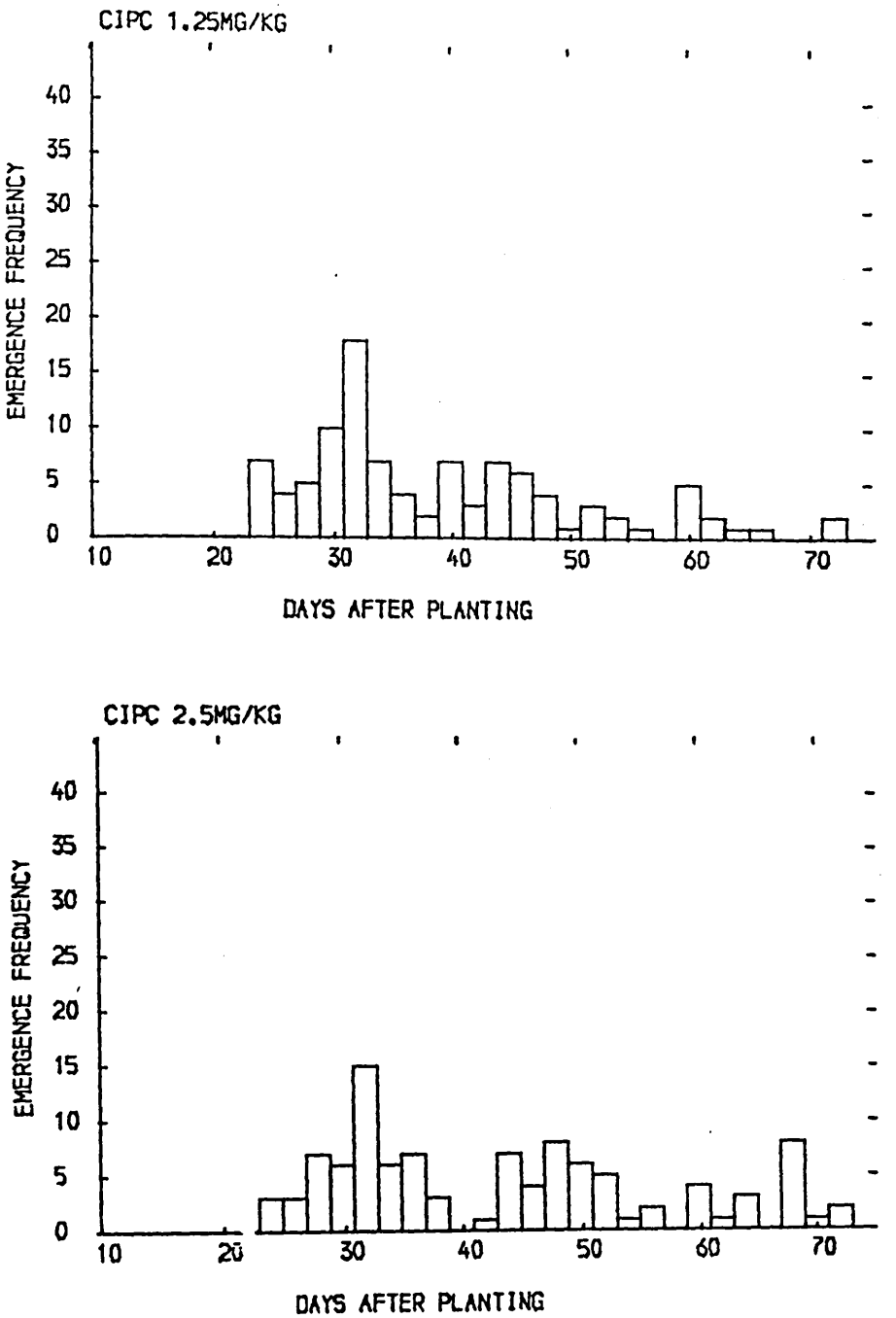


Fig. 3.3 Emergence profiles of chlorpropham treated seed tubers. cv. Pentland Crown. (1976-1977)

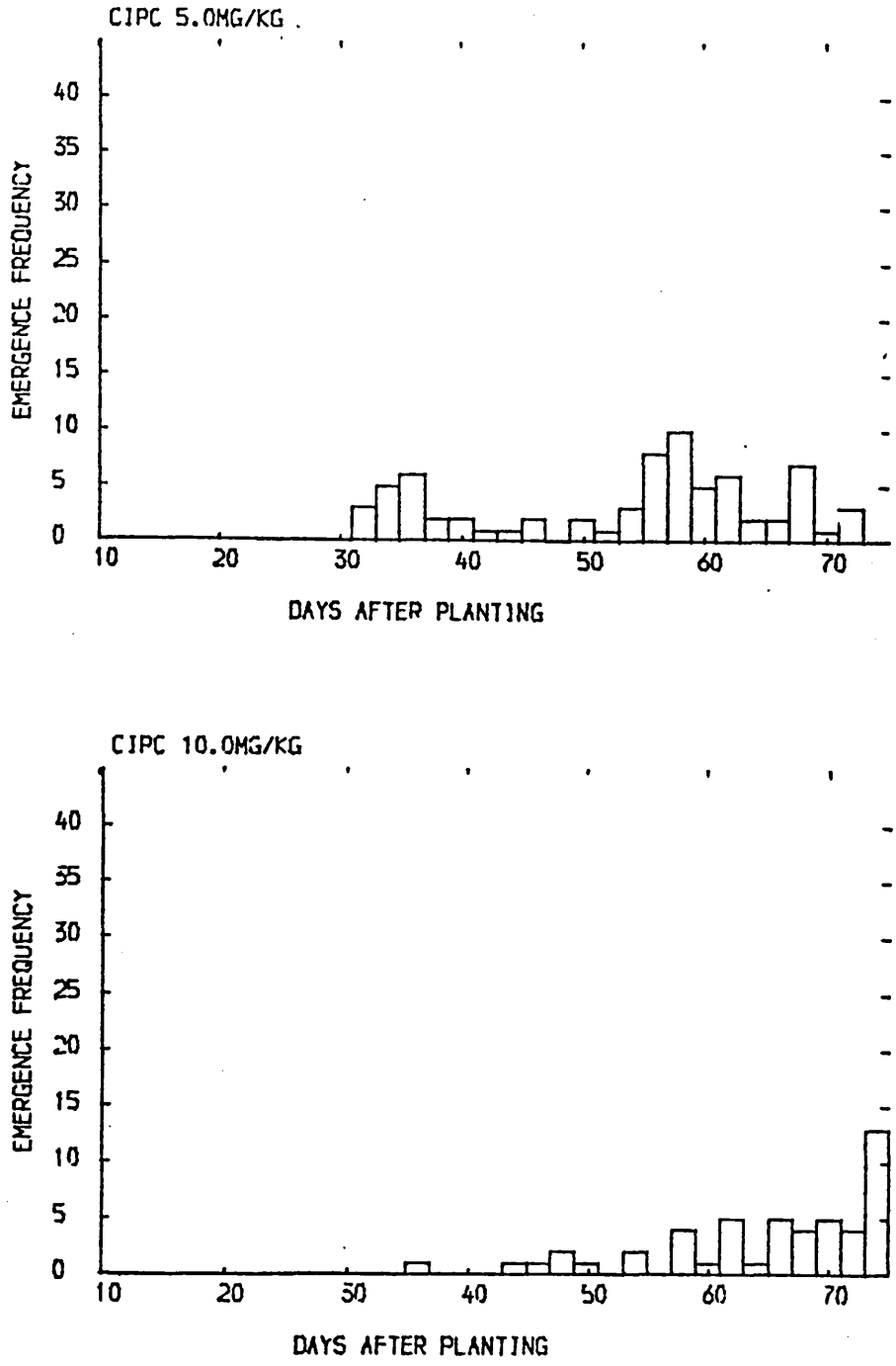


Fig. 3.4 Emergence profiles of chlorpropham treated seed tubers. cv. Pentland Crown. (1976-1977)

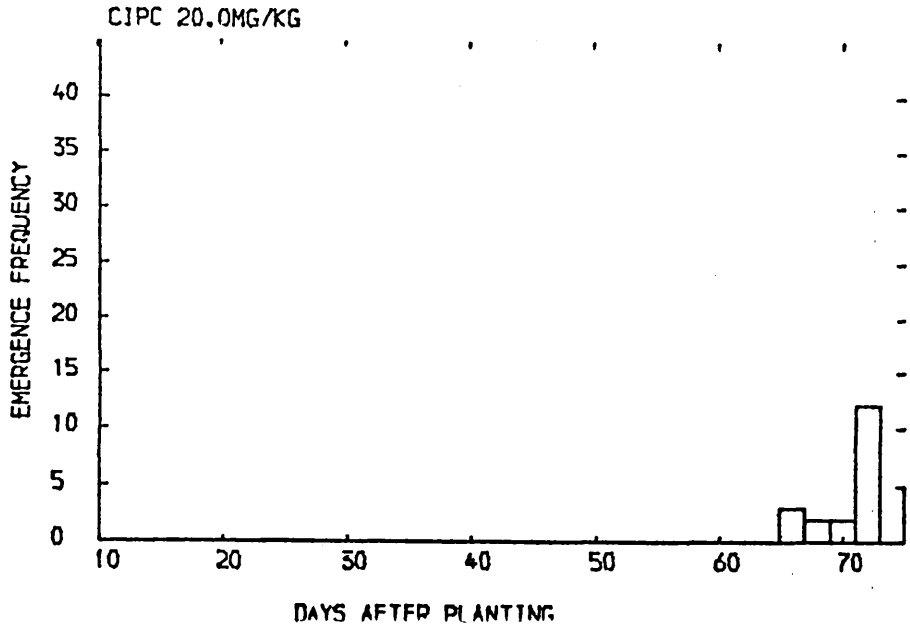


Fig. 3.5 Emergence profiles of chlorpropham treated seed tubers. cv. Desiree. (1976-1977)

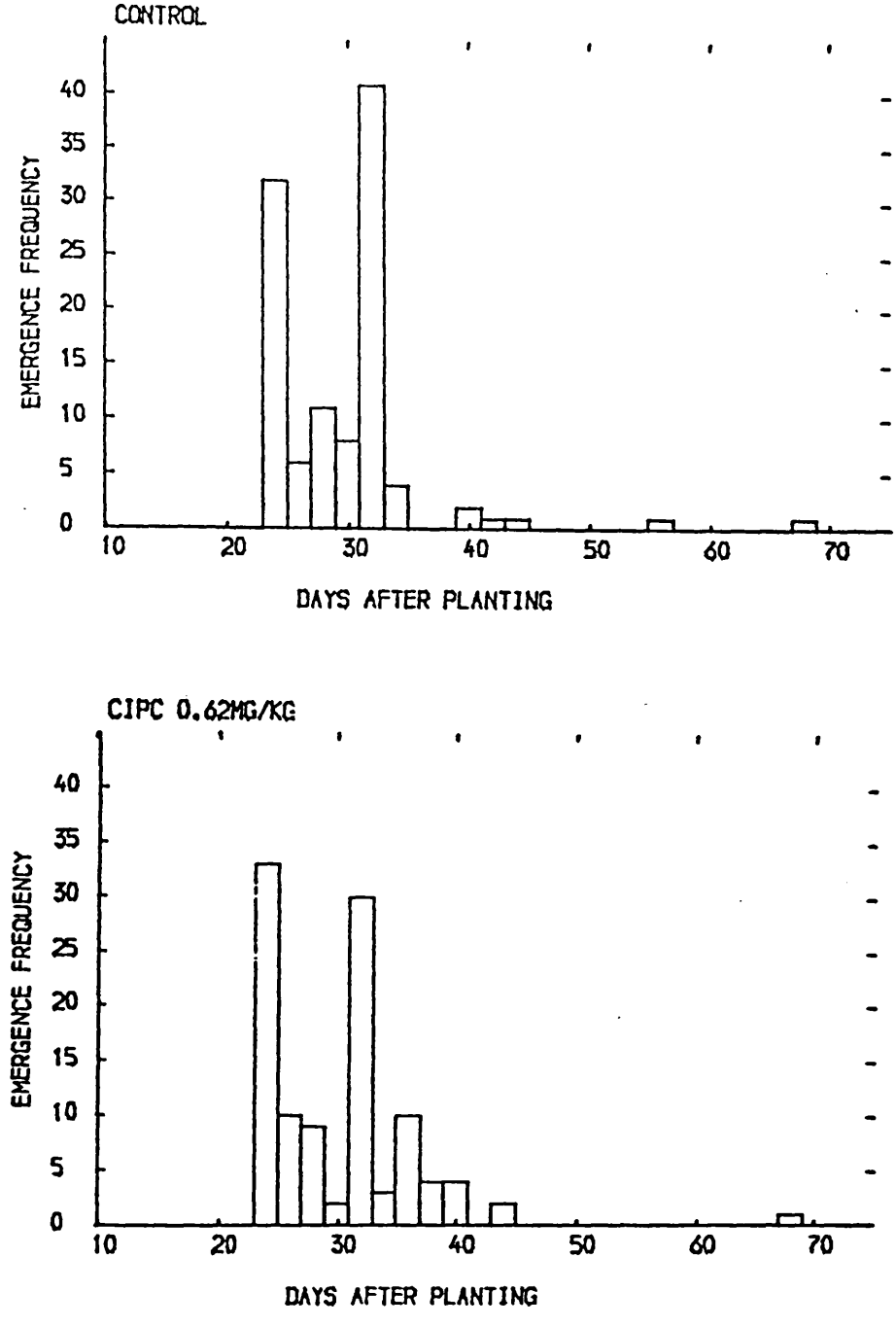
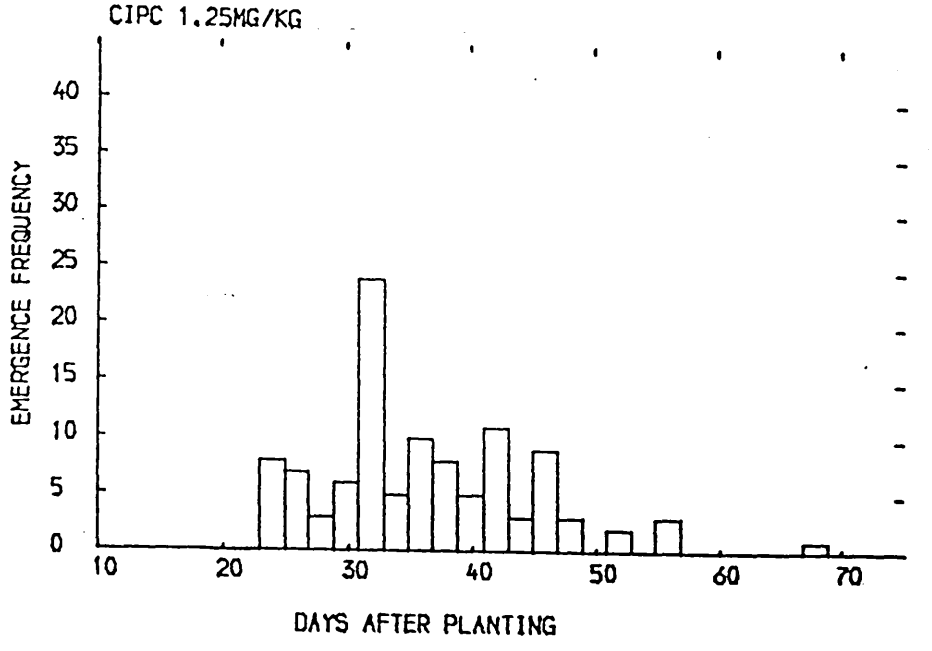


Fig. 3.6 Emergence profiles of chlorpropham treated seed tubers. cv. Desiree. (1976-1977)



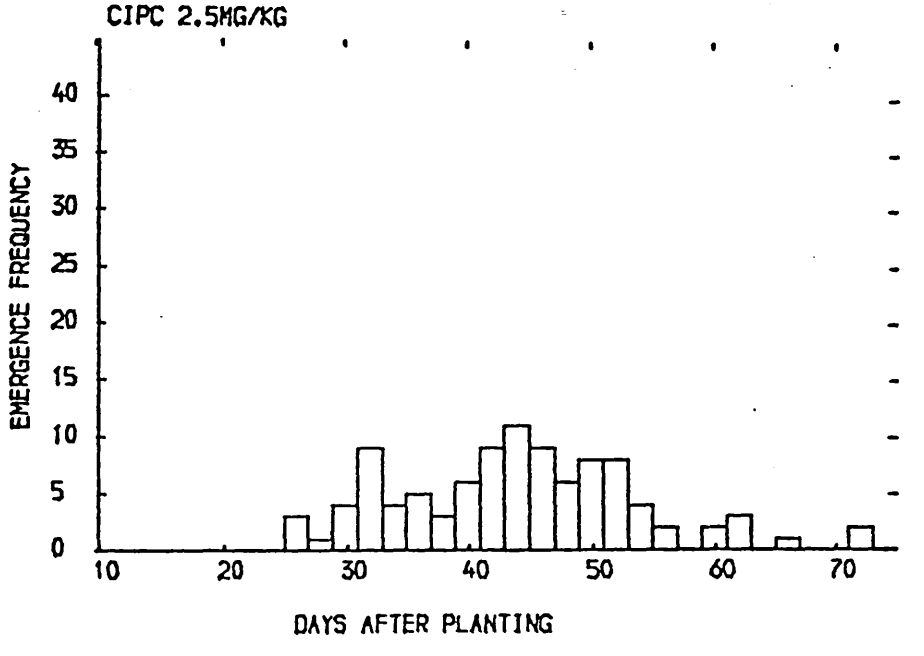
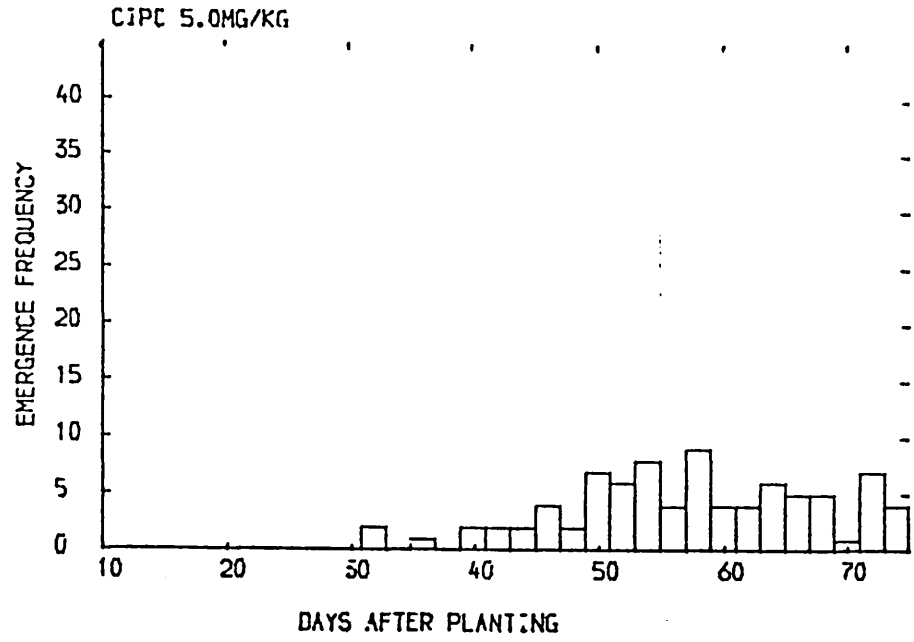


Fig. 3.7 Emergence profiles of chlorpropham treated seed tubers. cv. Desiree. (1976-1977)



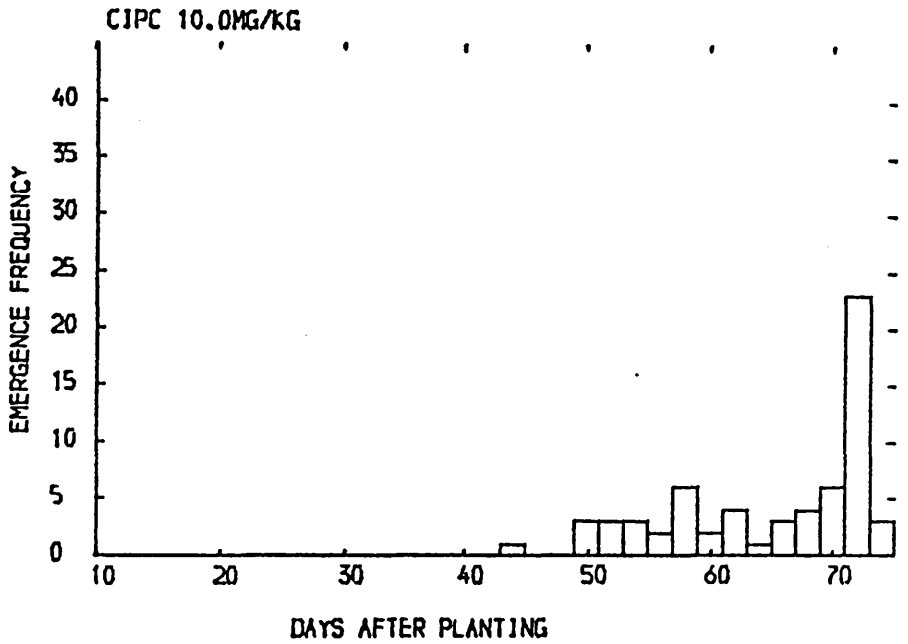


Fig. 3.8 Emergence profile of chlorpropham treated seed tubers. cv. Desiree. (1976-1977)

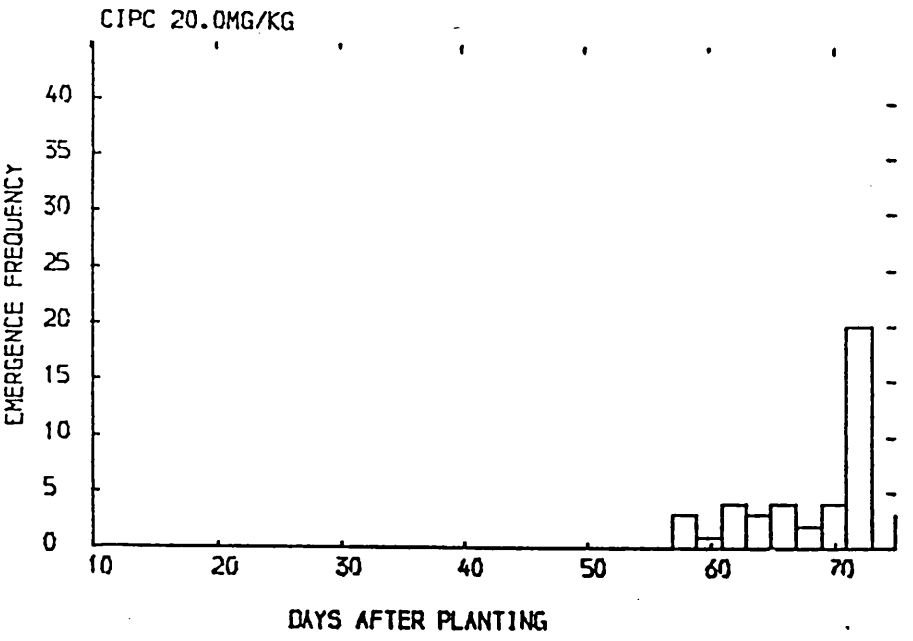


Table 3.2 The effect of chlorpropham residues on the emergence of seed tubers.

cultivar	chlorpropham applied ($mg\ kg^{-1}$)	residue at planting ($mg\ kg^{-1}$)	T25[a] (days)		T50[b] (days)		MET[c] (days)		TE[d] (days)	
			mean	sd[e]	mean	sd	mean	sd	mean	sd
Pentland Crown	0	0.10	26.7	1.48	29.7	1.32	30.6	1.51	100	0.0
	0.6	0.13	26.1	2.02	29.6	2.08	31.5	2.53	99.1	2.29
	1.2	0.21	30.8	2.52	37.4	4.92	39.1	3.91	94.4	6.10
	2.5	0.28	33.5	5.00	41.5	6.92	43.6	3.33	95.4	5.46
	5.0	0.52	48.5	7.92	57.1	4.01	51.1	5.60	73.2	8.89
	10.0	1.15	62.2	7.09	72.2	3.64	62.7	3.86	51.8	13.02
	20.0	1.77	73.8	2.52	75.0	0.0	68.2	1.79	24.1	5.73
Desiree	0	0.07	25.9	2.07	29.0	3.21	29.7	1.55	100	0.0
	0.6	0.06	25.1	1.21	28.9	3.29	30.1	1.84	100	0.0
	1.2	0.22	30.8	2.06	35.1	2.09	36.4	1.80	100	0.0
	2.5	0.46	39.0	3.93	45.3	4.03	44.2	1.24	92.6	6.73
	5.0	0.84	49.0	2.98	57.3	3.38	54.6	2.00	83.3	9.30
	10.0	1.55	63.1	3.89	69.0	3.25	61.4	1.61	60.2	10.19
	20.0	2.60	66.8	2.39	74.7	0.81	64.9	1.63	41.7	8.41
$LSD_{0.05}$[f]		0.36	6.6		6.1		4.8		12.0	

[a] Time (in days) to 25% emergence
[b] Time (in days) to 50% emergence
[c] mean emergence time in days
[d] The percentage of plants emerged by last scoring date
[e] Standard deviation
[f] Tukey multiple comparison procedure which allows simultaneous comparison between all pairs of treatments

Table 3.3 The effect of chlorpropham contamination on the yield of treated seed potatoes

Cultivar	Residue at planting	Yield per drill				
	(mg kg^{-1})	Large[a] (kg)	Medium[b] (kg)	Total* (kg)	%large	%medium
Pentland Crown	0.10	21.5	1.6	23.1	93.1	6.9
	0.13	21.2	1.9	23.2	91.4	8.2
	0.21	18.2	1.4	19.7	92.4	7.1
	0.28	17.3	1.5	18.8	92.0	18.0
	0.52	13.1	2.2	15.3	85.6	14.4
	1.15	7.3	1.5	8.8	83.0	17.0
	1.77	2.1	0.6	2.7	77.8	22.2
Desiree	0.07	16.8	3.2	20.1	83.6	15.9
	0.06	18.1	2.7	20.8	87.0	13.0
	0.22	15.2	2.2	17.5	86.9	12.6
	0.46	12.5	2.1	14.6	85.6	14.4
	0.84	9.2	1.7	10.9	84.4	15.6
	1.55	4.8	1.1	5.9	81.4	18.6
	2.60	3.9	1.3	5.2	75.0	25.0
$LSD_{0.05}$[c]	0.36	3.5	0.7	3.3		

[a] >50 mm
[b] 32-50 mm
[c] Tukey multiple comparison procedure which allows simultaneous comparison between all pairs of treatments

* The total yield per drill included some small tubers which did not fit into the large or medium category and therefore the %large and %small tubers do not necessarily add up to 100%

3.4 Discussion.

3.4.1 Sprout length.

Sprout lengths were measured on a 50 tuber sample / replicate on two different dates. The control tubers had sprouted to approx. 50 mm in length by the first sample date; in contrast, the treated tubers showed little sprouting. As tubers with sprouts as long as 50 mm are not generally planted out, sprouts longer than 2 mm were removed from tubers in all treatments and the tubers stored under illuminated conditions to produce short sturdy sprouts as in normal 'chitting' practice. Had the sprouts been allowed to grow unchecked for the remaining three weeks prior to planting, then control sprouts would have been so long that sprout breakage even with careful hand planting would have increased. It was decided that desprouting the seed prior to airing would be a suitable compromise. Sprout lengths before chitting and before planting are shown in Table 3.1. The higher the application of chlorpropham, the smaller the sprout length. When the sprout length figures were checked for normal distribution, controls followed a normal distribution but treated material did not. The reasons for this will be discussed in more detail in the next chapter.

The rossette type of sprouting and general lack of apical sprout dominance noted by Kim et al., (1972) could be seen in some of the tubers sampled although not all. Generally when the sprout tip is damaged by treatment with chlorpropham, there is an increase in multiple sprouting at that eye if regrowth does take place. Kim et al., (1972) concluded that one rough method to check for chlorpropham contamination was to observe the sprouting pattern of the seed. If eyes other than the apical eye sprouted then contamination should be

suspected if there were no obvious signs of disease. This work was carried out in the U.S.A. and in the U.K. another possibility must be considered. Tecnazene also induces lack of apical dominance leading to rosette growth at eyes. Cold storage of seed ($4^{o}C$) could also lead to lateral eyes sprouting at chitting.

3.4.2 Emergence.

To assess how the seed tubers would perform in the field, it was necessary to plant the material and observe a number of growth parameters. Details of emergence of seed tubers treated with chlorpropham are shown in Table 3.2 together with experimentally determined chlorpropham residue values.

The time taken to reach 25% (T25), 50% (T50), 75% (T75), and total (TE) emergence was calculated for each treatment if possible. Also the mean emergence time for each replicate drill was calculated. With many treatments the time taken to reach T25, T50, T75 and TE could not be presented because the emergence on the final day of scoring was too low. As plants were meeting across the drills, more damage was being caused by walking through the drills every 2 days than was acceptable and so scoring was discontinued. It was possible that a few more plants emerged later but they would have been smothered by the larger plants around them.

As expected, treatment with chlorpropham produced a highly significant ($P<0.01$) effect on mean emergence time (MET) and total emergence (TE). Photographs of the plots were taken on July 13, 1977 and these are shown in Plates 3.2 and 3.3 for the cultivars Pentland Crown and Desiree respectively. The plates give an impression of the

degree of blanking associated with each of the chlorpropham treatments.

It is interesting to note that the plants that did emerge in the treated plots were not obviously affected by the chemical treatment in any way other than the delayed emergence and seemed comparable to the control plants. Had the controls not been desprouted prior to chitting to deal with the problem of overlong sprouts at planting, then some effect on stem numbers caused by loss of apical sprout dominance might have been seen. Discoloration of stem and leaves and unusual growth behaviour has been reported with tecnazene use on seed (Nash, 1978) but was not noted in this study with chlorpropham.

Histograms of the interval emergence of the two cultivars are shown in Figures 3.1 - 3.8. From these it can be clearly seen that treatment with chlorpropham flattened the emergence profile in comparison with that found in control plots so that the MET gradually increased with increasing chlorpropham treatment (Figs. 3.9 and 3.10). However, in addition to the delay in emergence there was also the problem of the failure of some plants to emerge at all. The severity of this effect was proportional to the amount of chlorpropham applied and the chlorpropham residue level at planting. A cultivar effect was also seen because the effects of chlorpropham on the cv. Pentland Crown were more severe despite it having generally lower chlorpropham residue levels than Desiree. In similar experiments with tecnazene, Dalziel (1978) showed that, in comparison with the emergence of other cultivars, Pentland Crown was affected to a greater extent. The difference in cultivar susceptibility to chlorpropham treatment could have been due to a number of factors such as vigour of sprout growth

Fig. 3.9 The effect of chlorpropham residues on mean emergence time. cv. Pentland Crown

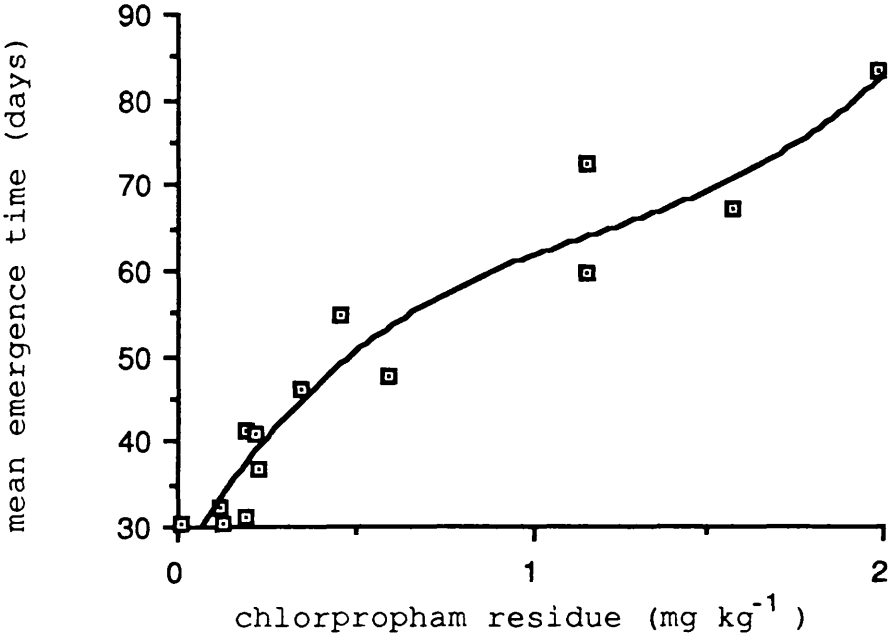
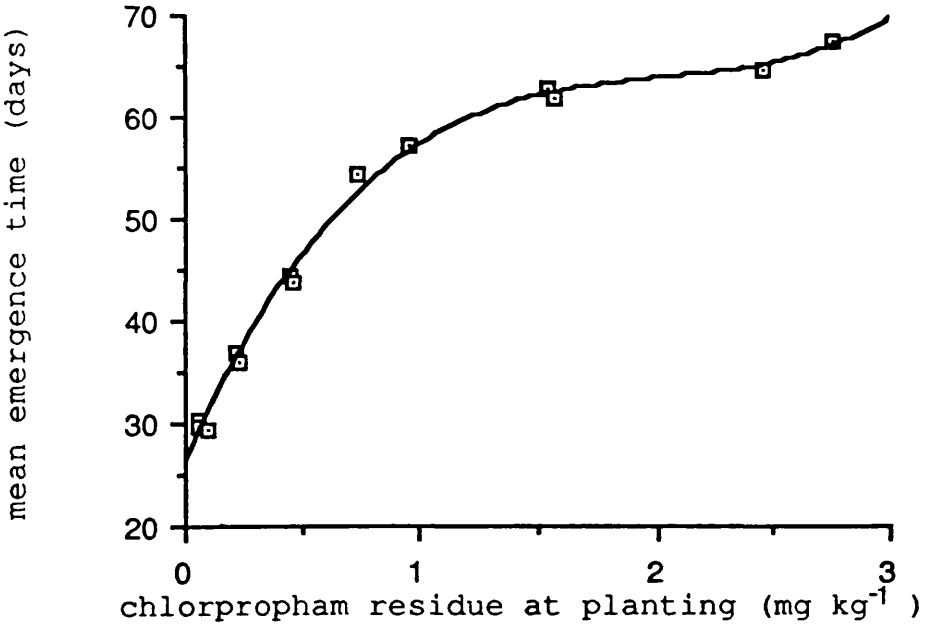


Fig. 3.10 The effect of chlorpropham residues on on mean emergence time. cv. Desiree



and, as chlorpropham tends to concentrate in the peel area, periderm thickness and possible compositional differences in the periderm.

3.4.3 Yield.

Dalziel (1978) had found that in the case of tecnazene, emergence and yield characteristics had to be noted because the relationship between the two was not strong (r^2 (co-efficient of determination) =0.34). He recommended that the crop be followed through to harvest. In this experiment, the relationship was much stronger (r = 0.96 and 0.98 for cv. Pentland Crown and Desiree respectively) and is shown in Fig. 3.10. The effect of chlorpropham on large (>50 mm) medium (32-50 mm) and total yield is shown in Table 3.3.

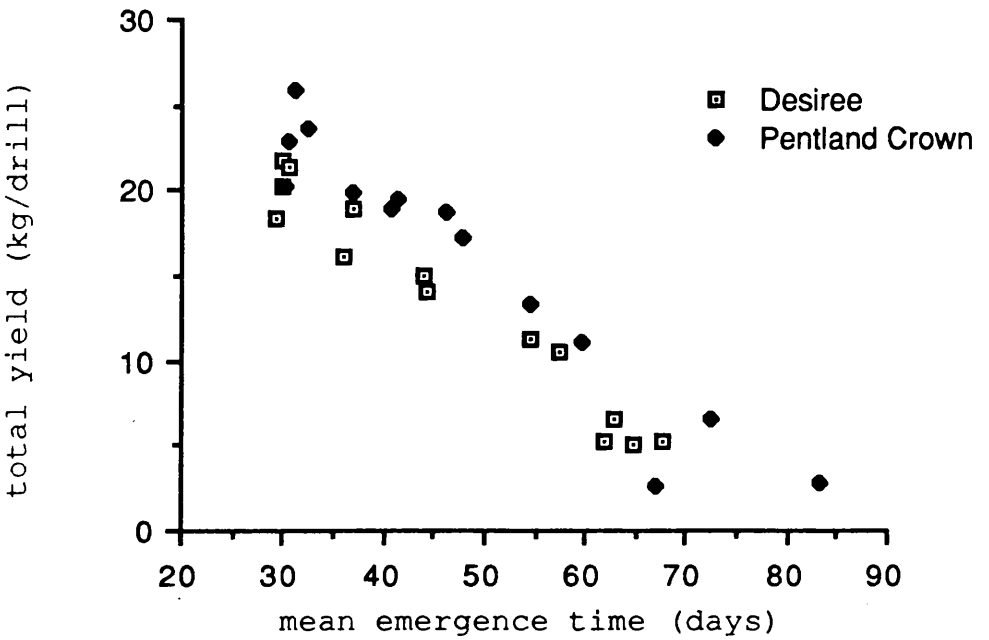
Analysis of variance revealed significant treatment, cultivar and interactive effects on the yields of large and medium tubers and on the total yield. Again, at higher chlorpropham levels these effects were particularly severe.

The proportion of large to medium tubers decreased with increasing chlorpropham residues. This effect was expected as the plants that did grow in the treated plots, although appearing normal, had been delayed by up to 5 weeks compared with controls.

3.4.4 Chlorpropham tuber residues.

The chlorpropham residue values associated with each treatment are shown in Table 3.2. Analysis of variance of the residue values revealed significant ($P<0.01$) cultivar, treatment and interactive effects. Generally, the cv. Desiree contained higher chlorpropham

Fig.3.11 The relationship between MET and total yield.



residues than Pentland Crown. Similar cultivar differences have been reported by Dalziel (1978) using tecnazene. The differences in residues between cultivars may be due to factors such as peel thickness and whether the cultivar is a vigorous sprouter or not.

Although this study was carried out once only, the residues and the subsequent performance of the seed give some guidelines to be followed when advice is sought on chlorpropham contamination problems. Firstly, the control tubers which had to be aired near the treated tubers in this experiment, picked up a trace of chlorpropham despite efforts to minimise this possibility. This gives some idea of how vapour drift of chlorpropham from treated material to seed tubers could present such problems. However, if exposed for a short period of time resulting in a residue at planting of <0.1 mg kg^{-1} then performance should not be affected.

After this study was completed, ADAS reports of field studies on chlorpropham effects on seed tuber performance were published (Anon, 1978; 1979). They had also attempted to link chlorpropham residues at planting with seed performance and concluded that concentrations of chlorpropham >0.3 mg kg^{-1} tuber peel could cause problems in the field. ADAS determine both tecnazene and chlorpropham in the peel of the tuber as most of the chemical is concentrated in this region (see Chapter 2 for the method of analysis employed by ADAS chemists). To convert tuber peel residue figures to approximate to whole tuber residues which are used throughout this thesis for reasons already discussed, it is necessary to divide by 10 (Corsini et al., 1978). ADAS therefore conclude that any chlorpropham residue >0.03 mg kg^{-1} whole tuber can cause problems in the field. Our study suggests a residue of approx. 0.2 mg kg^{-1} whole tuber is necessary before

Table 3.4 Parameters of the linear regression model ($y = \alpha + \beta x$) describing the relationship between chlorpropham residues and total yield, and mean emergence time and total yield.

cultivar	slope (β)	sd	intercept (α)	sd	r^2
	total yield = $\alpha + \beta$ chlorpropham residue				
Pentland Crown	-11.46	1.21	22.75	1.02	0.88
Desiree	-6.31	0.79	18.80	0.96	0.84
	total yield = $\alpha + \beta$ mean emergence time				
Pentland	-0.51	0.04	39.90	2.07	0.96
Desiree	-0.44	0.20	33.63	0.97	0.98

[a] co-efficient of determination

problems with delayed emergence and blanking become noticeable in the field. This figure is further substantiated by a field experiment carried out the following year and described in Chapter 4. As the cultivars used in the ADAS field trials were Desiree and Pentland Crown no simple explanation can be put forward to explain this disparity. It might have been possible that the weather conditions in the year their field trial was carried out were severe and enhanced any effect of chlorpropham contamination. Their potatoes were dipped in an unstated aqueous formulation of chlorpropham and this may have contributed to some difference in residues as found by van Vliet and Sparenberg (1970). However, the ADAS field trials did conclude that if emergence was reduced then chlorpropham residues could be detected up to six weeks later. This is important as many of the cases of possible sprout suppressant contamination investigated by ADAS involve the digging up of suspect seed some weeks after planting.

Conclusions.

A summary of the effects of chlorpropham on seed performance is shown below.

1) Seed tubers containing chlorpropham residues of <0.2 mg kg^{-1} grew sucessfully in the field with little if any effect on MET and total emergence. With residues of 0.2 mg kg^{-1}, the total yield decreased by approx. 15%. Most of this decrease was due to a drop in large tuber yield.

2) Seed tubers containing chlorpropham residues of 0.3 to 1 mg kg^{-1} showed increasing effects of the chlorpropham on seed performance ranging from a delay in MET of 14 days, decreased total emergence and subsequently a decrease in medium and large yield and in total yield.

These effects worsened with increasing chlorpropham residues at planting

3) In cases where the seed tubers contained chlorpropham residues of >1 mg kg^{-1}, drastic effects on total emergence (approx. 50%), mean emergence time, total yield and yield of large and medium tubers were noted. However, it took full commercial dose applications of chlorpropham to the tubers to produce residues as high as this at planting time.

CHAPTER 4

THE EFFECT OF TEMPERATURE AND DURATION OF AIRING ON THE PERFORMANCE OF CHLORPROPHAM TREATED SEED TUBERS

4.1 Introduction

The previous chapter showed how exposure to very small amounts of chlorpropham could badly affect the performance of seed tubers. With the knowledge gained in that chapter advice could be given when seed tubers were analysed and found to contain chlorpropham. However, when traces of chlorpropham e.g. 0.2 mg kg^{-1} were found in samples some growers asked if there was a procedure to reverse or overcome the effect of chlorpropham if the contamination was discovered early enough in the storage season. The other chemical, tecnazene, commonly used for sprout suppression in this country has been applied to seed tubers as a fungicide and any sprout suppressing effect reduced before planting by airing. Some problems associated with inadequate airing have lead to the Scottish Colleges recommending a ban on its use on seed despite several other advantages in its use. Like tecnazene, chlorpropham is also classed as a volatile pesticide and although active at a much lower concentration, its effect could be reduced by airing. Previous studies in our Dept. using tecnazene which had shown that its effect on sprouting could be reduced on airing lead us to attempt a similar approach with chlorpropham (Dalziel, 1978).

Studies in large commercial stores where chlorpropham was applied to suppress sprouting revealed the need for reapplication during the storage season to maintain sprout control. Chlorpropham residues were shown to decrease with time (Corsini et al., 1979) and assumed to be lost mainly through volatilisation; however, metabolism of the compound or binding to some component in the tuber and subsequent inability to extract this bound material could also lead to this effect. In these studies the chlorpropham was applied at a level sufficient to maintain sprout control in ware stores i.e. 10-20 mg kg^{-1}. Although this application rate is generally regarded as necessary to control sprouting, the application of the chemical is often uneven and in practice many tubers receive less than this and are controlled adequately for some time. This aspect will be discussed in some detail in the next chapter. Many seed emerged after low level treatment but much slower than controls. In effect they behaved as if planted weeks late, leading to yield reductions. Dyke (1956) showed that, in England and Wales generally, a 1 week delay in planting cost 0.75 tonnes ha^{-1} every week after the end of May. At the stage this work was carried out it was not known whether a treatment to reduce chlorpropham residues in lightly contaminated tubers would result in a marked improvement in growth performance in the field. To answer this question the study was set up. As well as airing duration, the temperature at which it was carried out was also investigated as it could affect the reduction of chlorpropham either through volatilisation or perhaps increased metabolism of the compound.

There are many problems associated with setting up of this type of study. Different airing and temperature combinations will affect

the physiology of the tuber in different ways, leading to the need for a separate control for each of the treatments in the study. Temperature and airing regimes themselves will have an effect on sprout growth, emergence and yield which are all parameters being assessed in the study. In addition, limitations on what can practically be carried out must be borne in mind in the development of the field trial. In the field trial in the previous chapter, the controls and treated tubers were desprouted after storage and allowed to chit for some time prior to planting. If this had not been carried out and the controls had been planted out with sprouts 400-500 mm long then they may have rotted in the field and any haulm growth would result from regrowth from the tubers at a later stage. Attempts to maintain what could be referred to as normal practice must be considered as important.

The level of chlorpropham treatment was chosen to represent what would be expected after a short exposure to the chemical and from residue figures obtained in Chapter 3; treatment with chlorpropham at a rate of 5 mg kg^{-1} produced tuber residues at planting in the order of 0.5 mg kg^{-1} and when these were planted out, there was an obvious reduction in yield compared with controls.

4.2 Experimental

4.2.1 Materials

Chlorpropham, glass distilled hexane, anhydrous sodium sulphate and alumina were as previously described (Chapter 3). Seed of cv. Desiree (AA1 grade) was purchased from J and E England, Scotland. Grammoxone was purchased from ICI (England). Sanspor was purchased from ICI (England). Temik 10G was purchased from Union Carbide U.K. Ltd. (England)

4.2.2 Methods

Chemical treatment

As in Chapter 3, the chlorpropham was applied to the potatoes on an alumina carrier. On 15 December 1977, 10 kg batches of cv. Desiree were dusted with 25 g carrier to give treatment rates of 0 (control - no chlorpropham added) and 5 mg kg^{-1}. There were four replicate boxes per treatment; each treated box had a corresponding control, resulting in eight boxes at each temperature and airing combination. Planting was assumed to be near the end of April, weather permitting. In case weather conditions postponed or accelerated the planting date, a number of extra boxes were treated and stored to ensure a 0 weeks airing figure could be included in the study (96 boxes in all were stored).

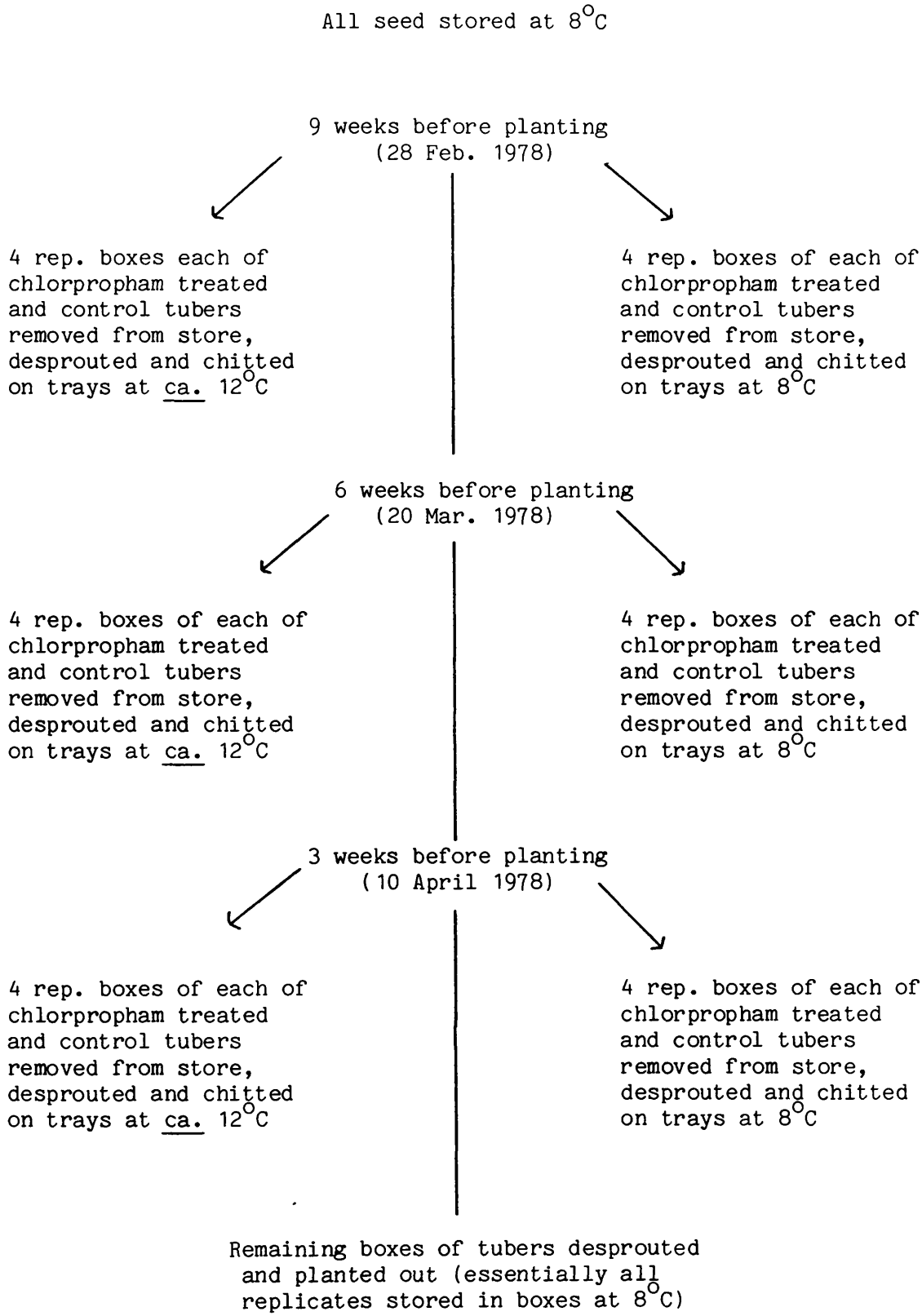
All boxes were stored at $8^{o}C \pm 1^{o}C$ until approx. 9 weeks before planting time and on 28 February 4 replicate treated boxes and their corresponding controls were removed from the cold room and any sprouts >5 mm removed. The tubers were then placed on open wooden trays of 5

kg capacity and stored under illuminated conditions at approx. 12°C until planted out. A large wooden box which could be held at approx. 12°C by the use of fans and heaters was used to store the trays of potatoes. The same type of fan as used in the 8°C storage regime was employed to ensure similar airing was carried out. Although the box was well insulated, the 12°C storage regime could not be as accurately controlled as the 8°C regime. 12°C was the minimum temperature recorded but for a few hours each afternoon the temperature could rise to approx. 14-15°C before dropping again. This was monitored by a thermal hydrograph (Cassell, U.K.) situated next to the trays.

Another 4 treated boxes with corresponding controls were placed on trays after desprouting and stored at 8°C under illuminated conditions until planted out. The procedure was repeated on 20 March (approx. 6 weeks before planting) and again on 10 April (approx. 3 weeks before planting). The 0 weeks airing treatments at 8°C and 12°C were essentially replicates because they had been stored at 8°C in boxes until planting i.e. no airing (see Diagram 4.1).

During airing the problem of chlorpropham vapour transfer from treated tubers to untreated controls was minimised by wrapping the eight replicate trays in plastic sheeting to restrict air movement, as far as possible, to an upward direction through the boxes to the top of the trays where the air was swept away by fans. Although not entirely suitable, it was felt this storage arrangement was preferable to storing the controls and treated tubers separately with the accompanying problems of matching humidity, temperature, light etc. exactly. With the equipment available at the time, this would have been impossible to achieve and therefore the controls would no longer

Diagram 4.1 Plan of temperature and airing duration of chlorpropham treated seed tubers.



be controls in the strictest sense. Any transfer of chlorpropham if it did occur could be quantified by analysis of the controls for chlorpropham and taken into account.

The degree of sprouting was quantified immediately before planting by measuring the longest sprout on each of a 50 tuber sample of each replicate and a 1 kg sample was removed from each replicate, washed and stored at -18^{o}C until required for analysis.

Planting

The experimental area was situated as before at Hattrick Farm, Bridge of Weir, Renfrewshire (NS 355673) and consisted of a sandy loam soil which had previously been treated with FYM. Immediately prior to planting, potato fertiliser (SAI) was applied at a rate of 1400 kg ha^{-1}. Temik 10G (aldicarb) granules were applied at the recommended rate (by hand using appropriate precautions) at planting time.

Experimental design

The site was planted out in four replicate blocks with the constraint that each of the eight treatments (4 airing times at 2 temperatures) in each block was planted beside its corresponding control to give a split plot type design. This resulted in 64 experimental plots in total. The blocks were protected by guard drills and as all treated tubers were cv. Desiree, cv. Pentland Crown (white tubers) was used to separate plots i.e. drills between treatments and 2 m lengths between plots in each drill. The 2 m length was chosen as the maximum length a tuber would be carried forward along the drill by a harvester, ensuring tubers would be assigned to the correct plots during lifting.

Husbandry

A combination of a Grammoxone (paraquat) spray at emergence time and hand weeding when necessary was practised to control weeds which if left unchecked could have been a serious problem in treated plots. As in the previous experiment, blight was contolled by Sanspor (captafol) sprays applied at fortnightly intervals from August onwards. Emergence scores were taken at 4 day intervals from 15 May - 6 July 1978 when it was considered too damaging to walk through the crop checking emergence. Plants emerging at this late stage would presumably contribute little to the final yield. Each drill was assessed individually and results subsequently pooled.

Yield

The crop was mechanically defoliated at the end of September, 1978 and harvested on 10 October, 1978. As before, each drill was assessed individually by being placed in 12 kg capacity nets, stored overnight and mechanically graded over 52 and 32 mm riddles on 11 October, 1978 when the yield of each drill was recorded.

Residue determination

The washed tubers were dealt with as previously described (Chapter 3).

4.2.3 Analysis of results

Sprout length

Preliminary analysis of the data to check that it was normally distributed and variances were not related to means so that statistical procedures could be carried out showed that the data was not normally distributed.

Emergence and yield

As the data collected in this section fullfilled the requirements of analysis of variance, this technique was used as a preliminary check for significant differences in replicates, drills and blocks before t tests were carried out to determine significant differences if any in treated tubers and untreated tubers under the same storage regime.

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the sample means

s_1 and s_2 are the sample standard deviations

n_1 and n_2 are the sample sizes

Table 4.1 Sprout length and emergence characteristics of tubers treated with chlorpropham (5 mg kg^{-1}) compared with controls aired under the same conditions after storage.

Treatment				
Aired at 12°C	airing (weeks)	mean sprout length (mm)	MET[a] (days)	TE[b] (%)
control	9*	101.5	22.4	99.6
chlorpropham	9*	23.77	36.9	94.0
control	6*	19.2	26.0	100
chlorpropham	6*	2.9	44.6	88.3
control	3*	14.4	32.5	99.6
chlorpropham	3*	3.7	43.4	90.8
control	0	453.8	35.6	99.2
chlorpropham	0	25.4	47.5	84.6
Aired at 8°C				
control	9*	30.7	22.1	100
chlorpropham	9*	4.8	45.7	87.9
control	6*	19.2	28.6	100
chlorpropham	6*	2.9	46.2	93.7
control	3*	7.8	34.0	100
chlorpropham	3*	3.6	42.2	92.1
control	0	411.2	36.0	97.9
chlorpropham	0	23.8	47.2	85.8

[a] mean emergence time

[b] total emergence

* tubers were desprouted before airing (see Diag. 4.1)

Table 4.2 Summary of yield data obtained from controls and chlorpropham treated seed tubers stored at 8°C and aired at different airing time - temperature combinations

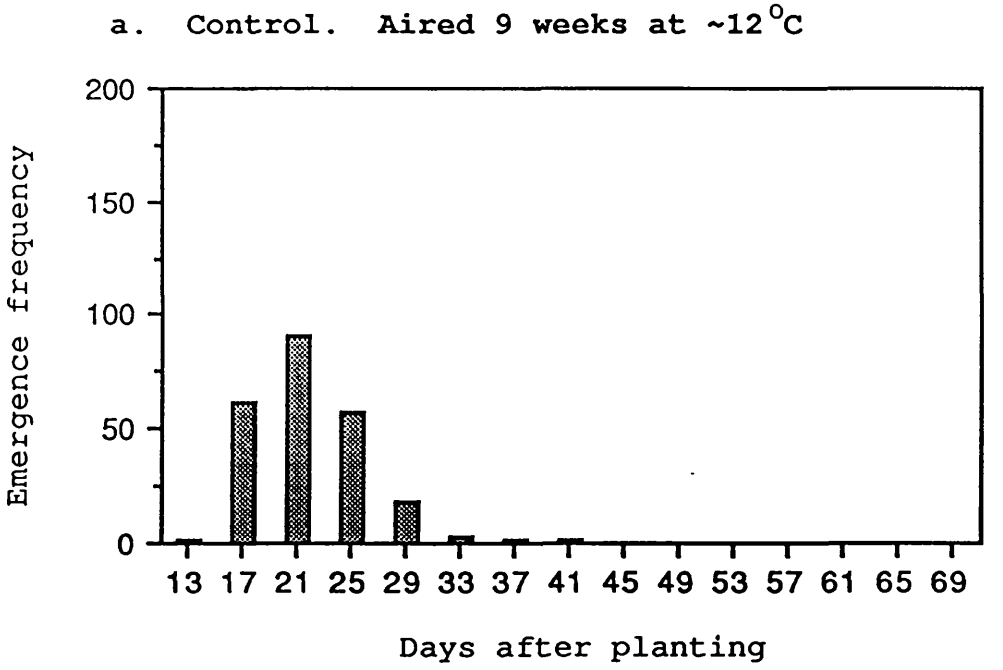
Treatment	airing time (weeks)	large[a] (kg/drill)	medium[b] (kg/drill)	total[c] (kg/drill)	%large	%medium
Aired at 12°C						
control	9	22.7	1.6	24.3	93.4	6.6
chlorpropham	9	18.3	1.3	20.0	91.5	8.5
control	6	21.7	2.3	24.0	90.3	9.7
chlorpropham	6	13.3	2.3	15.5	84.9	15.1
control	3	21.8	2.0	23.8	91.6	8.4
chlorpropham	3	15.4	2.5	17.9	85.7	14.3
control	0	18.7	2.2	20.9	88.9	11.1
chlorpropham	0	12.1	2.4	14.5	82.9	17.1
Aired at 8°C						
control	9	21.3	2.1	23.4	91.1	8.9
chlorpropham	9	12.9	2.7	15.6	82.3	17.7
control	6	21.2	2.3	23.5	90.2	9.8
chlorpropham	6	12.6	2.8	15.4	81.4	18.6
control	3	21.2	2.2	23.4	90.4	9.6
chlorpropham	3	13.0	2.2	15.2	85.4	14.6
control	0	17.9	1.9	19.8	90.2	9.8
chlorpropham	0	12.6	2.3	14.9	83.8	16.2

[a] > 50 mm

[b] 32-50 mm

For conditions of airing see Diag. 4.1.

Fig. 4.1 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



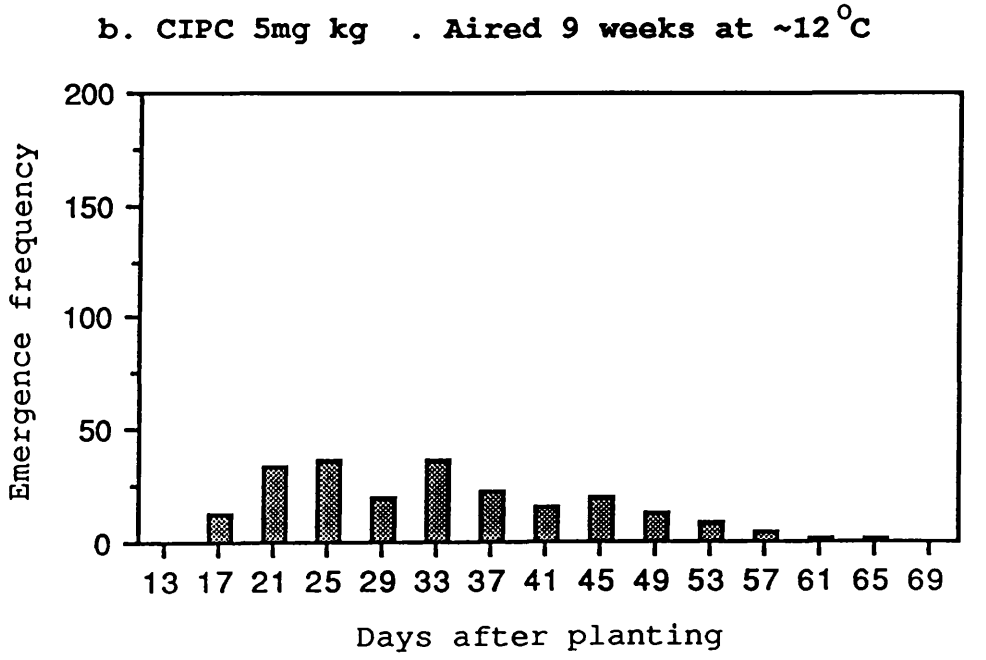
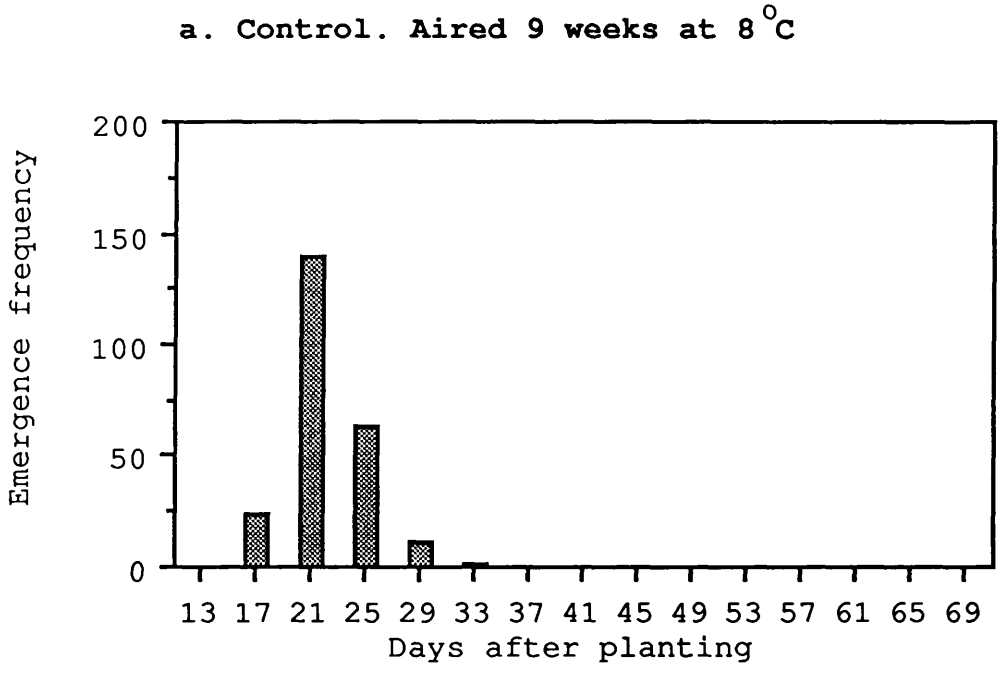


Fig. 4.2 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



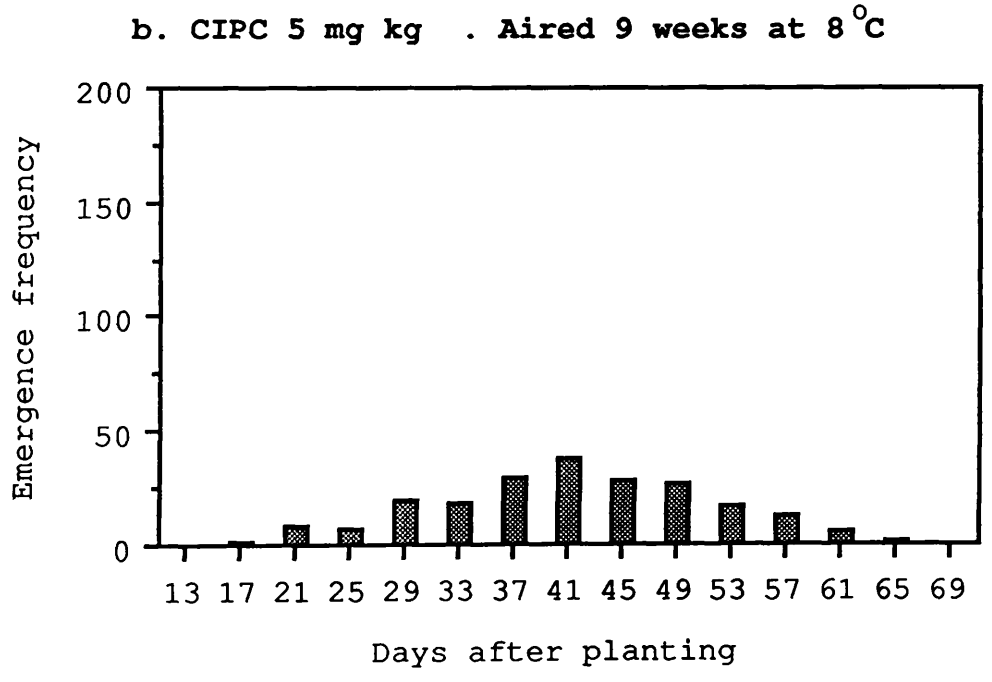
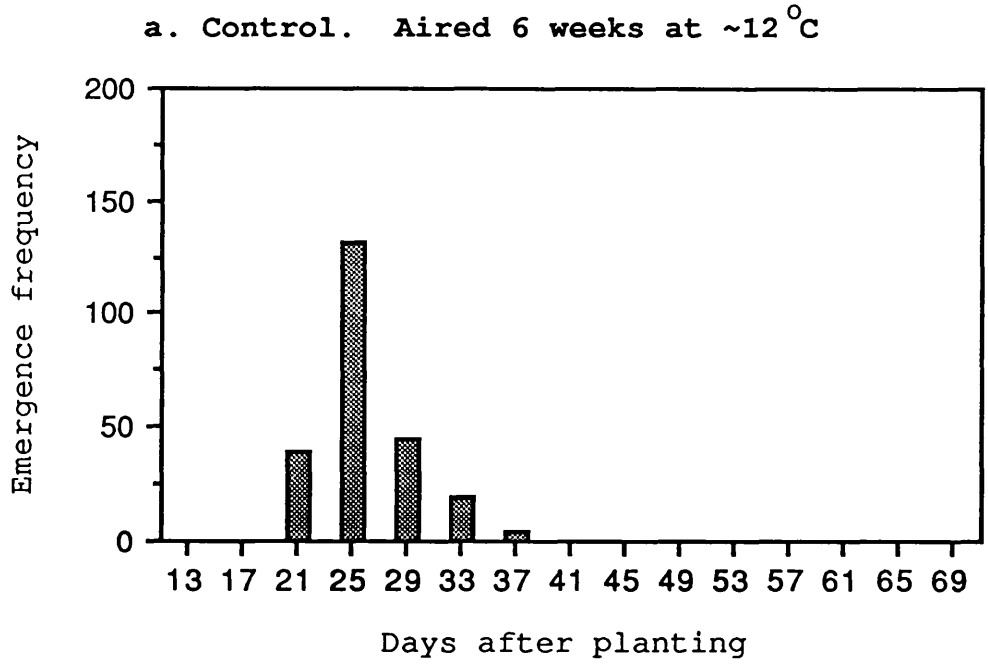


Fig. 4.3 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



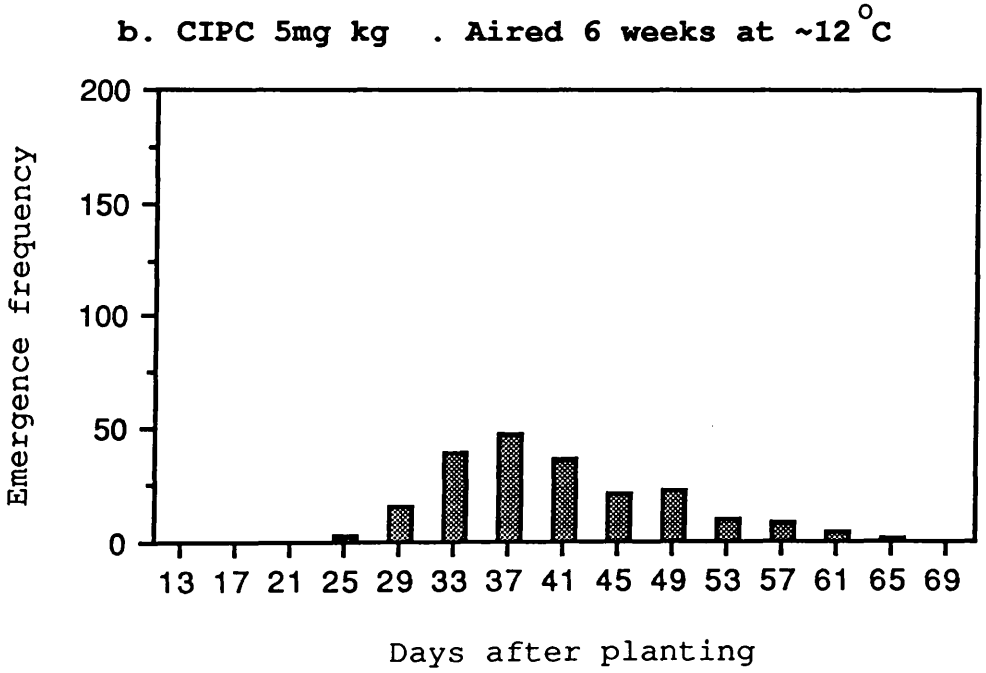
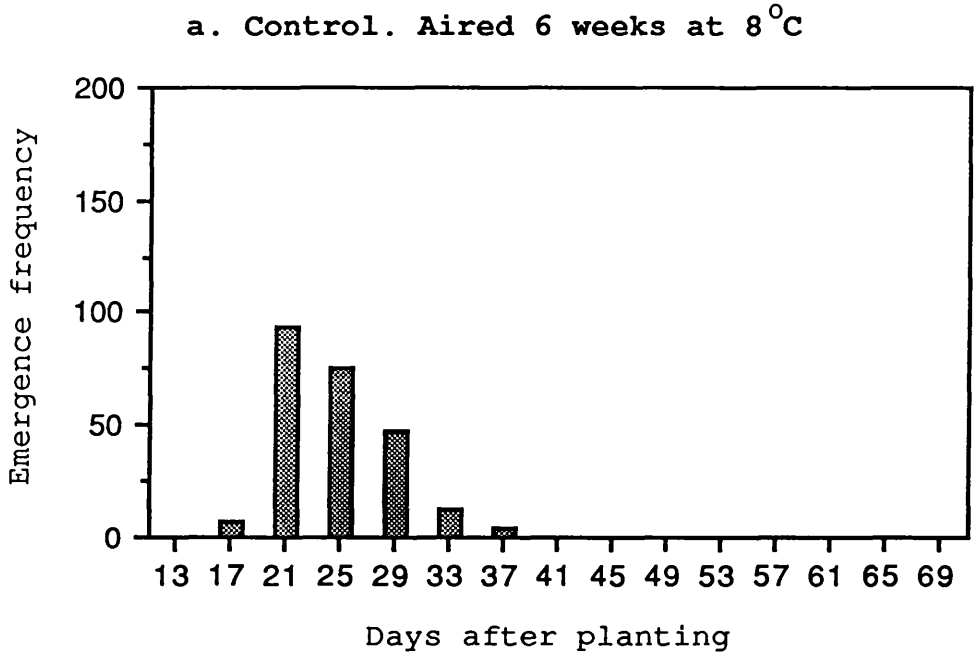


Fig. 4.4 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



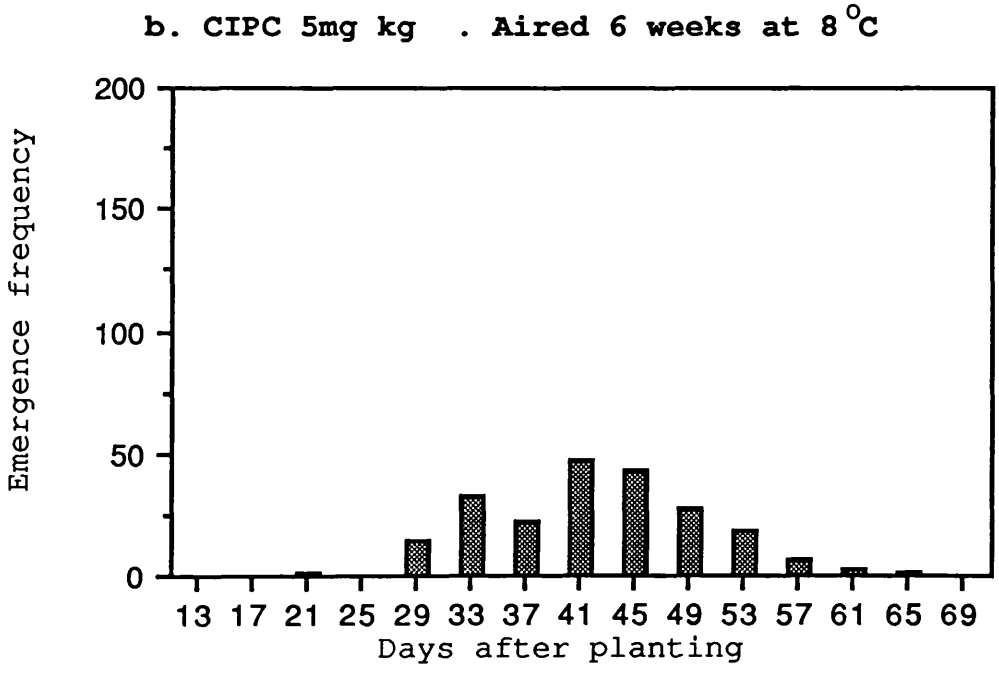
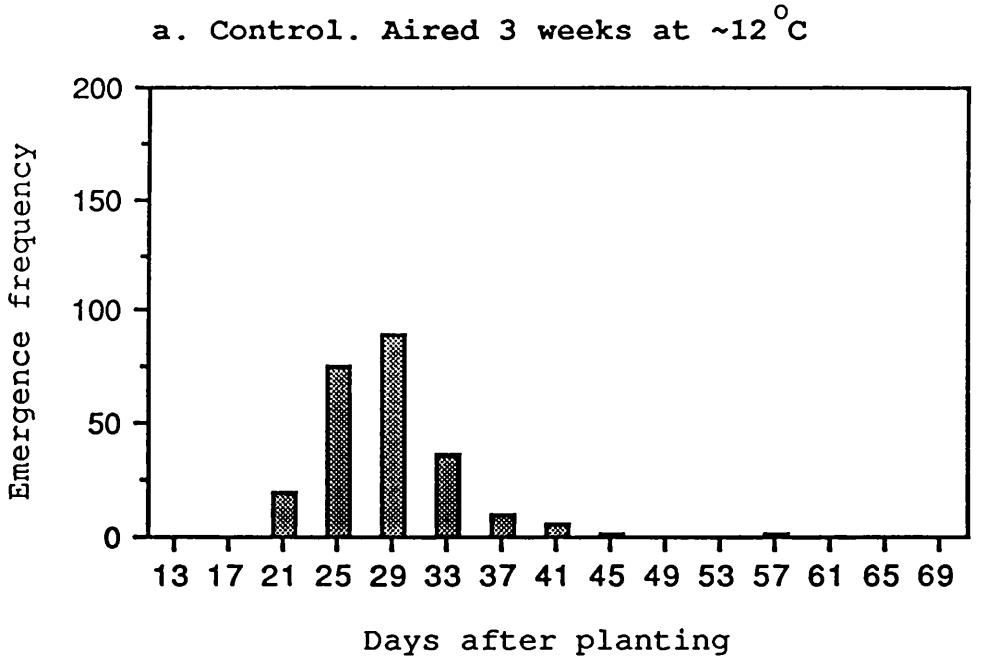


Fig. 4.5 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



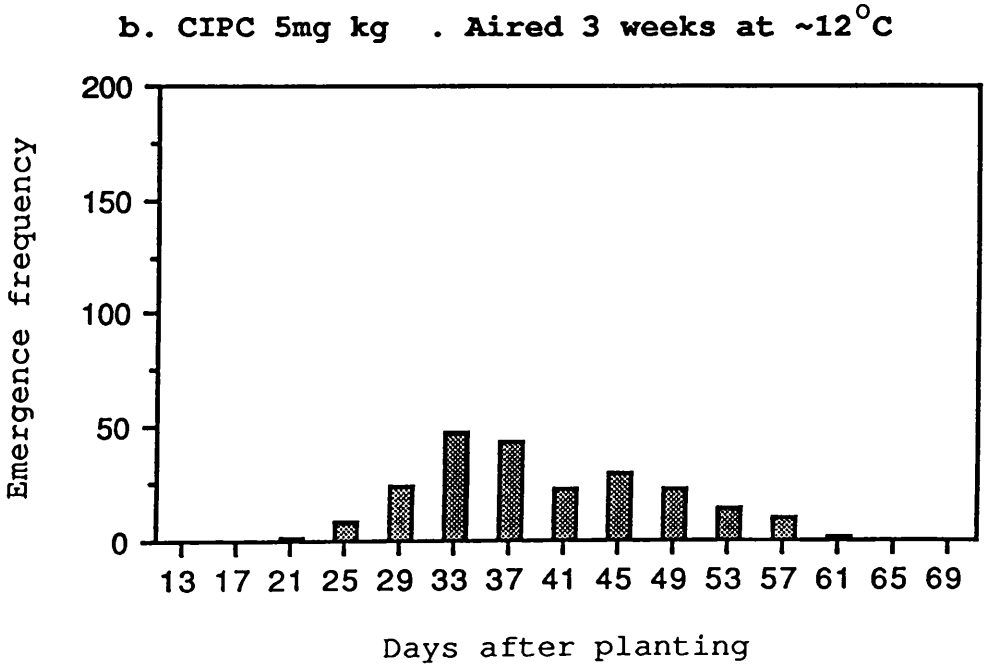


Fig. 4.6 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.

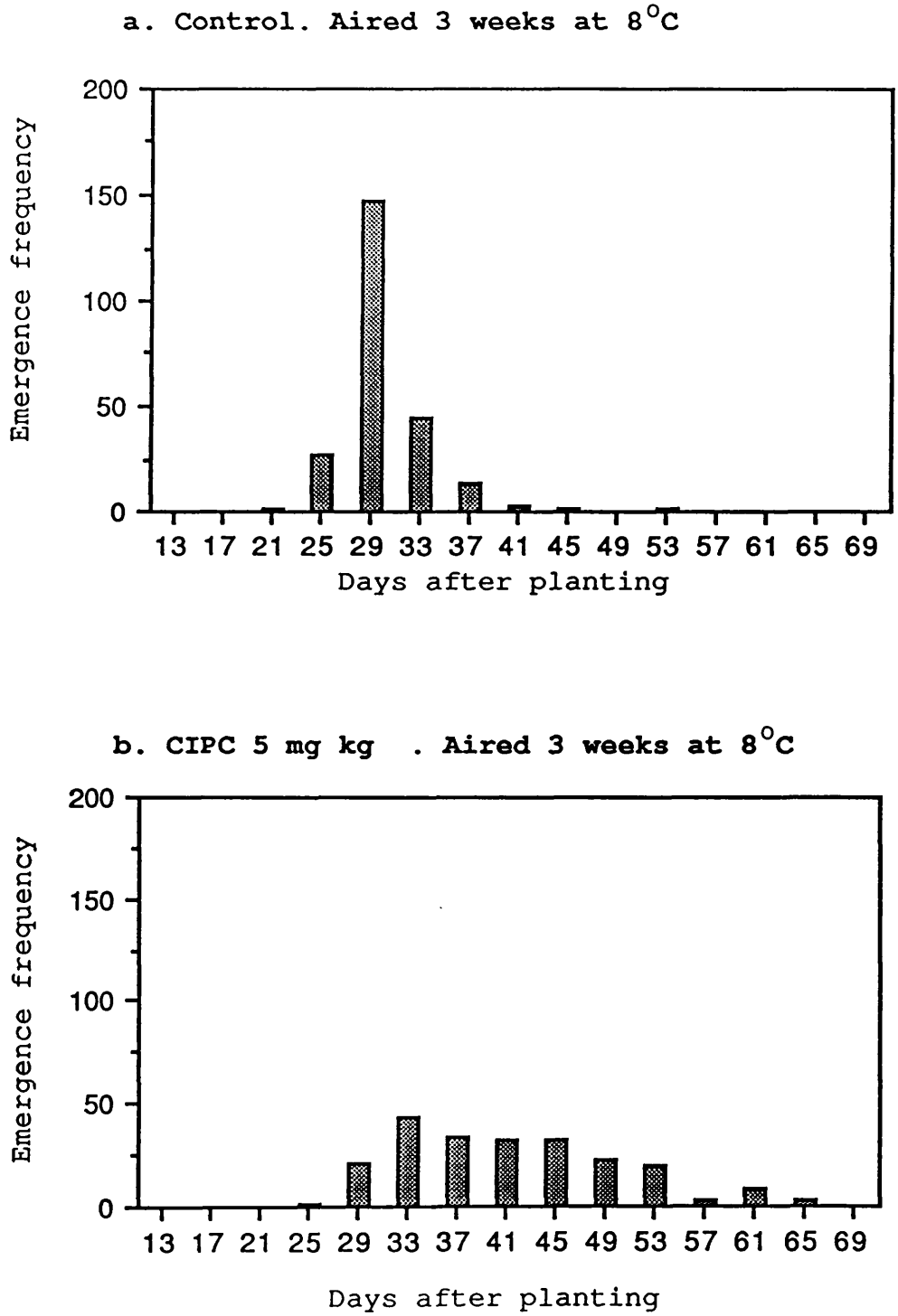
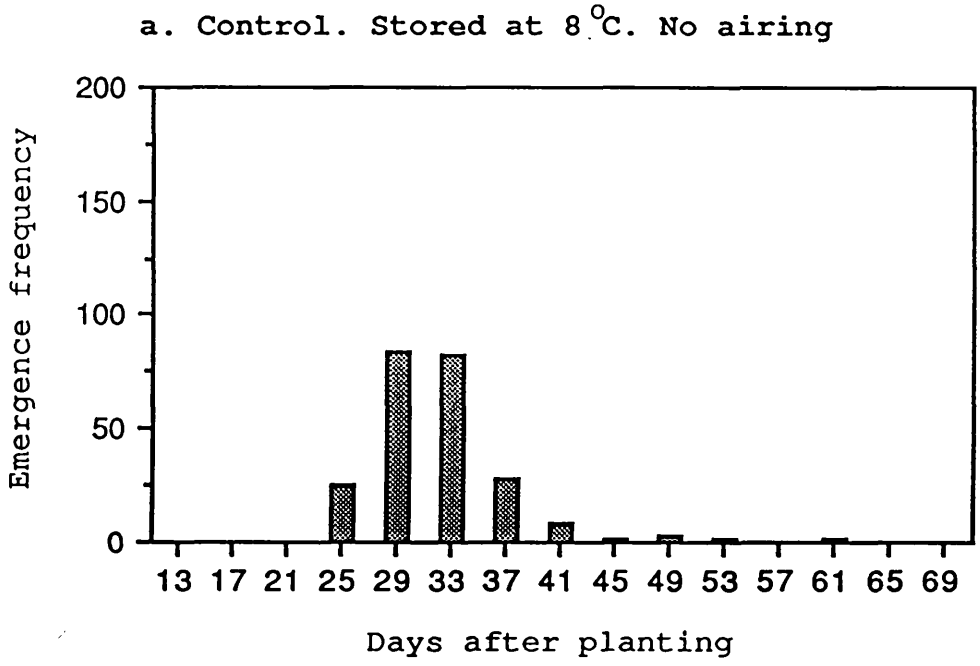


Fig. 4.7 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.



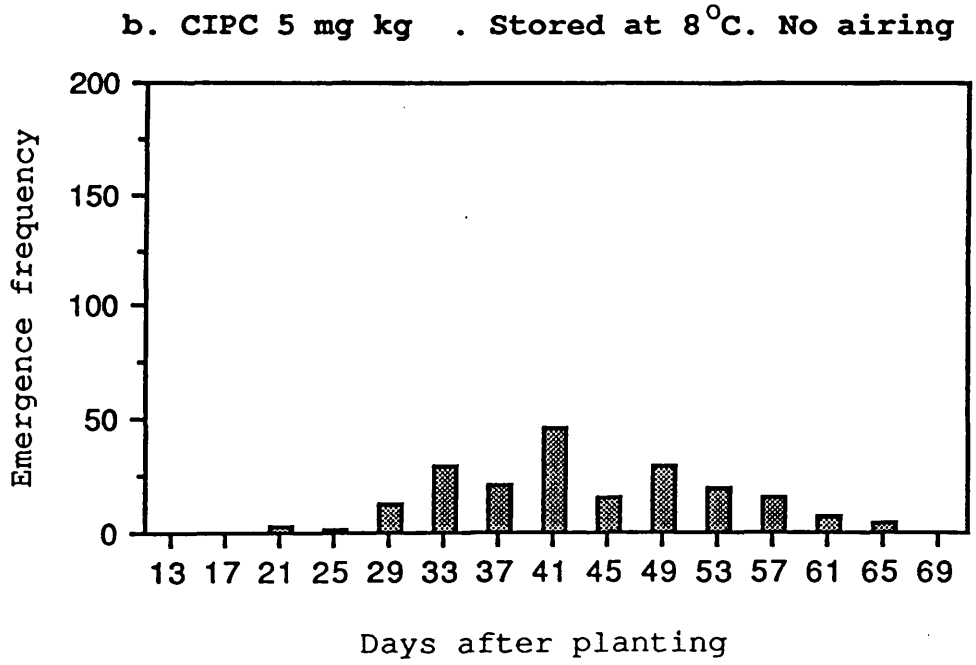
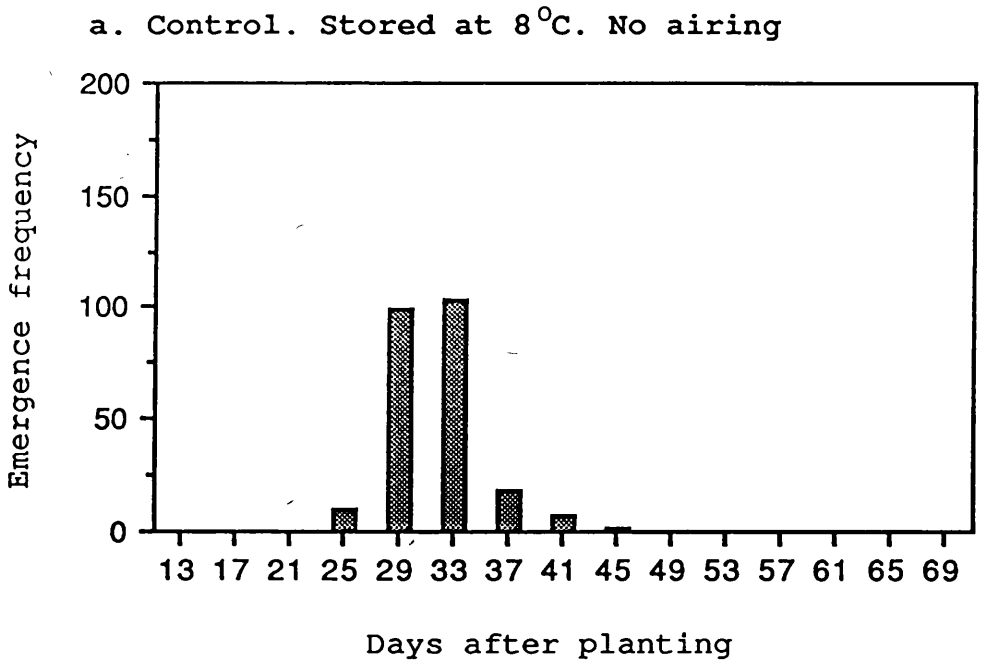
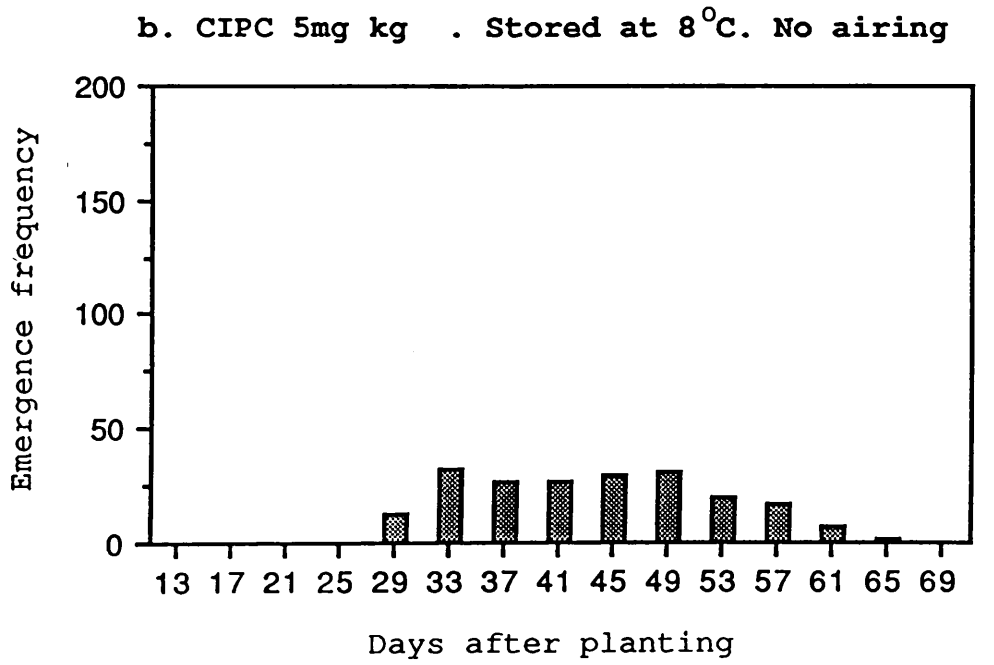


Fig.4.8 Emergence profiles of cv. Desiree seed stored in boxes at 8°C from treatment on 15 December 1977 until airing. Planted on 26 April 1978.





4.4 Discussion

Sprout length

1. As expected, after sprouts were removed on commencement of airing, the regrowth in controls and treated material increased with longer airing time. Temperature had an effect on the amount of regrowth.

2. The mean sprout length of control tubers allowed to sprout unchecked in boxes with no airing and stored at $8^{o}C$ was approx. 400 mm, showing cv. Desiree to be a vigorous sprouter. In the case of treated tubers, the mean sprout length was approx. 25 mm under the same conditions.

At this point a problem was encountered with the statistical analysis of sprout length data because the data obtained from treated material was not normally distributed. The requirements for most statistical routines used in agricultural experiments are, in general,

1. sampling should be random
2. the data obtained is normally distributed
3. variances are not related to means.

Most statistical packages on main frame or microcomputers allow testing for 2 and 3 above. The design and operating procedures in trials must cope with requirement 1, i.e. when collecting samples or data care must be taken to avoid any bias.

There are various methods available to allow the testing of the assumption of normality e.g. plotting the data on normal probability paper, histograms and boxplots (a way of representing the distribution of data within a sample). Two extreme cases to illustrate the problem

Fig. 4.12a Histogram of sprout length data from control tubers

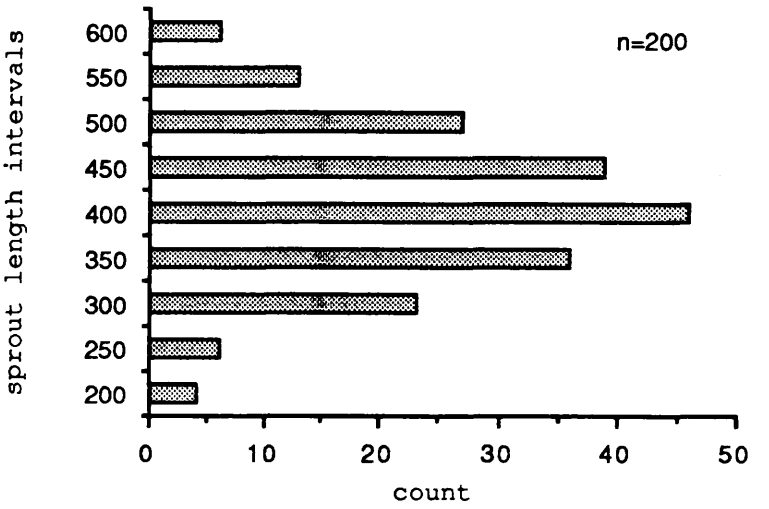
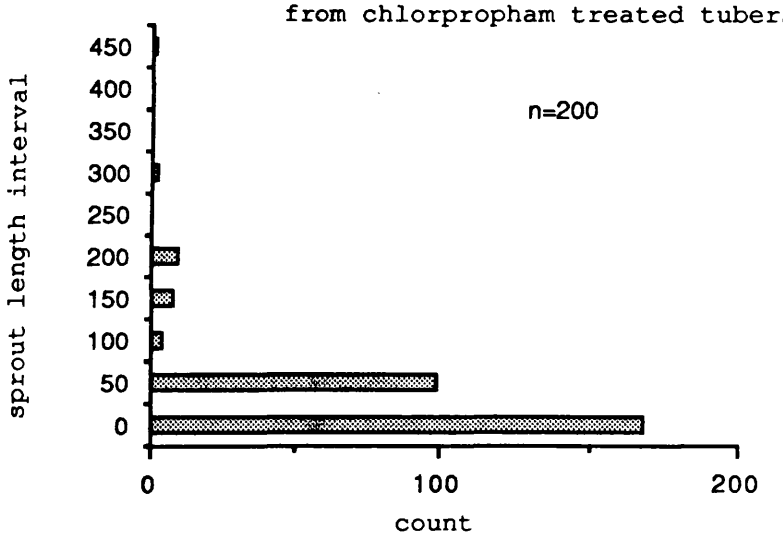


Fig. 4.12b Histogram of sprout length data from chlorpropham treated tubers



found with the data are shown in Fig 4.12 where the control data (4.12a) follows an obviously normal distribution and the treated data (4.12b) does not.

Usually, deviations from normality can be corrected through transformation of the data; commonly used transformations are square root, square or reciprocal but attempts to use these resulted in the control data failing to fulfil normal requirements.

The main problem was a direct result of unusual sprouting in treated tubers in stored boxes. Visual inspection of treated boxes revealed that while sprout control was good in the centre of the box, some tubers near the edges of the box had long sprouts on them (> 50 mm). As random sampling was carried out on these tubers and the 50 tuber sub-sample was a large proportion of what was in the box (Desiree seed was relatively large), some of these tubers were inevitably sampled. They had the effect of drastically altering the mean tuber sprout length for that box although <10% of material sampled. Fig. 4.12b shows this effect well; in a sample of 200, 20 are regarded as outliers by the statistical package in this particular case. Workers using other volatile chemicals have reported similar problems (Beveridge, 1979). The fact that these sprouting tubers are commonly found at edges of boxes suggests that airflow fluctuations or chlorpropham adsorption to the cardboard could be involved. This is substantiated by reports of irradiated tubers failing to show similar effects (Muir, 1987). Sprout length results with outliers are reported in Table 4.1.

In all cases, controls differed from treated tubers. With the longest airing regimes, especially at 12°C, treated tubers showed some sprouting indicating growth in the field was possible. No unusual patterns in sprout growth were observed. With tecnazene, thick bulbous sprouts etc. can be present. Also some blackening of the growing tip can occur after chlorpropham treatment but was not found here.

Emergence

The mean emergence time and total emergence were calculated as previously described (Chapter 3) and are summarised in Table 4.1.

Emergence profiles (Figs. 4.1-4.8) drawn up from emergence data show that in general, controls aired at 12°C emerged faster than those aired at 8°C. The same was true of treated tubers. Airing for 9 weeks allowed new growth of sprouts in controls and plants emerged quickly, irrespective of temperature. In contrast, the treated tubers stored for 9 weeks at 12°C emerged faster and more completely than those aired at 8°C.

The emergence profiles for 6 weeks airing at the two temperatures suggested the same general pattern as 9 weeks airing i.e. increased temperature of airing had a small positive effect on emergence of control tubers; the treated tubers emerged more slowly and with more blanking in the field. The 8°C storage regime resulted in greater delayed emergence than 12°C.

Three weeks airing after desprouting provided less time for new sprout growth on control tubers so at both airing temperatures, the

MET was delayed compared with controls at longer airing time - temperature combinations. The treated tubers aired at both temperatures showed a slight improvement in MET.

The last two pairs of histograms (Figs. 4.7 and 4.8) are essentially replicates as no airing was carried out i.e. they were stored in boxes at $8^{o}C$ until planting time when all sprouts >5 mm were removed. This desprouting resulted in a delay in MET compared to desprouted controls allowed to air and recover (from approx. 26 days for desprouted controls allowed to air to approx. 36 days for desprouted controls planted out immediately after desprouting). The MET of treated tubers planted out with no previous airing was delayed to approx. 47 days with an associated reduction in total emergence to 85%.

In only one treatment was a MET recorded which was similar to any of the controls - 9 weeks airing at $12^{o}C$ produced an MET of 36.9 days and controls which had been stored at $8^{o}C$ with no airing and had been desprouted prior to planting produced an MET of 36 days.

Yield

Emergence data has been collected by members of the Agricultural Chemistry Dept. in a number of studies involving different chemicals. The data has, in the past, been linked to eventual yield of the crop but the relationship between the two has not been sufficiently close (r^2= 0.34) to allow predictions of subsequent yield from emergence data (Dalziel, 1978). Because of this, the crop was grown, harvested and the yield data recorded. In all cases, the chlorpropham treated seed yielded significantly less in terms of large (>52 mm) and total tubers at harvest compared to controls; this effect occurred with each

airing time - temperature combination studied in the experiment. The yield of seed from treated tubers was not significantly different from that of corresponding controls but when looked in terms of the proportion of the total amount harvested from each of the temperature - airing combinations, there was an increase in percentage of seed harvested from chlorpropham treated plots. Delayed emergence for any reason would would result in a reduction in yield and an increase in the proportion of seed in the crop; the longer the delay in emergence the greater the effect on yield and the size distribution within the crop (Burton, 1966).

All airing time - temperature combinations studied in this trial failed to reduce the effect of chlorpropham to a level which could not be seen in the growing crop and in its yield. A summary of yield data is presented in Table 4.2. Although airing at 12^{o}C for 9 weeks did improve the performance of the treated seed, there was still approx. 5 kg/drill yield penalty compared to the corresponding control. Control tubers stored at 8^{o}C and desprouted immediately prior to planting gave similar yields to the treated tubers described above.

As discussed earlier, emergence data could not be used to predict yield performance in previous trials carried out but examination of the data produced in this study and the field trial reported in the previous chapter showed that this was not the case when working with a range of chlorpropham levels on a limited number of cultivars. With cv. Desiree, the relationships between MET and total and large yields in this experiment were found to be linear; the co-efficient of determination for MET and large yield was 0.75 i.e. 75% of the variability in yield could be attributed to emergence time. Linear

regression of all MET data and yield data obtained per plot (64) produced the equations shown in Table 4.3.

Table 4.3 Parameters of the linear model ($y = \alpha + \beta x$) describing the relationship between mean emergence time and various yield characteristics.

	slope (β)	sd	intercept (α)	sd	r^2
ware yield	-0.476	0.04	33.58	1.24	0.75
seed yield	0.022	0.006	1.45	0.22	0.17
total yield	-0.454	0.035	35.04	2.24	0.74

Residues

To minimise the amount of residue analysis, 2 from 4 replicate samples were randomly chosen and and analysed for chlorpropham. The figures obtained varied greatly, chlorpropham residue values ranged from 0.42 - 0.76 mg kg^{-1} washed whole tuber and no obvious relationship between airing duration and temperature was found and no obvious relationship between residues and MET or yield (Figs. 4.13 and 4.14). It is possible that the act of airing increased the range of values because the airing trays were stacked eight high and the sides of the trays wrapped in plastic sheeting to minimise chlorpropham vapour transfer from treated tubers to controls. This was successful from the point of view of minimising transfer of chemical because < 0.035 mg kg^{-1} was found in 5 control samples analysed for chlorpropham, but it may have contributed to greater variation among treated samples.

Fig. 4.13 The effect of chlorpropham residues on MET (1977-78). cv. Desiree

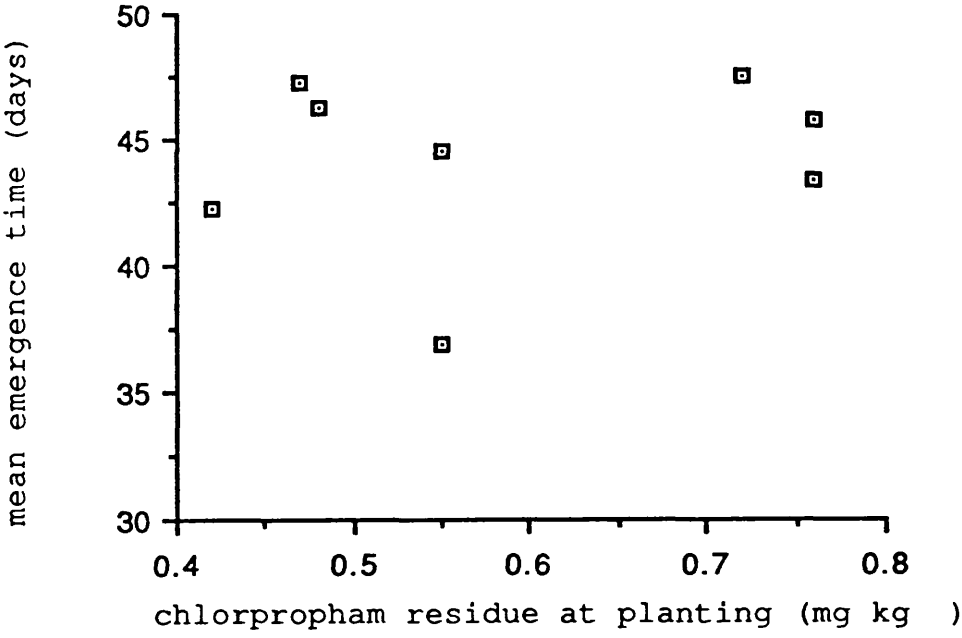
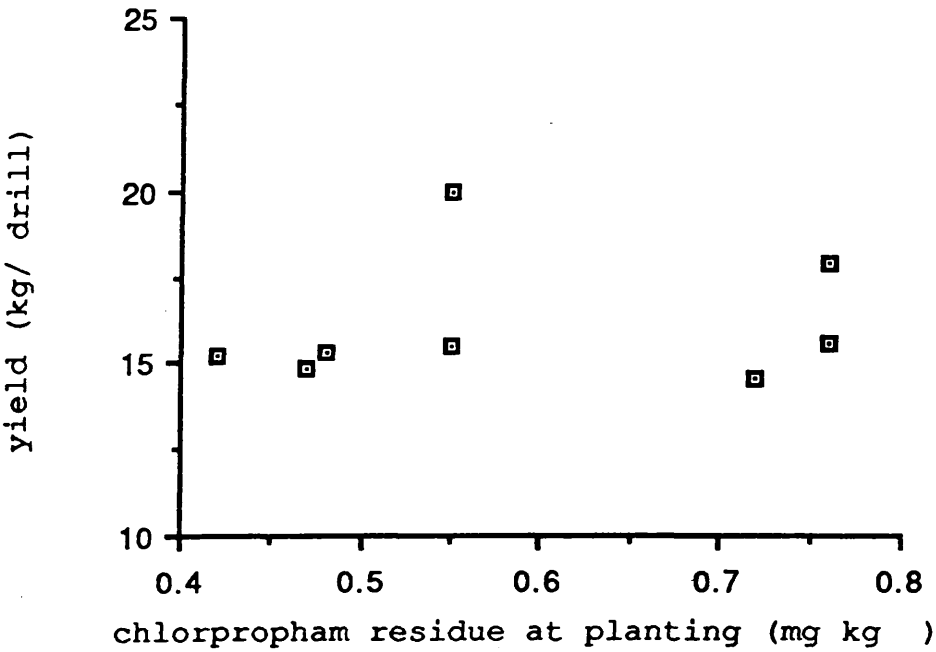


Fig. 4.14 The effect of chlorpropham residues on yield. (1977-78). cv. Desiree.



In conclusion, once seed has been contaminated with chlorpropham, changes in storage conditions are unlikely to completely overcome the effect of the chemical.

CHAPTER 5

DISTRIBUTION OF CHLORPROPHAM IN A COMMERCIAL BOX STORE

5.1.1 Introduction

Ware potatoes intended for processing into crisps are stored at 8-10°C to prevent the accumulation of reducing sugars in the tubers, high levels of which result in a dark brown coloured product on frying. Long term storage at these temperatures is only practicable through the use of sprout suppressant chemicals (van Vliet and Schriemer, 1963).

Crisp manufacturers in the UK and USA use either chlorpropham or maleic hydrazide to control sprouting in their potato stores (Burton, 1966; Sawyer, 1967; Burton, 1978) although on the Continent chlorpropham/propham mixtures are more popular. In addition to the advantages over the alternative sprout suppressant, tecnazene, in terms of price and activity, chlorpropham can be applied to potatoes in store as an aerosol. The various formulations of chlorpropham and methods of applying these formulations were briefly discussed in Chapter 1. Applications are usually carried out by specialised contractors and reapplication can be easily accomplished when needed. These aerosol applications should result in a relatively even distribution of the chemical throughout the store. When this study was carried out, very little information was available on sprout suppressant residues in stores except for studies where samples were taken from the top of the potato stack or when the potatoes were being

unloaded. These studies were mainly concerned with checking that residue levels did not infringe any laws or studying the effect of chlorpropham residues on the severity of internal sprouting (Sawyer and Dallyn, 1956; Sawyer and Dallyn, 1964; van Vliet and Sparenberg, 1970; Martens et al., 1971).

Wilson and Hunter (1965) attempted to examine the effects of airflow on chlorpropham distribution by sampling the piles of potatoes in experimental bins at different levels and sites. Their study was hampered by the fact that in two years out of three, the residues of chlorpropham at all sites in the bins were below the limit of the analytical method (0.5 mg kg^{-1}). They did state that droplet size as well as airflow must have an effect on chlorpropham distribution.

In 1979, Corsini et al., described the distribution of chlorpropham in a large modern bulk store in the USA Their analytical method was sensitive and precise enough to record differences in peel residues of chlorpropham at a number of sites in the store. The level of chlorpropham in the tubers at these sites was determined over a number of weeks and they noted that, in general, chlorpropham residues were high at the top and bottom of stores but the central area contained less. The method of application in bulk stores is via ventilation ducts and up through the bulk of potatoes. The thermal fog has no other obvious route to travel. However, the problem of chemical application is much greater in box stores as it is much easier for the fog to follow air passages such as the small area between the top potatoes in one box and the bottom of the next box.

The chlorpropham fog is not forced through the potatoes as in a bulk store or bins.

Adapting application methods to overcome these problems can be difficult because little information is available on the subject.

Chlorpropham can be applied to potatoes as granules or, more commonly, as an aerosol. Granular applications can only be carried out at the beginning of the storage period and rely on a delayed and slow release of chlorpropham to avoid wound healing problems during the 'curing' period. If the potatoes are wet going into store, granules can clump together on the tubers giving locally high levels of the chemical. It has been shown that skin spot (Polyscytalum pustulans) can be aggravated by high levels of chlorpropham (Ives, 1955) and for this reason and the possibility of only one application of the chemical, granular formulations are not very popular with processors. In bulk stores, they have been spread over the top potatoes as a precautionary measure because condensation tends to encourage sprouting in the top layer of tubers. Dusts are used in many European countries, especially in France where they are applied directly after harvest.

Aerosol applications of chlorpropham are by far the most popular method of application available, for a number of reasons. The application can be delayed until after the tubers have cured and because re-application is easy, flexibility in deciding which potatoes can be used for long term storage is possible. Box, bulk stores or bins can all be treated with the chemical although specialised machinery is needed and slightly different practices must be followed in each case.

To produce an aerosol (< 50 microns) of chlorpropham a fine stream of the chemical dissolved in a suitable solvent is exposed to a heat source resulting in a chemical 'fog' thick enough to obscure vision. Two main types of machine are available to produce these aerosols :

1. The Swingfog or Pulsejet
2. The TIFA (Todd Insecticidal Fog Applicator).

The Swingfog or Pulsejet can be easily identified by the long exhaust pipe. The machine consists of two tanks, one for fuel and the other for the pesticide mixture, a pump, carburettor and a long exhaust pipe. Once the engine has started and warmed up, the pesticide mixture is introduced at the end of the pipe. Different nozzles are available to give a range of flow rates and, therefore, a range of droplet sizes.

The TIFA machine is a modified version of a smoke generator used in World War 2. After the war the generators were adapted for use in applying pesticides. The machine consists of a petrol engine which operates an air blower and two pumps. Air is heated to 500-600^{o}C and passed to a distributer head where the chemical is introduced. At the point where the fog is created the temperature has been measured as 265^{o}C with a flow rate of 95 litres hour^{-1} (Matthews, 1979). As the chemical is separated from the spark plug of the engine, the fire risk is considerably reduced.

Any number of either of these machines can be used at a time to treat stores quickly. Large stores may need 3-5 machines to treat them.

To date, there have been no reports of chlorpropham breakdown during exposure to the high temperatures found in fogging machines, although it would be possible considering the thermal instability of chlorpropham above 230°C reported by Romagnoli and Bailey (1966) with analysis by GC. Presumably, any exposure to air and metal surfaces at high temperatures would hasten any breakdown of the compound. However, pyrethroid breakdown has been investigated and although these compounds have also been shown to be thermally unstable at temperatures around 230°C, little breakdown of these chemicals has been found (Matthews, 1979). The duration of exposure to the high temperatures found in fogging machines is presumably too short to allow breakdown to take place and we must assume that chlorpropham would behave similarly. Also the lack of oxygen in the exhaust would result in reducing rather than oxidising conditions.

The investigation reported in the first section of this chapter was prompted by reports from processors of localised sprouting problems in box stores after chlorpropham application. Analysis of preliminary samples of sprouting and non-sprouted tubers showed differences in the chlorpropham residue levels which could be large enough to explain the localised sprouting problems. Previously, sprouting problems had been attributed to the fact that the tubers in store had been grown under a wide range of conditions and therefore covered a wide range of physiological ages.

The aim of the investigation was to study the distribution of chlorpropham in a large box store some eight weeks after chlorpropham application with a view to pin-pointing areas of the store where

sprouting would be most likely to occur due to lack of chlorpropham. It was hoped that once these areas were identified, contractors could modify their application procedures so that more attention would be paid to these areas during reapplication of the chemical.

5.1.2 Experimental

The store chosen for these studies was a modern box store, designed for long term storage of potatoes at 10°C, with refrigeration facilities and environmental control units closely monitoring humidity and temperature. Full, the store held approximately 2,000 tonnes. It was part of a six store complex built for United Biscuits (UB) Foods Division at Grimsby, England.

The potatoes were stored in wooden 0.5 tonne boxes, grouped in units (9 by 10 by 5). Ten of these units were arranged in the store so that five units ran down each side of the store with a wide central corridor to permit removal and replacement of boxes.

The potatoes were sampled at the beginning of April (8 weeks from last chlorpropham application) and prior to another application of the chemical as sprouting had commenced at various sites in the store.

1977/78 storage season	History of chlorpropham applications to store
23rd Nov. 1977	20ppm + 25% overdose for wooden boxes. TIFA machine using a methanol formulation of chlorpropham (Stored Crop Conservation Ltd.).
12th Dec. 1977	14ppm + 25% overdose for wooden boxes. TIFA machine. Formulation as above.
end Jan. 1978	20ppm + 25% overdose for wooden boxes. Swingfog machine. Methanol formulation (Mirfield Chemical Co.).

All formulations were 50% chlorpropham in solvent (w/v).

5.1.2.1 Sampling

A plan of the store is shown in Diagram 5.1 with boxes where tuber samples were taken marked with an asterisk. The samples were taken at three heights i.e. top, middle and bottom boxes at each sampling point in each unit, resulting in 60 samples in all. Originally, a comparison of chlorpropham residues from samples taken at the centre of the units with those obtained from samples at an outside edge was envisaged. In attempting to remove boxes from the central corridor edge of the units, the units became unstable. Samples had to be taken, therefore, from the third box in from the corridor and not the actual centre of the unit as previously hoped. Considering the time and effort taken by the store manager to achieve even this, it was obvious that our original sampling plan could never have been achieved in a commercial store during the storage season.

At each of the sample sites at least 3 kg tubers, as near the centre of the box as possible, were removed, placed in thick polythene bags and sealed and labelled for subsequent chlorpropham analysis. Care was taken to ensure that the tubers lying at the surface of the boxes were not included in the samples as surface deposition of chlorpropham during fogging may have resulted in unrepresentative residues on these tubers.

5.1.2.2 Assessments

While collecting the tuber samples, assessments were made of

1. The degree of sprouting within the box being sampled. An arbitrary 1 to 5 scale was chosen in which 1 = no obvious sprouting and 5 = over 75% tubers with sprouts >20 mm (this amount of sprout growth is considered excessive by processors).

Sprout assessment key

1 - over 75% of tubers not sprouted
2 - " " " " with 1-5 mm sprouts
3 - " " " " " 5-10 mm sprouts
4 - " " " " " 10-20 mm sprouts
5 - " " " " " >20 mm sprouts

2. The amount of soil adhering to the tubers was noted as either minimal (1), average (2) or excessive (3).

5.1.2.3 Residue determination

Whole, unwashed tubers were minced, the mixture stirred and stored at -18°C until needed for analysis. Chlorpropham was determined as previously described (Chapter 2). In nearly all cases, an alumina column clean-up of the hexane extract prior to GC was considered unnecessary due to the relatively high levels of chlorpropham present on the tubers. Regular checks were made to ensure that the peak being measured was indeed chlorpropham and not a contaminant.

5.1.3 Results

Figs. 5.1a-e show diagrammatic representations of the bays within the store with associated chlorpropham residues.

In distribution work, there is more justification for using peel samples rather than whole tuber samples for chlorpropham analysis as most of the chlorpropham applied will be on or near the surface of the tuber. However, unless otherwise stated, all residues reported in this chapter are on a whole unwashed tuber basis. Unwashed tubers were used because it was felt that a figure for chlorpropham in the vicinity of the tubers was needed. Other workers have used statements such as "loose soil brushed off" but it is difficult to control the amount of soil removed in this way as it is dependent upon the type and condition of the soil at harvest and initial storage.

To investigate the possibility of a link between the chlorpropham residue in a sample and the amount of soil adhering to that sample, assessments were made of soil cover on the tubers taken from each sampling point. The relationship between the two is shown in Fig. 5.2. The relationship between sprout length on tubers and the chlorpropham residues in those samples was also investigated; the results are detailed in Fig. 5.3.

Diagram 5.1 A diagram of the box layout of the store showing sampling points (*)

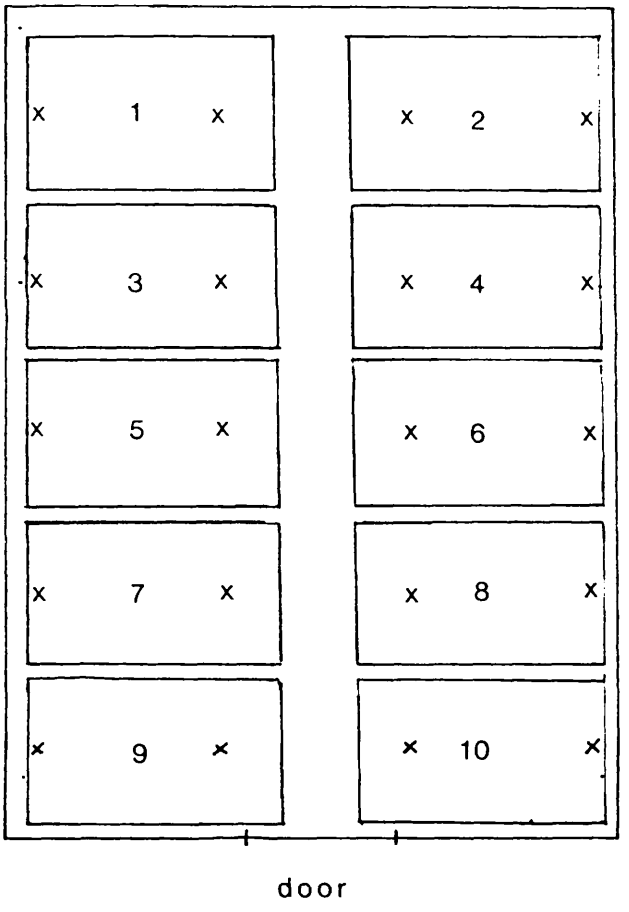


Fig. 5.1a Diagrammatic representation of chlorpropham residues (mg kg^{-1} unwashed tubers) found at various sampling sites in bays 1 and 2 of Grimsby store (1977-78)

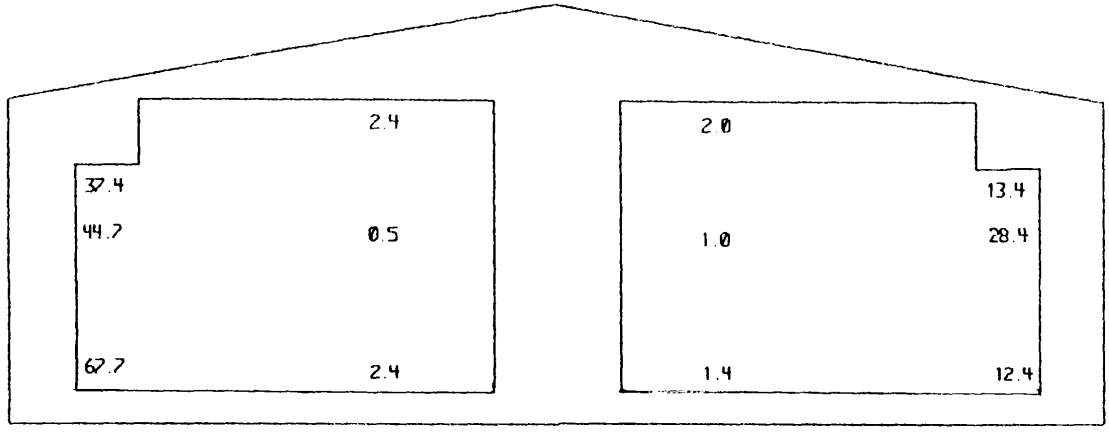


Fig. 5.1b Diagrammatic representation of chlorpropham residues (mg kg^{-1} unwashed tubers) found at various sampling sites in bays 3 and 4 of Grimsby store (1977-78)

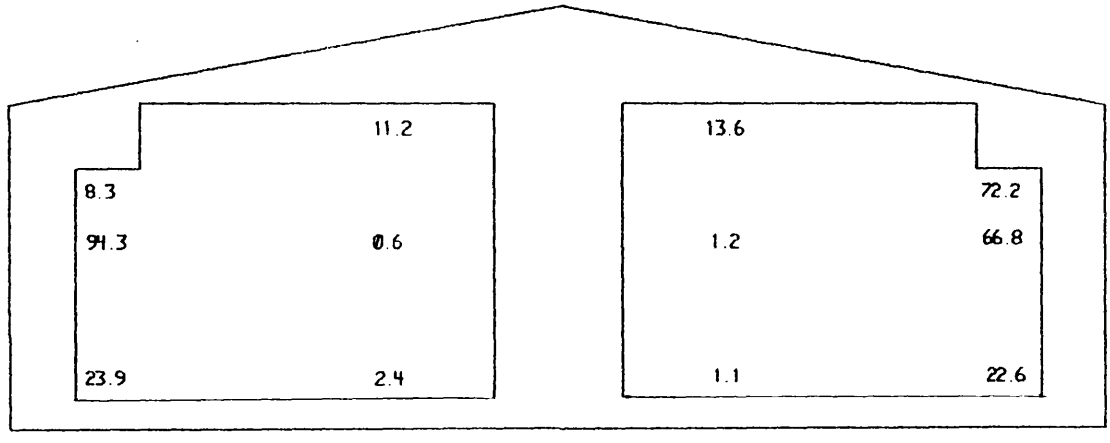


Fig. 5.1c Diagrammatic representation of chlorpropham residues (mg kg^{-1} unwashed tubers) found at various sampling sites in bays 5 and 6 of Grimsby store (1977-78)

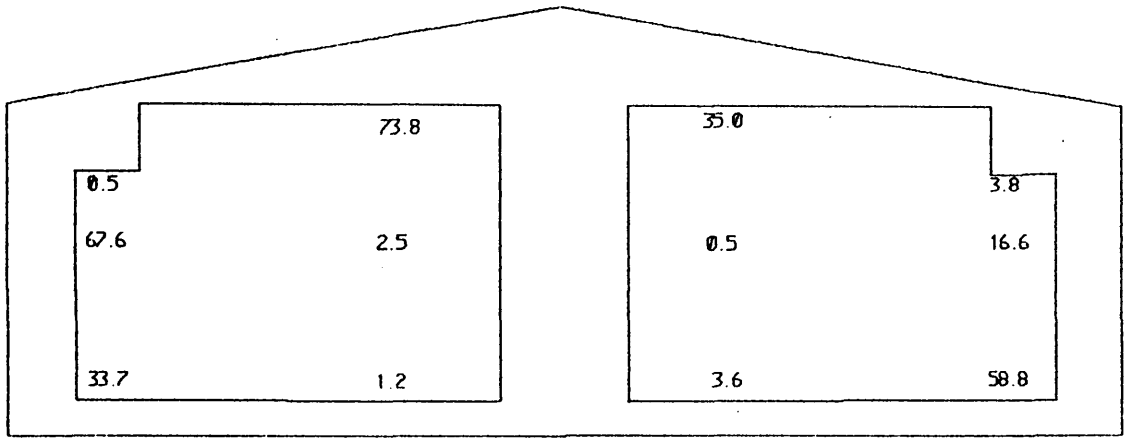


Fig. 5.1d Diagrammatic representation of chlorpropham residues (mg kg^{-1} unwashed tubers) found at various sampling sites in bays 7 and 8 of Grimsby store (1977-78)

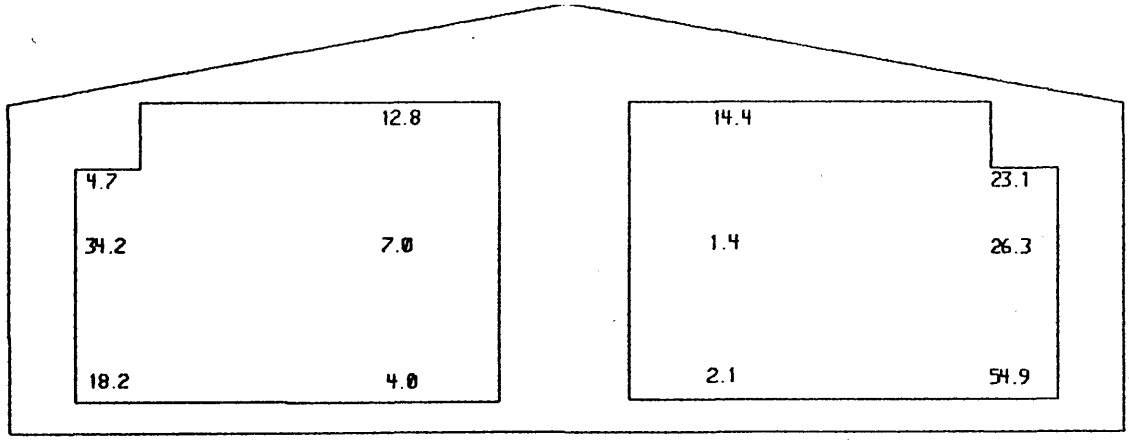


Fig. 5.1e Diagrammatic representation of chlorpropham residues (mg kg^{-1} unwashed tubers) found at various sampling sites in bays 9 and 10 of Grimsby store (1977-78)

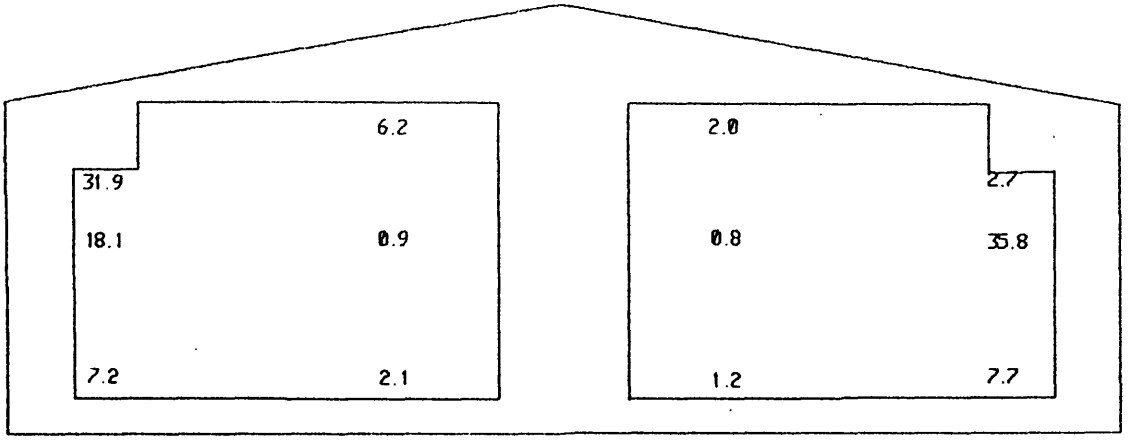


Fig. 5.2 Mean chlorpropham residue associated with soil assessment codes

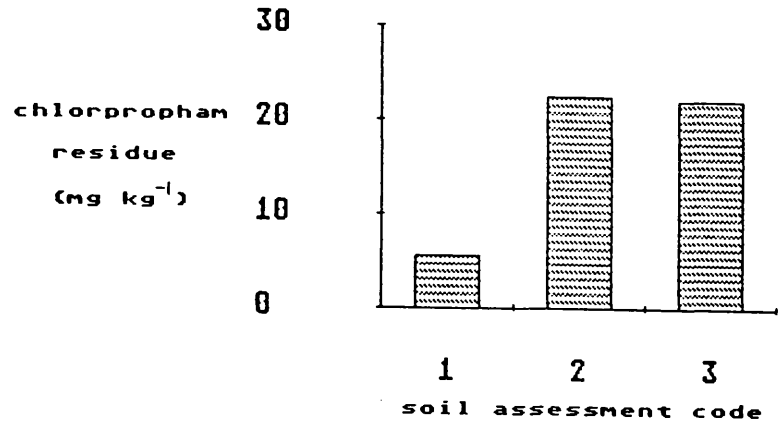


Fig. 5.3 Mean chlorpropham residues associated with sprout assessment codes

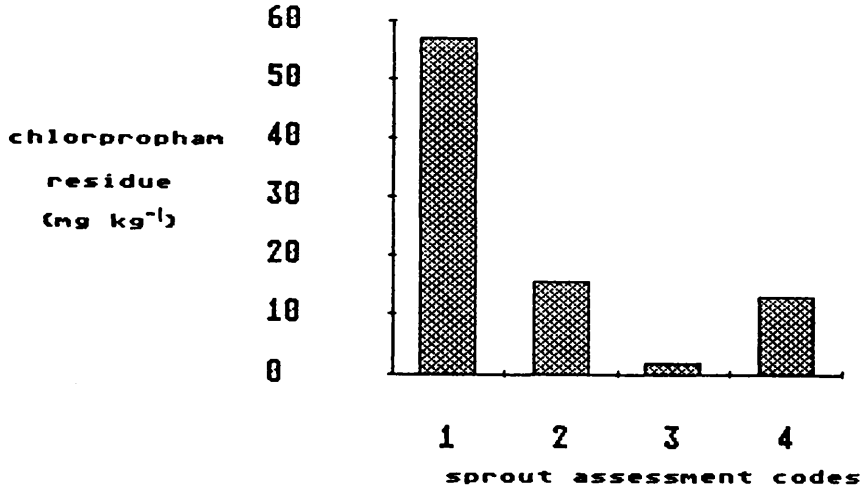


Plate 5.1 Chlorpropham encrusted on a duct wall in a commercial store (material removed for analysis >90% chlorpropham).

5.1.4 Discussion

From an examination of residues detailed in Fig. 5.1a-e it can clearly be seen that chlorpropham residues varied considerably throughout the store sampled. Obviously, the challenge of evenly applying a chemical to a store at a rate of only 20 mg kg^{-1} and in the presence of many wooden surfaces and concrete is a difficult one.

The processor involved in this trial actually treated the store with 20 mg kg^{-1} + a 25% overdose "for the wooden boxes". When asked for the reason for this, the justification given was that with new stores, sprout suppression was particularly difficult in the first few years and extra chlorpropham treatments were applied. It was assumed that in this time some, if not most, of the chlorpropham was absorbed by new boxes and concrete surfaces etc. As stores and boxes were aired each season, the 25% overdose was always needed. Also, chlorpropham losses through deposition on metal surfaces as well as boxes and floors are possible. The extent of these losses can be seen from Plate 5.1 showing chlorpropham encrusted on the main duct of a potato store. Additional chlorpropham is now commonly used in box stores for these reasons (Anon, 1985)

Tuber samples taken from boxes at ground and mid-level height inside the stacked units (bays) contained significantly less chlorpropham than those from samples taken from outer wall sites. Chlorpropham residues from samples taken from the tops of the bays failed to show the same pattern. Residues found at the top were more dependent upon the position of the Swingfog applicator used to apply the chemical.

A possible explanation for the general pattern of residues found in the store could be the procedure adopted during chlorpropham application when the operators aimed the machine down the side passages between the bays and towards the side walls. The high chlorpropham levels found on tubers stored at the side walls suggested that backlash of the aerosol towards these boxes had occurred. Observation of a similar chlorpropham application showed backlash of the fog which could be seen in the early stage of application before vision was obscured. As more aerosol was directed towards the walls, the fog appeared over the top of the bays and rolled towards the central corridor.

Enhancement of the residue pattern obtained by uneven application could be possible through redistribution of the chlorpropham during eight weeks storage. Even small changes in temperature and differences in air flow would enable redistribution to take place.

Generally, higher levels of chlorpropham were associated with low levels of sprouting and as chlorpropham levels decreased, the associated level of sprouting tended to increase (Fig. 5.3). At higher sprouting levels (ca. 20 mm sprouts) some chlorpropham levels were higher than expected but this could be explained by the fact that in some cases, the sprout growth had probably resulted from a lack of chlorpropham at a previous treatment.

It was thought that the amount of soil adhering to the tubers would have an effect on the quantity of chlorpropham adsorbed. Chlorpropham adsorption and desorption has been investigated as the chemical is used as a soil applied herbicide (Hance, 1980). In this study, no obvious link between the amount of soil and chemical residue

could be found (Fig. 5.2). As the assessment was very simple, only large differences would have been picked up. The type of soil would also be important; the organic matter fraction of the soil plays a major role in herbicide adsorption. The potatoes in this study had been grown in a sandy soil. The main conclusion to be drawn from this exercise was that chlorpropham varied with position in store, eight weeks after application. Previously, store managers etc. believed that any uneveness in application would be levelled out within a few weeks by volatilisation.

The question that still remained was whether the distribution found in this box store was due mainly to uneven application or a redistribution of the chemical during eight weeks storage or a combination of both of these.

5.2 An assessment of chlorpropham in store immediately after application.

5.2.1 Introduction

This assessment was carried out in a store adjacent to the one described earlier in the chapter. As it was part of a complex it was very similar to the store described earlier. Originally the idea was to sample the store in as similar a manner as possible to the previous study but, because of the difficulties experienced by the store manager in returning his store to normal after the previous trial, this could not be done. As disturbance to the store had to be minimised the idea of placing material with an absorbent surface at various locations in the store, to monitor the chlorpropham during and immediately after an application of the chemical, was investigated. Filter papers were chosen because they were cheap, easy to position and remove. It was hoped that the filter papers would show whether chlorpropham was evenly applied or not.

To enable a comparison to be drawn with the previous results (section 5.1), one bay of the store also had tuber samples removed as before.

5.2.2 Experimental

The store used was as previously described with the exception of the box lay-out (see Diagram 5.2). This had been changed by the processors because of the difficulty of getting chlorpropham into the middle of the bays shown by our earlier work.

Application of chlorpropham was carried out by a different group of contracters using five TIFA machines instead of Swingfogs. Whatman No.1 9 cm filter papers (Whatman Ltd., England) were placed in position throughout the store as shown in Diagram 5.3 before the chlorpropham was applied. The application rate was 20 mg kg^{-1} + 25% overdose for wooden boxes i.e. 25 mg kg^{-1}. A 50% w/v formulation in methanol was used (contracters own formulation - H. Green and Sons, England). Fans in the store were running at full capacity until approximately ten minutes before fogging started. The simultaneous use of five TIFA machines ensured the chlorpropham was applied quickly. After 45 minutes, the machines were stopped and the store left overnight. In the morning, the fog had cleared sufficiently to allow removal of the filter papers which were then placed in labelled bags, sealed and removed to the laboratory.

Potato samples from one bay were taken as before (section 5.1). These tubers had been exposed to one previous application of chlorpropham.

Analysis

The filter papers were placed in filter funnels and the chlorpropham washed through into 100 cm^3 volumetric flasks with methanol. Samples of these solutions were injected into a G.C. under conditions previously described (Chapter 2). Potato samples were dealt with as described in the same chapter.

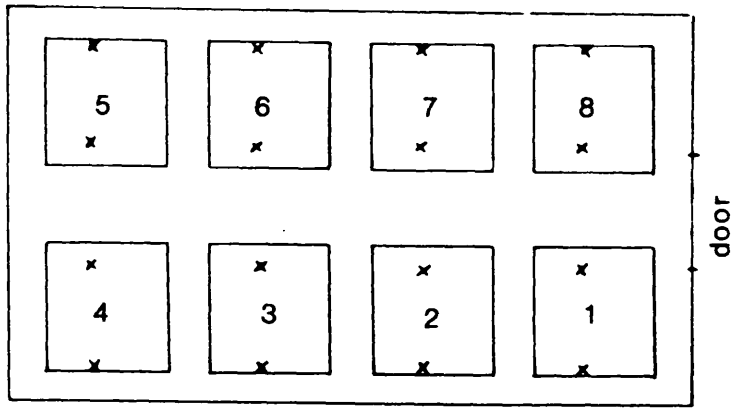


Diagram 5.3 Layout of box store showing sampling points (x).

Fig. 5.4a Diagrammatic representation of chlorpropham levels (mg/filter paper) found at sites in bays 1 and 8 in store after fogging.

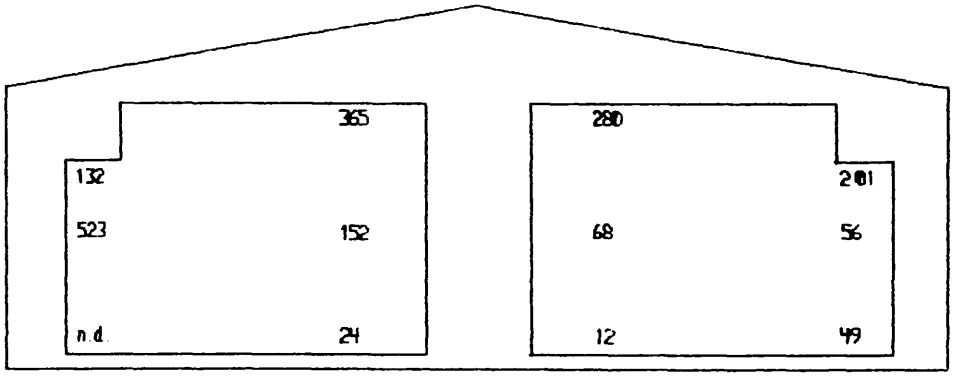


Fig. 5.4b Diagrammatic representation of chlorpropham levels (mg/filter paper) found at sites in bays 2 and 7 in store after fogging.

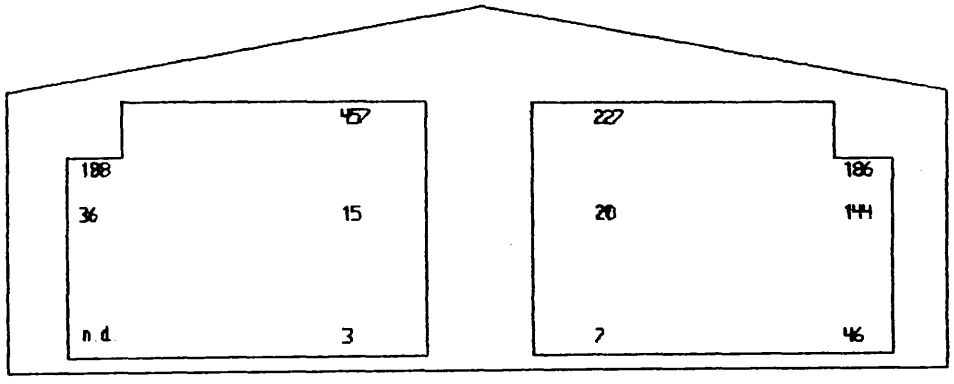


Fig. 5.4c Diagrammatic representation of chlorpropham levels (mg/filter paper) found at sites in bays 3 and 6 in store after fogging.

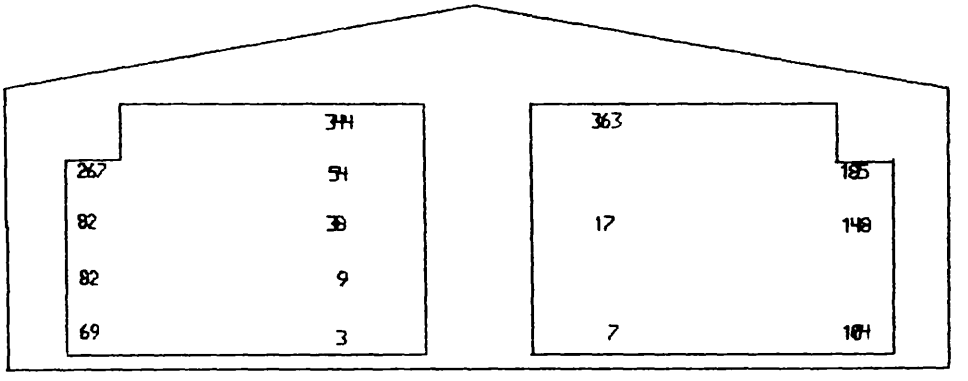


Fig. 5.4d Diagrammatic representation of chlorpropham levels (mg/filter paper) found at sites in bays 4 and 5 in store after fogging.

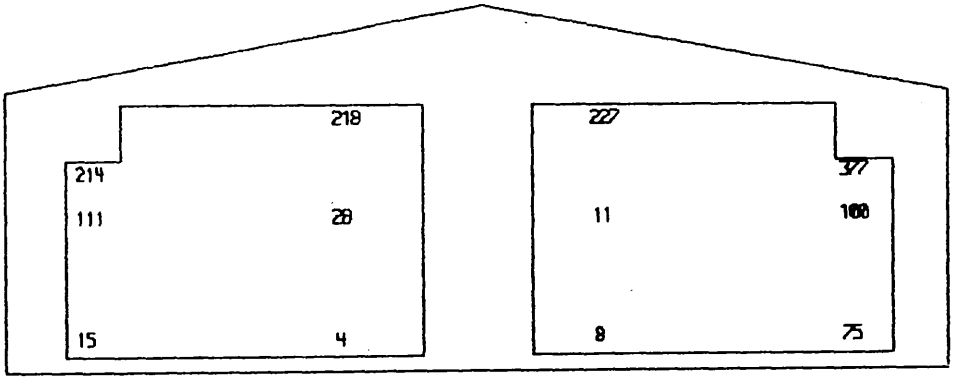


Table 5.1 Chlopropham residues found on tubers after fogging (bay 3)

	chlorpropham (mg kg^{-1} unwashed whole tubers)				
box height	1	2	3	4	5
wall	12.2	32.7	54.9	69.5	-[a]
near central	18.4	19.4	19.0	7.9	n.d.[b]

[a] only 4 boxes high at wall

[b] not determined

5.2.4 Discussion

Deposition of chlorpropham onto filter papers varied throughout the store. As in the previous section, chlorpropham levels were highest along the back wall of the store and lower in filter papers placed on the top of the second box in from the central corridor (Fig. 5.4). The lowest deposits consistently occurred at the inner boxes at ground level. This could be partly explained by the fact that during fogging, the operators generally aimed the nozzles of the TIFA machines towards lateral gaps separating bays and filter papers were always placed at a point mid-way along the bay. This position was as distant as possible from the general flow of chlorpropham.

Potatoes in bay 3 were sampled; the bay was chosen at random and the chlorpropham residues found are reported in Table 5.1. Again, less chlorpropham was found on the tubers in the inner boxes indicating low penetration. The chlorpropham level found in potatoes from the inner ground box was less than expected from the quantity of chlorpropham deposited on the corresponding filter paper from this box, despite the potatoes being exposed to one previous chlorpropham treatment. In general, the same trend in chlorpropham variability was found in potato samples as on filter papers but residue levels of chlorpropham on tubers could not be predicted from filter paper levels.

No observations on sprout length of tubers were attempted because it was early in the storage season and little sprout growth had occurred. As the amount of soil had been shown in the previous study to have little relationship to chlorpropham residues on the tubers, this assessment was not carried out.

Monitoring chlorpropham deposition by means of filter papers leads of course to making assumptions of how chlorpropham behaviour on tubers would relate to chlorpropham behaviour on filter papers. One of the main influences would be dimension - a flat thin filter paper bears no resemblance to a round potato. Air passing over a filter paper would hardly be disturbed in the same manner as passing over a potato or group of potatoes. Also the problem of deposition of chlorpropham onto an essentially inert cellulose paper compared with deposition onto the surface of a tuber with its waxes etc. must be considered.

Of these two problems, the one of dimension is probably more important during and immediately after the application of the chemical and although the use of filter papers can only suggest the extent of chlorpropham deposition, they can indicate problems with uneven application. Therefore, using filter papers as an indicator of chlorpropham deposition in the potato store, variability in chlorpropham with position in store could be shown during and in the fifteen hours after application. Enhancement of the chlorpropham variability by redistribution of the chemical during further storage still remains a possibility. When this work started, it was assumed that as chlorpropham was volatile uneven application would not lead to problems because within a few weeks, volatilisation of the chemical would occur, leading to redistribution. The only occasion when uneven distribution was acknowledged to occur was as the result of a build up of soil cones during potato loading. This can be a problem in both box and bulk stores stores. The build up of soil into a cone in the box, directly below the loader could slow down or even halt the

penetration of chlorpropham into tubers in the vicinity (Anon, 1985). Apart from this physical problem, which could be countered by more careful loading procedures, chlorpropham should be able to penetrate the store relatively evenly and completely. Little information is available on the behaviour and uptake of volatile sprout suppressant chemicals such as chlorpropham, tecnazene or DMN. They have all been shown to be active in the vapour phase (chlorpropham-Danielson, 1959; Slater et al., 1969; Steinbeiss et al., 1972; tecnazene-Reavill, 1954; DMN-Beveridge, 1979), and in the case of chlorpropham, studies using labelled material indicated the main route of uptake via the vapour phase was into the peel with particular concentration of the chemical at the eyes of the tubers (Peisker et al., 1972). As tecnazene and DMN are both concentrated in the peel in vapour non contact studies, it may be assumed the same general processes apply.

Studies of vapour phase activities of pesticides although quite common, are mainly restricted to studies of herbicidal drift (e.g. esters of phenoxyacetic acid) and of activity of some insecticides which are highly volatile compared to the compounds discussed here.

Other volatility studies have usually considered the estimation of the loss of chemical in field applications. An excellent series of two volumes which reviews the subject in depth is provided by Hartley and Graham-Bryce (1980). One of the main problems resulting from the linking of uneven residues of chlorpropham with sprouting of tubers is the fact that other workers, including one from our own laboratory, have shown that headspace levels of chlorpropham are remarkably consistent in a potato store at any given time, although the level will slowly decrease with time since last application (Filmer and Land, 1978; Boyd, 1984). It is difficult to reconcile the fact that

an even headspace level of chlorpropham can be associated with pockets of sprouting and good sprout control in store but this does appear to be the case. Air samples taken in these experiments were carefully chosen to avoid sampling too near potatoes and boxes and the headspace concentration obtained could be described as an average in the freely circulating store air. Sampling the air in between and immediately above the batches of potatoes would give different results (Boyd, 1984). The difference in the chlorpropham concentration in air circulating freely through the store and in air in a thin layer surrounding a treated tuber could be important.

Reports of chlorpropham headspace levels in store ranging between 0.3 - 1.0 mg m^{-3} (Boyd, 1984) and 0.1 - 4 mg m^{-3} (Filmer and Land, 1978) suggest that the freely circulating store air was saturated with chlorpropham because the calculated SVC at 10°C was 0.54 mg m^{-3} (see Chapter 1). The SVC was calculated from the vapour pressure measurement of chlorpropham; these measurements have often been extrapolated from higher temperatures and are liable to error, so the fact they are in general agreement is quite surprising. Aleksandrova and Klisenko (1982) gave the maximum possible concentration of chlorpropham in air as 0.1 mg m^{-3} and cautioned against not distinguishing between small droplets and true vapour in many studies. Our experiences of headspace analyses in dusty stores where fine dust particles were trapped in Tenax columns showed that this dust had a great effect on the level of chlorpropham subsequently detected because the dust particles adsorbed quantities of chlorpropham and tecnazene which had been used to treat the store. It may be necessary to fit some kind of removable filter to columns used for headspace analysis of this type to reduce this problem.

In addition, the SVC assumes that no interaction between chlorpropham and the surface it evaporates from exists, but Coxon and Filmer (1985) reported less chlorpropham in the air above potato tubers than expected and concluded that some adsorption of chlorpropham onto the periderm must have taken place. Many surfaces in a potato store are thickly coated with crystalline chlorpropham (see Plate 5.1) and the release of chlorpropham from these areas would approximate very closely to laboratory measurements of saturated headspace concentrations above a crystalline surface. The effect of these areas may be to swamp the store air with chlorpropham.

The degree of variability in chlorpropham residues found in the studies described in this chapter could give rise to concern over excessive amounts of the chemical being ingested. The majority of potatoes sampled contained residues within the limits currently imposed by several European countries (e.g. Holland - 5 mg kg^{-1} in whole tubers and 0.5 mg kg^{-1} in peeled tubers). Only in a few cases were potatoes sampled which exceeded the limits mentioned above and on peeling the residues were reduced considerably. So although high levels of the chemical could be detected on some potatoes, very little had penetrated to the flesh of the tubers and should, therefore, pose little problem from a toxicological point of view. However, a more even application of the chlorpropham to a store or a reduction in redistribution of the chemical would enable store managers to assess the need for reapplication of the chemical more easily and also ensure that certain sections of stores were not being overdosed to allow others to receive the appropriate dose. A reduction in the number of chemical applications in a given storage season could be envisaged, leading to reduced costs and an overall reduction of chlorpropham in

the store environment and consumer exposure to the chemical would be minimised.

In the light of recent legislation concerning statutory limits for pesticide residues in foods, any procedure to reduce the variability in addition to total concentration of residues in food must be investigated. Coxon and Filmer (1985) showed that a substantial portion of chlorpropham applied was rendered insoluble or unextractable within weeks of treatment and a knowledge of the fate of chlorpropham in a store and during processing would be of great value in assessing methods of reduction. Large scale balance studies can be carried out but a more detailed assessment can be gained through the use of radiolabelled chlorpropham. The next chapter assesses the knowledge of chlorpropham behaviour in potato stores and products to date and suggests some future studies to help increase our understanding of the processes involved.

CHAPTER 6

CONCLUSIONS

Until recently, the UK operated a voluntary pesticide control scheme, voluntary in the sense that once a chemical was approved for use, the onus was with the applier to ensure that the method of application and use was within guidelines set by the PAS (Pesticide Approval Scheme). If the recommended guidelines were adhered to, no problems with excessive residues would be encountered (the Good Agricultural Practice system). This situation contrasted sharply with that in the USA, Canada and EEC member countries. To bring the UK into line with the rest of the EEC, new legislation (the Food and Environment Protection Act (FEPA), 1985) is being implemented at present and will result in statutory maximum residue limits (MRLs) for specific compounds.

Enforcement of the new MRLs will involve detailed sampling and analysis to ensure the levels are being adhered to. Problems have already been encountered with the proposed MRL for tecnazene in potatoes. Most European countries do not permit the use of tecnazene as a sprout suppressant but it is in widespread use in the UK. The implementation of the proposed MRL of 1 mg kg^{-1} whole tuber has been postponed for 2 years perhaps indicating tecnazene used under present conditions would result in tubers or potato products with greater than 1 mg kg^{-1}. Publicity in the press over a decision by a retailer, (Marks and Spencer plc) to introduce its own limit immediately has fuelled public disquiet over pesticide and other xenobiotic residues

in food generally. It would be fair to say that tecnazene is not the sole chemical with this type of problem e.g. implementation of proposed MRLs of 5 mg kg^{-1} of dithiocarbamates on celery and lettuce has also been delayed.

Chlorpropham residues have been determined in potatoes at various stages of processing (Martens et al., 1971; Ritchie et al., 1983). A major problem has been the determination of chlorpropham in crisps or crisp frying oil because of the difficulty of separating chlorpropham from the oil components. Partitioning the extract between a series of two solvents although cumbersome and time-consuming did result in samples clean enough to allow the determination of chlorpropham in commercial samples by GC (Ritchie et al., 1983).

As expected, chlorpropham residues could be found at all stages of processing including the final crisp product but it was also found in the frying oil used (0.4 mg kg^{-1} oil). Earlier work had failed to detect a build up of chlorpropham in the oil and it was assumed that chlorpropham was lost through volatilisation at 180°C from the oil during cooking. However, it now appears that chlorpropham can be concentrated in the oil and whether or not an equilibrium is reached between chlorpropham added in potatoes and removed in the form of crisps and/or volatilisation from the frier is certainly worthy of further investigation.

Basically, this thesis concerned itself with practical problems arising from the use of chlorpropham on potatoes. The difference between the amount of chlorpropham applied to stored potatoes and the residue found cannot be explained simply by a combination of volatilisation and precipitation onto the fabric of the building

although large quantities have been found adsorbed on concrete (Boyd and Duncan, 1984) and crystallised on metal surfaces within the store. What is the fate of the remainder of the chlorpropham applied? The possibilities that exist include

(1) bound intact to cellular material (swep can be directly incorporated into lignin but so far no evidence to suggest the same process occurs with chlorpropham)

(2) metabolism and/or binding to either the tuber periderm itself or soil in contact with the tuber.

Most extractable chlorpropham can be found in the peel or periderm of the tuber and so far, studies using radioactive labels have suggested the same for ^{14}C labelled chlorpropham or its metabolites; little penetrates into the tuber.

The work in this thesis showed that approx. 10% of the original amount of chlorpropham applied on an inert carrier formulation was present at planting. Studies of potatoes exposed to chlorpropham vapour alone (van Vliet and Sparenberg, 1970) resulted in smaller residues than those obtained with powder or aerosol applications of the chemical. It is possible that the use of an aqueous dip formulation of chlorpropham could result in larger residues of chlorpropham being found although effects on sprouting would not be much greater. Further work on types of formulations and their effect on residues and penetration of chlorpropham into the tuber is needed to clarify matters.

Temperature and duration of airing were investigated as a possible means by which chlorpropham residues could be reduced and therefore their effect on seed tubers minimised. Although some success

in reducing the effect in terms of emergence and yield was noted, no chlorpropham treated tubers performed as well as controls. With hindsight, bearing in mind the adsorption of chlorpropham on the periderm noted by Coxon and Filmer (1985), it was unlikely that airing and temperature changes would result in large differences in response; more probably, the results that were obtained could be attributed to an increase in general metabolic activity as a result of pre-sprouting the seed. Results in Chapter 3 had already shown that cv. effects were noticeable; the more vigorous sprouter tending to have on average larger residues of chlorpropham for a corresponding MET or yield than the less vigorous sprouter.

Duration and temperature of airing reduced the effect of chlorpropham on the performance of treated seed but alternative approaches such as treatment with a dormancy breaking compound e.g. ethylene chlorhydrin might have been as, if not more successful; dormancy breaking compounds have been used successfully on MH treated tubers in an attempt to overcome sprout inhibition (Ratikin, 1973). However a problem associated with this type of approach is that adding another chemical to the list of treatments already given would be open to criticism even if on seed potatoes. The straightforward approach of manipulating storage conditions to minimise the effect has much to recommend it, especially in the light of the impending introduction of statutory limits on chemicals added to food crops.

To minimise the effect of chlorpropham residues on performance in the field, seed should be pre-sprouted to enable the grower to assess the vigour of sprouting. Seed that does not pre-sprout well is unlikely to perform well in the field, although it should be noted

that it often performs better than expected from sprouting data alone (Kim et al., 1972). The Scottish Colleges have supported the practice of high temperature storage (ca. 20°C) of seed samples some weeks before planting and note the response. This practice was introduced to decrease the incidence of delayed emergence and yield due to tecnazene treatment. Airing the seed before planting can in some cases be the stage at which the material is contaminated so contamination cannot be ruled out totally at this point.

Changing practices in stores or processing.

In the last few years, much emphasis has been placed on lowering average fat intake in the diet. Crisps are fried products and to reduce criticism of the product, crisp processors have adopted a number changes to ensure their product remains popular. Low-fat crisps (30% rather than 33%) were introduced as a healthier alternative to normal crisps and more recently, the introduction of crisps produced from unpeeled tubers have become available. They have the advantages of extra fibre and less fat per unit weight because the slices they are made from are thicker than normal crisp slices. Concern must be expressed over their introduction as the marketing techniques employed by the companies involved, advertise them as a healthier alternative to ordinary crisps. Throughout this thesis, it has been emphasized that when excessive levels of chlorpropham occur in some samples of treated tubers, peeling reduces the residues to a level acceptable on the Continent (< 0.5 mg kg^{-1} peeled tuber). The two sprout suppressant chemicals in use in the UK concentrate in the peel and this aspect of their use was considered an advantage when they were first introduced because potatoes were commonly peeled

before use and most of the chemical was discarded with the peel. Although chlorpropham residues in crisps have been determined and found to be in the order of 0.4 mg kg^{-1}, preliminary analysis of crisps made from unpeeled tubers showed chlorpropham residues at least a factor of 10 higher. Presumably a similar process occurs with tecnazene and as residues of this chemical are generally higher (0.3-0.6 mg kg^{-1} product (Dalziel, 1978)) than those of chlorpropham a similar increase is to be expected with unpeeled material.

Metabolites

Early work on chlorpropham metabolism in potatoes failed to find any metabolites present in sufficient quantities to isolate and determine. Evidence of "bound" or unextractable labelled material was found by Coxon and Filmer, (1985) but no polar metabolites were noted. These authors specifically investigated the possibility of 3-chloro-aniline and 3,3'-dichloroazobenzene production and failed to find any although as TLC radio-scanning was involved the sensitivity of the method was not as great as GC-MS used in more recent studies. Heikes (1985) published a study which showed the presence of isopropyl-N-(3-chloro-4-methoxyphenyl)carbamate (methoxy-chlorpropham) in chlorpropham treated tubers. Identification was confirmed by comparison with a standard on different GC columns and by GC-MS. The metabolite was found in market basket survey samples at a concentration of 0.004 mg kg^{-1}(baked potatoes), 0.008 mg kg^{-1}(chips) and 0.0063 mg kg^{-1} with corresponding levels of chlorpropham of 0.25, 2.4 and 1.6 mg kg^{-1} respectively.

That methoxy-chlorpropham was a metabolite was confirmed in a small study where a number of potatoes were dipped in a 5% aqueous

solution of Sprout-Nip EC (a formulation produced by PPG Industries, USA). Normal treatment with Sprout-Nip EC involves spraying washed potatoes with a 1% solution of the formulation so it is possible the potatoes were treated with chlorpropham at a rate of 100 mg kg^{-1} with a metabolite level of 0.17 mg kg^{-1} 6 weeks after treatment. This would imply an approximate conversion rate of 0.2%. The conversion factor would result in potatoes treated with the normal application rate containing after some weeks, approx. 0.04 mg kg^{-1} of methoxy-chlorpropham, which would be at the limit of detection with our GC-FID system.

In Chapter 2, the possibility of hydrolysing chlorpropham to 3-chloroaniline with subsequent derivatisation with HFBI and detection by EC was discussed. Later, it was found that increasing the temperature of the EC detector resulted in a response to chlorpropham. This would imply that the mechanism of detection involved thermal dissociation and not molecular ion formation.

More work is needed to identify and quantify the presence of the methoxy metabolite under UK conditions. Jumar et al. (1968) investigated the sprout suppressant properties of a number of phenylcarbamate compounds as sprout suppressants and found that methoxy substituents on the ring removed sprout suppressant activity; methoxy-chlorpropham is unlikely to have sprout suppressant properties.

Recently, Worobey and Sun (1987) and Worobey et al., (1987) reported the presence of 3,3'-dichloroazobenzene (DCAB) in the peel of chlorpropham treated potatoes. The concentrations found were in the range 2 - 39 ug kg^{-1} peel and were only detected through the use of

sophisticated GC-MS facilities and GC with EC detection. The DCAB presumably formed from 3-chloroaniline (3-CA) released from hydrolysis of chlorpropham because none was found in control tubers. Although soil studies have shown that micro-organisms are capable of degrading chlorpropham to 3-CA, this transformation had not been reported previously in plants.

When potatoes are treated with chlorpropham, the tubers generally have a layer of soil adhering to the surface. It is possible that despite the low temperature (7 - 8^{o}C) and moisture content of the soil in store, microbial activity could result in 3-CA and subsequently DCAB production. Bordelau and Bartha (1972) studied the effect of a number of aniline derivatives on azobenzene production and concluded that the electronegativity of chlorine in the 3 position of the ring reduced the likliehood of DCAB production in the field. Further work on these metabolites is being carried out in the Agricultural Chemistry section on the influence of soil on the production of 3-CA and DCAB. It should be noted that Worobey et al., (1987) measured the chlorpropham concentration in treated tuber peel at 21 - 166 ug kg^{-1}, which is much lower than would be expected from commercially treated tubers.

Ritchie (1986) investigated the possibility of chlorpropham metabolism by common potato pathogens. Simple liquid culture systems were used and ^{14}C labelled chlorpropham was introduced to the flasks. Erwinia caratovora var. atroseptica (bacterial soft rot), Polyscytalum pustulans (skin spot) and Phoma exigua var. foveata (gangrene) were examined and no polar metabolic products were found in the cases of soft rot and skin spot. A polar metabolite was isolated from the

^{14}C chlorpropham treated Phoma culture but unfortunately was not characterised.

The study in Chapter 5 on the distribution of chlorpropham in a commercial store showed great variability in residues which was related to store position. An attempt in the second part of the chapter to open out the bays met with some success and a number of options for future work could be followed from these initial surveys. Little information is available on the effect of aerosol droplet size or formulations on application efficiency and from the preliminary evidence obtained in a small-scale trial in a bulk store (not reported in this thesis) this is an area worth investigating further. It must be emphasized that in the study reported here, preliminary talks and sample analysis indicated some of the degree of variability in sprouting in the store.

The method of chlorpropham application could be changed. There is evidence to suggest that chlorpropham vapour in the ventilation system can suppress sprouting (van Vliet and Sparenberg, 1970) but bearing in mind the amount of chlorpropham found crystallised out along ventilation ducts, this process is presumably already acting in most stores after chlorpropham aerosol applications.

As application of chlorpropham to box stores gives the greatest problems, perhaps a major change in store and box design is needed. Of the two, improved box design to allow greater ventilation through the box could be implemented more quickly because they have to be changed every few years anyway. Improved airflow through boxes would allow greater penetration of chlorpropham at treatment time.

These suggestions all assume that chlorpropham will remain on the market and be the method of choice for sprout control in processing stores for a number of years to come. For processors, alternative methods of sprout control are limited because temperature control and irradiation both result in reducing sugar accumulation. Other chemical sprout suppressants could be used but are not as popular as chlorpropham. Any chemical competing with chlorpropham would have to be non-toxic, cheap and readily available, as effective on a cost/tonne basis and have no effects on other potato components or aspects of processing quality. At present, only DMN has been sufficiently investigated to show that it is at present, a viable option. DMN unfortunately cannot be patented and this has hampered its further development. Other chemicals which can suppress sprouting had been dismissed earlier because of excessive volatility. If costs are not too high, an application system similar to the one employed with nonanol (3-5-5-trimethylhexan-1-ol) could be employed. Many of these compounds are naturally produced by the tuber (like DMN) but their extra volatility could result in lower residues when removed from store. Continuous or intermittent vapour application would, of course, require a greater degree of attention and awareness of ventilation rates than is the case with chlorpropham.

The possibility of breeding for low reducing sugar accumulation at low temperatures ($4^{o}C$) using gene manipulation techniques and breeding for long dormancy at $10^{o}C$ which would enable long term storage for processing without sprout suppressants are both being actively pursued. Some success with the second approach has been reported (Hughes et al., 1986). It may be that in future sprout suppressants will not be needed.

However, the introduction of long dormancy cvs. may produce problems in seed growth and it is possible that dormancy breaking compounds for use on seed will be needed in the future. Clones under investigation at present have a dormancy of up to 160 days and it is hoped to improve further improve this. At present, the chemicals available for breaking dormancy (e.g. ethylene chlorhydrin and gibberellin type compounds) are not always successful and others are toxic so further study in this area is needed.

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