SUBSTITUTED NAPHTHALENES

AND THEIR ROLE AS POTATO SPROUT SUPPRESSANTS

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This thesis describes work relating to the substituted naphthalenes and their use as potato sprout suppressant chemicals. Sprout suppressants are used to control the sprouting of potatoes during extended storage.

The study included estimating the volatility of certain substituted naphthalenes. This was attempted by developing a static method that allowed a simple, fast and reasonably accurate technique to be developed for screening the volatility of many substituted naphthalenes. The results obtained indicated that the headspace (concentration of chemical in the air surrounding the chemical source) increased with both time and temperature. However, the concentration remained relatively constant as the concentration of chemical was increased. The method was also used to relate the volatilities of selected chemicals - dimethylnaphthalene, ethylnaphthalene and chloromethylnaphthalene to their activities as potato sprout suppressants. These were selected from a previous potato storage experiment carried out by Stephen and Duncan (1984).

With realistic potato storage conditions in mind a dynamic model system was developed and used to study the headspace of ethylnaphthalene over a long period of time. This method enabled the behaviour of ethylnaphthalene to be predicted in a commercial potato store. The decrease of ethylnaphthalene headspace concentration with time indicated that ethylnaphthalene would need to be re-applied several times throughout the storage season.

The effect that substituted naphthalenes had on the healing of tuber wounds was measured by following the development of resistance to water loss in cut potato discs. A syringe method was developed and used as a quick assay. Using this system the substituted naphthalenes were not found to give an inhibiting effect compared to the controls. The effect of the organic solvent masked the effects of the substituted naphthalenes. Thus the true effect of the substituted naphthalenes on wound healing was unable to be seen.

The general indication from past work (McGee 1984; Wilson et al 1987) was that the substituted naphthalenes would have an inhibiting effect on the healing of whole wounded tubers under commercial conditions. Thus substituted naphthalenes could not be applied to potatoes entering the store immediately after harvest as it is necessary for wound healing to take place in advance to prevent water loss and the entry of fungal and bacterial pathogens.

So in order that substituted naphthalene residues could be determined, sensitive and accurate methods for the extraction, clean-up and quantification of dimethyl-naphthalene from potatoes were derived. Both Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) analysis methods were developed. The recovery of the GC method was calculated at 90.4% $\pm$ 2.6% and levels down to 0.006µg of dimethylnaphthalene could be detected and levels of 0.5mg kg^{-1} of potato residue. The recovery of the HPLC method was slightly lower at 85.9% $\pm$ 1.7% but was slightly more sensitive, detecting levels of dimethylnaphthalene down to 0.005µg and levels of 0.42mg kg^{-1} in potato residues.

The remainder of the work in this thesis described a potato storage experiment comparing different sprout treatments. It was concluded that diisopropylnaphthalene and ethylnaphthalene gave better control of sprout growth and the number of eyes opened than untreated tubers. However, as the storage period increased the treatments became less effective. This effect was thought to be related to the relatively high volatility of the substituted naphthalenes and the loss of chemical by dissipation with time. Correspondingly, the chemical residues also decreased with time. Critical residue values were found to be 0.4 - 0.6mg kg^{-1} (Beveridge 1981b).

CONTENTS

DECLARATION

Reports of much of the data in Chapter Two have already been published - O'Hagan, N., ; Boyd, I. M., and Duncan, H. J., (1986). Abstr - 10th Trienn. Conf. EAPR.

INTRODUCTION

1.1 The Potato Crop

The potato (Solanum tuberosum L.) was originally introduced to Europe from South America in the sixteenth century and has become a major food crop since then. It provides a staple diet and is an important source of complex carbohydrates with an average human consumption in Britain of around 110kg per head of population per year (Anon 1988).

The amount of potatoes being used by the potato processors (canners, crispers, dehydrators and chippers) comprises a significant proportion of the total human consumption - almost 24% during the growing season June 1986 - May 1987 (Anon 1988).

As there is a demand for raw and processed potato products throughout the year, it is often necessary to store harvested potatoes for periods up to nine months. After harvest, potatoes undergo a period of dormancy lasting between one and fifteen weeks. Burton (1966) agreed with Emilsson (1949) in regarding the dormant period as the time when the buds are not growing. The actual length of the dormancy period will depend upon factors such as: variety, temperature, maturity, time of lifting, mechanical damage and disease and infection.

In recent years potato storage has mainly been confined to two main methods although some older methods are still practised in localised areas.

1.2 Types of Potato Stores

1.2.1 Clamp

The old methods of storage include clamps, pits and Dickie pies but these have gradually been replaced by specialised buildings, although the clamp system is still used in some countries.

The clamp was originally constructed by digging pits in well drained soil, inserting the potatoes and covering them with a layer of straw for insulation and soil to protect against frost and wind. More recently sheets of plastic have been laid down between layers of straw to increase the resistance to freezing winds. Although the method is cheap, it has the disadvantage in that the environment cannot be controlled, especially the temperature during the different seasons (Nash 1985).

In the early 1960's I Dickie and his farmer co-workers improved on existing methods by introducing fans to allow ventilation and they surrounded the clamp with bales of straw sandwiched with sheets of plastic to prevent draughts; this was known as a "Dickie pie" clamp (Nash 1985).

However, in recent years there has been a changeover from outside storage to storing the potatoes within buildings. This has involved a cutback in the amount of labour needed and allowed a more controlled and efficient degree of store management which together improved the quality of the stored product. It also improved access to the potatoes.

Bulk and box stores are now the most popular methods of potato storage.

1.2.2 Box

The potatoes are loaded into half or one tonne wooden boxes which are stored in stacks up to six boxes high, in large concrete floored buildings. The minimum of labour is required as the boxes can be loaded in and out of the store using a forklift. Spaces are left between batches of boxes to allow easy access by machinery (Anon 1985).

The temperature is controlled by powerful fans positioned around the store that will circulate the air. There is no need for ducts. The main advantage of box storage is that it enables better control of the potatoes, it allows the identification of boxes from individual growers, it also enables disease and sprouting to be recognised early allowing steps to be taken to deal with the problem quickly and, if need be, the potatoes can be removed easily (Nash 1985).

1.2.3 Bulk

The potatoes are stored in bulk, in large specially constructed or adapted stores which have insulated walls which have been thickened to withstand the pressure generated by the potatoes being loaded into the store. The potatoes are often stacked to depths of over twenty feet (Anon 1985).

The temperature is controlled either by large fans that re-circulate the air around the store or by mixing the air in the store with the air outside as required.

The amount of air is dependent on the required temperature needed. If there is a possibility of the air outside being too warm then some modern stores have refrigeration units built in. There can often be a problem of temperature fluctuations within the potato pile, with temperature gradients building up between the bottom and top layers. This can be overcome, however, by a series of underground ducts that allow air to flow up through the potato pile. The main disadvantage of this type of storage system is that it is hard to know what is going on at the bottom of the potato pile. It can take a long time to detect rotting potatoes and to empty large sections of the store to remove the rotting tubers once identified (Nash 1985).

1.3 Sprout Control

Potato quality throughout storage is important to both the fresh and processing markets. The fresh market is concerned with appearance as well as cooking quality. The processing market is concerned with tuber characteristics which influence the quality of the processed product. Quality of the raw product is affected by both the environment and store management during the storage period.

Potato storage losses can exceed 20% of the total crop going into storage. The main causes of loss are sprouting, disease and damage (Nash 1985).

Sprouting results in a loss of water and energy and generates heat and surface moisture which encourages secondary infection. This affects the quality of the potato and lowers the nutritional value and eventually leads to a loss in saleable weight.

For these reasons, control of sprout growth during storage is important. This can be achieved by any of the following methods:

1. Control of storage temperature.

2. Sprout suppressant chemicals.

3. Irradiation of tubers.

4. Use of light.

1.3.1 Storage Temperature

Sprouting in tubers can be delayed by storing the potatoes at low temperatures between 0 - 3°C. This causes a problem in that it results in a buildup of reducing sugars which eventually gives a sweet taste to the potato and an undesirable dark brown/black product which is unacceptable to the processors (Sparks 1965; Zaehringer et al 1966).

This problem can generally be eliminated by raising the temperature of the store to 8 - 10°C which will decrease the amount of reducing sugars present (Anon 1985) but unfortunately will also encourage the tubers to sprout. Hence another method of sprout control will be required which is usually chemical.

1.3.2 Chemical Sprout Suppressants

There is only a limited number of chemicals available in the market and none of these were introduced primarily for their sprout suppressant properties.

In the UK there are currently three approved sprout suppressant chemicals being used on ware potatoes: tecnazene, chlorpropham and propham. A fourth chemical, maleic hydrazide, has gained limited clearance.

Figure 1.1 shows the structure and properties of these chemicals.

Tecnazene

Tecnazene (TCNB) is the name given to 1,2,4,5 tetrachloro-3-nitro-benzene. It was originally introduced as a fungicide in the 1940's. The recommended application rate as a sprout suppressant is 135mg kg^{-1} although lower rates are often used on potatoes intended for processing as an initial treatment, prior to applying chlorpropham.

It is applied to the potatoes either as a dust or as granules. Its mode of action is not yet known, but it does not prevent wound healing (Leonard et al 1986; Burton 1966) and can be applied to the potatoes as they are loaded into the store after harvest and before wound healing has taken place. Tecnazene is the only available sprout suppressant chemical on the market that can be used on seed potatoes provided they are well aired before planting.

The main disadvantages of tecnazene are its high cost and a question mark about its toxicity which is related to it being an organochlorine compound which could lead to it being resistant to breakdown in the environment (Leonard 1988). Hence there is concern that it may work its way into the food chain.

Chlorpropham and Propham

Chlorpropham (CIPC) is the common name given to isopropyl-N-(3-chlorophenyl) carbam which is often applied with its unchlorinated analogue, propham. It is applied to potatoes in the vapour phase either as a foggable liquid or as granules. Because of its high activity at low concentrations, it has a low recommended application rate of 10 - 20mg kg^{-1}. Chlorpropham was first introduced as a herbicide in 1951 and it acts by inhibiting mitosis (Corbett et al 1984). This creates problems with its use as it affects the wound healing of potatoes (Audia et al 1962; Reeve et al 1963; McGee 1984; Leonard et al 1986). Chlorpropham cannot be used on seed potatoes and cannot be applied to the potatoes as they are loaded into the store.

It needs to be applied after the potatoes have had significant time to heal which can extend to six weeks after loading. Other than these obvious disadvantages there is also a problem with high residue levels as levels of 1 - 80mg kg^{-1} have been reported and are not uncommon in unwashed whole tubers (Dalziel and Duncan 1980).

McGee (1984) suggests that the initial chlorpropham application should be delayed for as long as possible in order to avoid early development of a problem known as "blemish", which is thought to be an abnormal form of skinspot . In recent years, it has become common practice to control the sprouting early in the storage season with tecnazene, followed by application of chlorpropham when needed throughout the storage period.

Maleic Hydrazide

Maleic hydrazide (MH) is the common name given to 6-hydroxy-3-(2)-pyridazinone. It was first introduced in 1949 as a growth retardant on grass and has been used as a growth regulant in the USA on onions, tobacco and potatoes. Maleic hydrazide is sprayed on the growing potato crop, taken up by the leaves and translocated through the plant and into the tuber, with the assimilates in the phloem. It is normally applied as the diethanolamine salt or more recently as potassium salt at a rate of 1.7kg/hectare about six weeks before harvest. This treatment is usually sufficient to retard sprouting.

However, the UK climate often is unfavourable for efficient uptake and translocation of the chemical to the tubers and often leads to varying residues in the tubers, some of which may not be sufficient to control sprouting throughout the storage season. Like chlorpropham, maleic hydrazide affects cell division (Corbett et al 1984) and cannot be used on seed potatoes.

Residue levels of 6 - 40mg kg^{-1} have been reported (Franklin and Longhead 1964) and because the maleic hydrazide has been translocated, the residues are inevitably higher in the tuber flesh than those resulting from chemical sprout suppressants applied to the tuber surface after harvest. It should be noted that maleic hydrazide residues cannot be reduced by peeling as is the case with the alternative chemicals.

1.3.3 Light

Light can be used to control the rate of sprout growth, the degree of control depending on the wavelength of light used. McGee et al (1987) showed that light did not affect the length of the dormant period but it did affect the rate of sprout elongation once dormancy had been broken.

The suppression of sprout growth by light resembles the high irradiance class of photomorphogenic reactions, in that it requires moderate light intensities over an extended period. It is not photoreversible and has a complex action spectrum with peaks of activity in the red (707nm) and blue (400-500nm) regions (Smith 1975; Gaba and Black 1983).

In Europe light is used commercially to prevent the sprouts on seed potatoes growing too long. This is known as chitting. The potatoes on wooden trays are allowed to sprout in order to secure earlier growth and earlier bulking, reductions in losses from disease and higher yields. In tropical climates the development of a diffuse-daylight seed storage system allows the seed potatoes to be in a plantable condition, at relatively high temperatures, for 6 - 8 months between cropping seasons (McGee et al 1987).

Other advantages of diffuse daylight are that the sprouts may be stronger as well as shorter (Wassink et al 1950), bacterial disease is reduced (Anon 1982) along with a reduction in storage losses (Anon 1982) and there is a potential increase in yield (McGee et al 1988).

The main disadvantage is that it is not applicable to large scale storage situations and must be confined to seed as there is a greening effect on the potato causing a high buildup of alkaloids which are poisonous to humans and animals.

1.3.4 Irradiation

Sprouting can be delayed or prevented by irradiating the tubers with doses in the range 30 - 150Gy. The usual dosage rate is 100Gy. Normally the inhibition is irreversible and unlike chemical sprout suppressants, a single radiation treatment is sufficient, irrespective of post irradiation storage conditions. The mode of action is not yet known but Urbain (1986) proposed several mechanisms for the sprout inhibition action of irradiation including:

1. Interference of nucleic acid and nucleotide synthesis in the meristematic tissue of the potato buds.

2. Disturbance in the phosphorylation process.

3. Interference in the synthesis of auxins.

4. Chromosomal disorder in cells of the meristematic tissue which prevent normal cell division and growth.

Irradiation interferes with the wound healing process. If irradiation was carried out immediately after harvest then it could result in pathogenic infections entering tubers via injuries and wounds resulting from harvest. Thus irradiation needs to be delayed till after wound healing. The effect of gamma-irradiation causes a marked but temporary increase in the level of reducing sugars, which is dose dependent (Burton et al 1959; Muir et al 1987). This can cause a problem for the processor but the amount of reducing sugar can be reduced by reconditioning at a high temperature. Irradiated potatoes, regardless of how long they are stored, undergo blackening during or after cooking. This is thought to be related to the increase in polyphenol content of the potato tissue and interaction of the polyphenols with ferrous salts present in the potato (Urbain 1986).

In addition to the problems mentioned above, the main disadvantages are the cost and accessibility of the irradiation equipment.

The potatoes need to be transported to and from the reactor for irradiation which creates extra expense. There is also an additional problem of gaining the public's confidence with respect to reactor safety and also with regard to eating irradiated food.

In summary the methods available at present for the long term storage of potatoes have disadvantages:

1. Low temperatures produce an undesirable product.

2. Irradiation has several drawbacks which were mentioned earlier in this chapter.

3. Light can be used to control sprouting in seed but not ware.

4. There is only a limited number of chemical sprout suppressants available and these have various drawbacks, especially nowadays as there is an increase in the public's general awareness and anxiety regarding the use of chemicals on foodstuffs. This will inevitably lead to a reduction and the eventual banning of certain chemicals due to the buildup of chemical residues. With this in mind, alternative sprout suppressant were sought.

1.3.5 Natural Sprout Control

Dormant potato tubers were found by Meigh et al (1973) to give off volatile substances that had a suppressing effect on the growth of sprouts in potatoes.

The idea of using natural volatile chemicals has many advantages:

1. The chemical could be applied directly to the potato as a vapour.

2. Re-application could be carried out throughout the storage season, whenever needed.

3. Toxicological problems should be minimised as the chemical is naturally produced by the potato and is concentrated mainly in the skin which can be removed easily by peeling.

4. Most of the chemical should be removed by airing the potatoes particularly at an elevated temperature prior to use.

The volatile components produced by the potatoes were later identified 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 such as carvone, pulegone and borneol were too volatile for use commercially because the effect wore off quickly as the chemical dissipated (See Figure 1.2).

Dimethylnaphthalene was the only chemical tested (see Figures 1.3 and 1.4 for structure and properties) that could control the sprouting over a storage period and when tubers were aired before planting they had little effect on emergence or yield (Beveridge et al 1981a).

In development work (Beveridge et al 1981a) an initial application of dimethylnaphthalene isomer mixture was not adequate to control sprouting under commercial conditions and for long term storage re-application or continuous application of the chemical would be required.

In these developmental experiments (Beveridge et al 1981a), a mixture of isomers consisting of mainly the 1,6 isomer with other isomers present in lesser amounts was used as it was considerably cheaper and therefore likely to be commercially acceptable.

As mentioned earlier, dimethylnaphthalene had no effect on the emergence or yield of seed potatoes at recommended application rates once aired. There has, however, been some contradictory results on the effect it has on the wound healing process. There is no obvious problem when it is applied directly to whole tubers, but adverse effects were noted on the rate of wound healing using potato discs (McGee et al 1983). Its mode of action is not really known but it is thought to interfere with the production of auxins, especially indoleacetic acid which is involved in cell elongation (personal communication Dr H Duncan).

There has not been a lot of work done on its toxicity but data produced by the British Industrial Biological Research Association in May 1978 suggested an oral LD_{50} figure of 5000mg kg^{-1} in rats for the 1,6 dimethylnaphthalene (Anon 1976).

As well as dimethylnaphthalene, the trimethylnaphthalenes, especially the 1,4,6-trimethylnaphthalene, were shown to have similar sprout suppressant activity as that given by the commercial sprout suppressants, chloropropham and tecnazene (Filmer and Rhodes 1985) using a simple bioassay. However, further development of these chemicals by a commercial firm was hampered due to the difficulty in obtaining a patent due to prior publication. Studies were then diverted towards its analogues or derivatives which could be patented and many of them revealed to have sprout suppressant activity (Stephen and Duncan 1984).

One such chemical which may have potential as a commercial sprout suppressant is diisopropylnaphthalene (Stephen and Duncan 1984) which has already had a lot of toxicological data published (Iwahara 1974; Hasegawa 1982) showing it to have a low toxicity. It also has the advantage of being available in large amounts, at a lost cost, due to its present use as an industrial solvent in carbon paper in Japan.

In general, most of the substituted naphthalenes will suppress sprout growth to some extent. The reason for the variation in activity between compounds is not yet known but the degree of activity does vary between isomers.

1.4 Objectives

With the drawbacks of existing sprout suppressant methods, there is a need for alternative sprout suppressant methods to be used. Interest has increased in natural chemicals, especially the substituted naphthalenes. The objective of the work described in this thesis was to study the potential of substituted naphthalenes as commercial sprout suppressants.

The work described in Chapters Two and Three concentrated on the development of headspace methods that could allow the volatility of the substituted naphthalenes to be studied under static and dynamic systems. It was hoped that the results could be used to predict the activity of the substituted naphthalenes on potatoes in a commercial potato store.

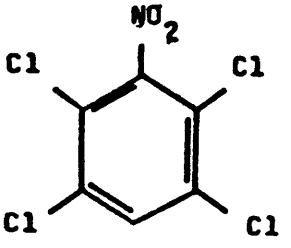
In Chapter Four a study on the effect of the substituted naphthalenes on the healing of tuber wounds was carried out and the results discussed. This is an important point as an early application of a wound inhibiting compound to tubers results in water loss and allows the entry of fungal and bacterial pathogens.

With an increase in public awareness of chemical residues in foodstuffs and the recent introduction of Government legislation, the work in Chapter Five concentrates on the development of analytical methods for the analysis of substituted naphthalenes in potatoes.

In Chapter Six the effect of the substituted naphthalenes on the growth of potato sprouts and residue levels were assessed and compared to commercial applications.

FIGURE 1.1 STRUCTURES AND PROPERTIES OF SPROUT SUPPRESSANT CHEMICALS

TECNAZENE



1,2,4,5-TETRACHLORO-3-NITRO-BENZENE

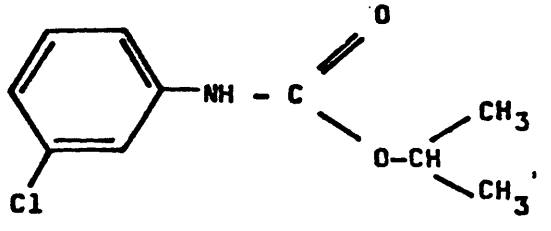
$C_{10}H_{12}C_1NO_2$

M Wt 213.68

mp 40.7-41.1°C

bp 229°C

CHLORPROPHAM



(3-CHLOROPHENYL) CARBAMIC ACID 1-METHYLETHYL ESTER

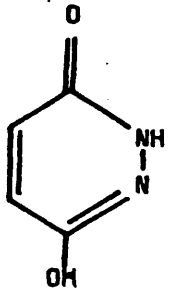
$C_6HCl_4NO_2$

M Wt 260.89

mp 90-101°C

bp 304°C

MALEIC HYDRAZIDE



1,2-DIHYDRO-PYRIDAZINE-DIONE

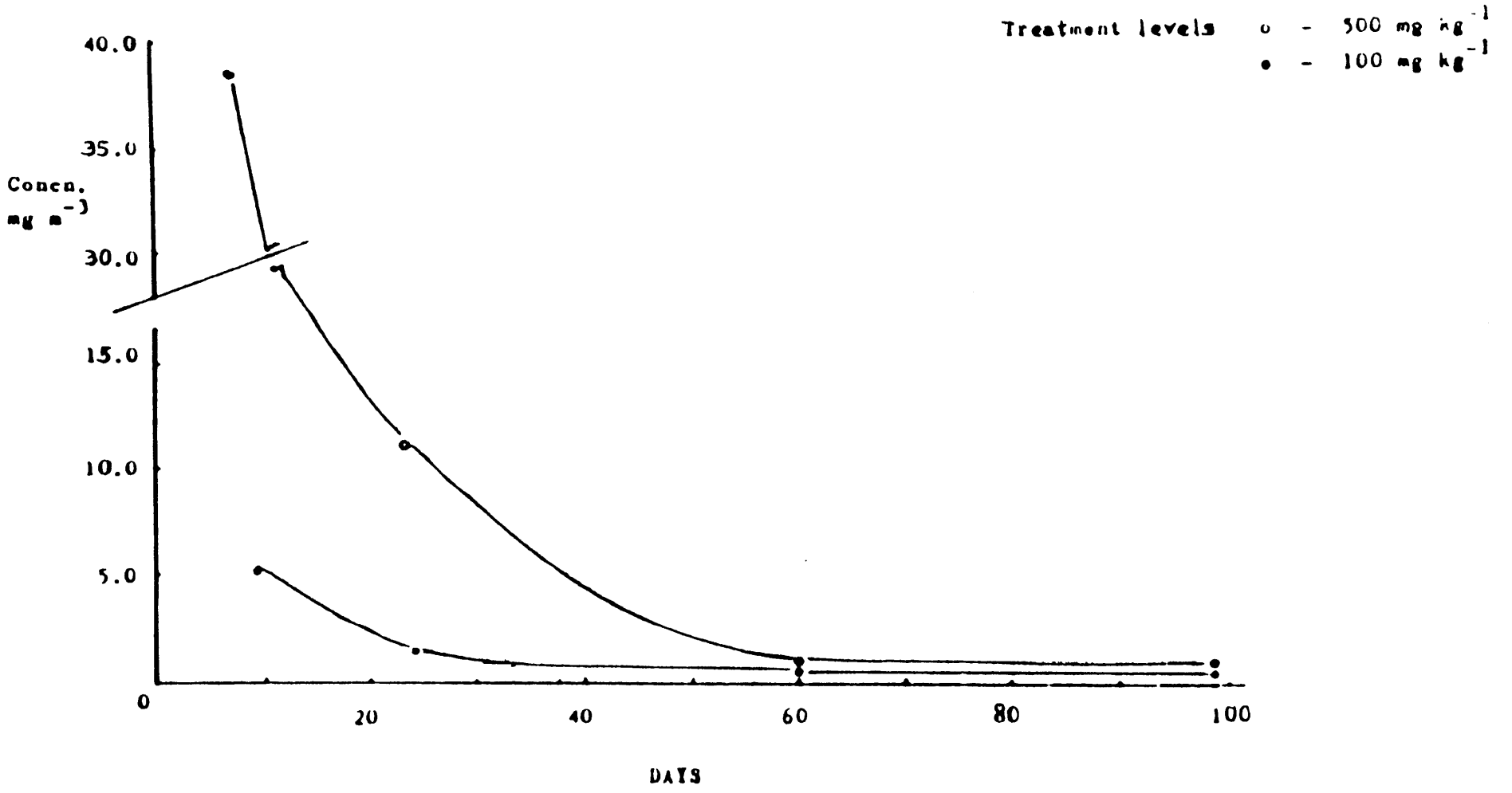
$C_4H_4N_2O_2$

M Wt 112.1

mp 292-298°C

bp

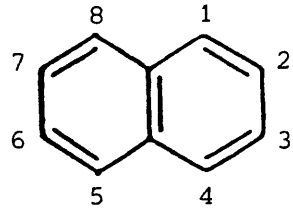
FIGURE 1.2 ANALYSIS OF PULEGONE IN THE HEADSPACE ABOVE POTATOES (Boyd and Duncan 1985)



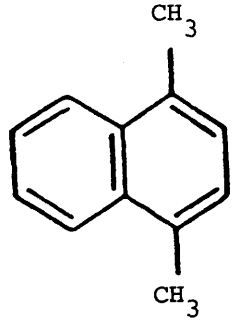
Headspace levels - Pulegone

FIGURE 1.3 STRUCTURE AND PROPERTIES OF SUBSTITUTED NAPHTHALENES

NAPHTHALENE RING STRUCTURE



1,4 DIMETHYLNAPHTHALENE



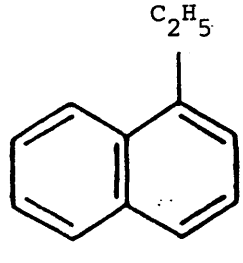
$(CH_3)_2\ C_{10}H_6$

M Wt 156.23

mp 7.6°C

bp 268°C

ETHYLNAPHTHALENE



$C_{12}H_{12}$

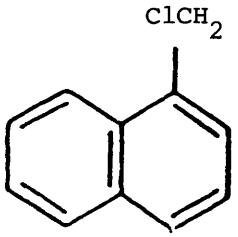
M Wt 156.23

mp -13.8°C

bp 258.67°C

FIGURE 1.4 STRUCTURE AND PROPERTIES OF SUBSTITUTED NAPHTHALENE

CHLOROMETHYLNAPHTHALENE



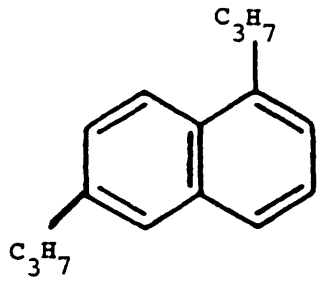
$(ClCH_2)C_{10}H_7$

M Wt 176.65

mp 32°C

bp 291.2°C

DIISOPROPYLNAPHTHALENE



$(C_3H_7)_2C_{10}H_7$

M Wt 212

mp -30°C

bp 290-299°C

HEADSPACE STUDIES ON DIMETHYLNAPHTHALENE ANALOGUES AND THEIR ACTIVITIES AS SPROUT SUPPRESSANT CHEMICALS

2.1 Introduction

Dormant potato tubers were found to evolve volatile substances that had an inhibiting effect on the growth of potato sprouts and this could extend dormancy (Burton 1952; Burton and Meigh 1971).

Some of these volatile substances were later identified by combined gas chromatography and mass spectrometry (GC-MS) and shown to be 1,4 dimethylnaphthalene and 1,6 dimethylnaphthalene (Meigh et al 1973). Meigh et al (1973) measured the potency of each volatile compound by treating batches of tubers with the chemical and measuring the effect on both the total weight of sprouts produced by the tubers and on the length of the longest sprout on each tuber. The 1,4 dimethylnaphthalene and 1,6 dimethylnaphthalene were demonstrated to be volatile enough to inhibit growth of sprouts on the potatoes if they were allowed to build up a high enough concentration. Meigh and his co-workers (1973) noted that the addition of methyl groups seemed to increase sprout inhibiting activity but the position of the groups on the aromatic ring seemed to be somewhat irrelevant. However, it was thought that this effect was attributable to increasing solubility in non-polar substances, since the potency of the volatile compounds is dependent on at least two factors: 1) the intrinsic activity at the point of action in the cell, and 2) the rate of transport from the vapour phase (in air) to the non-polar phase in the cell.

Beveridge et al (1981b) carried out work to investigate further the activity of 1,4 dimethylnaphthalene under more realistic storage conditions by studying the effects of the chemical on the storage and growth of seed tubers. The method used involved applying the 1,4 dimethylnaphthalene at a rate of 100mg kg^{-1} on an alumina carrier to 10kg batches of tubers, stored in cardboard boxes with loosely fitting lids at 10°C for a 12 week period before planting. This method has been demonstrated by Beveridge (1981b) to simulate a potato store. The results showed that 1,4 dimethylnaphthalene was as effective as tecnazene (120mg kg^{-1}) in controlling sprouting and that 1,4 dimethylnaphthalene had little effect on the emergence or yield of seed tubers when they had been aired for a 3 - 6 week period before planting. Thus the 1,4 dimethylnaphthalene has an advantage in being highly volatile, thereby reducing residue concentration in seed tubers which minimise the effect on the subsequent growth of ware potatoes and has obvious advantages for the consumer.

Further work carried out by Beveridge et al (1981b) demonstrated that when 1,4 dimethylnaphthalene was applied to potatoes as one initial application, it was not adequate to inhibit sprouting over a long period of time at a temperature of 10°C in a well ventilated potato store where the potatoes were intended for processing and that it would be necessary on such occasions to re-apply the chemical at intervals throughout the storage season.

Previous work with substituted naphthalenes mostly involved studying the dimethylnaphthalenes but Stephen and Duncan (1984) investigated the different activities of other substituted naphthalenes and their role in inhibiting potato sprout growth.

Stephen and Duncan (1984) used a similar method to that mentioned earlier (Beveridge et al 1981b) where 10kg batches of tubers were treated with various substituted naphthalenes applied on an alumina carrier at three concentration levels - 20mg kg^{-1}, 100mg kg^{-1} and 500mg kg^{-1}. The results were then calculated based on the mean length of the longest sprout. The results in Table 2.1 showed that mono and di substituted alkyl naphthalenes had sprout suppressant properties similar to dimethylnaphthalene and that halogen substituents could also give sprout suppressant activities although the appearance of the sprouts were subsequently different. A few of the chemicals used in this experiment were active at the three treatment levels but most of them were active at levels of 100mg kg^{-1} and above. At these higher levels, the chemicals were found to be more effective at controlling the sprouting over a longer period of time. The conclusion to be drawn on these chemicals is that they are less effective at lower concentrations.

However, some of the chemicals were found to be ineffective at controlling sprouting. This could be related to work carried out by Beveridge et al (1981b) mentioned earlier in this chapter, which showed that some of the chemicals proved to be ineffective when applied as a single application under commercial conditions because the chemicals were too volatile, resulting in short persistence and could not maintain an adequate headspace concentration. Alternatively, the chemicals had a low volatility resulting in an inadequate vapour concentration.

Meigh and co-workers (1973) carried out work using volatile chemicals and showed that there was a minimum effective headspace concentration for each below which sprout suppression activity will progressively decrease.

Meigh (1969) used a method to study the effects of a constant known headspace of a compound on potatoes which involved adding a known amount of chemical in vapour form to a stream of ventilating air which was then passed over a sample of tubers. This was a slightly modified method to that used previously by Meigh (1967) which had allowed the chemicals to be applied at a constant rate through silicone rubber tubes into the airstream. The headspace concentrations were then calculated by measuring the rate of ventilation and loss in weight of the test chemical.

More recently, Beveridge et al (1983) studied the headspace concentrations of substituted naphthalenes by applying initial concentrations of chemicals to potatoes and following the headspace over a period of time. The chemicals were applied on an alumina carrier and dusted onto 10kg batches of potatoes that were stored in cardboard boxes. The headspace levels were taken over a 14 week period. The results showed that as the application rate increased, then the headspace concentration also increased, but over the 14 week period the general trend was for the headspace level to decrease with time. The rate of decrease varied between the substituted naphthalenes, depending on their volatility.

Some of the chemicals were thought to be too volatile for use over a prolonged period of time as their headspace levels decreased at a fast rate and other chemicals were not volatile enough to build up a minimum effective headspace (see Figure 2.1). Beveridge (1983) calculated that 100mg kg^{-1} was the minimum effective application rate for 1,4 dimethyl-naphthalene which gave a minimum effective headspace between 3 - 6mgm^{-3} over a 12 week period.

Since the substituted naphthalenes have been assessed by several laboratory methods, they have been applied as vapours introduced directly into air surrounding tubers and as solids distributed among the tubers. It was decided that the objective of the experimental work which will now be described was to develop suitable headspace methods that could be carried out under laboratory conditions with no tubers present. There were two objectives to this chapter:

The first objective was to develop a suitable method that would allow the volatility of many potential sprout suppressant compounds to be studied under a static system over a short period of time, under controlled conditions. The intention was to try and relate the volatility of each chemical to its activity as a sprout suppressant. Based on this method, three chemicals were then chosen from previous potato experiments and their headspace concentrations were measured under different headspace time intervals, temperatures and concentrations of chemical.

The second objective was to study the substituted naphthalenes under more realistic potato storage conditions. It was hoped to trap the volatiles present in the headspace using a porous polymer absorbent, over a long period of time at low temperatures.

2.1.1 Development of a Small Scale Static Headspace System

The experiment was designed to allow the volatility of many potential sprout suppressant compounds to be studied under a static system over a short period of time, under controlled conditions and to relate this volatility to the chemical's sprout suppressant

activity. Compounds which were shown to be suitably volatile could then be tested further for sprout suppressant activity on a more realistic and larger scale.

The setting up of the experiment was based on a technique used by food chemists to study the flavour of food (McCarthy et al 1963; McMullin et al 1975; Aspelund and Wilson 1979; Ehler et al 1979). This involved placing a small amount of the food sample in a closed container which was sealed air tight with a rubber septum. The container was then allowed a period of equilibration before headspace samples were withdrawn with a gas tight syringe and injected directly onto a packed gas chromatographic column equipped with a flame ionisation detector.

It was decided to use this method as it involved using small amounts of chemical that needed very little sample preparation, merely a short period of equilibration in a closed container with very little opportunity for loss of volatiles. On account of the relatively high volatility of the substituted naphthalenes, their headspace concentrations could be measured directly on a GC equipped with an FID unlike tecnazene and chlorpropham where a preconcentration step was required using porous polymer precolumns.

The experiment was set up initially based on the technique designed by McCarthy et al (1963) who followed the change in the volatile component produced during the ripening of two varieties of banana.

Triplicate flasks were set up by adding 100µg 1,4 dimethylnaphthalene made up in hexane to the bottom of narrow necked 120cm^3 flasks and sealed air tight with alloy septum lids. The flasks were allowed to stand at room temperature for 24 hours, which was thought initially to be enough time to allow the system to reach equilibrium. Triplicate 0.6ml headspace samples were withdrawn from each flask using a 1ml gas tight syringe which were injected directly onto a gas chromatograph (GC) equipped with a flame ionisation detector (FID) and the results compared to standards. The resulting headspace levels (see Table 2.2) were very low and had poor reproducibility. The average reproducibility of headspace injections from the same flask lies within 70% and the headspace concentrations between separate flasks had a reproducibility of 49%. It was decided that if the method was going to be used to screen a lot of compounds over a short period of time, a more reproducible method would be needed with less variation within and between flasks. With this in mind it was decided to try and improve the method by making it static.

2.1.2 Improvements in Methodology

Solvent

Initially, 1,4 dimethylnaphthalene was added to the flask in a volatile, organic solvent - hexane, both of which are volatile enough to enter into the headspace in substantial amounts.

Consequently, the headspace samples taken with the gas tight syringe contained solvent molecules as well as 1,4 dimethyl-naphthalene molecules which undoubtedly influenced the results. There was also the problem that as 1,4 dimethylnaphthalene is soluble in hexane the 1,4 dimethylnaphthalene molecules will remain in hexane rather than enter the headspace thus reducing the concentration of 1,4 diemthylnaphthalene molecules in the headspace (see Table 2.2) therefore giving low headspace concentrations.

It was therefore decided to add the 1,4 dimethylnaphthalene to the flask on its own, thus removing any interfering solvent molecules that may reduce or cause a variation in the headspace concentration of the 1,4 dimethylnaphthalene. To increase the sensitivity of the method, a larger amount of chemical was added to the bottom of the flask which would speed up equilibration over a given time and possibly increase the concentration of 1,4 dimethylnaphthalene in the headspace over a time period and thus allow significant amounts of the chemical to be analysed. An alternative method to increase the headspace concentration was to take a larger headspace volume using a larger air tight syringe, but this would lower the efficiency of the chromatographic analysis and would lower the sensitivity of the method.

Using this modified method, three flasks were set up on separate days by pipetting 1000mg of 1,4 dimethylnaphthalene into the bottom of each flask. The flasks were then sealed and stored at room temperature for 24 hours before analysis. This was done to measure the change in headspace concentrations from day to day.

From Table 2.3 it can be seen that by adding larger amounts of chemical the headspace concentration has increased and the variation between replicate injections has been reduced. The average reproducibility of headspace injections from the same flask lies within 93% and the headspace concentrations between separate flasks were within 69% reproducibility. However, in spite of this advance there is still a problem of variation between different flasks especially those set up on different days.

Temperature

In the experiments mentioned above, the 1,4 dimethylnaphthalene had been added to the flasks and left at room temperature for 24 hours to allow the 1,4 dimethylnaphthalene to reach equilibrium within the flask. The time taken for equilibration within a static system depends upon the nature of the chemical and also the temperature. It was thought that the variation between flasks in Table 2.3 was high due to the change in temperature between the flasks especially when they were set up on different days. Susceptibility of the flasks to temperature changes, brought about by heaters, draughts from windows and doors and diurnal changes, will in turn affect the volatility of the compounds from day to day and cause a variation in headspace concentrations. However, the variation in results was minimised by submerging the flasks in a temperature controlled water bath where they were held in position with clamps so that only their necks were above water (see Plate 2.1). The temperature of the water bath was set at 31°C $\pm$ 1°C unless stated otherwise.

This temperature was chosen for speed as it allowed a high concentration of chemical to enter the headspace in a short period of time.

Gas-tight Syringe

An early problem which led to a loss in efficiency of the gas-tight syringe was the presence of a small leak between the barrel and the plunger, which led to a significant sample loss.

It was also found to be important to have a routine injection technique and to handle the syringe as little as possible as body heat increased the temperature of the syringe barrel which interfered with the headspace levels.

The accuracy of the 1ml gas-tight syringe (Hamilton, Series 700) was 99±1% (Phase Separation Ltd, England).

With the improvements mentioned above, an experiment was initiated where three flasks were set up on the same day at a controlled temperature adding in turn 1000mg of 1,4 dimethylnaphthalene to the bottom of each flask (see Table 2.4). The average reproducibility of headspace injections from the same flask was 96% and the headspace concentrations between separate flasks were within 95% reproducibility. It should be noted that the chemical was assumed to reach equilibrium within the time period. However, as seen later in this Chapter, this was not found to be the case.

2.1.3 Materials and Methods

Having developed a reliable and reasonably reproducible method for headspace analysis of 1,4 dimethylnaphthalene, it was decided to use this method to study the volatility of a few selected chemicals under controlled conditions. The chemicals were chosen from the results of the experiment carried out by Stephen and Duncan (1984) which was described earlier in this chapter. The compounds chosen were 1,4 dimethylnaphthalene, ethylnaphthalene and chloromethylnaphthalene, the structures of which are shown in Figures 1.3 and 1.4. The 1,4 dimethylnaphthalene was chosen as it has been shown to be effective as a potato sprout suppressant, ethylnaphthalene was shown to be promising in results by Stephen and Duncan (1984) whereas chloromethylnaphthalene gave poor control over sprouting. Plates 2.2 - 2.4 show the effects of 1,4 dimethylnaphthalene and ethylnaphthalene on the potato sprout.

It was decided to study the headspace concentrations of these three chemicals under controlled conditions at various temperatures, time intervals and amounts of chemical added to the flasks, to see what affect these individual factors had on the headspace concentration.

Triplicate analysis was carried out on the flasks. Significant differences were calculated using a T test, calculated as follows:

$$S^2 = \frac{\{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2\}}{(n_1 + n_2 - 2)}$$

$$t = \frac{(x_1 - x_2)}{S\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

x_1 and x_2 - sample means

S_1 and S_2 - sample standard deviations

n_1 and n_2 - number of replicates

t has $(n_1 + n_2 - 2)$ degrees of freedom

$p < 0.05$

Time

Triplicate flasks were set up for each compound and involved 1000mg of 1,4 dimethylnaphthalene (Aldrich Chemical Co., Dorset, England) being added to the bottom of separate flasks then sealed air tight using crimpers (Jones Chromatography Ltd., Mid Glamorgan, Wales) and alloy septum lids. 1000mg of chemical was added to each flask as it was thought that it was a large enough concentration to give a detectable headspace concentration when working with compounds with a low volatility. The temperature of the water bath was maintained at 31°C $\pm$ 1°C which was a temperature easy to maintain in the water bath and it also allowed a relatively high concentration of the chemical to be studied over a short period of time. The time taken for each chemical to equilibrate with the system was not known and no doubt varied depending on the nature of the chemical. To study the effect of time on the headspace concentration, flasks were set up as mentioned above and analysed after 24, 48 and 72 hours of equilibration. A fresh set of flasks was set up for each analysis day (see Table 2.5 and Figure 2.2).

Temperature

Triplicate flasks were set up for each compound and involved 1000mg of 1,4 dimethylnaphthalene, ethylnaphthalene and chloromethyl-naphthalene being added to the bottom of separate flasks and sealed air tight using crimpers and alloy septum lids. The flasks were equilibrated for 72 hours which was thought to be long enough to allow the chemicals to reach equilibration within the system.

It was decided to study the headspace concentrations at 21°C, 31°C and 39°C. These temperatures were chosen to allow the effects of temperature on headspace to be followed over a large range. These temperatures were used as the water bath was most easily adjusted to these temperatures but each temperature could vary within ± 1°C (see Table 2.6 and Figure 2.3).

Concentration

Triplicate flasks were set up for each concentration and chemical. It involved 1000mg, 500mg and 100mg of 1,4 dimethylnaphthalene, ethylnaphthalene and chloromethylnaphthalene being added to separate flasks and sealed air tight using crimpers and alloy septum lids. The flasks were maintained for 72 hours at 31°C ± 1°C (see Table 2.7 and Figure 2.4).

It is important to note that each set of flasks was set up in triplicate and replicate headspace samples were taken from each flask. Since it was felt that the rubber septum was not completely air tight after headspace samples were taken, a fresh set of flasks were set up for each analysis date.

It should also be noted that since the chemicals being used were all in liquid form, they were pipetted directly into the bottom of the flasks.

Gas Chromatography Conditions

All the results being reported were obtained using a PYE PU 4500 gas chromatograph with a flame ionisation detector.

The headspace samples were injected into a GC equipped with columns that were packed with a Gas Chrom Q diatomaceous earth support (Applied Science Laboratories Inc., Penna., USA) coated with either OV17 or OV101 stationary phase (Phase Separations Ltd., England). The OV17 was a semi-polar phase and the OV101 a non-polar phase.

Conditions used:

Temperatures :	column oven	180°C
	injector	220°C
	detector	250°C

Gas Flow Rate :	Nitrogen (carrier gas)	$30cm^3/min$
	Air (to FID)	$180cm^3/min$
	Hydrogen (to FID)	$30cm^3/min$

The GC was then calibrated with 100µg ml^{-1} standard injections. The data was then collected, calibrated and integrated using a Shimadzu C-RIB recorder/integrator.

Typical chromatographs for 1,4 dimethylnaphthalene and ethylnaphthalene standards are shown in Figure 2.5.

2.1.4 Results

TABLE 2.1 Influence of some substituted naphthalene on sprout growth during storage (Stephen and Duncan 1984).

Chemical	Mean length of longest sprout (mm)		
Untreated control	175		
	20mg/kg	100mg/kg	500mg/kg
2,3 dimethylnaphthalene	141	15	25
1 bromo 2,3 dimethylnaphthalene	132	85	64[a]
1,4 dimethylnaphthalene	59	10	1
1 chloro-4-methylnaphthalene	81	4	1
1 bromo-4-methylnaphthalene	63	10	2
1 bromo-2-methylnaphthalene	41	25	8
1 chloromethylnaphthalene	182	147	124
1 chloromethyl-2-methylnaphthalene	170	184	185
1,4 dibromonaphthalene	120	11	26
1 acetyl-4-methylnaphthalene	156	85	83[b]
2 methylnaphthalene	162	121	4
1 methylnaphthalene	139	129	8
1 ethylnaphthalene	24	9	0
mixed methylnaphthalene	172	174	6
LSD (5% level)	16	13	12

[a] level employed 161mg/kg

[b] level employed 173mg/kg

TABLE 2.2 The concentration of 1,4 dimethylnaphthalene ($ngcm^{-3}$) above 100µg 1,4 dimethylnaphthalene in hexane, in the air (static system) at room temperature after 24 hours.

HEADSPACE CONCENTRATION ($ng\ cm^{-3}$)		
Flasks	Headspace Samples	Mean Headspace
A	9.1 4.7 6.9	6.9 ± 1.8
B	2.2 3.8 4.2	3.4 ± 1.0
C	3.5 5.3 6.2	5.0 ± 1.3

mean ± standard deviation from triplicate headspace samples taken from each flask

TABLE 2.3 The concentration of 1,4 dimethylnaphthalene (ng cm^{-3}) above 1000mg of 1,4 dimethylnaphthalene in the air (static system). Headspace samples were taken from separate flasks on different days, after 24 hours at room temperature.

HEADSPACE CONCENTRATION (ng cm^{-3})		
Flasks	Headspace Samples	Mean Headspace
Day One A	32.5 30.9 33.2	32.2 ± 1.2
Day Two B	23.5 24.5 22.8	23.6 ± 0.9
Day Three C	22.1 21.4 24.2	22.5 ± 1.5

mean ± standard deviation from triplicate headspace samples taken from each flask

TABLE 2.4 The concentration of 1,4 dimethylnaphthalene (ng cm^{-3}) above 1000mg 1,4 dimethylnaphthalene in the air (static system). The flasks were maintained at 31°C ± 1°C for 48 hours.

HEADSPACE CONCENTRATION (ng cm^{-3})		
Flasks	Headspace Samples	Mean Headspace
A	77.8 77.1 74.0	76.3 ± 2.0
B	77.9 81.4 77.6	78.9 ± 2.1
C	76.9 75.4 71.5	74.6 ± 2.8

mean ± standard deviation from triplicate headspace samples taken from each flask

TABLE 2.5 The concentration of dimethylnaphthalene analogues (ng cm^{-3}) above 1000mg of compound in air (static system) over 72 hours at 31°C ± 1°C.

CHEMICAL	TIME (HOURS) 24	48	72
1,4 dimethylnaphthalene	108.5 ± 6.0	76.7 ± 5.0	74.5 ± 5.1
ethylnaphthalene	98.5 ± 5.5	115.7 ± 5.5	68.4 ± 4.4
chloromethylnaphthalene	1.2 ± 0.3	4.4 ± 0.3	6.8 ± 0.4

mean ± standard deviation taken from triplicate flasks; triplicate analysis on each flask

TABLE 2.6 The regression of the results of DMN analogues (ng cm^{-3}) above 1000mg of compound in air (static system) over 72 hours at 31°C ± 1°C.

CHEMICAL	R^2	SLOPE
1,4 dimethylnaphthalene	99.9%	3.11*
ethylnaphthalene	99.9%	3.46*
chloromethylnaphthalene	89.6%	0.23

* These steps were significantly different from zero at $p<0.05$

TABLE 2.7 The concentration of dimethylnaphthalene analogues (ng cm^{-3}) above 1000mg of compound in air (static system). The flasks were maintained at 21°C, 31°C and 39°C for 72 hours.

TEMPERATURE (°C)	CHEMICAL		
	1,4 Dimethyl-naphthalene	Ethyl-naphthalene	Chloromethyl-naphthalene
21	44 ± 3.2	35.5 ± 3.4	3.6 ± 0.3
31	74.2 ± 5.2	68.4 ± 4.8	6.8 ± 0.4
39	100 ± 5.6	97.3 ± 5.1	7.8 ± 0.4

mean ± standard deviation taken from triplicate flasks; triplicate analysis on each flask

TABLE 2.8 The concentration of dimethylnaphthalene analogues (ng cm^{-3}) above 1000mg, 500mg and 100mg of compound in air (static system) maintained at 31°C for 72 hours

CHEMICAL	CONCENTRATION ADDED (mg)		
	1000	500	100
1,4 dimethylnaphthalene	74.2 ± 5.0	75.9 ± 4.0	86.9 ± 6.4
ethylnaphthalene	68.4 ± 4.3	66.5 ± 4.2	75.0 ± 5.7
chloromethylnaphthalene	6.8 ± 0.4	5.4 ± 0.3	6.2 ± 0.4

mean ± standard deviation taken from triplicate flasks; triplicate analysis on each flask

FIGURE 2.1 THE HEADSPACE CONCENTRATION OF DIMETHYLNAPHTHALENE ABOVE POTATOES (Boyd and Duncan 1985)

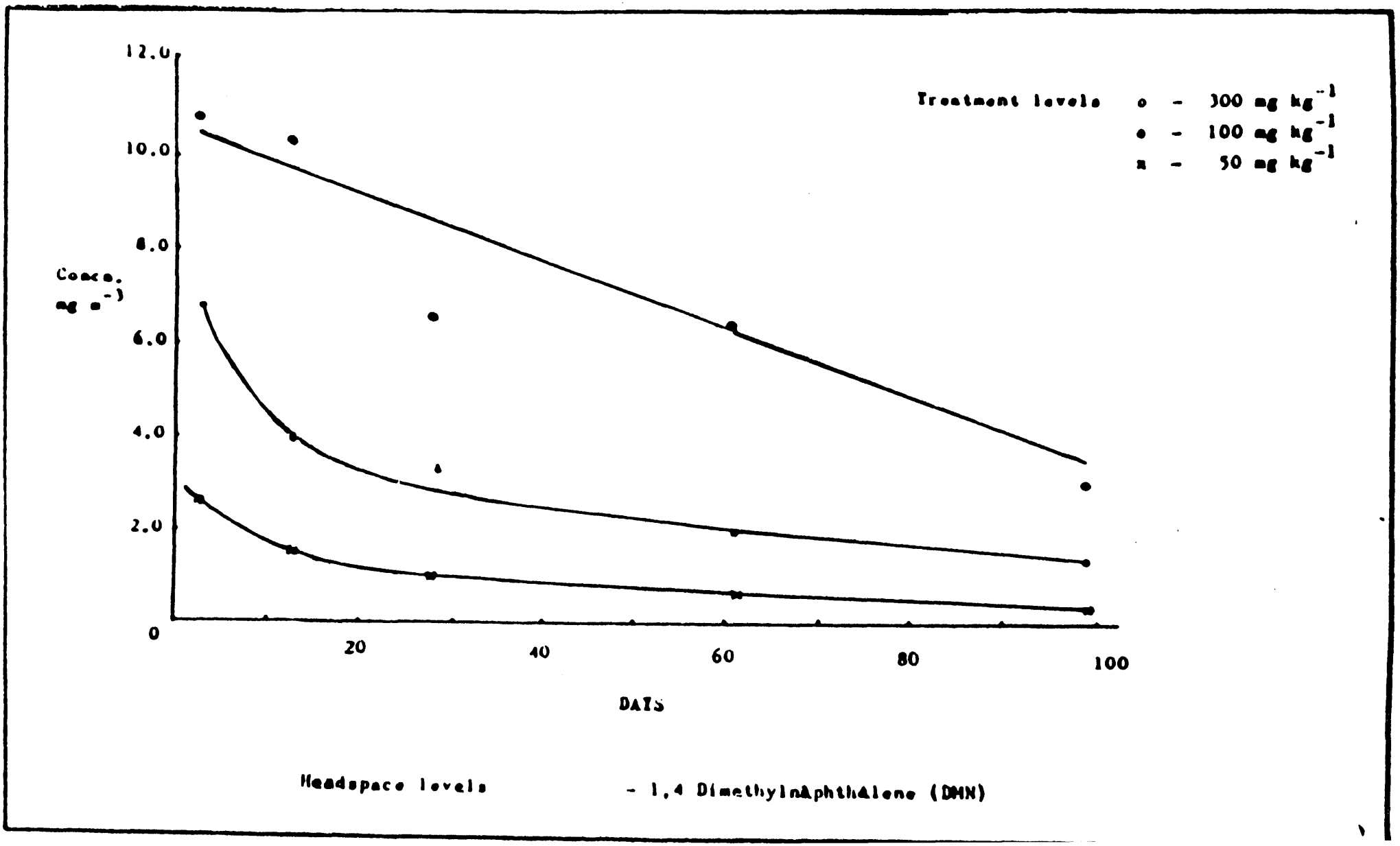


FIGURE 2.2 THE HEADSPACE CONCENTRATION OF DIMETHYLNAPHTHALENE ANALOGUES OVER 72 HOURS AT A TEMPERATURE OF 31°C ± 1°C

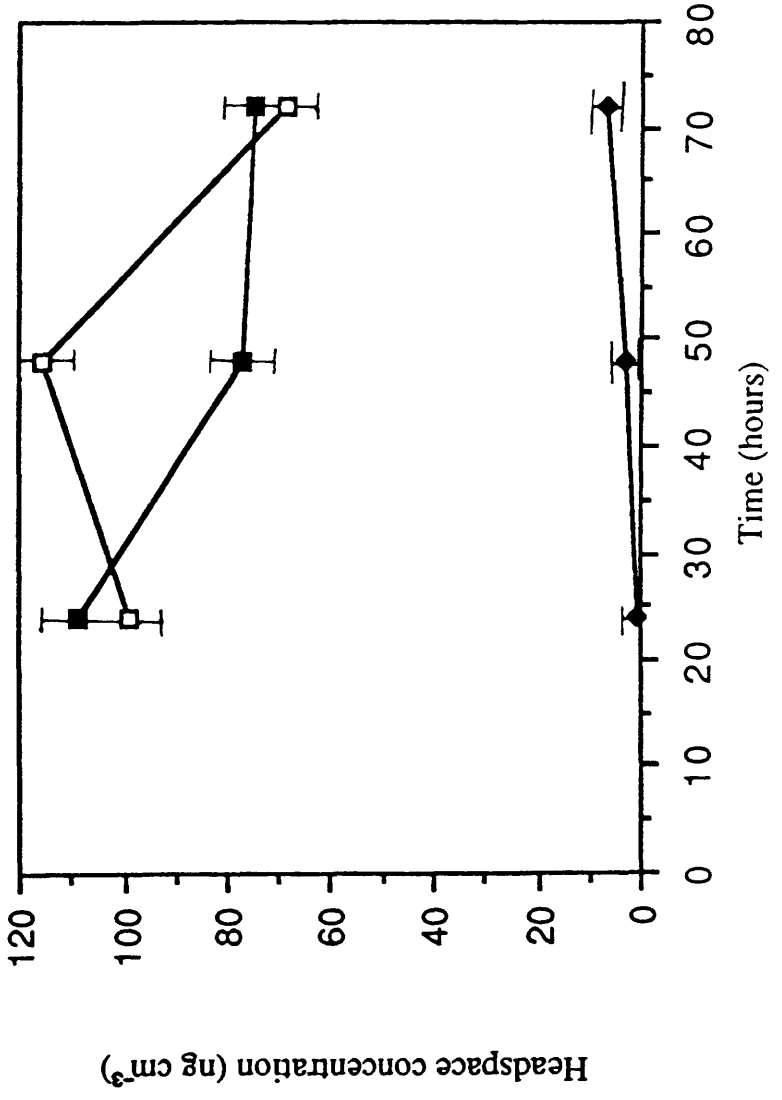
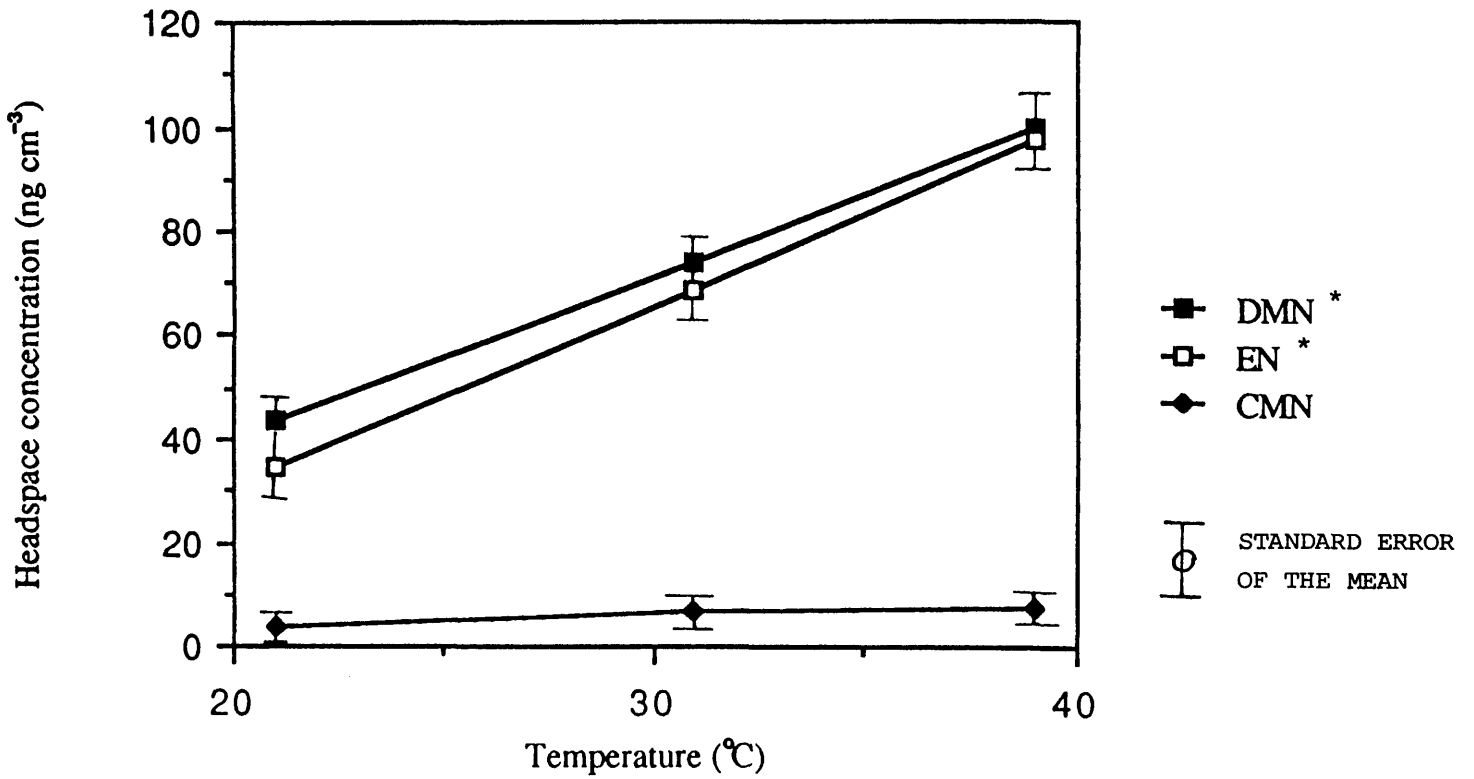


FIGURE 2.3 THE HEADSPACE CONCENTRATION OF DIMETHYLNAPHTHALENE ANALOGUES AFTER 72 HOURS OVER A TEMPERATURE RANGE OF 21°C - 39°C



* These slopes were significantly different from zero at $p<0.05$.

FIGURE 2.4 THE EFFECT OF THE AMOUNT OF DIMETHYLNAPHTHALENE AND ITS ANALOGUES ON HEADSPACE LEVELS. FLASKS EQUILIBRATED FOR 72 HOURS AT 31°C ± 1°C

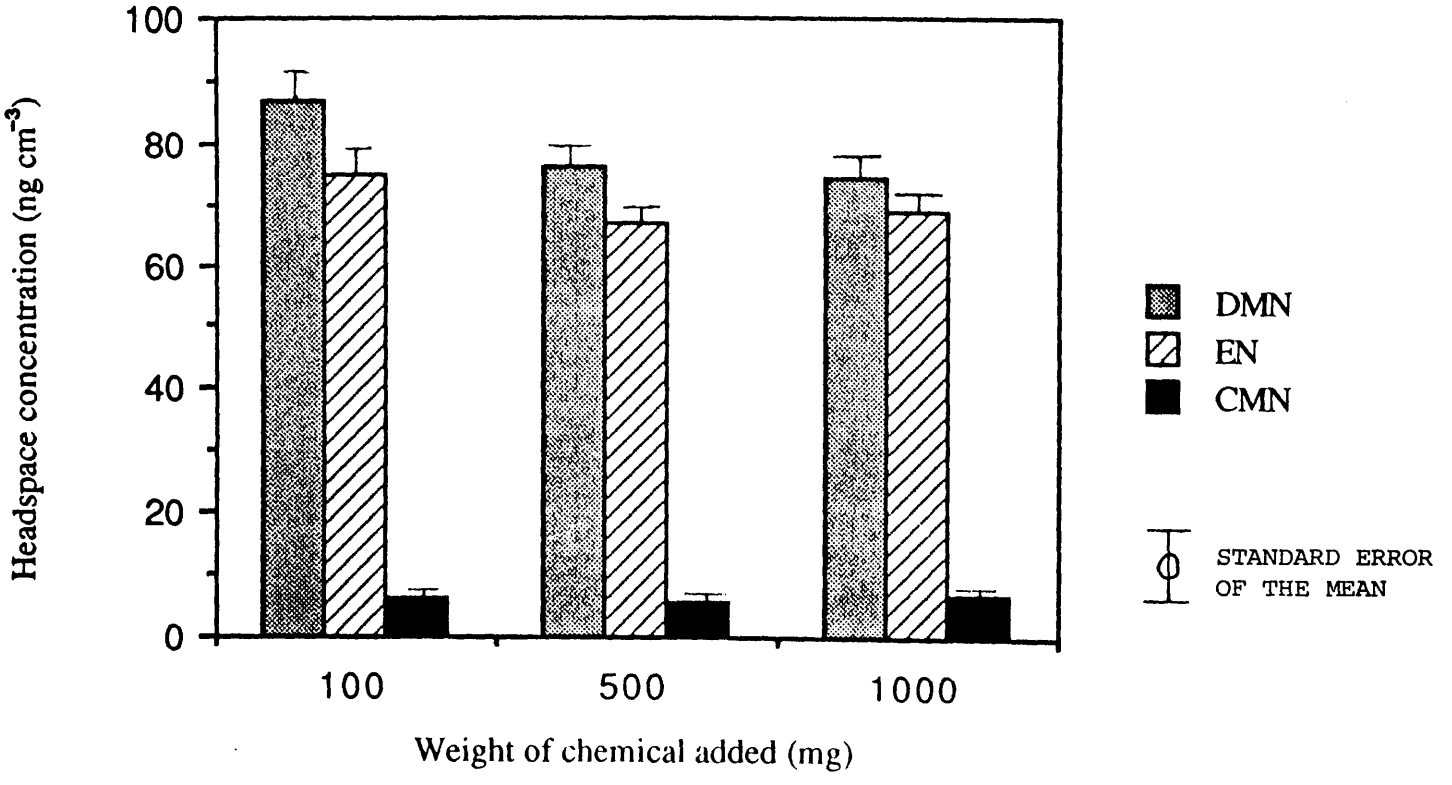


FIGURE 2.5 GAS CHROMATOGRAMS OF:

A) DIMETHYLNAPHTHALENE STANDARD

B) ETHYLNAPHTHALENE STANDARD

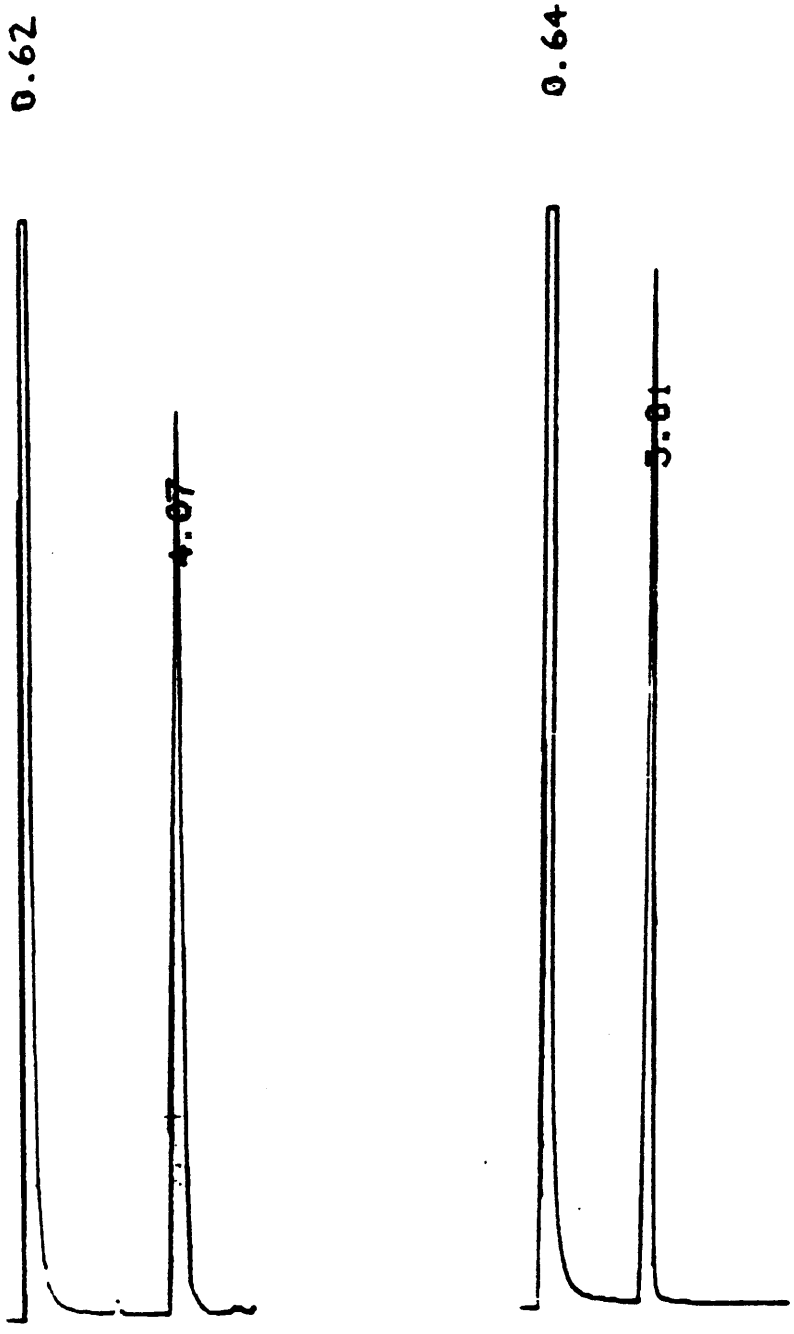


FIGURE 2.6 EFFECT OF DIMETHYLNAPHTHALENE ANALOGUE AND APPLICATION RATE ON THE SPROUT GROWTH OF TUBERS STORED FOR 12 - 14 WEEKS AT 10°C (STEPHEN AND DUNCAN 1984)

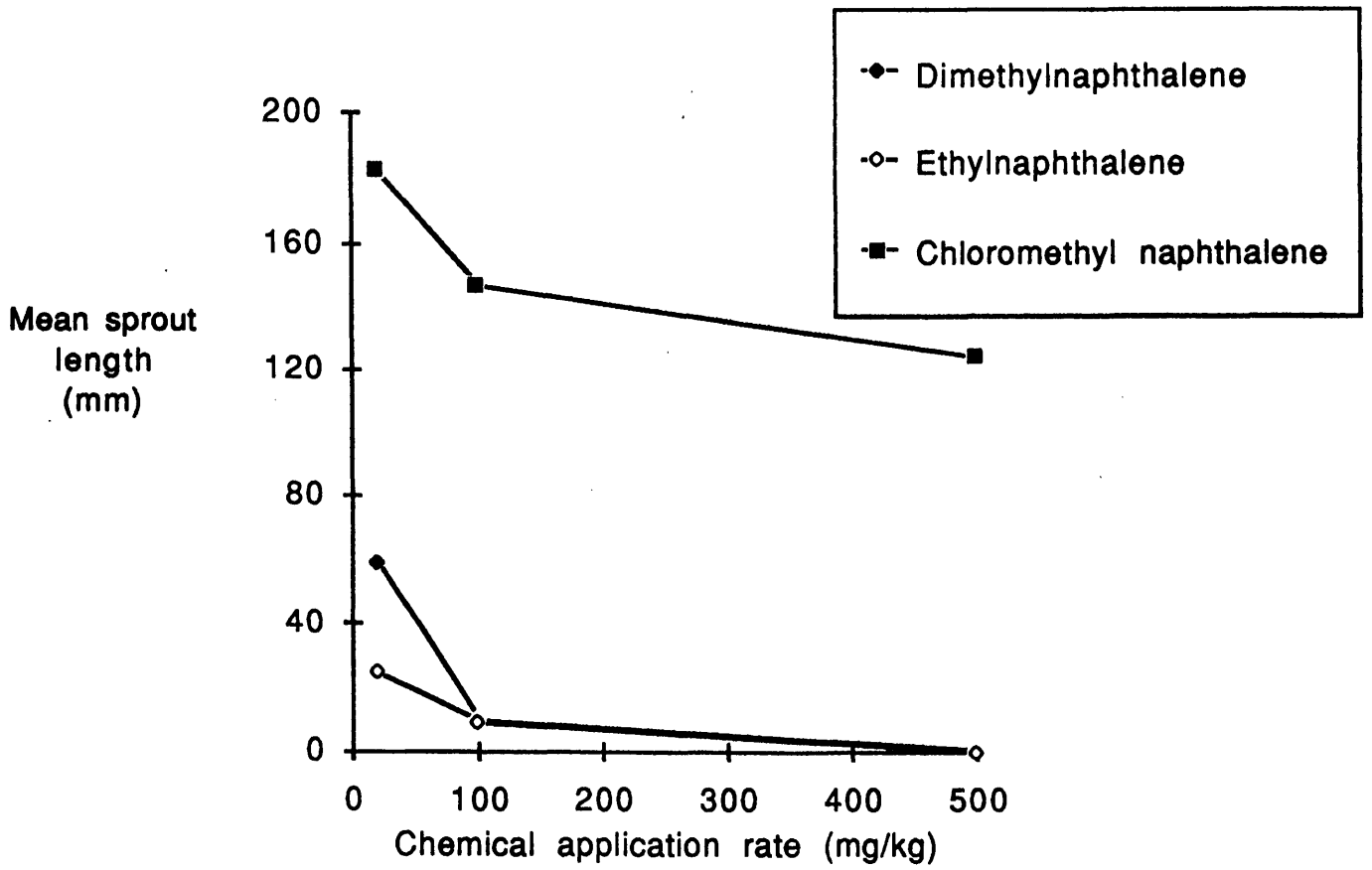


PLATE 2.1 FLASKS IN TEMPERATURE CONTROLLED WATER BATH

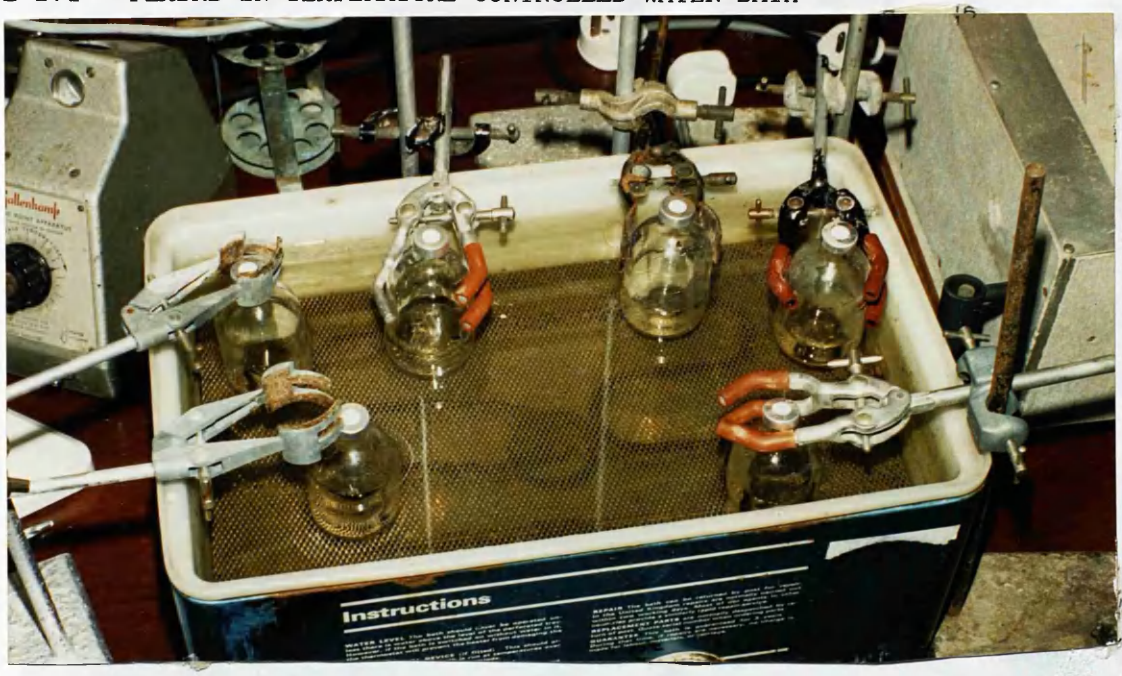


PLATE 2.2 SPROUT LENGTH OF UNTREATED MARIS PIPER TUBERS AFTER 18 WEEKS (STEPHEN AND DUNCAN 1984)

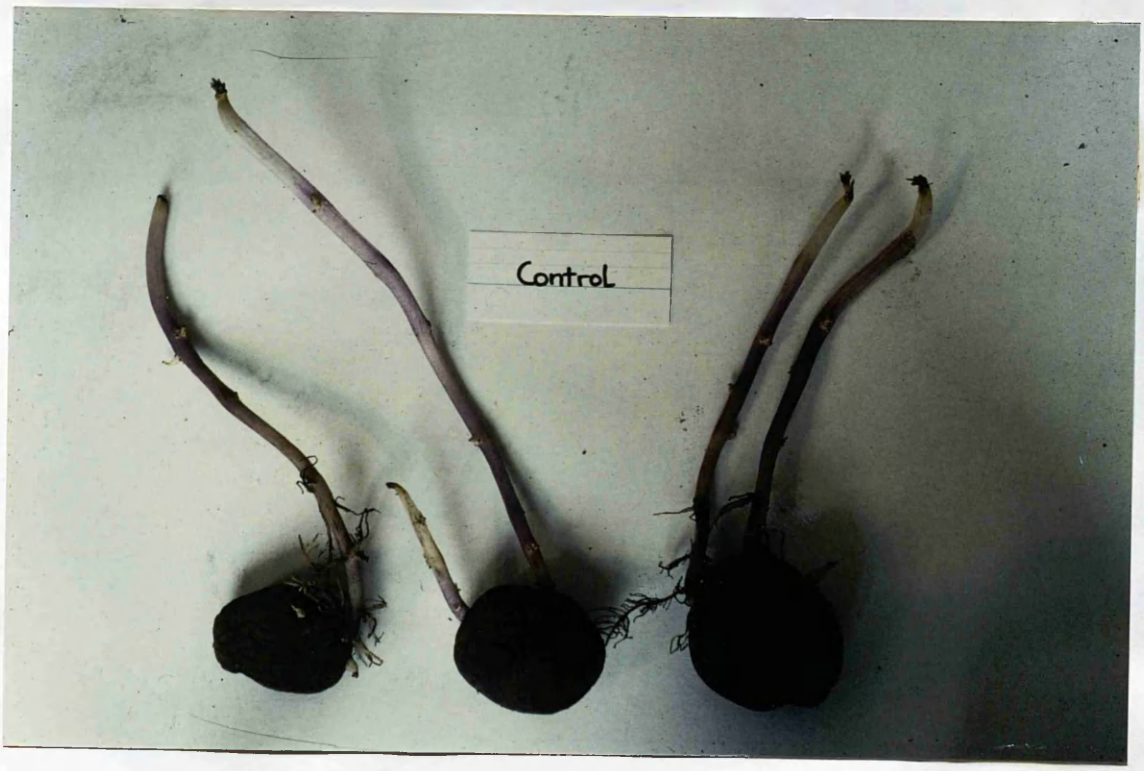
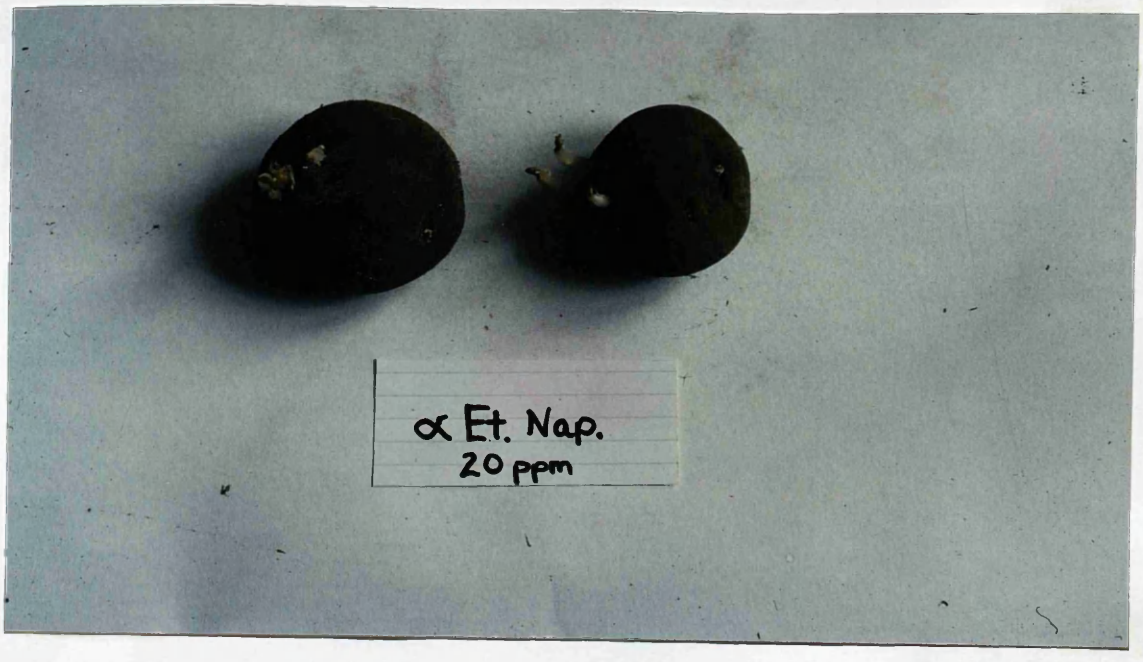


PLATE 2.3 SPROUT EFFECT ON DIMETHYLNAPHTHALENE (100mg kg^{-1})
TREATED MARIS PIPER TUBERS AFTER 14 WEEKS (STEPHEN AND DUNCAN 1984)

PLATE 2.4 SPROUT EFFECT OF ETHYLNAPHTHALENE (20mg kg^{-1})
TREATED MARIS PIPER TUBERS AFTER 14 WEEKS (STEPHEN AND DUNCAN 1984)



2 2.5 DISCUSSION

Effect of Time

Figure 2.2 and Table 2.5 show that the headspace concentration of dimethylnaphthalene and ethylnaphthalene fluctuate initially. The chloromethylnaphthalene has a very low headspace concentration which after 24 and 48 hours is close to the FID detection limits. To allow a more detailed study of chloromethylnaphthalene a more sensitive detector would be needed. The headspace concentrations of dimethylnaphthalene and ethylnaphthalene after 72 hours are at least 10 times greater than that of chloromethylnaphthalene.

The results from this experiment seem to suggest that the headspace varies within the flask until equilibrium has been reached, which is assumed in this work to be around 72 hours. This seems to be the time when it reaches a relatively stable concentration at 31°C. However, there is a possibility that the time was underestimated and the headspace concentrations varied as they had not yet reached equilibrium. The exact time for equilibrium to take place could be investigated in work in the future.

Effect of Temperature

Table 2.7 and Figure 2.3 show the effect of temperature on the headspace concentration of 1,4 dimethylnaphthalene, ethylnaphthalene and chloromethylnaphthalene. As the temperature is increased from 21°C to 39°C then as expected the headspace concentration also increases significantly for dimethylnaphthalene and ethylnaphthalene.

As the temperature is increased then the amount of heat entering the system also increases correspondingly and more chemical molecules will reach their Latent Heat of Vaporisation and thus the headspace concentration will also increase. However, the latent heat of vapourisation varies between chemicals and the lower the heat of vapourisation then the higher the headspace concentration (Fine 1978).

Figure 2.3 shows the effect of temperature on the headspace concentrations of both 1,4 dimethylnaphthalene and ethylnaphthalene. Both increase proportionally to an increase in temperature and at similar rates. The headspace concentration of chloromethylnaphthalene was low and only increased non significantly with the temperature increase which indicates that it has a very low volatility unlike dimethyl-naphthalene and ethylnaphthalene.

Effect of Concentration

The results of studying the headspace concentration against concentration of chemical added are shown in Table 2.8.

Effect of Concentration (cont)

Figure 2.4 shows that at three different concentrations of chemical added, the headspace concentration of dimethylnaphthalene and ethylnaphthalene are at least 10 times greater than that of chloromethylnaphthalene after 72 hours at 31°C ± 1°C.

The headspace concentrations remain relatively constant for each compound at the different concentrations of chemical added. There was no significant difference found for the headspace of each compound at different concentrations of chemical added.

Conclusion of Results from Static System

A summary of the development work leading to the final static headspace method used in Chapter Two is as follows:

1. A low concentration of dimethylnaphthalene (100µg) in hexane was added to the flasks. The resulting headspace concentrations were low, 2 - 9ng cm^{-3}. A reproducibility of 70% was achieved from headspace samples taken from the same flask and a poorer reproducibility of 49% taken from separate flasks. The solvent molecules were thought to interfere with the headspace concentration.

2. A higher concentration of dimethylnaphthalene (1000mg) was added on its own to the flasks. The headspace concentrations were fairly high, 21.4 - 33.2ng cm^{-3}. The reproducibility of the method was tested by setting up similar flasks on separate days. A good reproducibility of 93% was obtained from headspace samples taken from the same flask. However, a poorer reproducibility of 69% was achieved between the flasks set up on separate days. This was probably due to changes in temperature between days.

3. The reproducibility of headspace samples between flasks was increased by using a temperature controlled water bath, to minimise the temperature changes from day to day. 1000mg of dimethylnaphthalene was added to the flasks which were held at 31°C $\pm$ 1°C for 48 hours. The corresponding headspace concentrations were high, 71.5 - 81.4ng cm^{-3}. The reproducibility of the headspace concentrations within and between sample flasks were high with values of 96% and 95% respectively.

The development of this methods allows a simple, fast and reasonably accurate screening method to study the volatility of compounds. The method in this chapter was used to study the volatility of sprout suppressant chemicals at various temperatures, time intervals and amounts of chemicals added to the flasks. It has shown that 1,4 dimethylnaphthalene and ethylnaphthalene are fairly volatile compounds and chloromethylnaphthalene has a low volatility.

These chemicals had already been used in potato experiment trials by Stephen and Duncan (1984) and a look at Figure 2.6 reveals how effective each chemical was at controlling the sprout growth in the potatoes.

Dimethylnaphthalene and ethylnaphthalene gave good control over sprouting especially at levels above 100mg kg^{-1} and chloromethyl-naphthalene gave poor control at all levels.

It could be feasible that the low volatility of a compound and the activity of the compound as a sprout suppressant are connected. It is thought that the poor control of chloromethylnaphthalene at inhibiting sprout growth could be related to its low volatility.

Although this method cannot predict accurately the activity of a compound on potatoes in a potato store, it could be used as a screen for chemicals and then those shown to be sufficiently volatile could be tested further by applying them to potatoes in larger scale experiments.

This method need not be restricted to sprout suppressant chemicals but can also be used to study the volatility of a wide range of chemicals which have different used in many industries eg food and beveridge industries.

2.2 Introduction to Dynamic Headspace

Beveridge et al (1981a) demonstrated that some chemicals, known to be active sprout suppressants when applied to potatoes as a constant vapour, were not so active when applied on a solid carrier. Further work by Beveridge and his co-workers (1983) showed that after applying volatile chemicals to potatoes in an initial application that the headspace of volatile chemicals decreased over a period of time and the headspace concentration often fell beneath the minimum effective headspace for dimethylnaphthalene of 3.5mg m^{-3} below which sprout suppressant activity will progressively diminish with time as demonstrated by Beveridge (1979).

The aim of this experiment was to study the concentration of a volatile chemical under realistic conditions over a long period of time. It was decided that only one chemical should be studied due to the limitation of time and resources. Ethylnaphthalene was chosen since it has been shown by Stephen and Duncan (1984) to be active as a sprout suppressant and was shown earlier in this chapter to be volatile under a static system.

The experiment was based on a system used previously by Boyd (1984) to collect, separate and identify the volatiles present in the headspace surrounding the raw tubers. Potatoes were stored in an aluminium tank and air was swept from the bottom of the tank over the potatoes and trapped onto a porous polymer.

2.2.1 Method

The aluminium tank was washed and dried before being placed in a temperature controlled room at 10°C ± 0.5°C. A headspace sample was taken over seven days to measure any background interferences. The ethylnaphthalene/alumina mixture was then applied evenly over the bottom of the aluminium tank and headspace samples were taken over 124 days. The precolumns were attached to the tank for periods of time ranging from 4 to 15 days. Once the headspace sample had been taken the columns were removed and sealed at both ends with PTFE caps and stored in sealed bags in a fridge till analysis by thermal desorption onto a GC.

2.2.2 Experimental

Material

Chemical Application

Most of the sprout suppressant chemicals are applied to potatoes on a solid carrier. In this experiment ethylnaphthalene was applied to the bottom of the tank on alumina (alumina grade 0). The 5000mg formulation was prepared by dissolving 5g of ethylnaphthalene (Aldrich) in 250ml diethyl ether (Analar Grade) and mixed with 125g of alumina on a rotary evaporator for two hours to allow the solvent to evaporate off.

Aluminium Tank System

The aluminium tank used in the experiment was the same tank constructed for and used by Boyd (1984) to store potatoes. The tank was based on a system previously used for the collection of headspace volatiles from large samples of fruit and vegetables (Cole 1980; Ismail et al 1980; Heydanek and McGorrin 1981).

The tank is illustrated in Figure 2.7 (further details can be obtained from Boyd's thesis).

The tank was washed and dried before being attached to the air supply which was used to sweep the headspace volatiles from the tank onto the precolumn. The air supply was supplied by a pressurised air cylinder (BOC Glasgow Ltd) which was fitted with a pressure regulator (CS Milne Ltd., Glasgow). The air flow to the tank was controlled using a needle valve flow controller (Model 8744, Brooks Instrument Division).

Porous Polymer Pre-column

Porous polymer adsorbents have been widely used in environmental, food and medical research for the concentrating of trace organic compounds from dilute media (Zlatkis et al 1973; Bertuccidi and Montedora 1974; Micketts and Lindsay 1974; Pellizzari et al 1975; Williams et al 1978; Cole 1980; Ismail et al 1980; Simpson 1980).

The adsorbent Tenax GC (Phase Separation Ltd., Wales) which is a porous polymer based on 2,6-diphenyl-p-phenylene oxide, has been used to trap the volatiles produced by potatoes (Varns and Glynn 1979) and to study the headspace concentrations of commercially applied potato sprout suppressants in large scale potato stores (Filmer and Land 1978; Beveridge et al 1983; Boyd and Duncan 1986a; Boyd and Duncan 1986b).

Boyd (1984) considered Tenax GC to be the best available adsorbent for general use because of its wide range of applicability, its thermal stability (stable up to a temperature of 375°C) and its low affinity for water (Russell 1975).

Work carried out by Pellizzari et al (1976) demonstrated that Tenax GC can be used repeatedly with no decrease in trapping efficiency (even after recycling 15 times). They studied the effects of transportation and storage on samples trapped on the Tenax GC and showed that there was no loss of trapped volatiles up to a storage period of four months.

Precolumns

The Tenax GC is generally used to trap the headspace volatiles packed in a precolumn. The method used by Boyd (1984) was followed to prepare and pack the precolumns.

Eight Tenax GC adsorbent pre-columns were made from eight lengths of borosilicate glass tubing 100mm long and 5mm in diameter. These were soaked in concentrated HCI (Analar, Analar Standards Ltd., Poole, England) for 36 hours, then the tubing was washed thoroughly with deionised water followed by acetone (Analar) and then dried in a 220°C oven for two hours. Each length of tubing was then packed with 100mg of Tenax which was held in place with silanised wool.

Conditioning Pre-columns

The precolumns were conditioned to remove any adsorbent volatiles from the precolumns (the method used by Boyd was followed). The precolumns were heated under a flow of nitrogen at 30cm^3 min^{-1} at a temperature of 320°C for two hours. An aluminium block that had been built and used by Boyd (1984) was used to condition the pre-columns throughout the experiment. After the columns had been conditioned they were allowed to cool before being sealed with PTFE caps at both ends and stored in sealed polythene bags until ready for use.

2.2.3 Headspace Analysis

Thermal Desorption

All the results reported here were obtained by coupling the pre-column directly onto the top of an analytical column and thermally desorbing the trapped volatiles on the precolumn using a heating

block designed by Boyd and constructed from a block of aluminium which had a 7mm diameter hole. The block was heated with a 150W cartridge heater which had been inserted into the block and was maintained at a temperature of 240°C.

Analysis was carried out as follows:

The pre-column was connected to the packed column in the GC with a coupling. The desorption block was placed round the precolumn and the carrier gas was connected to the top of the precolumn. All these operations were carried out quickly, usually within 30 seconds, as during these operations the carrier gas flow to the column was interrupted. A cross sectional diagram of the pre-column, heating block and packed columns are shown in Figure 2.8. The heat flushed the volatiles off the precolumn and onto the packed column of the GC.

Ethylnaphthalene standards were made up using glass distilled hexane and injected onto an empty heated glass precolumn on the desorption injection unit. The areas of samples obtained were compared to the standard areas.

2.2.4 Gas Chromatography

Gas Chromatograph

All of the results reported were obtained using a PYE 104 gas chromatograph fitted with an FID.

Column

The column used throughout this experiment was a glass column packed with a Gas Chrom Q Support and coated with a 5% ovioi stationary phase (Phase Separations Ltd., England).

Temperatures

Column Oven	165°C
Detector	250°C
Injection Port (heating block)	240°C

Gas Flow Rates

Nitrogen (carrier gas)	30cm^3/min
Air (to FID)	100cm^3/min
Hydrogen (to FID)	30cm^3/min

Data Collection

Data collection, calibration and integration was achieved using a Spectra Physics SP 4290 Integrator (Burke Electronics Ltd., Glasgow).

2.2.5 Results

The results were expressed in graphical form. The graph was drawn from the best fit straight line determined by linear regression analysis of analytical data (Miller and Miller 1984). The best fit straight lines were determined for:

headspace concentration against time,
log headspace concentration against time, and
natural log headspace concentration against time.

In this case, the best fit straight line was obtained using headspace concentration against time (see Figure 2.9).

FIGURE 2.7 DIAGRAM OF ALUMINIUM TANK

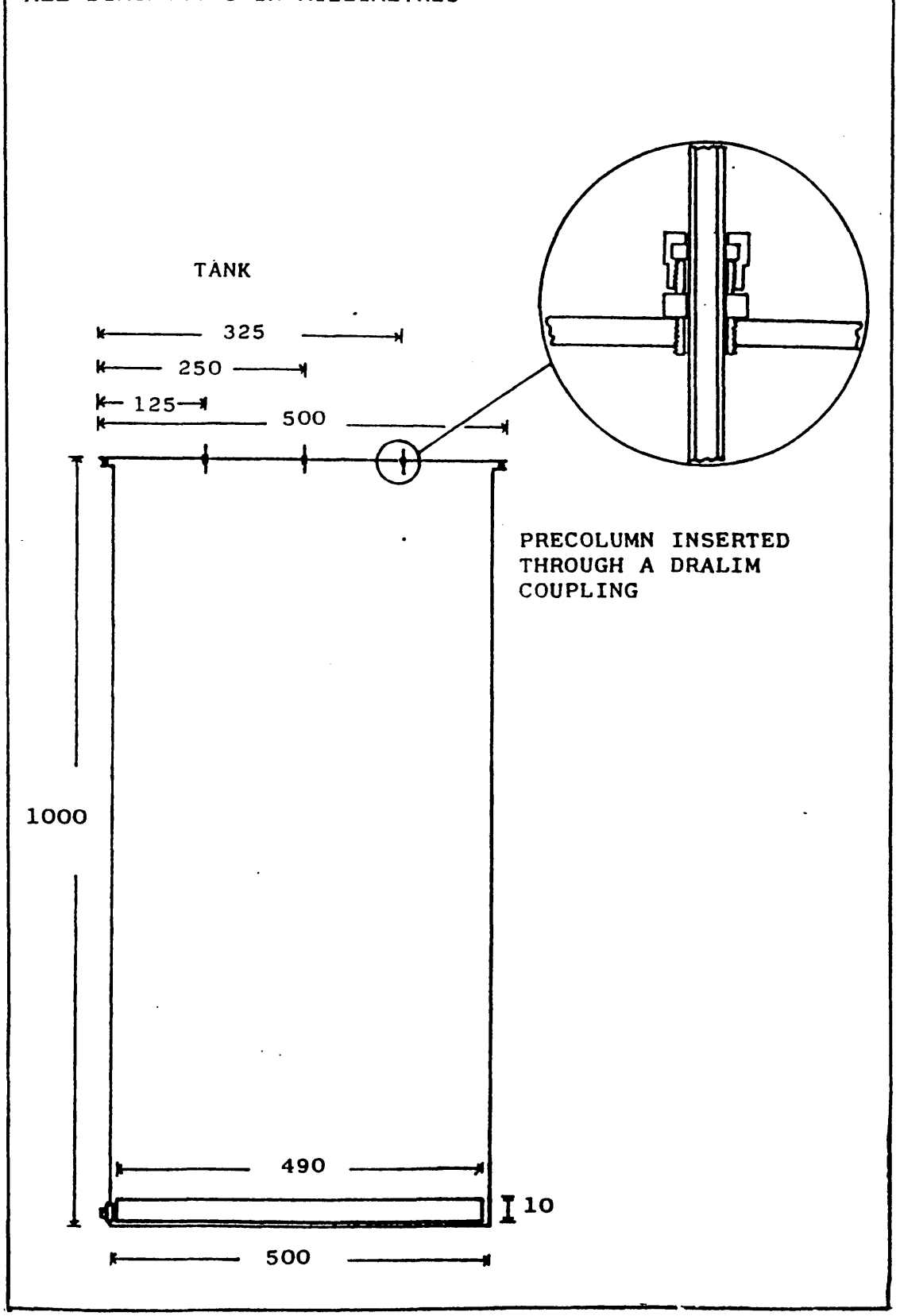


FIGURE 2.8 CROSS SECTION OF DESORPTION APPARATUS

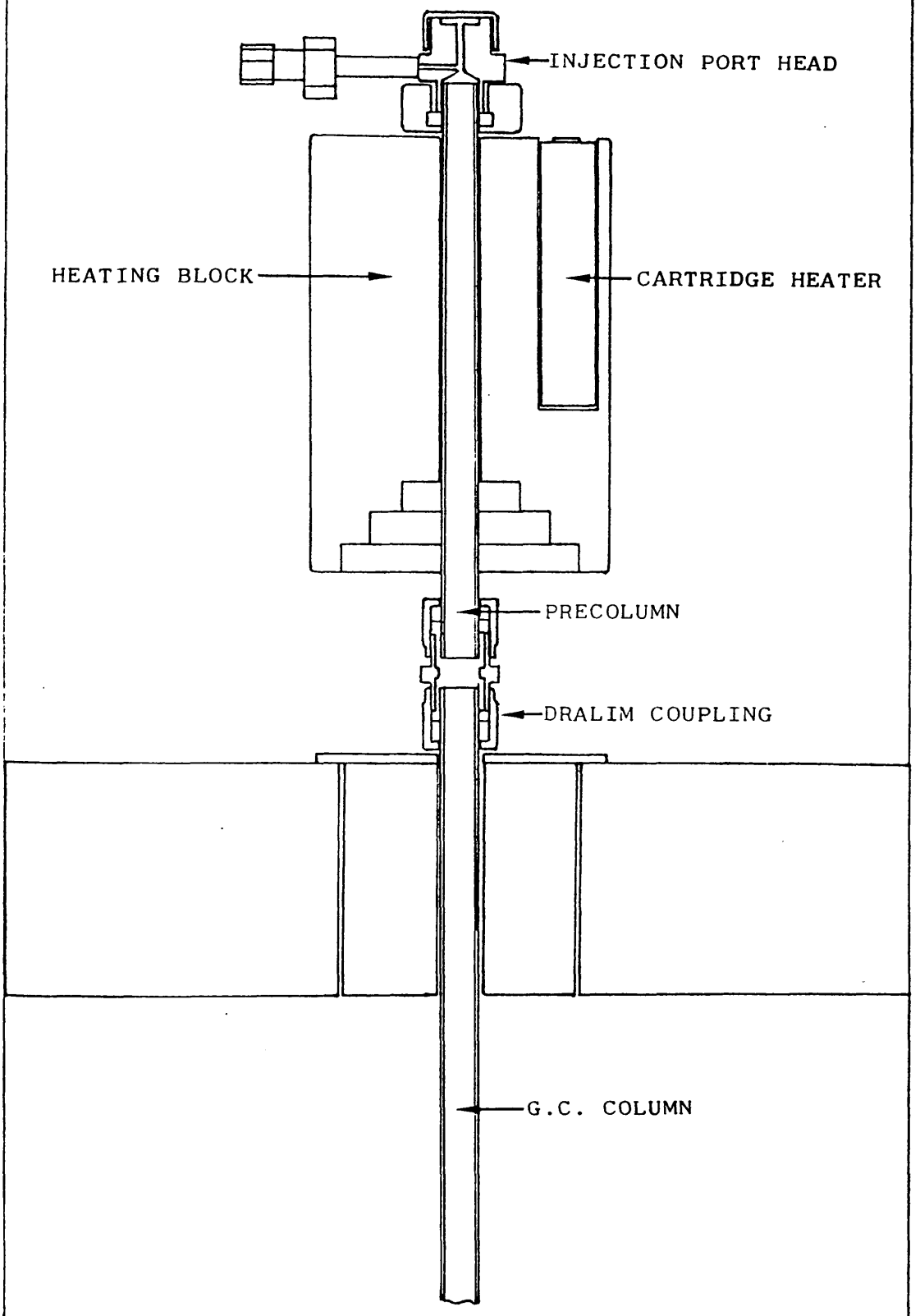
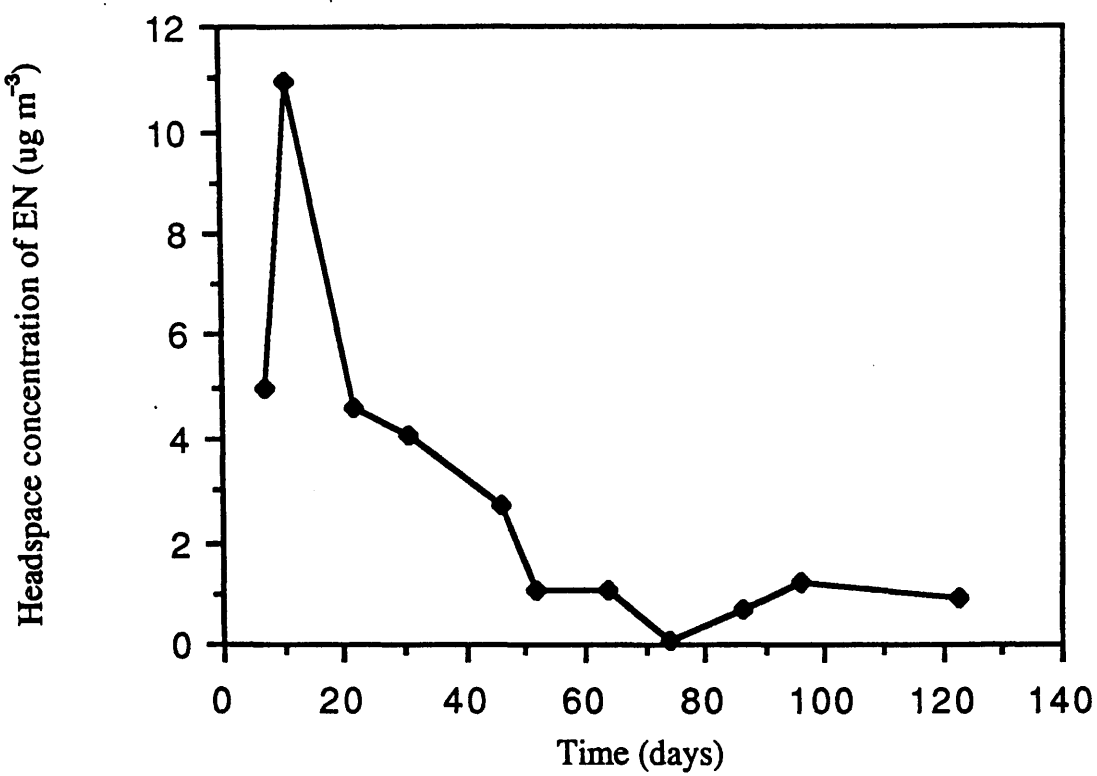


FIGURE 2.9 TENAX PRECOLUMNS WERE USED TO MEASURE THE HEADSPACE CONCENTRATION (μg m^{-3}) OF ETHYLNAPHTHALENE IN AN ALUMINIUM TANK, OVER 124 DAYS AT 10°C ± 1°C



2.3 Discussion

The experiment is unreplicated because of the size and complexity of the apparatus. More definite conclusions could have been drawn if the results could have been replicated.

Figure 2.9 can be described best by splitting it into two parts.

In part one (days 0 to 11) there was a sharp increase in the headspace concentration after the ethylnaphthalene was added to the tank. It continued to increase until day 11 when the highest headspace concentration was reached, after which the headspace started to decrease. This part of the graph can be explained by the relatively high volatility of the ethylnaphthalene. After it was added initially in a high concentration to the tank, a larger number of the ethylnaphthalene molecules volatilised rapidly which caused the headspace to increase at a fast rate.

The rate of volatilisation was higher than the rate of dissipation and so the headspace continued to increase until day 11 when the maximum amount of ethylnaphthalene molecules were present in the headspace and no more molecules were able to enter. This is known as the saturation point.

In the second part of the graph the headspace decreased after day 11 till day 52. The decrease is probably due to the loss of chemical from the tank. It is either lost by the gradual dissipation of the ethylnaphthalene from the tank to the surrounding air or by being trapped onto and removed by the Tenax precolumn. There is also a gradual decrease in chemical concentration at the bottom of the tank.

The headspace continued to decrease until day 52, by which time it had settled down to give a low steady headspace concentration until it started to decrease slightly from day 74 till day 86. It then increased till day 96 before settling down to give a steady headspace again.

After day 52, due to the loss of ethylnaphthalene from the tank which was described earlier, the concentration of ethylnaphthalene left in the tank was very low and the ethylnaphthalene that was left was held strongly onto the alumina. A small amount may have been absorbed onto the sides of the tank. This meant that the ethylnaphthalene left in the tank would have been released very slowly giving a low headspace concentration. At day 70 the air cylinder could not be obtained till day 86 due to a strike by the company that supplied the cylinders. Over the period of time that the cylinder was empty there was no air flowing over the ethylnaphthalene which was still being released and a headspace concentration built up, but was not carried to the Tenax precolumn due to no air flow being present.

Thus less chemical was trapped and the concentration decreased. It increased again when the full cylinder was attached on day 86, as the air flow was able to carry the ethylnaphthalene, which had built up in the headspace above the chemical at the bottom of the tank while the cylinder was empty, to the Tenax precolumn.

Another possible reason for the increase in headspace concentration between days 86 and 96 could be due to a slight increase in temperature in the temperature controlled room. This was caused by people working in the room and the door was opened allowing the temperature to increase and therefore increasing the volatilisation of the ethylnaphthalene which would increase the headspace concentration.

After day 96 the headspace decreased slightly but this may be due to the effect of the air flow and temperature settling down to the previous conditions.

The results from this experiment were in agreement with the results from work carried out by Beveridge et al (1983) who studied the headspace of volatile chemicals on potatoes in cardboard boxes. They found that the chemical built up a high headspace concentration initially and then decreased. The rate of decrease depended on the volatility of the compound and the reservoir present. If the headspace fell below the minimum effective headspace concentration then sprouting would occur.

2.4 Conclusion

This experiment allowed the headspace of volatile chemicals to be studied under realistic storage conditions. It enables the behaviour of the chemical to be predicted over a long period of time in a commercial potato store.

The results indicated that ethylnaphthalene is a volatile compound that would need to be re-applied several times throughout the storage season to maintain the minimum effective headspace concentration. Because of its high volatility it could be introduced easily as a vapour that could be distributed throughout the store via ventilation ducts.

The method could have been more accurate if the results had been replicated and more headspace samples taken over a regular time interval but, as mentioned earlier, time and resources were limited.

HEADSPACE STUDIES ON DIISOPROPYLNAPHTHALENE USING A MODIFIED STATIC HEADSPACE METHOD

3.1 Introduction

The need for alternative potato sprout suppressant chemicals was mentioned earlier, in Chapter One as was the possibility of several substituted naphthalenes having the potential to control potato sprout growth. However due to patent problems the synthesis of these chemicals for the purpose has proved difficult.

This led to a study of other substituted naphthalenes including diisopropylnaphthalene (the 2,6 isomer) which was demonstrated by Mike Everest-Todd (personal communication) and Stephen and Duncan (1984) to give good control of sprouting over a 17 week period. More work is needed to be carried out, especially on a large scale before this could be seriously considered for commercial use (see Figure 1.4).

Diisopropylnaphthalene has been submitted to toxicological tests, as it is used presently in large amounts as a solvent in the carbon paper industry (in Japan). It was found not to accumulate in mice during metabolic and distribution studies and can be released rapidly from organisms (Iwahara 1974; Hasegawa 1982). These results were repeated in similar tests carried out on fish (Yashida and Kojima 1978a). Diisopropylnaphthalene was found to be broken down easily by micro-organisms. A possible microbial oxidation pathway has been proposed by Yashida and Kojima (1978b) which suggests that there is no threat of environmental pollution.

It has been classified as a non-irritant and there is no evidence of carcinogenic or teratogenic effects (Anon 1979).

Large scale studies including field trials and treatments of potatoes in commercial potato stores are more needed on diisopropylnaphthalene. However it does seem to have potential as a commercial potato sprout suppressant. The reasons behind this opinion are as follows:

1. It has been shown in preliminary work to give good control of potato sprout growth over a reasonable length of time.

2. Toxicological tests have shown it to have a low toxicity to humans and animals. It also creates no threat to the environment.

3. It is available in large amounts at a relatively low cost.

The objective of the experimental work which will now be described was to study the volatility of diisopropylnaphthalene using a slightly modified static headspace method to that used previously in Chapter Two. The modified method will be compared to the previous established method.

3.2 Materials and Method

In this Chapter modifications were introduced to try and improve the existing static headspace analysis method and use it to study the volatility of diisopropylnaphthalene.

In the modified method the apparatus, conditions and analysis by GC are the same as those used previously.

However, at time of analysis, instead of taking the headspace sample directly by inserting the gas tight syringe into the septum, a disposable 5cm Yale Microlance needle (Becton Dickinson, UK Ltd., England) was inserted into the septum in advance which acted as a needle guide to the gas tight syringe (see Plate 3.1).

It was thought this would give more consistent results for the following reasons:

1. The needle guide prevents contamination of the syringe needle with particles from the septum. These particles from the septum may absorb some of the chemical giving unreliable results or may just have a distorting effect on the resultant chromatograms.

2. The sample is taken from the same point in the flask on each occasion keeping conditions as constant as possible each time.

3.2.1 Experimental

Experiment One

The initial experimental work was carried out mainly by a final year undergraduate student (Robin Oliver) who worked under my close supervision throughout.

The aim of this experiment was to compare the volatilities of different concentrations of 1,4 dimethylnaphthalene (Aldrich Chemical Co) and 2,4 diisopropylnaphthalene (Aldrich Chemical Co) which was used in this preliminary work, due to the unavailability of the 2,6 isomer at that time. Both volatilities were studied under a static headspace system using disposable syringes as needle guides.

Triplicate flasks were set up for each chemical and concentration which involved adding 1000mg, 500mg and 100mg of chemical to the bottom of a series of flasks. Again the flasks were allowed to stand for 24 hours which was thought to be long enough for the system to reach equilibrium, and maintained at 31° $\pm$ 1°C.

Experiment Two

The aim of this experiment was to compare the old and new analysis methods of static headspace analysis, over two separate time intervals under similar experimental conditions.

1,4 dimethylnaphthalene was used as a reference compound. It was used because of its high volatility and because of previous work carried out with this chemical, studying its headspace concentration under static systems.

The experiment was set up using two sets of flasks, each set contained six flasks which each had 500mg of 1,4 dimethyl-naphthalene added to the bottom.

This amount of chemical (500mg) was chosen as it gave as high a headspace concentration as 1000mg. Since a lot of flasks were being set up it was therefore more economical to use half the amount. Using lower amounts (100mg) tended to increase the headspace variation slightly (See Table 2.7). On each occasion the flasks were then sealed and maintained at 31°C $\pm$ 1°C. The first set of flasks were analysed after three days and the second set after seven days.

On the day of analysis each set of flasks had headspace samples taken from three flasks. In the one case, the samples were taken by inserting the gas tight syringe through the needle guide which remained inserted into the septum. Numerous headspace samples can be taken with the gas tight syringe when this method is adopted. Samples were taken from the remaining three flasks by inserting the gas tight syringe directly through the septum. After, a maximum of five samples from each flask, reproducibility was lost.

Experiment Three

The aim of these experiments was to compare the old and new analysis methods using 2,6 diisopropylnaphthalene (supplied by Willowbank Research) and to study the volatility of 2,6 diisopropylnaphthalene using different concentrations of chemical left to equilibrate over different time intervals.

A) This experiment was set up to compare the new and old analysis methods using 2,6 diisopropylnaphthalene. In the preliminary experiments described in Experiment One (Section 3.2.1) the diisopropylnaphthalene was shown to be much less volatile than 1,4 dimethylnaphthalene. To make sure that a detectable amount of diisopropylnaphthalene appeared on the chromatograph it was decided to add 1000mg of diisopropylnaphthalene to the bottom of the six flasks. These flasks were then sealed and maintained at 31°C $\pm$ 1°C for three days.

On analysis day, triplicate headspace samples were taken from each of the three flasks by inserting the gas tight syringe through the needle guide as mentioned earlier and from the other three flasks by inserting the gas tight syringe directly through the septum.

B) This experiment was set up to observe the effect that time and concentration had on the headspace concentration of 2,6 diisopropylnaphthalene.

Two sets of six flasks were set up, each set was prepared by adding 200mg of diisopropylnaphthalene to three flasks and 1000mg to the remaining three flasks. The flasks were maintained at 31°C $\pm$ 1°C and the first set of flasks were removed after two days for analysis and the second set after seven days. The flasks were all analysed using the needle guide.

3.3 Results

Experiment One

Table 3.1 The concentration (ng cm^{-3}) of 1,4 dimethylnaphthalene and 2,4 diisopropylnaphthalene in air (static system) after 24 hours at 31°C ± 1°C.

Chemical	Concentration Added (mg)		
	1000	500	100
Dimethylnaphthalene	103.5±2.13	102.4±16.6	117.1±19.2
Diisopropyl-naphthalene	2.03±0.29	2.48±0.68	2.65±1.18

mean and standard deviation from three flasks. Each flask had triplicate headspace samples taken.

Experiment Two

Table 3.2 The concentration (ng cm^{-3}) of 500mg 1,4 dimethylnaphthalene in air (static system) after three and seven days at 31°C ± 1°C.

Time (days)	Without needle guide	With needle guide
3	9.96±1.44	14.50±1.73
	14.50±3.66	13.10±3.50
	18.81±4.40	9.18±2.50
7	49.84± 7.25	72.60± 8.72
	72.57±18.26	65.60±17.34
	93.80±22.00	45.84±12.62

mean ± standard deviation from triplicate headspace samples taken from each flask.

Experiment Three

Table 3.3 The concentration (ng cm^{-3}) of 1000mg 1,4 diisopropyl-naphthalene in air (static system) after three days at 31°C ± 1°C.

Amount of chemical added (mg)	Without needle guide	With needle guide
1000	2.40±0.32	1.80±0.34
	1.71±0.59	2.44±0.60
	2.02±0.83	1.83±0.47

mean ± standard deviation of triplicate headspace samples.

Table 3.4 The concentration (ng cm^{-3}) of diisopropylnaphthalene in air (static system) after two days at 31°C ± 1°C.

Chemical	Concentration Added (mg)	
	200	1000
	2.11±0.69	2.75±0.61
Diisopropylnaphthalene	2.10±0.49	1.51±0.03
	1.58±0.48	2.03±0.59

mean ± standard deviation of triplicate headspace samples.

Table 3.5 The concentration (ng cm^{-3}) of diisopropylnaphthalene in air (static system) after seven days at 31°C ± 1°C.

Chemical	Concentration Added (mg)	
	200	1000
	30.61±27.55	14.75±8.98
Diisopropylnaphthalene	12.60± 8.20	21.11±5.98
	17.80±11.31	16.35±9.51

mean ± standard deviation of triplicate headspace samples.

FIGURE 3.1 GAS CHROMATOGRAM OF A DIISOPROPYLNAPHTHALENE STANDARD

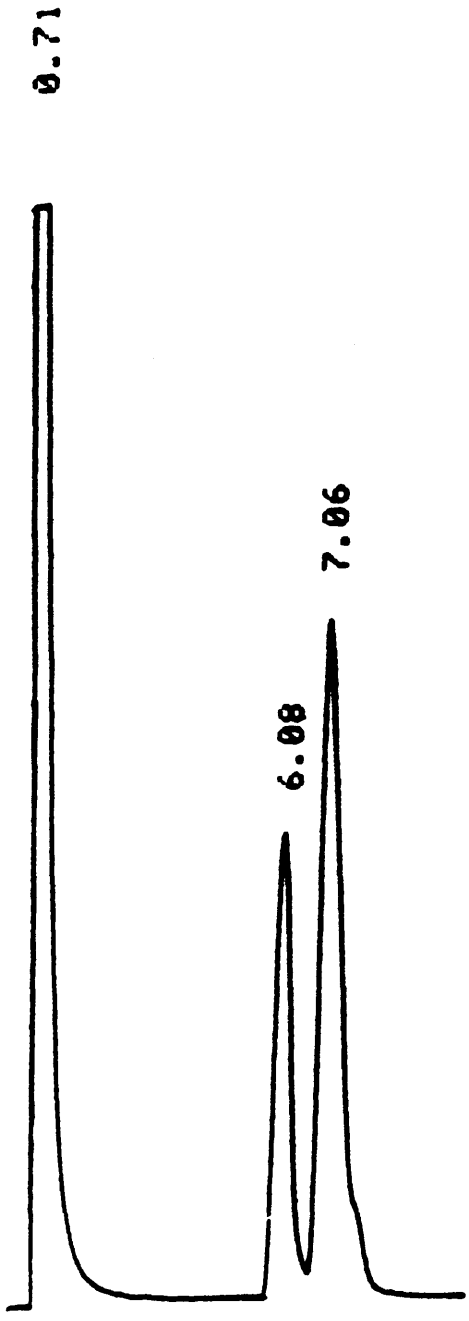


PLATE 3.1 HEADSPACE FLASKS WITH AND WITHOUT A NEEDLE GUIDE

3.4 Discussion

Experiment One

Results are shown in Table 3.1. The results show that 1,4 dimethylnaphthalene is roughly fifty times more volatile than 2,4 diisopropylnaphthalene at the three concentration of chemical added.

In Chapter Two the results from experimental work showed that there was a high correlation between a high headspace and good sprout activity for ethylnaphthalene and dimethylnaphthalene. However, the low headspace of 2,4 diisopropylnaphthalene does not necessarily mean that 2,4 diisopropylnaphthalene would give poor sprout control. Stephen and Duncan (1984) demonstrated that the 2,6 isomer of diisopropylnaphthalene gave good control of sprout growth over a seventeen week period. The low volatility of diisopropylnaphthalene could account for it being an active sprout suppressant as less compound is lost to the atmosphere and more chemical is left at the site of action. Although a volatile chemical as mentioned earlier has the advantage of building up a high headspace concentration, if the chemical is too volatile eg pulegone (see Figure 1.2) then the headspace concentration will fall rapidly below the effective headspace concentration for sprout suppression.

Experiment Two

Results are presented in Table 3.2. The results show that the headspace levels, under similar experimental conditions, are significantly higher ($p < 0.01$) using the old method of analysis rather than the modified method, after seven days. However, there was no significant difference found after three days. There is slightly less variation between similar headspace samples using the modified method after both three and seven days.

The reason for the modified method giving a lower headspace concentration after seven days is possibly due to the chemical condensing on the syringe of the needle guide. This will interfere with the equilibration system within the flask and reduce the amount of chemical in the vapour phase thus making it less saturated and reducing the concentration of the chemical in the headspace.

The results showed that after seven days the headspace concentration was significantly higher ($p < 0.01$) than after three days which would suggest that equilibration had not yet been reached between the 1,4 dimethylnaphthalene and the flask after three days.

Although the degree of variation within and between flasks was reduced slightly using the modified method, it was still present due to:

1) Difficulty of obtaining reproducible headspace samples from the flasks using a gas tight syringe and the technique used to inject the headspace sample into the GC for analysis. Human error and the accuracy and reproducibility of the gas tight syringe (99%) led to a variation between headspace samples from the same flask and give a low correlation between samples from different flasks.

2) Accuracy in adding the exact and the equivalent amounts of chemical to the bottom of each of the flasks. Human error and the accuracy of the graduated pipette are responsible for inaccuracies that occur as the graduated pipette was used to add the chemical to the flask in most cases.

3) Temperature fluctuations still occur between and within flasks. A better system is needed to regulate the temperature.

Experiment Three

Results are presented in Tables 3.3, 3.4 and 3.5.

The results shown in Table 3.3 show that the headspace levels under similar experimental conditions were not significantly different. This was probably due to the low volatility of diisopropylnaphthalene. The small scale of experiment meant the headspace concentration was susceptible to any small changes in conditions.

The results in Table 3.4 showed that the headspace after 2 days is slightly higher for 1000mg diisopropylnaphthalene than 200mg. However, in this experiment as mentioned above, the headspace concentrations were very low and the differences between results were not significant. However, the results presented in Table 3.5 showed that the headspace concentration has built up to give a significantly higher ($p < 0.01$) headspace after seven days than two days (Table 3.4). Because of the low volatility of the diisopropylnaphthalene it took a long time for the diisopropylnaphthalene to come to equilibrium with the system.

3.5 CONCLUSION

In conclusion, the modification to the existing small scale headspace analysis did not increase the sensitivity of the method. Although the method was good for testing the volatility of volatile compounds, it was not very sensitive at measuring the headspace concentration of non volatile compounds which gave a low headspace concentration. Alternative methods to increase the sensitivity of the method are:

1) A larger headspace sample volume should be taken from the flasks. However this would cause a dilution effect and would generally result in a loss of chromatographic efficiency.

2) Increase the length of time allowed for the flasks and chemical to come to equilibrium, but this would take a long time for a low volatile compound and one of the advantages of this method is its speed.

3) Increase the temperature of the waterbath which would increase the saturated vapour pressure of the chemical and hence its concentration in the vapour phase.

This experiment was designed to test the volatility of chemicals. However, it cannot predict the volatility of the chemical in storage and in contact with potatoes which contain lipids, proteins, starches, cellulose and other polymers which can all interact with the volatile chemical and influence the vapour phase concentration. This was also a problem for food chemists trying to measure the concentration of volatile compound present in food.

In the future, work could be done to improve the sensitivity of the existing static headspace method which would allow a wider range of chemicals to be studied, under more controlled conditions.

It would also be useful to devise an experimental method that would allow routine headspace analysis of chemicals in situ with potatoes under laboratory conditions.

THE EFFECT OF SUBSTITUTED NAPHTHALENES ON THE WOUND HEALING CAPACITY OF TUBERS

4.1 Introduction

Potato tubers are surrounded by a protective skin, known as a periderm, which acts as a barrier against water loss and the entry of fungi and bacteria. A large proportion of the potato crop is damaged during and after the harvest period by mechanical injury when loading and unloading the tubers into the store. Damage can lead to serious losses due to desiccation and rotting of the tubers caused by bacterial and fungal pathogens. However, the damage can be reduced by good management and a mechanical harvester that will remove stones.

Wounded potatoes are stimulated and undergo numerous complex biochemical processes that lead to a new periderm. The nature of the stimulus is not known, but it initiates a rapid increase in the rate of nucleic acid and protein synthesis in the cells beneath the injured surface (Borchert and McChesney 1973; Sadava and Crispeels 1978; Sato et al 1979). This is accompanied by a breakdown of membrane followed by a deposit of suberin into the cell walls (Artschwager 1927). Suberin is a complex polymer which is thought to consist of phenolic and aliphatic compounds (Cottle and Kolattukudy 1982). It is thought to play an important role in water diffusion resistance (Kolattukudy and Dean 1974; Soliday et al 1979). After the initial 2-3 cell layers have been suberized the cells immediately beneath start to divide to form a periderm which in turn is suberized to produce the equivalent of a new potato skin.

Immediately after harvest, the process of wound healing must be allowed to take place if satisfactory storage of the tubers is to take place.

This is known as the 'curing period' which normally lasts for 2-3 weeks in commercial stores. Wounds on potatoes heal faster under high temperature (15°C) and high humidity (90% RH) conditions (Anon 1985).

The rate at which the wounds heal depends on a variety of factors.

1. **Temperature**

As for most biological processes, cell division is temperature sensitive (Lipetz 1970) hence temperature plays an important role in the wound healing process. The optimum temperature for wound healing is reported at between 20°C and 25°C (Ali et al 1975; Walker and Wade 1978; Thomas 1982; Dean 1989).

2. **Relative Humidity**

High relative humidity (78%) is generally considered to be the optimum for wound healing (Artschwager 1927) although very high relative humidities may result in proliferation within the cells at the wound surface expanding (Lange et al 1970). If the humidity is low then the cells at the surface will dry out and crack (Werner 1938).

3. **Cultivar**

Rate of wound healing has been noted to vary with cultivar and with the thickness of the periderm (McGee et al 1985b).

4. Sprout Suppressant Chemicals

It has been shown that the sprout suppressant chemical chlorpropham does inhibit wound healing (Audia et al 1962; Reeve et al 1963; McGee 1984; Leonard et al 1987). Tecnazene, on the other hand, does not affect wound healing (McGee 1984; Leonard et al 1987). Dimethylnaphthalene has also been shown to retard wound healing (McGee 1984; Hartmans and van Es 1986).

Methods of Studying Wound Healing in Potatoes

In the past, the most common method of studying the wound healing process was by examining sections of tuber under a microscope (Nielsen 1968). However, this involved a lot of preparative work in sectioning and staining the potato tissue and the necessary degree of replication was not often achieved.

In recent years, the rate of wound healing has been measured by estimating the rate of water loss from the wounded potato. Kolattukudy and Dean (1974) showed that the permeability of cut potato surfaces decreased as suberin was laid down during wound healing. This estimate was calculated by exposing discs of potato tissue to air and measuring the loss in weight after the discs had partially been dried out. Similar work has been carried out on the transpiration from leaf surfaces (Monteith 1965; Weatherley 1965; Grace 1975) which also involve the movement of water through plant tissue (cuticle). In these studies most workers divided the resistance to water loss into two types.

1. External Resistance

External resistance to water loss which is the rate at which water vapour is removed from the tuber surface and is dependent on the environmental factors such as temperature, relative humidity and the rate of air circulation.

2. Internal Resistance

Internal resistance to water loss is influenced by the free diffusion of water to the tuber surface and is controlled by the development of a suberized periderm.

The method for assessment of wound healing which was employed in this work and will be presented later in this chapter was developed by Jarvis and Duncan (1979). It involved measuring the rate of water loss from small potato discs, the analysis time was reduced by exposing the discs to a stream of air which minimised the external resistance and allowed factors which affected the development of internal resistance to water loss ie wound healing to be studied.

The objective of this chapter was to study the effects that ethyl-naphthalene and diisopropylnaphthalene have on the wound healing process using a slightly modified method to that used by McGee et al (1985a). These chemicals were chosen for their sprout suppressant activity shown by Stephen and Duncan (1984) and their potential as commercial sprout suppressants. It was decided not to study dimethylnaphthalene as McGee (1984) had already shown it to have an inhibiting effect on potato discs.

4.2 Materials and Methods

The set of experiments were carried out over a twelve month period, thus the potatoes were usually obtained in batches shortly before use. It also meant that various cultivars (Maris Piper, Desiree) of different maturities were being used although all the potatoes were dormant with no visible sprouts at time of inspection. As only one experiment could be set up at a time, the potatoes were bought in as needed. This caused a limitation to the comparisons that could be made between experiments.

Medium sized tubers were washed and left overnight at laboratory temperatures. The tubers were then surface sterilised by flaming three times with ethanol. Discs of tuber tissue 11mm in diameter and 4mm deep were prepared under asceptic conditions by taking tuber cores 11mm in diameter with a No 7 cork borer. The cores were then sliced into equivalent sized discs using a hand made device consisting of 10 metal blades bolted together at 4mm intervals.

The potato discs were then transferred to the lids of previously prepared petri-dishes. Six discs were allowed per dish. The bases of the dishes contained a thin layer of water agar. The dishes were then inverted. The purpose of water agar was to maintain 100% relative humidity which encouraged wound healing. The agar was prepared by autoclaving a 1.2% w/v solution using deionised water of Agar Technical (Oxoid Agar No 3) for 2 hours. The agar solution was allowed to cool slightly and then poured onto the base of each petri-dish.

A method of application was chosen that would allow a known amount of chemical to be brought into direct contact with a wound surface.

This was done by pipetting 25µl of distilled water onto the disc surface and then injecting 5µl of the chemical solution which was made up in methanol (Analar) onto the water droplet. Methanol was used as it did not seem to affect the potato at the concentration used.

In the first two sets of experiments, three levels of both diisopropyl-naphthalene and ethylnaphthalene were investigated : 10µg, 50µg and 100µg of chemical applied per disc. In the third set of experiments two levels of chlorpropham were investigated : 10µg and 100µg of chlorpropham per disc. Two levels were used in this case as chlorpropham was included as a standard. Chlorpropham has already been shown to have an inhibiting effect on potato discs (McGee 1984; Leonard et al 1986).

Four replicate dishes were prepared for each treatment, on each of the analysis days at intervals between 0 and 21 and then stored in cardboard boxes in the laboratory at a temperature of 22°C $\pm$ 2°C until they were analysed.

On each analysis day, the resistance to water loss was calculated by measuring the weight loss of the discs, when placed under an air stream for a certain period of time. The method was similar to that described by Jarvis and Duncan (1979).

The discs were transferred to a previously weighed, dry, clean petri-dish lid. These were weighed again before being exposed to a ninety second airstream obtained from an industrial hair dryer which was clamped 30cm above the petri-dish.

The dish and discs were weighed after ninety seconds and again a further three times, each time after a 20 second period under the airstream. By using the airstream the external resistance to water loss was reduced allowing the development of internal resistance to water loss to be studied over a short period of time.

During the initial ninety second exposure to the airstream the rate of water loss is non-linear (Jarvis and Duncan 1979) and weight loss between this period is discounted, but the weight differences between the twenty second exposures to the airstream were recorded and gave three replicate values for the rate of water loss.

The dishes were stored in the same room where the analysis was to take place. The airstream temperature and relative humidity were recorded at the start and changes were noted throughout the analysis. On each analysis day, water loss was recorded from freshly prepared material which had no treatment. This was so that compensations for changes in humidity and temperature could be made, as well as providing a measure for external resistance to water loss.

The results obtained were incorporated into the following equations allowing the internal resistance of the tissue to water loss to be calculated. This was carried out by a computer programme which was written to handle the large number of data generated.

$$r_{ext} = \frac{d(1-r.h.)}{E_o}$$

$$r_{int} = \frac{d(1-r.h.)}{E-r_{ext}}$$

r_{ext} - total external resistance to water loss

r_{int} - total internal resistance to water loss

E - rate of water loss per unit area of aged discs

E_o - rate of water loss per unit area of fresh discs

d - saturation vapour density of water vapour in the air at the air-stream temperature

r.h. - relative humidity

The equation assumes that the external resistance to water loss is constant but as the tuber discs age the external resistance to water decreases and the internal resistance increases. In order to overcome this problem, total resistance to water loss will be reported.

The internal resistance to water loss in aged discs can be considered a direct comparison of the tissue's ability to wound heal. The higher the internal resistance in the disc to water loss, then the further the wound healing process has progressed.

Three experiments were set up using this method some of which were carried out twice.

4.3 Results

The results are shown in Tables 4.1, 4.2 and Figures 4.1, 4.2 and display the values obtained for total resistance to water loss in duplicate sets of discs treated with 0µg, 10µg, 50µg and 100µg of ethylnaphthalene over a period of twenty-one days.

The results are shown as the means of duplicate dishes in Tables 4.3, 4.4 and Figures 4.3, 4.4 and display the values obtained for total resistance to water loss in duplicate sets of treated discs with 0µg, 10µg, 50µg and 100µg of diisopropylnaphthalene over a period of twenty-one days. The data was also calculated as the internal resistance which was similar to the total resistance. As both results gave the same statistical results, to be consistent the data will be reported as the total resistance. The treatment level means were compared with the untreated control on two separate days and significant differences were tested by using an F test on the analysis of variance. The least significant difference between the means of the treatments and control were calculated by Fischer's Least Significant Difference method for multiple comparisons, calculated as:

$$LSD = t_{o.os,\ a(n-1)} \times \sqrt{\frac{2\ mse}{n}}$$

t has a(n-1) degrees of freedom

a = number of treatments

n = number of replicates

mse = mean square of error

The results shown in Table 4.5 and Figure 4.5 display the results obtained for total resistance to water loss in a set of discs treated with 0µg, 10µg and 100µg of chlorpropham over a period of twenty-one days.

TABLE 4.1

ETHYLNAPHTHALENE TREATED MARIS PIPER DISCS - SET ONE

Days After Treatment	Total Resistance to Water Loss (mg^{-1} cm^{-2} 5)				
	Control	0µg $disc^{-1}$	10µg $disc^{-1}$	50µg $disc^{-1}$	100µg $disc^{-1}$
0	0.24 ± 0.03	0.21 ± 0.01	0.20 ± 0.04	0.21 ± 0.03	0.21 ± 0.03
4	0.26 ± 0.03	0.23 ± 0.02	0.25 ± 0.04	0.22 ± 0.04	0.23 ± 0.05
6	0.46 ± 0.06	0.32 ± 0.08	0.36 ± 0.10	0.27 ± 0.07	0.49 ± 0.10
8	0.33 ± 0.06	0.26 ± 0.06	0.26 ± 0.09	0.27 ± 0.04	0.28 ± 0.05
12	0.30 ± 0.06	0.26 ± 0.04	0.31 ± 0.07	0.31 ± 0.08	0.34 ± 0.11
14	0.37 ± 0.05	0.31 ± 0.05	0.30 ± 0.04	0.42 ± 0.10	0.38 ± 0.06
19	0.53 ± 0.11	0.27 ± 0.01	0.30 ± 0.03	0.38 ± 0.03	0.50 ± 0.02
21	0.65 ± 0.09	0.23 ± 0.03	0.25 ± 0.06	0.34 ± 0.04	0.41 ± 0.05

mean ± standard deviation

TABLE 4.2

ETHYLNAPHTHALENE TREATED DESIREE DISCS - SET TWO

Days After Treatment	Total Resistance to Water Loss (mg^{-1} cm^{-2} 5)				
	Control	0µg $disc^{-1}$	10µg $disc^{-1}$	50µg $disc^{-1}$	100µg $disc^{-1}$
0	0.22 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.19 ± 0.04	0.20 ± 0.02
3	0.21 ± 0.03	0.22 ± 0.05	0.19 ± 0.04	0.23 ± 0.05	0.26 ± 0.03
7	0.47 ± 0.06	0.47 ± 0.07	0.39 ± 0.07	0.49 ± 0.10	0.37 ± 0.09
10	0.47 ± 0.09	0.31 ± 0.06	0.45 ± 0.01	0.41 ± 0.10	0.37 ± 0.08
14	0.48 ± 0.07	0.40 ± 0.06	0.49 ± 0.13	0.65 ± 0.05	0.49 ± 0.08
17	0.44 ± 0.07	0.33 ± 0.09	0.45 ± 0.10	0.54 ± 0.10	0.21 ± 0.06
21	0.43 ± 0.08	0.37 ± 0.08	0.31 ± 0.05	0.35 ± 0.03	0.41 ± 0.06

mean ± standard deviation

TABLE 4.3

DIISOPROPYLNAPHTHALENE TREATED MARIS PIPER DISCS - SET ONE

Days After Treatment	Total Resistance to Water Loss (mg^{-1} cm^{-2} 5)				
	Control	0µg $disc^{-1}$	10µg $disc^{-1}$	50µg $disc^{-1}$	100µg $disc^{-1}$
0	0.15 ± 0.02	0.15 ± 0.03	0.15 ± 0.02	0.16 ± 0.02	0.12 ± 0.01
3	0.18 ± 0.02	0.12 ± 0.01	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.05
6	0.24 ± 0.04	0.16 ± 0.03	0.20 ± 0.04	0.18 ± 0.03	0.21 ± 0.04
9	0.42 ± 0.08	0.26 ± 0.06	0.21 ± 0.03	0.24 ± 0.04	0.24 ± 0.05
13	0.66 ± 0.08	0.48 ± 0.11	0.32 ± 0.05	0.38 ± 0.08	0.33 ± 0.05
15	0.66 ± 0.12	0.36 ± 0.05	0.35 ± 0.01	0.38 ± 0.09	0.29 ± 0.04
18	0.75 ± 0.14	0.45 ± 0.12	0.40 ± 0.06	0.32 ± 0.06	0.29 ± 0.04
21	0.41 ± 0.06	0.26 ± 0.03	0.23 ± 0.03	0.25 ± 0.05	0.36 ± 0.05

mean ± standard deviation
Day 9 LSD = 0.105 at $p<0.05$
Day 15 LSD = 0.12 at $p<0.05$

TABLE 4.4

DIISOPROPYLNAPHTHALENE TREATED DESIREE DISCS - SET TWO

Days After Treatment	Total Resistance to Water Loss (mg^{-1} cm^{-2} 5)				
	Control	0µg $disc^{-1}$	10µg $disc^{-1}$	50µg $disc^{-1}$	100µg $disc^{-1}$
0	0.24 ± 0.03	0.26 ± 0.04	0.25 ± 0.03	0.23 ± 0.05	0.21 ± 0.01
3	0.27 ± 0.04	0.26 ± 0.05	0.27 ± 0.12	0.28 ± 0.03	0.27 ± 0.04
6	0.37 ± 0.05	0.34 ± 0.03	0.30 ± 0.05	0.35 ± 0.09	0.33 ± 0.06
10	0.47 ± 0.05	0.35 ± 0.08	0.33 ± 0.05	0.33 ± 0.06	0.50 ± 0.11
13	0.47 ± 0.08	0.35 ± 0.07	0.32 ± 0.06	0.35 ± 0.01	0.43 ± 0.08
17	0.78 ± 0.13	0.41 ± 0.06	0.36 ± 0.05	0.27 ± 0.05	0.36 ± 0.02
21	0.58 ± 0.13	0.37 ± 0.08	0.26 ± 0.04	0.30 ± 0.03	0.45 ± 0.07

mean ± standard deviation
Day 10 LSD = 0.085 at $p<0.05$
Day 17 LSD = 0.097 at $p<0.05$

TABLE 4.5

CHLORPROPHAM TREATED DESIREE DISCS

Days After Treatment	Total Resistance to Water Loss (mg^{-1} cm^{-2} 5)			
	Control	0µg $disc^{-1}$	10µg $disc^{-1}$	100µg $disc^{-1}$
0	0.22 ± 0.03	0.21 ± 0.01	0.20 ± 0.02	0.24 ± 0.03
3	0.27 ± 0.03	0.22 ± 0.01	0.24 ± 0.02	0.24 ± 0.02
6	0.41 ± 0.13	0.34 ± 0.06	0.28 ± 0.03	0.46 ± 0.12
10	0.51 ± 0.07	0.36 ± 0.14	0.47 ± 0.11	0.70 ± 0.12
13	0.71 ± 0.10	0.43 ± 0.08	0.76 ± 0.13	0.56 ± 0.09
17	0.44 ± 0.05	0.45 ± 0.06	0.55 ± 0.13	0.62 ± 0.12
20	0.81 ± 0.14	0.54 ± 0.09	0.37 ± 0.07	0.76 ± 0.13

mean ± standard deviation

FIGURE 4.1 THE EFFECT OF ETHYLNAPHTHALENE ON THE DEVELOPMENT OF RESISTANCE TO WATER LOSS IN AGED TUBER DISCS - SET ONE

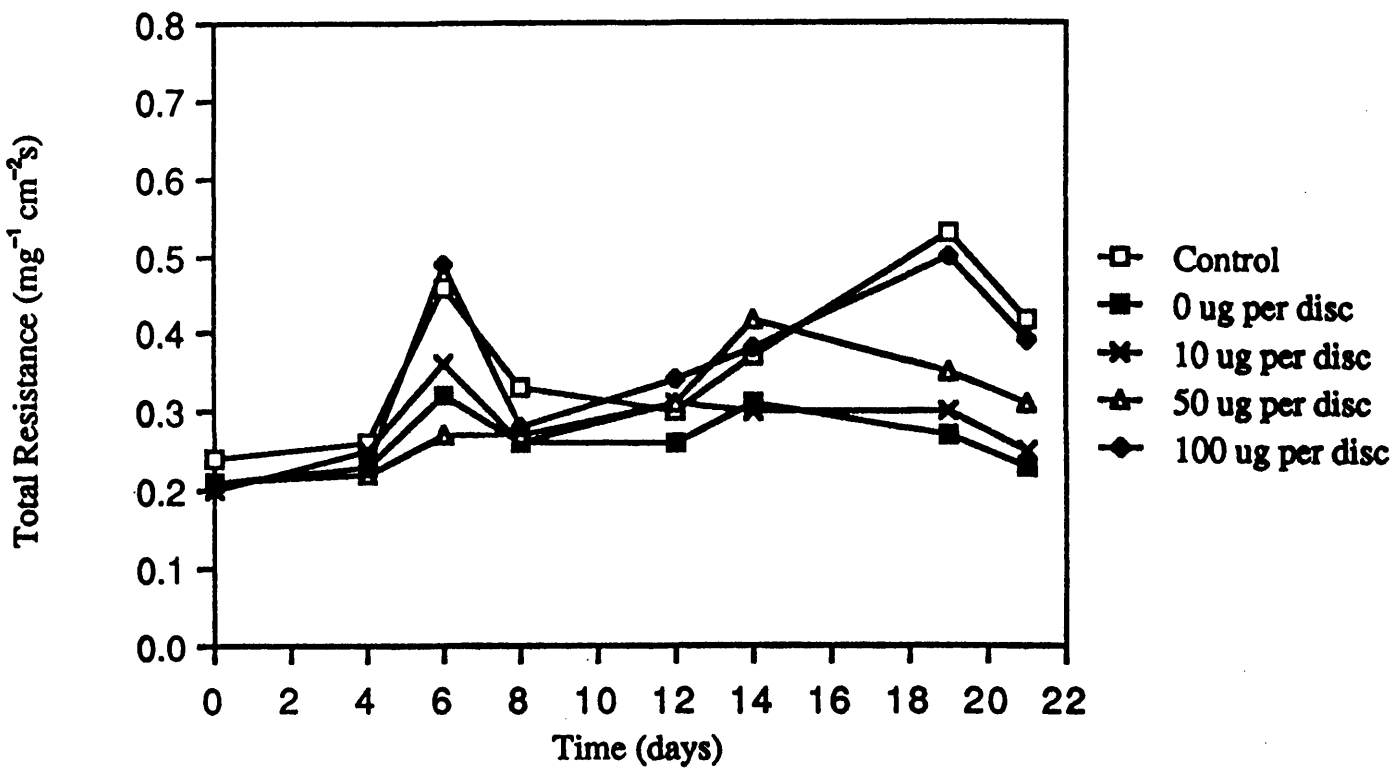


FIGURE 4.2 THE EFFECT OF ETHYLNAPHTHALENE ON THE DEVELOPMENT OF RESISTANCE TO WATER LOSS IN AGED TUBER DISCS - SET TWO

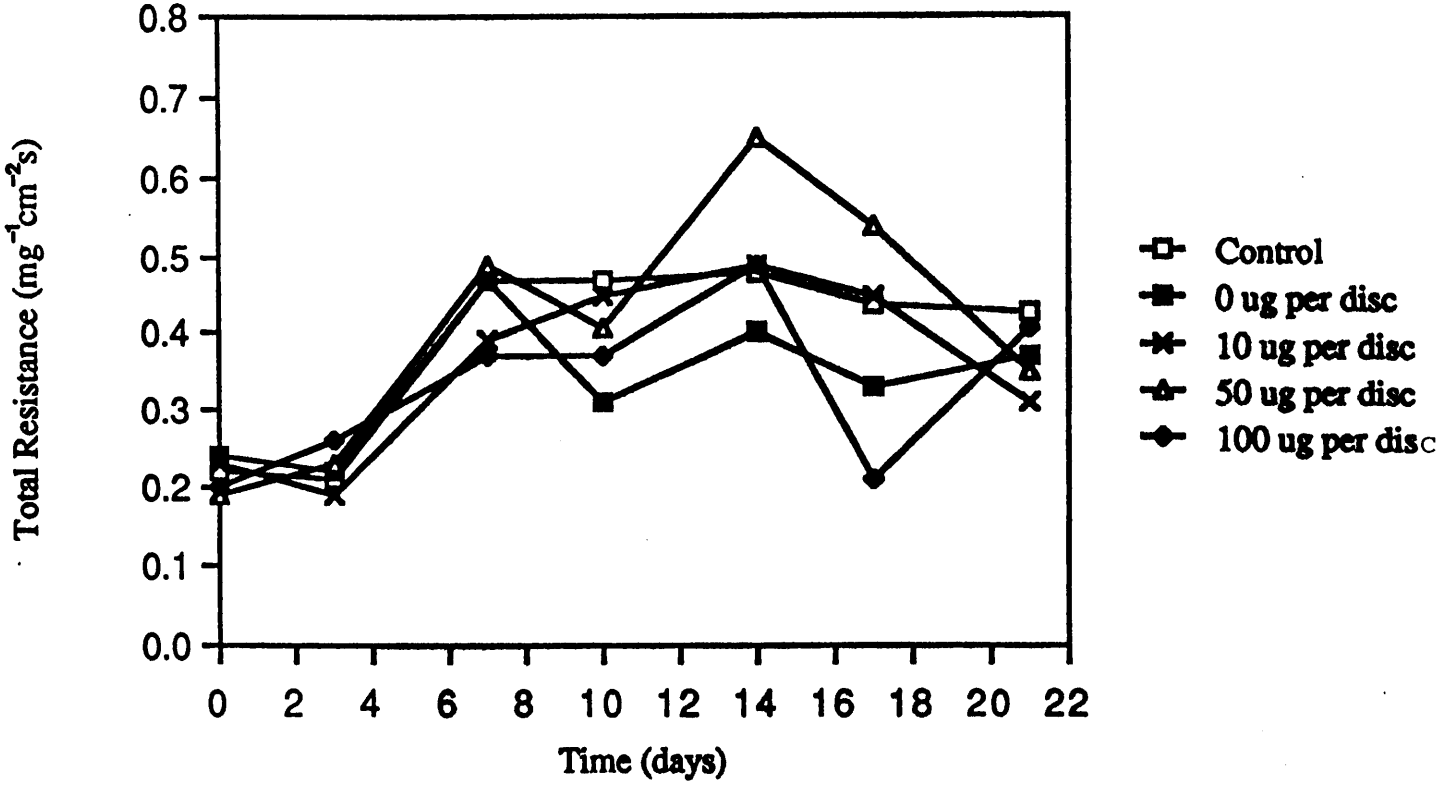
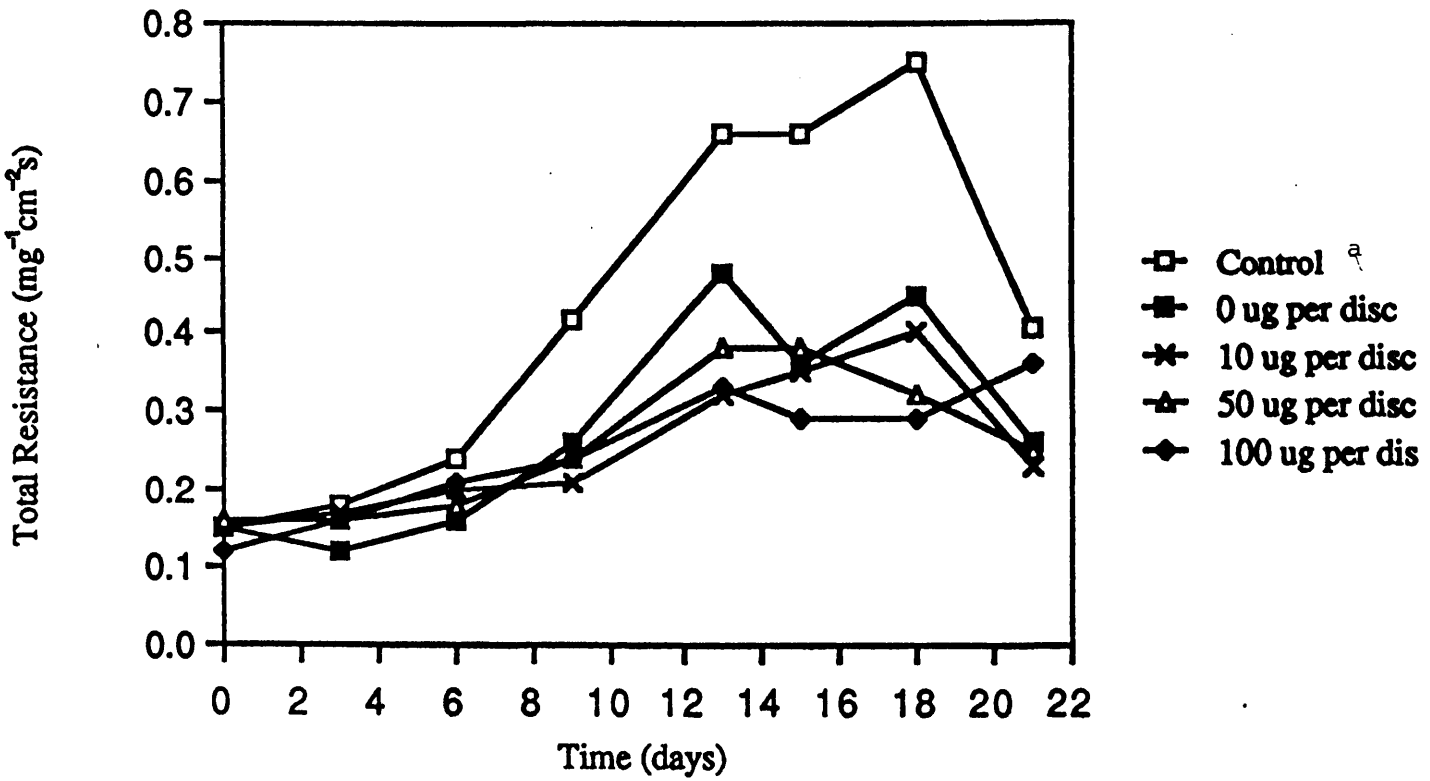
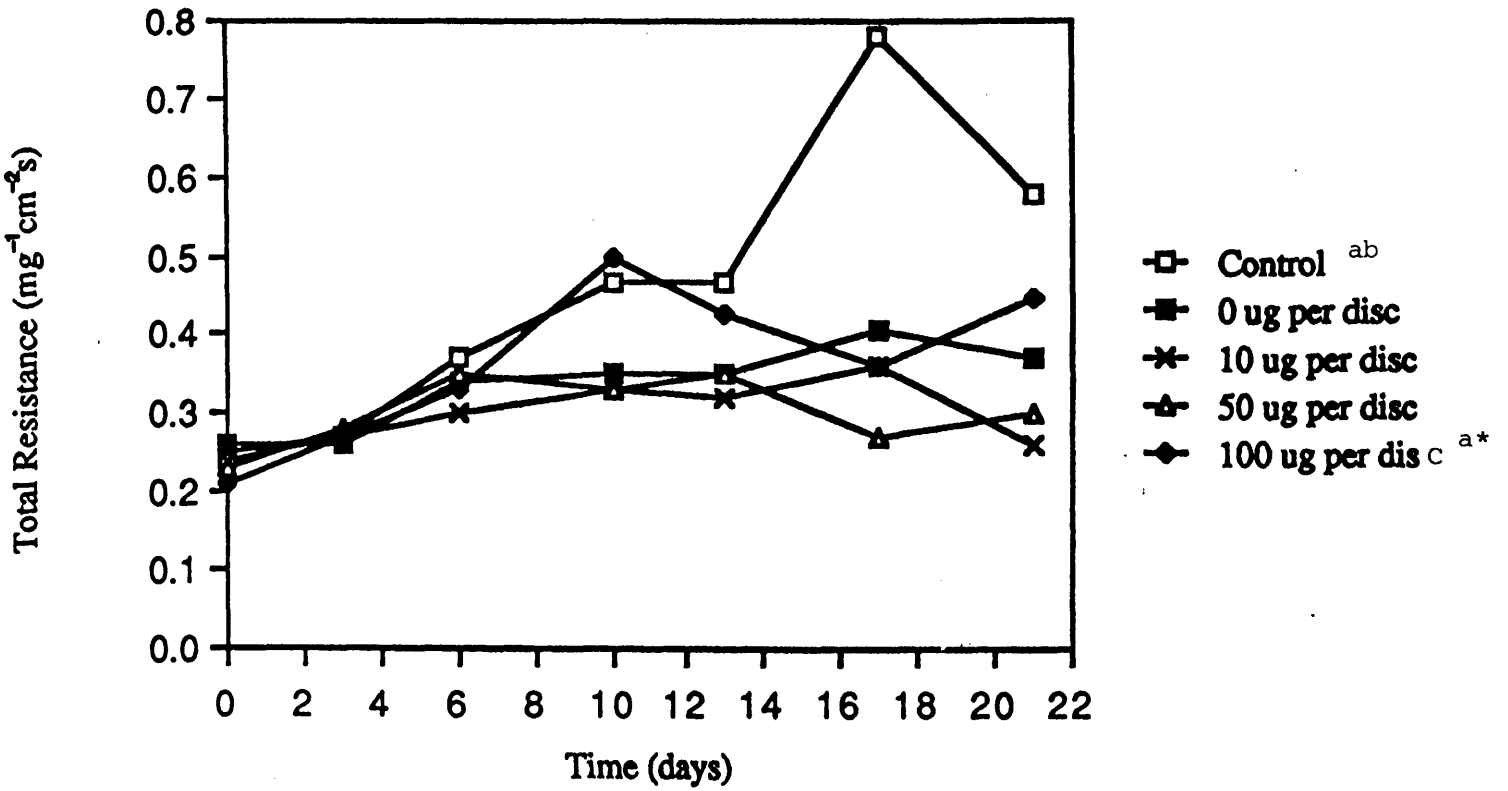


FIGURE 4.3 THE EFFECT OF DIISOPROPYLNAPHTHALENE ON THE DEVELOPMENT OF RESISTANCE TO WATER LOSS IN AGED TUBER DISCS - SET ONE



a At days 9 and 15 these discs were significantly different from the other treatments at $p<0.05$.

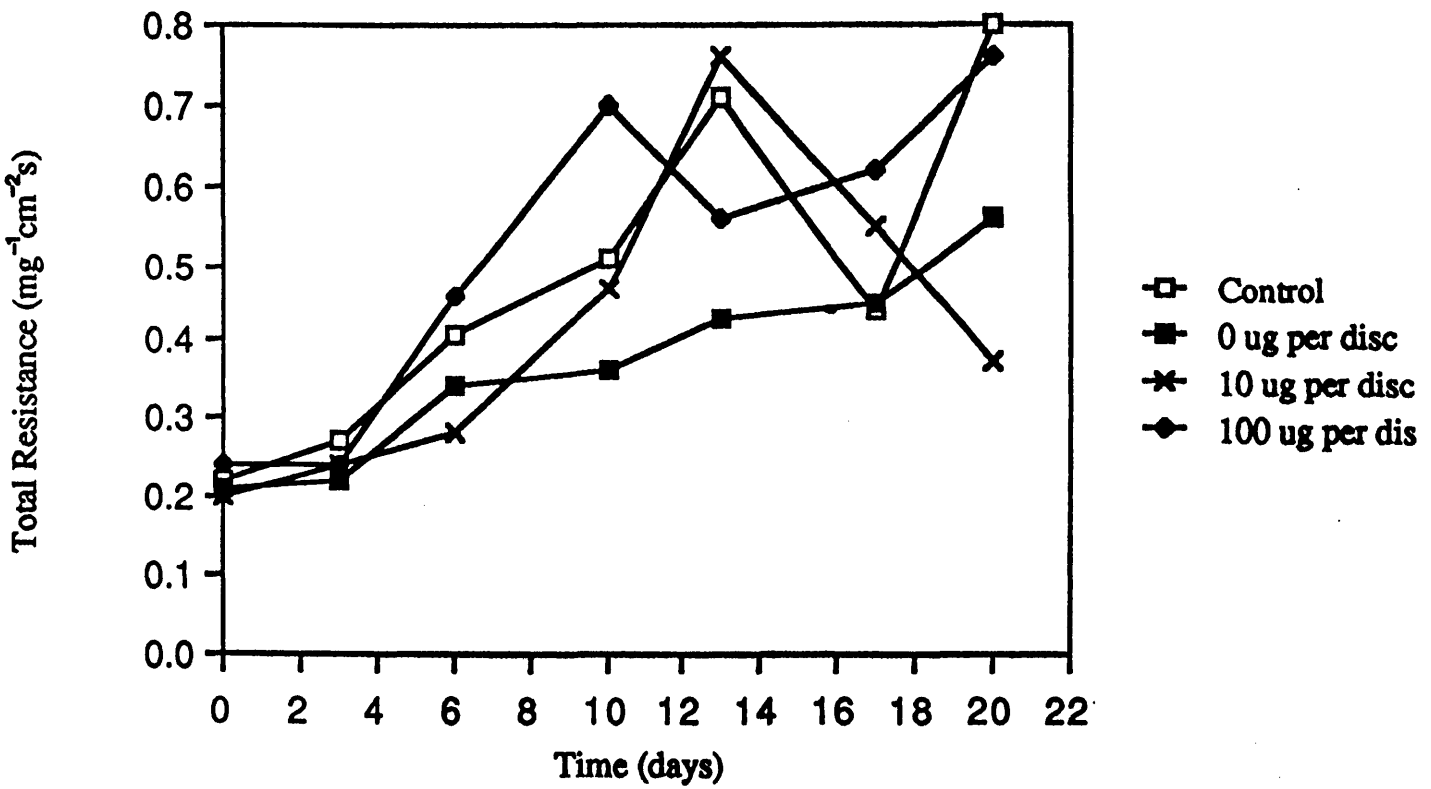
FIGURE 4.4 THE EFFECT OF DIISOPROPYLNAPHTHALENE ON THE DEVELOPMENT OF RESISTANCE TO WATER LOSS IN AGED TUBER DISCS - SET TWO



* a At day 10 these discs were significantly different from the other treatments at $p<0.05$.

b At day 17 these discs were significantly different from the other treatments at $p<0.05$.

FIGURE 4.5 THE EFFECT OF CHLORPROPHAM ON THE DEVELOPMENT OF RESISTANCE TO WATER LOSS IN AGED TUBER DISCS



4.4 Discussion

For the three treatments it seems evident from Figures 4.1 - 4.5 that the exposure of the chemicals to the discs had an adverse effect on the wound healing process, as measured by the rate of water loss and comparing it to the control. Before mentioning the chemical treatments it is important to note that the treatment 0μg $disc^{-1}$ also had an adverse effect on the wound healing of the potato discs. In all sets of treatments it gave a lower resistance than some of the chemical treatments. It is thought that both water and organic solvent can have a deleterious effect on the wound healing process (Leonard 1988). Thus when considering the effect of the chemical on the potato discs, the effect of the 0μg $disc^{-1}$ will need to be taken into consideration. The method of application is also rather unrealistic considering that in a commercial situation the substituted naphthalenes would rely on their vapour phase distribution for their activity.

Ideally, the treatments should reach their maximum resistances within a few days. A fast build up of resistance is important especially after harvest when the chemicals are applied immediately as the potatoes go into storage. Any delay in the development of the periderm formation may be sufficient to allow fungal and bacterial rotting to take place.

There has been no previous work carried out on the wound healing effects of diisopropylnaphthalene and ethylnaphthalene but work has been carried out with dimethylnaphthalene and its effect on wound healing of potatoes (McGee 1984; Wilson et al 1987).

McGee (1984) studied the effect of dimethylnaphthalene on potato discs where he found that at high levels of application 100μg $disc^{-1}$ and 200μg $disc^{-1}$ wound healing was inhibited but at lower levels of 10μg $disc^{-1}$ there was no significant difference with respect to increasing the resistance to water loss between the treatment and control. Wilson et al (1987) also found dimethylnaphthalene to have a negative effect on the wound healing of potato periderms.

In Figures 4.1 - 4.4 it is not known why the resistance to water loss drops and rises again after it has reached its maximum resistance. Jamieson (1988) thought that the drop in resistance to water loss was due to the discs themselves. It was thought that as time progressed, the discs became dried out in appearance when the relative humidity within the petri-dish was high (~ 100%). Under these conditions diffusion of moisture into the discs may occur. This water would readily be lost in the determination of resistance to water loss and so give exaggerated water loss values. Since water loss is inversely proportional to resistance to water loss, a low resistance value will be recorded and so this may account for the fall in resistance to water loss. However, in all cases the resistance increased slightly again before tailing off. The reason for this cannot yet be explained and obviously this area requires further investigation.

The results from Figures 4.1 - 4.4 seem to suggest that the chemical treatments have an adverse effect on the discs. It was decided to use the data for diisopropylnapthalene from Tables 4.3 and 4.4 to test for significant differences between treatments.

In Table 4.3 there was a significant difference in water loss between the control and treatments at both 9 and 15 days. In Table 4.4 at day 10 both the control and 100μg $disc^{-1}$ were significantly different from the other treatments, but on day 17 only the control was significantly different. It is clear from these results that water and methanol treated discs have an adverse effect on the discs. With an exception of $100\mu g^{-1}$ at day 10 of Set Two diisopropyl-naphthalene treated discs there is no significant difference between the water and methanol treated discs and the diisopropylnaphthalene treated discs. The water and methanol mask any effect that the chemical may have on the discs. This makes it very difficult to note the effect that the chemical itself has on the potato discs. For future work it would be advisable to apply the substituted naphthalene to the potato discs as a vapour.

The effect of chlorpropham on the discs are shown in Table 4.5 and Figure 4.5. These results were inconsistent and the effect of the chemical on the discs could not be noted accurately. However, work has been carried out earlier by McGee (1984) and Leonard et al (1987) in which chlorpropham was found to have an inhibiting effect on the healing of wounded potatoes. Leonard et al (1987) found that tuber discs treated with chlorpropham delayed the appearance of resistance to water loss through the disc surface. It made the discs more susceptible to infection by gangrene (Phoma exigua var foveata) when inoculated during a limited period about four to six days after wounding. They also carried out work on whole tubers on a commercial scale and found that chlorpropham inhibited wound healing.

However, slight stimulatory effects were noted, at low application levels of chlorpropham on water loss resistance of tuber discs. However, McGee (1984) noted that chlorpropham inhibited periderm formation but not suberization.

A major disadvantage to the set up of this experiment was that it was carried out over a twelve month period which created problems in obtaining the same variety of potato throughout the experiment. Different varieties were often used for different sets of treatments and when the same variety was used the potatoes were probably from different batches. Because the experiment was carried out over a twelve month period, potatoes of different physiological ages were often necessarily used. McGee (1984) has shown that the potato variety plays an important role in influencing the rate of wound healing. He also found that freshly harvested potatoes had a lower resistance to water loss than potatoes which were more mature. These factors will obviously contribute to the differences between similar sets of treatments.

In conclusion the work described in this chapter has not been able to show that the substituted naphthalenes had an inhibiting effect on the wound healing of potato discs. If the substituted naphthalenes did have an inhibiting effect on whole tubers under commercial conditions. Then when used on a large scale by the processing industry, a delay in application of the substituted naphthalene would be necessary to allow the potatoes to wound heal. If a sprout suppressant was necessary then tecnazene which has been shown (Reavill 1954; McGee 1984; Leonard 1988) to have little effect on wound healing and has the added benefit of controlling certain fungal species could be applied immediately followed later by applications of substituted naphthalenes throughout the storage period.

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR DIMETHYLNAPHTHALENE RESIDUE ANALYSIS

5.1 Introduction

In recent years there has been an increase in importance of quantifying residues in food and crops. The Pesticide (Maximum Residue Levels in Food) Regulations 1988 has recently been introduced and is partially concerned with setting up maximum residue levels of pesticides in food commodities. This enables the Government to set limits with respect to the maximum permissible residue levels in food and crops. Thus if the substituted naphthalenes were introduced commercially as potato sprout suppressants, then among other things a fast and reliable method would be needed for residue analysis.

As a result of this, and the limited information currently available regarding the extraction and analysis of substituted naphthalenes from potato residues, it was decided in this chapter to investigate and develop several analytical methods which could be used to quantify substituted naphthalenes in potato residues. Due to a limitation on time and resources, dimethylnaphthalene was the only substituted naphthalene considered in this chapter. In 1979, Beveridge adapted an existing extraction method used for tecnazene residue analysis (Dalziel and Duncan 1975) to suit the requirement of dimethylnaphthalene.

In this method, a suitable representative potato sample was macerated and the chemical extracted from the plant tissue using ethanol.

The extract was then partitioned with solvents (hexane-ethanol) to remove interfering molecules. The hexane was then collected and reduced. It was cleaned-up using an alumina column. The extract was finally reduced to a small known volume and quantified.

Beveridge (1979) reported a UV spectrophotometric method which was capable of detecting dimethylnaphthalene down to levels of 0.1μg cm^{-3} in solution. The dimethylnaphthalene absorbed strongly at 228nm. However, the method was found to be unsuitable for the analysis of plant tissues which cause interferences at 228nm and consequently lower the sensitivity of the method.

Most of the substituted naphthalenes that Beveridge (1979) worked with were analysed by a GC equipped with an FID. This procedure was capable of detecting down to 1 - 10μg kg^{-1} levels of dimethylnaphthalene in residues. No work has yet been reported on the High Performance Liquid Chromatographic (HPLC) analysis of substituted naphthalene residues. This procedure is commonly used in the residue analysis of foods and crops. It is popular for its high speed and selective analysis of samples. The relevant details of both GC and HPLC are reported below.

Gas Chromatography

Gas chromatography is a process by which a volatile mixture of compounds can be separated by adsorption (or partition) between two phases, one of which is mobile (carrier gas) and the other stationary (column packing).

The sample which needs to be volatile and thermally stable is injected onto a column, through a septum in the injection port by means of a microlitre syringe. The carrier gases commonly used are nitrogen, argon, helium and hydrogen. The choice is governed principally by availability, cost and the detector to be used. The carrier gas passes through the injector and into the column. The resolution of the column is dependent on length and packing material as will the flow rate of carrier gas. The retention time of the sample is dependent on its volatility and its adsorption onto the column. When the compound is eluted from the column it passes through a detector. The detector produces a response which is proportional to the concentration of compound passing through it.

A number of detectors have been developed and used; however, the flame ionisation detector is the most popular and widely used. It gives good linearity up to 10^7 with a lower limit of detection of approximately 10^{-9}g ml^{-1}. It has the ability to respond to virtually all organic compounds. The detector consists of a small hydrogen-air flame, burning at a metal jet situated at the end of the column. The organic compounds eluted from the column are burned in the hydrogen-air flame and ions are produced. The ions which are proportional to the concentration of the compound are collected by a pair of polarised electrodes inside the detector and the current produced is amplified before being passed to a recorder.

High Performance Liquid Chromatography

HPLC is a method which is particularly effective for the analysis of non-volatile and thermally sensitive compounds.

The compounds are separated by interaction between two phases, one of which is mobile (liquid) and the other stationary (the column packing). The compounds to be separated are dissolved in a suitable solvent and introduced onto the column usually in a 10 or 20µl volume either by using a syringe or an injection valve. A pressurised flow of the liquid phase allows the compounds to pass through the column. A variety of different pumping systems are used in HPLC, the most common being the reciprocating piston pump system. This pump is capable of delivering a constantly reproducible and pulse free supply of the mobile phase to the column. The resolution of the compound is dependent upon the equilibration of the compounds between the mobile and stationery phases. However, the resolution of a compound can be manipulated by different choices of solvents and column packings. It is possible to use a mobile phase of a certain polarity by mixing two or more solvents eg methanol, water, acetonitrile. A typical HPLC column is made of stainless steel tubing. It is packed with an inert packing material which has a stationary phase bound to it. Common non-polar stationary phases are based on phenyl and octadecyl (C_{18}) groups. This is mainly known as reverse phase chromatography. Semi-polar phases have cyano and alkyl amine groups. Ion exchange packings are also available, allowing cationic and anionic exchange eg sulphonated styrene divinyl benzene and cross linked polymethacrylate particles. As a result HPLC acquires a high degree of versatility not found in other chromatographic methods.

There are many detectors available for use with HPLC. The most widely used, however, is the UV absorbance detector. It gives a linearity range of approximately 10^5 with a minimum detection limit of 10^{-9}g ml^{-1}.

UV light is supplied either be a mercury discharge lamp or by a deuterium lamp. The emitted UV light passes through a flow cell which is connected to the column effluent. Changes in UV absorbance of the mobile phase are detected by a reference flow cell which monitors the light intensity. The absorbance is linearly proportional to the sample concentration (Beer and Lambert Law). This enables the chromatogram to be used for quantitative analysis.

5.2 Extraction and Analysis of Dimethylnaphthalenes by GC

Modifications to the method used by Beveridge (1979) were adopted. The details are as follows:-

A representative 100g of macerate was taken from approximately 1kg of treated tubers. The 100g sample was then homogenised for one minute with 100cm^3 ethanol (Burroughs Ltd, Witham, Essex) in a Waring blender. 100cm^3 hexane (Rathburns Chemicals Ltd., Walkerburn, Scotland) was added to the homogenate and blended for a further one minute. The homogenate was filtered under reduced pressure through Whatman No 1 filter paper. The blender cup was rinsed out with two separate 50cm^3 portions of hexane which were also passed in turn through the filter paper. The filtrate was then quantitatively transferred to a 500cm separating funnel. 100cm^3 of saturated sodium chloride was added and shaken vigorously in order to increase the ionic nature of the aqueous ethanol phase and thus encourage a greater partition of dissolved substituted naphthalenes from the ethanol phase to the hexane phase.

The aqueous/ethanol layer was discarded and the hexane layer was washed and shaken vigorously with 200cm^3 of 10% (w/v) sodium carbonate (BDH Ltd., Poole, England) solution, followed by 200cm^3 deionised water in order to remove any remaining interferences, such as fatty acids. The hexane layer was then collected and dried over anhydrous sodium sulphate (BDH Ltd., Poole, England). After filtering off the sodium sulphate and washing it with 15cm^3 hexane, the combined filtrate was reduced in volume to 5cm^3 using a rotary evaporator.

Clean-up of Residue Extract

This involved the preparation of an alumina column. This was done by pouring a slurry of alumina in hexane into a glass column with dimensions 300cm^3 by 9cm^3 diameter. The column was packed to a depth of 150cm^3.

The residue/hexane extract was applied to the column and the flow rate regulated to 1cm^3 min^{-1} with hexane as an eluting agent. The theory was that any interfering compounds would either be eluted immediately or adsorbed onto the alumina allowing dimethylnaphthalene to be separated and eluted. The first 25cm^3 of effluent was discarded and the subsequent 100cm^3 was collected. The volume was finally reduced to under 5cm^3 by removing the solvent using a rotary evaporator. The volume was finally adjusted to 5cm^3 by quantitative transfer of the reduced volume to a 5cm^3 volumetric flask. The extract was then analysed using gas chromatography.

Efficiency of GC Extraction Method

The percentage recovery of dimethylnaphthalene was calculated by spiking 100g of macerated tubers with 10mg dimethylnaphthalene. Extraction, clean-up and quantitative analysis was carried out.

The percentage recoveries ranged from 51.4% to 66.0% with a mean value of 56.3% $\pm$ 6.1% from five replicate samples.

The recovery values reported above are fairly low with a poor reproducibility. The principal sources of loss of dimethylnaphthalene were thought to be at the clean-up and rotary evaporation states.

In an attempt to improve the percentage recovery of the method and increase the reproducibility the percentage recovery for individual stages was calculated and modifications were introduced to reduce these losses.

Alumina Column

The clean-up method was investigated initially due mainly to the numerous steps involved and the length of time taken (approximately 2 hours) for the dimethylnaphthalene to be eluted.

The percentage recoveries were calculated by adding 10mg of dimethylnaphthalene to an alumina column and calculating the percentage of dimethylnaphthalene eluted and present in the final 5cm^3 volume. The percentage recoveries ranged from 60.0% to 79.4% with a mean value of 68.6% $\pm$ 8.4% from five replicate samples.

Bonded Silica Adsorption Cartridges

Recent years have seen the introduction of disposable mini cartridges Sep-pak pre-columns that have improved residue clean-ups. After testing a variety of columns with different packing materials and bonded phases, a Sep-pak silica cartridge (Waters Assoc., Milford, USA) was found to give the highest percentage recovery of dimethylnaphthalene from residue extracts. Two cm^3 of hexane were run through each mini Sep-pak cartridge before $1cm^3$ of extract was introduced to the cartridge. The cartridge was then leached with approximately $4cm^3$ of hexane. The dimethylnaphthalene was collected in a 5cm volume. Interfering molecules including green pigment were retained on the adsorbent.

The Sep-pak cartridges have many advantages over the traditionally packed alumina columns. These advantages are:-

1. Lower cost - it involves less apparatus and a lower solvent consumption.

2. Faster - a clean-up with a Sep-pak cartridge takes less than 5 minutes. In contrast alumina packed glass columns require 3 hours to pack the column, set the flow rate, elute the dimethylnaphthalene and reduce the extract volume.

3. Greater accuracy - there is less chance of cross contamination due to less handling and fewer steps involved in the process.

The percentage recovery from the Sep-pak silica cartridge was calculated using standard dimethylnaphthalene solutions made up in hexane: 10mg, 5mg and 1mg concentrations were added to the cartridges. The percentage recovery was calculated from the $5cm^3$ collected (see Table 5.1). An average of 95.6% $\pm$ 1.0 of dimethylnaphthalene applied to the cartridge was recovered. However, this percentage recovery was lowered slightly to 95.2% (SD $\pm$ 1.2%) when spiked potato juice was used instead of standard solutions.

Solvent Removal

Solvent is removed using a rotary evaporator which operates under vacuum. The temperature of the solvent is controlled by a thermo-statically controlled water bath. The higher the temperature of the water bath, the faster the solvent is removed. During the removal of solvent from dimethylnaphthalene the temperature was maintained at 28°C $\pm$ 1°C. This allowed the rapid evaporation of hexane.

However, owing to the volatile nature of dimethylnaphthalene, there was a possibility of the compound being lost during evaporation along with the hexane. The effect of temperature on the loss of dimethylnaphthalene was therefore followed. This involved spiking $50cm^3$ of hexane with 10mg of dimethylnaphthalene and then reducing the volume to $5cm^3$. This was carried out at different temperatures and the percentage recovery was calculated. Table 5.2 shows the results. As the temperature increased the recovery of dimethylnaphthalene in general decreased. The lowest temperature 20°C $\pm$ 1°C gave the highest recovery of 97.7% dimethyl-naphthalene.

However, this step took approximately 40 minutes. The optimum temperature was found to be 24°C ± 1°C which gave a 96.8% recovery of dimethylnaphthalene in less than 20 minutes.

Percentage Recovery Using Modified Method

The percentage recovery of dimethylnaphthalene from potato residues was calculated using the modifications to the method eg clean-up and rotary evaporation stage as mentioned earlier (see Section 5.2).

This involved spiking 100g of macerated tubers with 1mg of dimethylnaphthalene before extraction, clean-up and quantitative analysis.

The percentage recoveries ranged from 86.5% to 92.8% with a mean value of 90.4% ± 2.6% from five replicate samples. It was possible to detect levels of dimethylnaphthalene down to 0.006μg and levels of 0.5mg kg^{-1} in potato residues on a conventional GC packed column. The sensitivity could however be increased by using a capillary column, which unfortunately was not available for use during this study.

It is thought that the remaining percentage lost during recovery was during the homogenising stage when heat generated by the high speed blender valve could have caused evaporative loss of dimethylnaphthalene.

5.3 Extraction and Analysis of Dimethylnaphthalene by HPLC

As there is no work published on the HPLC analysis of the substituted naphthalenes, it was hoped to develop a suitable extraction, clean-up and quantification method for the analysis of dimethylnaphthalene in potato residues.

The extraction method used previously for the analysis of dimethylnaphthalene by GC is not suitable for use with HPLC. The main reason for this was that hexane was not very miscible with the polar solvents used as the mobile phase in HPLC and would result in poor resolution. Another extraction method was used that was based on a method by Tepe and Scroggs (1967) to extract trifluralin (α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) from crops. Trifluralin is an effective herbicide and work is being carried out at present (at Glasgow University) to assess its sprout suppressing effect on potato tubers. The method used was a modification of the method used by Sattar Mohammad (personal communication) to extract trifluralin from potato residues.

A representative 50g of macerate sample was homogenised for two minutes with 150cm^3 of methanol (Analar grade) in a Waring blender. The homogenate was filtered under reduced pressure through a Whatman No 1 filter paper. The blender cup was rinsed with two separate 25cm^3 portions of methanol which were also passed through the filter paper. The filtrate was then quantitatively transferred to a 500cm^3 separating funnel. 150cm^3 of 10% sodium chloride was added and shaken vigorously. The aqueous/methanol layer was then shaken with 50cm^3 of methylene dichloride.

This encouraged the chemical to leave the aqueous/ methanol phase and enter the methylene dichloride phase. The methylene dichloride layer was collected and dried using anhydrous sodium sulphate. The remaining aqueous/methanol phase was washed a further two times with two separate $50cm^3$ volumes of methylene dichloride. The methylene dichloride washings were then collected and dried along with the previous $50cm^3$ of methylene dichloride. After filtering off the sodium sulphate and washing with $100cm^3$ of methylene dichloride, the combined filtrate was reduced to dryness using a rotary evaporator at 30°C $\pm$ 1°C. The residue remaining at the bottom of the flask was then dissolved in $5cm^3$ of hexane or methanol. Interfering molecules were removed from the extract by passing through a Florisil packed glass column with dimensions of $400cm^3$ by $25cm^3$ diameter. The effluent was then analysed by GC.

This method was adapted slightly for the extraction of dimethylnaphthalene. The extraction was followed as mentioned above until the rotary evaporation stage. The solvent was then evaporated at 24°C in the case of dimethylnaphthalene rather than the 30°C used for trifluralin. Due to the high volatility of the dimethylnaphthalene, it was also important not to reduce the extract volume below $4cm^3$. If it was evaporated to dryness then a lot of the dimethylnaphthalene would be lost. Beveridge (1979) suggested that this was due to the heat of evaporation of hexane keeping the solution cool.

The extract was then submitted to a clean-up treatment using Sep-pak silica cartridges as mentioned earlier except methylene dichloride was used instead of hexane. The extract was then analysed by HPLC.

HPLC Analysis

Due to the non-polar nature of the dimethylnaphthalene molecule, it was decided that the best separation would be achieved by 'reverse phase' chromatography. This involved using a mobile phase which was more polar than the stationary phase. A Perkin Elmer series 400 solvent delivery system was used with a Perkin Elmer 155-100 sampling system. The stationary phase used was a C_{18}, octadecylsilane packed column and a mobile phase of methanol/water (70/30) mixture with 1ml of glacial acetic acid added to improve the resolution of the dimethylnaphthalene. The flow rate was set at 2ml min^{-1} and the dimethylnaphthalene was eluted from the column after 5 minutes and detected using a Perkin Elmer UV detector at 230nm. A typical trace of dimethylnaphthalene is shown in Figure 5.1.

Percentage Recovery

Percentage recovery of dimethylnaphthalene was determined by spiking 50g of macerated tubers with 1mg of dimethylnaphthalene before extraction, clean-up and quantitative analysis by HPLC.

The percentage recoveries ranged from 83.2% to 87.5% with a mean value of 85.9% $\pm$ 1.7% from five replicate samples. It was possible to detect levels of dimethylnaphthalene down to 0.005µg and levels of 0.42mg kg^{-1} in potato residues. It was thought that the remaining dimethylnaphthalene not recovered was lost during the partitioning stage in the separating funnel.

A poor separation of the methylene dichloride and aqueous/methanol layers was thought to cause some of the dimethylnaphthalene to be discarded with the aqueous/methanol phase. Work could be carried out in the future to increase the efficiency of the method by improving the partitioning of the solvents and also to increase its sensitivity by lowering the detection limits.

5.3 CONCLUSION

The GC analysis of dimethylnaphthalene was successfully improved. The percentage recovery and reproducibility of the method was increased. This was accomplished by using a Sep-pak silica cartridge for cleaning-up the dimethylnaphthalene residue extract and also by controlling more closely the temperature of the rotary evaporator when removing solvents.

A method was developed for the extraction, clean-up and analysis of dimethylnaphthalene residues by HPLC. The percentage recovery of the HPLC method was lower (85.9% $\pm$ 1.7%) than the GC method (90.4% $\pm$ 2.6%). However the reproducibility of the HPLC method was slightly higher. The HPLC was more sensitive with a detection limit of 0.005µg whereas the GC could only detect levels down to 0.006µg.

GC and HPLC are both used for their high speed and selective analysis of samples. However HPLC analysis has many advantages. It analyses a large range of organic molecules ie non-volatile and thermally sensitive. It is non-destructive in that the sample remains intact unlike GC where the sample is thermally broken down during detection (FID).

The latest technology allows much automation for HPLC, where a high degree of reproducibility and accuracy is obtained. This allows many samples to be analysed with a reduction in man hours.

TABLE 5.1

PERCENTAGE RECOVERY OF SEP-PAK SILICA CARTRIDGES

Concentration of Chemical Added (mg)	Percentage recovery of Dimethylnaphthalene in $5cm^3$ Volume (%)	Average Percentage Recovery ± Standard Deviation (%)
1	95.4, 93.2, 96.4, 95.9, 95.0	95.2 ± 1.2
5	96.2, 96.5, 95.0, 93.8, 95.8	95.5 ± 1.1
10	96.5, 95.5, 95.8, 96.2, 97.0	96.2 ± 0.6

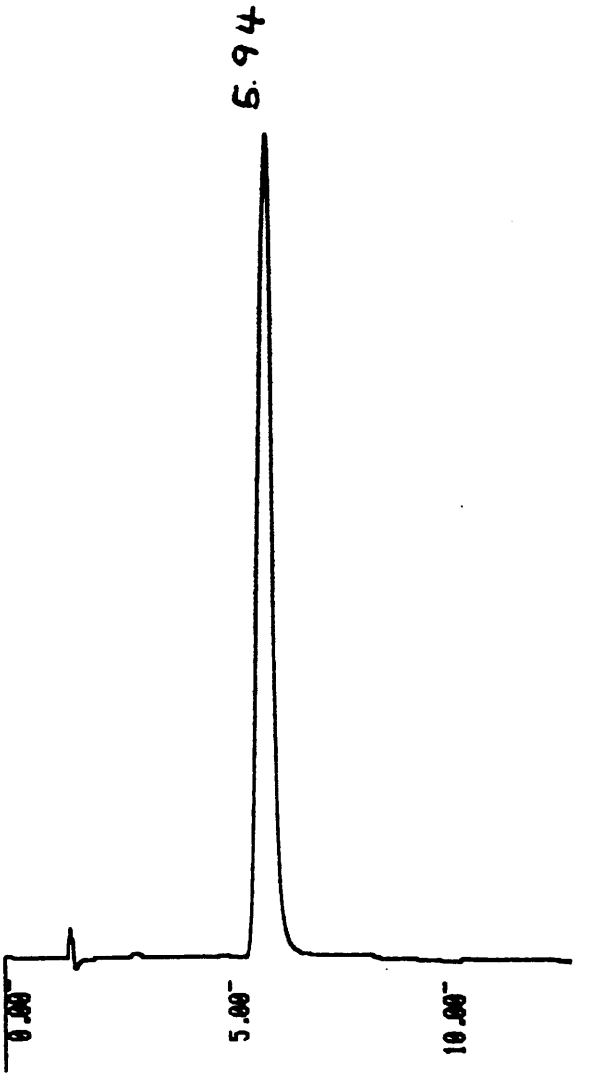
TABLE 5.2

EFFECT OF ROTARY EVAPORATOR TEMPERATURE ON PERCENTAGE RECOVERY OF DIMETHYLNAPHTHALENE

Rotary Evaporator Temperature (°C)[a]	Percentage Recovery of Dimethylnaphthalene	Mean Percentage Recovery
20	97.6, 97.8, 98.0	97.8 ± 0.2
22	96.3, 98.1, 97.4	97.2 ± 0.9
24	96.0, 96.4, 97.8	96.7 ± 0.9
26	96.5, 96.2, 95.8	96.2 ± 0.4
28	95.8, 94.6, 95.0	95.1 ± 0.6
30	93.6, 94.9, 93.9	94.1 ± 0.7
32	95.8, 95.3, 94.8	95.3 ± 0.5
34	95.6, 95.4, 95.1	95.3 ± 0.3
36	93.8, 94.9, 95.5	94.7 ± 0.9
38	93.2, 93.7, 94.8	93.9 ± 0.8
40	90.9, 92.9, 91.7	91.8 ± 1.0

a Temperature ± 1°C

FIGURE 5.1 HPLC TRACE OF DIMETHYLNAPHTHALENE USING A C_{18} COLUMN AND UV DETECTION



THE EFFECT OF SUBSTITUTED NAPHTHALENES ON THE RESIDUE CONCENTRATION AND SPROUT GROWTH OF TUBERS

6.1 Introduction

As stated in Chapters One and Two, Meigh and his co-workers (1973) isolated and identified some dimethylnaphthalene isomers which were evolved from stored potatoes. Further work by Meigh et al (1973) using a simple bioassay showed that some of the dimethylnaphthalene isomers had sprout suppressant properties.

Beveridge (1979) carried out work on dimethylnaphthalene with the object of developing a new sprout suppressant that would be effective under commercial conditions. He designed an experiment that consisted of treating 10kg amounts of tuber samples at rates between 0 and 500mg kg^{-1}. The samples were stored in cardboard boxes with loosely fitting lids and were studied over 12 to 14 weeks at 10°C. Using this method he showed that dimethylnaphthalene when applied at 100mg kg^{-1} was as effective as the recommended application rate of tecnazene (135mg kg^{-1}) at controlling sprout growth. This technique was shown to reflect the behaviour of chemicals in potato stores fairly accurately (Beveridge 1979).

From further work carried out at Glasgow University (Dr H Duncan, personal communication) it became apparent that sprout suppressant activity was not confined to one or two isomers of dimethylnaphthalene, but that all isomers tested were active to some degree.

Stephen and Duncan (1984) decided to investigate this further using a similar method to that designed by Beveridge (1973). They demonstrated that other substituted naphthalenes gave good control of sprout length when held at 10°C for 15 - 17 weeks. The results showed that many mono and disubstituted alkyl naphthalenes are sprout suppressants and that halogenated substitutes also give active compounds.

The object of the work in this chapter was to assess the effect of ethylnaphthalene and diisopropylnaphthalene on the growth of potato sprouts and residue levels over a 24 week period and to compare them to controls of tecnazene and chlorpropham when applied at recommended application rates.

Ethylnaphthalene and diisopropylnaphthalene were chosen from the headspace results of work carried out in Chapters Two and Three. Ethylnaphthalene was chosen mainly for its slightly high volatility and diisopropylnaphthalene for its low volatility. For this reason it was decided to apply diisopropylnaphthalene at two different treatment levels. It was hoped to relate the treatment levels to varying residue concentrations and sprout growth over the 24 week period.

6.2 Experimental Methods and Materials

The assessment experiment for each chemical consisted of an untreated control, tecnazene and chlorpropham treatments and three substituted naphthalene treatments. Each treatment was applied to three 10kg batches of each potato cultivar.

Two cultivars were used throughout the experiment - Pentland Squire and Maris Piper. Both were obtained from a local farm on 25 October and stored in sacks at 10°C until 17 November when they were then treated.

The chemicals used for each treatment were obtained from Aldrich Chemical Co (Dorset, England) except diisopropylnaphthalene which was donated by Willow House Research. The solvents used were Analar grade unless stated otherwise.

The chemicals were applied to the potatoes on an alumina solid carrier (Alumina 'O', Spence and Son, Airdrie, Scotland) which was spread evenly over the potatoes. Each chemical treatment was prepared by adding the necessary amount of test chemical to a 300ml volume of hexane (except tecnazene which was dissolved in 600ml of hexane due to its low solubility at the amount used) and then mixed with 250g of alumina. The chemical was coated onto the alumina using a rotary evaporator which evaporated off the hexane leaving the chemical associated with the alumina. This was then transferred to a 500g screw top jar and shaken on an end over shaker for two hours to ensure a homogeneous mixture. The levels of treatment were as follows: tecnazene (135mg kg^{-1}), chlorpropham (12mg kg^{-1}), ethylnaphthalene (100mg kg^{-1}), diisopropylnaphthalene (50mg kg^{-1}) and diisopropylnaphthalene (100mg kg^{-1}).

The treatments, in the form of 25g lots of alumina, were applied to 10kg batches of potatoes which were stored in cardboard boxes (395mm x 300mm x 150mm) with overlapping lids. After the potatoes had been treated they were stored in 10kg batches in cardboard boxes in a temperature controlled cold room held at 10 $\pm$ 2°C. Powerful fans were present to circulate the air around the room.

See Diagram 6.1 for a summary of the potatoes throughout the experiment.

6.2.1 Sample Loss During Preparation of Treatment

It was thought that some of the volatile chemicals could be lost during the preparation of the treatment particularly under the vacuum of the rotary evaporator over a long period of time. In order to know the exact amount of chemical being applied to the potatoes a 10g subsample of each chemical treatment was shaken on an end-over-end shaker for two hours with 15ml of diethyl ether in a 50g screw cap jar. It was then filtered and further washed with diethyl ether and made up to a 25ml volume and injected onto a GC equipped with an FID and compared to standard solutions.

6.2.2 Measurement of Sprout Length and the Number of Eyes Open

On 22 January and again on 16 May the degree of sprouting and the number of eyes open on the tubers were quantified. Two boxes for each treatment were studied and 25 tubers were chosen at random from each box.

The mean length of the longest sprouts and the number of eyes open were recorded for each box and the pattern of sprouting was also noted for each treatment. At the end of the experiment on 20 May the % sprouting was calculated by weight, ie

$$\frac{\text{total weight of sprout}}{\text{total weight of tubers}} \times 100\%$$

6.2.3 Residue Levels

Potato samples were taken for residue analysis on 8 December, 14 February and 4 May. One kg of potatoes were chosen at random from each treatment box studied and washed to remove excess soil etc. The potatoes were then sliced before being sealed in polythene bags and stored in a freezer until analysis. Only two of the three samples for each treatment were analysed.

Potatoes that were either untreated or treated with tecnazene or substituted naphthalene were analysed by the GC method described in Chapter Five. Chlorpropham treated potatoes were analysed by the following method. A defrosted 50g subsample of potato was taken from 1kg of minced sample and blended at high speed for two minutes with $150cm^3$ hexane and 80g of anhydrous sodium sulphate. The mixture was transferred with washings to an aluminium bottle, shaken on a shaker for 30 minutes and filtered using a Buchner assembly. The residue was washed four times with $50cm^3$ hexane and the filtrate obtained was reduced in volume in a rotary evaporator and made up to $2cm^{-3}$ ie no clean-up method was used.

The samples were then injected into a GC and compared to a standard solution.

6.2.4 Analysis of Results - % Sprout Length and Eyes Open

The data was examined for cultivar and treatment effects for both sprout length and the number of eyes open.

Results were expressed as the means of the boxes. Significant differences between cultivar means were tested by an F test on the analysis of variance. If the difference was significant then treatment data for each cultivar was dealt with independently. Chemical treatment means were compared with the untreated control and significant differences were tested for by using an F test on the analysis of variance. The least significant difference between the means of the chemical treatments and untreated controls that would be significant were calculated by Fisher's Least Significant Difference method for multiple comparisons, see page 94 for calculation.

Example Result of Analysis of Variance on Sprout Length

Source	Degree of Freedom	Sum of Squares	Mean Square	F Statistics
Day	1	863	863	4.54
Error	46	8,752	190	
Total	47	9,615		

6.3 RESULTS

TABLE 6.1

PERCENTAGE OF CHEMICAL RECOVERED FROM 10g SUBSAMPLES OF CHEMICAL TREATMENTS

Chemical	Expected concentration extracted from 10g of alumina treatment (mg)	Actual concentration extracted from 10g of alumina treatment (mg)	% Recovery
Ethylnaphthalene ($100mg\ kg^{-1}$)	370	255	68.9
Diisopropylnaphthalene ($50mg\ kg^{-1}$)	185	139	75.1
Diisopropylnaphthalene ($100mg\ kg^{-1}$)	370	234	63.2
Chlorpropham ($12mg\ kg^{-1}$)	4.63	4.55	98.3
Tecnazene ($135mg\ kg^{-1}$)	500	397	79.4

TABLE 6.2

MEAN SPROUT LENGTH OF TUBERS 66 AND 176 DAYS AFTER TREATMENT STORED AT 10°C + 2°C

TREATMENT	SPROUT LENGTH (mm)			
	Maris Piper		Pentland Squire	
	Day 66	Day 176	Day 66	Day 176
Control	31.7 (32.5, 29.4)	31.1 (27.8, 34.4)	45.3 (36.0, 62.7)	35.9 (38.9, 31.6)
Ethylnaphthalene (100mg kg^{-1})	17.4 (20.7, 14.2)	27.4 (30.7, 24.4)	8.5 (10.2, 7.1)	40.0 (43.2, 37.9)
Diisopropylnaphthalene (50mg kg^{-1})	7.9 (9.1, 6.6)	20.7 (22.1, 19.4)	3.4 (2.1, 4.7)	25.8 (28.2, 22.7)
Diisopropylnaphthalene (100mg kg^{-1})	9.9 (7.9, 13.5)	20.8 (22.2, 19.6)	6.5 (11.1, 1.9)	22.9 (26.4, 19.8)
Tecnazene (135mg kg^{-1})	5.6 (6.6, 4.3)	9.5 (11.8, 6.7)	3.7 (3.3, 4.3)	12.9 (16.4, 9.5)
Chlorpropham (12mg kg^{-1})	0.3 (2.0, 0.4)	1.1 (1.2, 0.08)	0.5 (1.4, 0)	0.7 (0.6, 0.24)
5% LSD	10.58	6.92	10.58	6.92

mean (sprout values)

There was no significant difference between cultivars at Day 66 but the treatments were significantly different from the corresponding untreated control ($p < 0.05$). All the treatments except ethylnaphthalene were found to be as effective as chlorpropham at controlling sprout length.

There was no significant difference between cultivars at Day 176. It was noted that all treatments except ethylnaphthalene gave significantly shorter sprouts than the control.

TABLE 6.3

MEAN NUMBER OF EYES OPEN IN TUBERS 66 AND 176 DAYS AFTER TREATMENT STORED AT 10°C + 2°C

TREATMENT	NUMBER OF EYES OPEN			
	Maris Piper		Pentland Squire	
	Day 66	Day 176	Day 66	Day 176
Control	1.7 (2.0, 1.3)	2.0 (1.7, 2.0)	1.4 (1.8, 1.2)	1.6 (1.9, 1.3)
Ethylnaphthalene (100mg kg^{-1})	1.6 (1.5, 1.6)	1.9 (2.2, 1.6)	0.7 (0.5, 0.8)	1.5 (1.8, 1.2)
Diisopropylnaphthalene (50mg kg^{-1})	0.6 (0.7, 0.5)	1.5 (1.4, 1.6)	0.5 (0.4, 0.6)	1.2 (1.3, 1.1)
Diisopropylnaphthalene (100mg kg^{-1})	0.6 (0.6, 0.5)	1.5 (1.4, 1.8)	0.5 (0.5, 0.6)	1.2 (1.4, 1.0)
Tecnazene (135mg kg^{-1})	0.7 (0.8, 0.5)	1.2 (1.2, 1.1)	0.7 (0.7, 0.6)	0.2 (0.0, 0.1)
Chlorpropham (12mg kg^{-1})	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.3, 0.0)	0.2 (0.0, 0.1)
5% LSD	0.48	0.39	0.48	0.39

mean (number of eyes open)

There was no significant difference noted between cultivars at Day 66. However, all treatments gave a significantly lower number of eyes open than the control ($p < 0.05$). All the chemical treatments except ethylnaphthalene were as effective as chlorpropham at suppressing the sprout eyes open.

There was no significant difference noted between cultivars at Day 176. It was noted that only tecnazene and chlorpropham had significantly less eyes open than the control ($p < 0.05$).

TABLE 6.4

EFFECT OF TREATMENT AND TIME ON THE RESIDUE CONCENTRATION OF PENTLAND SQUIRE TUBERS

TREATMENT	RESIDUE CONCENTRATION (mg kg^{-1})		
	NUMBER OF DAYS AFTER TREATMENT		
	21	89	168
Ethylnaphthalene (100mg kg^{-1})	1.72, 1.26	0.48, 0.39	0.10, 0.21
Diisopropylnaphthalene (50mg kg^{-1})	2.77, 1.73	2.10, 1.64	0.95, 0.72
Diisopropylnapthalene (100mg kg^{-1})	2.49, 2.38	4.22, 3.41	1.58, 1.25
Tecnazene (135mg kg^{-1})	1.72, 1.26	1.85, 1.36	1.44, 0.77
Chlorpropham (12mg kg^{-1})	0.73, 0.69	0.91, 1.33	0.73, 1.08

TABLE 6.5

EFFECT OF TREATMENT AND TIME ON THE RESIDUE CONCENTRATION OF MARIS PIPER TUBERS

TREATMENT	RESIDUE CONCENTRATION (mg kg^{-1})		
	NUMBER OF DAYS AFTER TREATMENT		
	21	89	168
Ethylnaphthalene (100mg kg^{-1})	2.12, 1.84	0.40, 0.44	0.10, 0.10
Diisopropylnaphthalene (50mg kg^{-1})	4.20, 4.33	3.84, 2.97	1.83, 1.46
Diisopropylnapthalene (100mg kg^{-1})	4.16, 4.32	4.24, 3.57	2.92, 2.34
Tecnazene (135mg kg^{-1})	4.43, 3.36	3.24, 2.56	2.83, 2.04
Chlorpropham (12mg kg^{-1})	0.90, 1.04	1.63, 1.38	0.85, 0.36

TABLE 6.6

PERCENTAGE OF SPROUTING IN TREATED TUBERS AFTER 180 DAYS

STORED AT A TEMPERATURE OF 10°C ± 2°C

TREATMENT	REPLICATE	% SPROUTING	
		MARIS PIPER	PENTLAND SQUIRE
Control	1	13.6	13.4
	2	11.7	17.5
Ethylnaphthalene (100mg kg^{-1})	1	15.4	16.6
	2	13.2	12.4
Diisopropylnaphthalene (50mg kg^{-1})	1	11.1	10.8
	2	11.1	9.4
Diisopropylnaphthalene (100mg kg^{-1})	1	10.1	8.2
	2	11.0	10.3
Tecnazene (135mg kg^{-1})	1	5.2	10.5
	2	6.8	10.8
Chlorpropham (12mg kg^{-1})	1	0.12	0.09
	2	0.16	0.33

DIAGRAM 6.1

FLOW CHART OF POTATO HANDLING OPERATION

25 October

Potatoes purchased

Pentland Squire and Maris Piper obtained and stored in sacks in a 10°C temperature controlled room

17 November (Day 0)

Potatoes treated

Treatments dusted on 10kg batches of potatoes and stored in cardboard boxes at 10°C

8 December (Day 21)

First residue sample taken

22 January (Day 66)

Sprout length and number of eyes open observed

14 February (Day 89)

Second residue sample taken

4 May (Day 168)

Third residue sample taken

16 May (Day 176)

Sprout length and number of eyes open observed

20 May (Day 180)

Total % sprouting obtained for each treatment

6.4 Discussion

In Table 6.1 it can be seen that the amount of chemical recovered from the 10g subsample of treated alumina varies depending on the chemical. Chlorpropham gave the highest recovery of 98% and diisopropylnaphthalene (100mg kg^{-1}) gave the lowest at 63%. Other chemical recoveries varied in between these two extremes.

There are two possible reasons for the loss of chemical. The first is that not all of the chemical is extracted by the diethyl ether and depending on the nature of the chemical some of it is tightly bound to the alumina and unextractable. The second more probable reason is that some of the chemical is lost under the vacuum when using the rotary evaporator over a long period of time. The theory is that the more volatile the chemical then the larger the amount of chemical lost. This could explain the low recovery of the substituted naphthalenes which are known to be fairly volatile (Chapters Two and Three) and the high recovery of tecnazene and especially chlorpropham which are a lot less volatile.

It is evident that the chemicals are being applied at a concentration less than the optimum application rate. Table 6.1 gives a guide to the concentration of chemicals actually being applied and available to inhibit sprouting.

In Table 6.2 there was no significant difference found between the sprout growth of Pentland Squire compared with Maris Piper tubers after 66 days. Thus the effect of the chemical treatments could be described collectively for both cultivars.

It was clear from the results that all the chemical treatments gave significantly shorter sprouts than the control and that both treatment levels of diisopropylnaphthalene were as effective at controlling sprout growth as the commercial sprout suppressants, chlorpropham and tecnazene.

However, after 176 days, the sprouts of Maris Piper appear to be shorter than the sprouts of Pentland Squire. Possible reasons for this are that Pentland Squire may be a 'more vigorous' sprouter than Maris Piper or that the periderms of both cultivars differ in thickness or composition and this may affect the pathway of the chemical to the site of action. The difference in sprout length between the cultivars was not found to be significant. Thus the effect of the chemical treatments could be described collectively for both cultivars. It was found that all the chemical treatments except ethylnaphthalene gave significantly shorter sprouts than the control.

It should be noted that the sprout length of the controls decreased with time. This was thought to be due to light exposure when the box was opened earlier, which stunts the sprout growth. The sprout growth was also restricted by the lids of the cardboard boxes which made the sprouts curl and the tips turned slightly black and withered.

In Table 6.3 there was no significant difference found between cultivars and the number of eyes open per tuber. Therefore the effect of the chemical treatments on the number of eyes open can also be described in general for both cultivars. After 66 days all treatments gave a significantly lower number of eyes open than the control. Most treatments gave values less than one which would suggest that may of the tubers had no eyes open.

The method of assessment (Section 6.2) involved counting the number of eyes open from 25 randomly chosen tubers and the results were presented as the mean. After 176 days only tecnazene and chlorpropham had significantly less eyes open than the control. However, the number of eyes open had increased with time which perhaps can be explained by the dissipation of the chemical with time, a situation described earlier.

However, it should be noted that there was a significant difference found between sprout lengths and number of eyes open on different days. It was thought that as the treatment time increased then the chemical treatments became less effective at inhibiting the sprout growth and number of eyes open (see Table 6.6). This is thought to be due to the dissipation mainly of the more volatile chemicals from the cardboard boxes especially in the presence of a powerful fan which was used to circulate the air around the temperature controlled room. This loss of chemical would reduce the concentration present in the headspace and lower the amount of active chemical available at the site of action. This in turn reduced the inhibiting effect on the potatoes and eyes open and sprouts started to grow and continued to grow with time. The conditions in this store were too severe. This was the first year since installation. In future this circulation will be reduced in line with commercial practice.

It is clear from Tables 6.4 and 6.5 that the tuber residues vary according to the chemical treatment applied and the length of time the sample was taken after treatment application.

It should be noted that the more volatile naphthalenes gave slightly higher residue concentrations initially than tecnazene and chlorpropham treatments but as the length of time after application increased the residue concentrations dropped for all treatments with ethylnaphthalene giving the lowest concentration after 168 days.

The variation between different chemical residues was thought to be related to the volatility of the chemical used, the more volatile the chemical then the faster an effective headspace can be built up and the more chemical will be available for adsorption by the periderm. However, as the time increased, a lot of the chemical was lost by dissipation which correspondingly reduced the chemical residue in the tuber. It was also found that as the application rate was increased for diisopropyl-naphthalene the residue also increased as expected.

Another point to note was that for the same chemical treatment and application rate the cultivar Maris Piper consistently contained a higher residue concentration than the cultivar Pentland Squire. Periderm differences between the cultivars was the most likely explanation. This was backed up by the opinion of Artschwager (1924) who indicated that the thickness of the periderm was usually a varietal feature although it can also be influenced by cultural factors. This can also be related to work done by Dalziel and Duncan (1980) which showed that tecnazene treated tubers had most of their residue concentrated in the periderm. This was thought to be the same for other chemicals used in the treatments (Dr M J Leonard - personal communication).

It was found that the two sample residues taken from each cultivar for individual treatments varied at random.

This could be explained by either (a) the uneven application of chemical to the 10kg of tubers resulting in an unrepresentative one kg sample taken for residue analysis, or (b) the position of the box in the temperature controlled room ie the position of the box in relation to the powerful fan.

In Table 6.6 it could be seen that there was a difference in the percentage of sprouting between different chemical treatments and cultivars. As mentioned previously this was probably due to the volatility of the chemical and thickness of the periderms. All treatments except ethylnaphthalene were shown to give a lower percentage sprouting than the control after 180 days giving evidence of their sprout inhibiting properties over a long time.

6.5 Conclusion

In conclusion, the work from this chapter has shown that diisopropylnaphthalene and ethylnaphthalene were significantly better than the control at regulating sprout growth and the number of eyes open in tubers. In fact, they were initially found to be as effective as the commercially used sprout suppressants. However, as the storage period increased, all of the treatments became less effective, especially the diisopropylnaphthalene and ethylnaphthalene treatments. This was thought to be due to some chemicals being more volatile than others and the loss of ethylnaphthalene and diisopropylnaphthalenes by dissipation with time.

A similar trend to that mentioned above was found with tuber residues.

Initially the treatments (with the exception of chlorpropham) gave relatively high residue concentrations which were found to decrease with time. It should be noted that the largest decrease in residue concentrations was found to be with ethylnaphthalene treated tubers. This treatment was also found to be the least effective at controlling sprout growth and the number of eyes open in the tubers.

This was almost certainly related to its being more volatile which was shown in earlier static headspace studies to be higher than that of diisopropyl-naphthalene.

The results from this experiment were in agreement with results from the dynamic headspace work carried out in Chapter Two and work carried out earlier by Beveridge et al (1983) which showed that the headspace concentrations of some volatile chemicals fell beneath the minimum effective headspace concentrations below which sprout suppressant activity ceases with time. In order to maintain sprout inhibition throughout the storage period, it was important to keep the concentration of volatile chemical above its minimum effective headspace. This could be done by re-applying the chemical throughout the storage period either as a dust or as a spray.

Residue concentration should not be a potential problem in commercial stores as naphthalene residue concentrations can be reduced by a period of airing (Beveridge 1979). This was also shown here from the results at the end of the storage experiments where it could be seen that the ethyl-naphthalene residue concentration had dropped from being the highest to one of the lowest.

The naphthalenes applied and used in the correct way have a lot of advantages and potential as future commercial sprout suppressants.

A small scale static headspace method was developed; this method was simple, fast and could easily be used in the laboratory. It was used in Chapters Two and Three to study the volatility of some substituted naphthalenes and to relate these volatiles to their sprout suppressant activities on whole tubers. This method need not be restricted to the study of sprout suppressant chemicals; it could be adapted for many uses in different industries. A similar method is used in the food industry to study food flavours and aromas.

However, this method was developed with the future purpose of being used to screen many potential sprout suppressant chemicals over a short period of time. The chemicals which would be shown to be fairly volatile could then be tested on a larger scale using whole tubers in a temperature controlled room, as described in Chapter Six. Due to the high volatility of certain substituted naphthalenes an initial application of chemical is not enough to control the sprouting over a long period of time. The results from Chapters Two and Six show that the headspace of some substituted naphthalenes build up quickly to give a high concentration of chemical around the tuber. This high concentration of chemical present at the site of action, suppressing sprout growth but with time the chemical would be lost to the atmosphere. The concentration of chemical at the site of action would be reduced and would eventually fall below the critical headspace level (the minimum concentration of chemical needed in the air to inhibit sprout growth).

If the substituted naphthalenes were to be used commercially in potato stores, then it would be recommended that the chemicals be applied as a vapour and be re-applied throughout the storage season. The store manager could use either the headspace or potato chemical residue as an indication when to re-apply the chemical.

The headspace can be measured using Tenax pre-columns which could be placed around the store. The columns can be replaced at regular intervals throughout the storage season. The headspace can be measured from the pre-columns using thermal desorption onto a gas chromatograph (as described in Chapter Two). When the chemical headspace drops and is near the critical headspace concentration, then the chemical can be re-applied. Tuber samples can be taken throughout the storage season, the residue concentration of the tubers can be extracted and analysed by GC and HPLC (as described in Chapter Five). When the chemical concentration falls and is near the critical residue level then the chemical can be re-applied.

Another advantage of applying the substituted naphthalenes is their high volatility. This would allow the chemical residue in potatoes to be reduced by a short period of airing towards the end of their storage period. This is important, especially in the future with the increase in Government legislation regarding chemical residue limits in food.

Wound healing studies were carried out on tuber discs in Chapter Four. The method was not suitable to show the effects of the substituted naphthalenes on the wound healing process. The effects were masked by the adverse effects of the organic solvent on the discs.

In the future, when using this method, it would be more realistic to apply the substituted naphthalenes as a vapour. The effect of the substituted naphthalenes should also be studied on whole tubers in storage conditions similar to those described in Chapter Six.

The work in this thesis has shown that certain substituted naphthalenes have potential to be used as a commercial potato sprout suppressant. The next step would be to set up trials in a commercial potato store using the most promising substituted naphthalenes from laboratory experiments.

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