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STUDIES ON THE POTATO SPROUT

SUPPRESSANT/FUNGICIDE TECNAZENE

MARTIN JOHN LEONARD

Thesis presented for the
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Agricultural Chemistry
University of Glasgow

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Summary

This thesis describes work relating to the use of potato sprout suppressant chemicals - compounds which are used to control the sprouting of potatoes during extended storage.

In particular, several implications of the use, in commercial situations, of one of these compounds, tecnazene, were assessed.

This included a study on the effects on subsequent field growth of a range of tecnazene residues in seed potatoes at planting - this chemical having previously been cited as a cause of delayed emergence and reduced yield. A storage experiment and field trial are described from which it was concluded that a specific effect on emergence and yield cannot be attributed to a given residue at planting, although this can be a useful guide in broader terms. It was considered that a residue at planting of less than 1 - 1.5 mg/kg on a whole tuber basis would have little effect on emergence and yield. It was also noted that tecnazene treatment resulted in an increased number of stems from each tuber, and in turn to a higher proportion of smaller tubers. This effect was noted for even the lowest application rate indicating that tecnazene treatment could perhaps be used to manipulate tuber size distribution while having little or no effect on emergence and total yield.

The method of residue analysis employed in the above work is described in a separate section, along with an assessment of tecnazene toxicology.

Two other commercial problems linked to the use of sprout suppressants were investigated, both of which are related to the use of tecnazene in large scale commercial potato stores:

The effect of vapour phase application of tecnazene on the healing of tuber wounds is discussed, based on results obtained using a rapid method for assessment of the degree of suberisation and periderm development. Tecnazene treatment appeared to have no detrimental effect on wound healing in contrast to the other major sprout suppressant chemical, chlorpropham, which clearly inhibited the process. This is an important point as incomplete healing of tuber wounds results in water loss and facilitates infection by fungal or bacterial pathogens.

The effect of tecnazene, either alone or in combination with chlorpropham, on the incidence of internal sprouting in treated tubers was also studied. This phenomenon occurs when sprouts grow into a tuber rather than out and away from tuber, and renders such tubers useless for processing into crisps. The results indicated that while internal sprouting can occur in the absence of tecnazene, the pattern of sprouting induced by tecnazene treatment would appear to increase the incidence of this disorder.

The remaining section of work in this thesis was based on a subject of less practical significance, but of much interest - the mode of action of tecnazene. It was considered that tecnazene might act by inhibiting gibberellin biosynthesis or action and for this reason bean seedling bioassays were conducted in which very dilute aqueous solutions of tecnazene were fed to the plants. This did result in some growth inhibition when compared to controls

although it was not nearly as marked as that effected by a known gibberellin biosynthesis inhibitor which was included in these assays, a fact which could possibly be partly explained by the very limited aqueous solubility of tecnazene. Any growth inhibition induced by tecnazene could be overcome by application of exogenous GA_3 , although, again partly for reasons of solubility, this need not necessarily implicate gibberellin biosynthesis inhibition or site blocking. Further work, probably at the cell constituent level, is required.

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CHAPTER 1

Introduction

1.1. The Potato Crop

The potato, as a staple dietary item, is an important source of complex carbohydrate in many countries throughout the world, with average human consumption in Britain alone around 110 kg per head of population per year.

This figure includes potatoes which have been processed in one way or another, and which comprise a significant proportion of total human consumption - almost 24% during season 1986-87 (Anon. 1988).

As the potato, under UK conditions, is an annual crop, storage of harvested tubers for a period of up to 9 months is necessary in order to meet demand throughout the year for both raw potatoes and processed potato products.

Tubers must be stored with the objective of maintaining product quality throughout the holding period. The main obstacles to this are sprouting following break of dormancy, and disease development.

1.2. Sprouting

On reaching maturity potatoes enter a period of dormancy during which sprout growth does not occur. Burton (1966a) found that this period varies between 5 and 14 weeks after harvest and that it depends on factors such as variety, climate during the growing season, and storage temperature. Dormancy breaks more readily when potatoes have grown during a season which has been hot and dry, or when the storage temperature is increased within the general range 4°C to 23°C. Pathogenic infection and mechanical damage to tubers can also lead to earlier dormancy break.

Tuber sprouting, the result of dormancy break, is undesirable as it can lead to:

1. Tuber softening due to moisture loss, and a concomitant loss of saleable weight.
2. Difficulties with the unloading and dressing of the potatoes.
3. Metabolic changes which can affect the quality of the potatoes e.g. an accelerated rate of sweetening in sprouted potatoes renders them unsuitable for processing; also, sprouted potatoes are of lower nutritional value than those which have not sprouted.
4. Overheating in the store associated with an increase in the respiration rate of the sprouted tubers.
5. Development of internal sprouts.

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

1. Control of storage temperature
2. Use of chemical sprout suppressants
3. Use of light
4. Irradiation of tubers

1.2.1. Storage temperature

While different potato varieties tend to vary in their response to different storage temperatures there is, in general, a common pattern of growth at a constant temperature. Storage at or below 5°C results in a very slow rate of sprout growth - likely to be imperceptible in varieties which sprout slowly even under favourable conditions. However, varieties which are more vigorous under favourable conditions may show slight sprout growth after prolonged storage at temperatures as low as 2°C (Burton, 1966b). An increase in the rate of sprout growth will occur as the storage temperature is increased above 2°C, reaching an optimum at round 15-18°C. Further increases in temperature lead to reduction in the growth rate over an extended period, although initially sprout growth may be more rapid at 23-25°C (McGee et al., 1986).

From the preceding it is obvious that low temperature storage (4°C or less) could be used to prevent significant sprout growth during storage. There are, however, problems associated with this type of storage in that it can lead to increased losses

due to gangrene and skinspot. Low temperature storage also results in a build up of reducing sugars - a factor which makes this type of sprout control impracticable for tubers which are to be processed into crisps as the product is usually unacceptably dark brown in colour. The Maillard reaction between the amino function of amino acids and the carbonyl function of reducing sugars is responsible for this discolouration (Gray and Hughes, 1978a).

Although short-term high temperature (ca. 15°C) reconditioning of potatoes stored at low temperature can sometimes lower the level of accumulated reducing sugars (Burton and Wilson, 1978; Storey and Shackley, 1987), the best method of avoiding this problem is to store the tubers at a temperature of 8 - 10°C (Anon. 1985a). This would obviously result in significant sprouting and thus necessitates the use of another method of sprout control - usually chemical.

1.2.2. Chemical Sprout Suppressants

In Britain three compounds are currently approved for use as sprout suppressants on ware potatoes. These are (Figure 1.1):

1. Chlorpropham (CIPC) or a mixture of chlorpropham and the related compound propham (IPC).
2. Tecnazene (TCNB).

Chlorpropham (isopropyl N-(3-chlorophenyl) carbamate) and its unchlorinated analogue propham are chemicals which are normally

applied as a thermal fog in bulk potato stores - usually at 10-20 mg of active ingredient per kilogramme of potatoes (Anon., 1985b). This is achieved by hot plate vapourisation of a methanol formulation of the chemical. A granular formulation of chlorpropham is also available and may be used in box stores. In general, application as a thermal fog is more efficient than application on a solid carrier, although this may be less true of box stores where the fog is not forced through the potatoes as in a bulk store and is thus more likely to follow air passages between boxes (Duncan et. al., 1986).

Chlorpropham, which also has herbicidal properties, acts by inhibiting mitosis (Corbett et al., 1984a) and as such, when present in sufficient concentration, is a very efficient inhibitor of sprout growth. The fact that it can be applied as an aerosol is an advantage in that the chemical can be re-applied during the storage season, while the only, admittedly significant, problem associated with use of the chemical is its detrimental effect on the healing of tuber wounds (McGee, 1984; Leonard et al., 1986; Audia et al., 1962; Reeve et al., 1963). This precludes the use of chlorpropham on tubers immediately after they have been loaded into store if the problems of pathogenic infection associated with incomplete wound healing are to be avoided. It is therefore recommended that chlorpropham only be applied after a sufficient period of "curing" (wound healing), which may extend six weeks into storage. In fact 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", and thought to be an abnormal form of skinspot. An increasing practice over recent years has been control of sprouting during the early part of the storage season by tecnazene application - a chemical thought to have no adverse effect on wound healing.

Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene), like chlorpropham, is a sprout suppressant chemical which is volatile and is active in the vapour phase. It is predominantly applied as a dust at the rate of 135 mg/kg (Anon., 1985b), although a liquid formulation is available for application as a thermal fog. Based on 1983 figures, the cost of tecnazene application is approximately four times that for chlorpropham (Anon. 1982), and is one of the reasons for its restricted use in bulk storage facilities. It is also more volatile than chlorpropham and controls sprouting by a mechanism which, although not yet fully understood, is certainly different to that of chlorpropham. For the latter reason tecnazene might be considered a less efficient sprout growth inhibitor than chlorpropham as it seems to reduce the rate of growth and, unlike chlorpropham, does not cause cessation of cell division in the meristematic region of the growing sprout. Tecnazene is, however, still used on a fairly large scale by individual growers (Anon., 1981) and is the only sprout suppressant chemical which can be used on seed potatoes, - albeit ostensibly in its alternate role as a fungicide for the control of *Fusarium* spp., the causal organisms of the condition known as dry rot. The use of tecnazene in this way, i.e. on seed potatoes, is a rather controversial practice as residues of the chemical have been implicated in the poor growth of

treated tubers.

One other sprout suppressant chemical, maleic hydrazide (1,2-dihydro-pyridazinedione, Fig. 1.1.), has recently been used on a trial scale in the U.K. It is completely different from the chemicals which have already been mentioned as it is not a storage treatment but is applied to foliage prior to harvest. Application in this way is normally as the water soluble sodium or potassium salt, usually at the rate of 2.5 kg per hectare 3-5 weeks prior to senescence or defoliation. If applied less than 3 weeks before death of the foliage inhibition may not be completely effective because of insufficient translocation of the compound into the daughter tubers. Application more than 5 weeks prior to senescence may affect yield and result in mis-shapen tubers (Burton, 1978a). The latter effect is attributed to the mode of action of maleic hydrazide which, like chlorpropham, affects cell division (Corbett et al., 1984b).

The major drawback to its use under U.K. conditions, apart from implication as a potential carcinogen, is that the climate is often unfavourable for efficient uptake and translocation. This can result in a residue in the tubers which may not be sufficient to control sprouting throughout the storage season. This is not a major problem where the store design facilitates application of chlorpropham, but can be serious in smaller stores used by individual growers. Maleic hydrazide, like chlorpropham, should never be used on seed potatoes.

1.2.3. Light

Exposure of tubers to light can be used to control sprouting, although the degree of control depends on the wavelength of light used. Wassink et al. (1950) found that a very considerable reduction in sprout growth could be achieved by exposure to light from the blue, violet, red and infra-red regions of the spectrum when compared to those on tubers held in the dark. A reduction in sprout elongation as a result of exposure to yellow and green light was also observed, but was not as marked as that at other wavelengths. In all cases the degree of growth inhibition was dependant on the intensity of the irradiation.

More recently McGee et al. (1987), using narrow-band width light sources, showed a peak of growth inhibition at 707 nm (in the red region), with additional inhibitory activity in the 400-500 nm range (blue region of the spectrum). They found that broad-band sources were generally less inhibitory and that their effects were non-additive. The influence of intensity of irradiation noted by Wassink et al. (1950) was also observed.

The inhibitory effect of light on sprout growth which, in general, results in sprouts which possess chlorophyll and are shorter and sturdier than those grown in the dark, is one which is not readily applicable to large scale storage situations. It is, however, made use of during chitting of seed potatoes in the U.K., and is becoming of more widespread use in the tropics for the control of sprout growth in small scale situations using natural light.

1.2.4. Irradiation

Potato sprout growth can be delayed or prevented by irradiating the tubers with Gamma-rays, electrons or X-rays (Burton, 1978b). Although doses less than 10 Gy stimulate sprouting, potatoes irradiated with doses in the range 30-150 Gy do not sprout when stored. The inhibition is usually irreversible and requires only a single treatment regardless of subsequent storage conditions.

Irradiation of potatoes, however, affects more than sprouting. It has a detrimental effect on the wound healing process and thus if conducted immediately after harvest can result in pathogenic infection of tubers via lesions which invariably occur during harvesting and handling operations, and which are likely to have incompletely healed. Irradiated potatoes also tend to undergo blackening during or after cooking - a phenomenon which has been linked to increased polyphenol content of the potato tissue and interaction of the polyphenols with ferrous salts present in the potato (Urbain, 1986). Another undesirable effect of irradiation to control sprout growth is that it results in changes in the reducing sugar content of the treated tubers. Gamma-irradiation causes a marked but temporary increase in the level of reducing sugars, which is dose dependant (Burton et al., 1959; Muir et al., 1987). Early and exacerbated senescent sweetening would also appear to be a consequence of this type of treatment.

Although the mechanism by which irradiation inhibits sprout growth is not yet clear, speculation has included:

1. Interference with nucleic acid synthesis in the meristematic region of sprouts.
2. Disturbance of the phosphorylation process.
3. Inhibition of the formation of the auxin, indole acetic acid.
4. Chromosomal changes in the cells of the meristematic tissue which prevent normal cell division and growth.

While it has been tested in some countries, large scale use of irradiation to control potato tuber sprouting would still appear economically non-viable because a radiation source of the size required is not readily portable and the cost of transportation of potatoes would be prohibitively expensive.

1.3. Thesis objectives

The work encompassed in this thesis addressed several problems of commercial significance in the potato industry, all of which were related to use of the sprout suppressant/fungicide tecnazene.

Interest in this chemical was first stimulated during vocational employment as a seed potato inspector for the Department of Agriculture and Fisheries (Scotland). This work involved contact with a number of seed potato growers, from which it was clear that tecnazene played an important role in the control of sprout growth during storage, particularly for smaller producers

using makeshift storage areas. However, it was also evident that the use of tecnazene in this way could be rather hazardous as residues of the chemical had on several occasions been cited as the reason for the poor field growth of treated seed. The work described in Chapter 3 was designed to assess the effect of a range of tecnazene residues in tubers at planting on aspects of subsequent growth. It was hoped that the results could be used to predict the effect of a given residue at planting on emergence and yield from the crop, and to determine a level below which there would be no detrimental effect on crop performance.

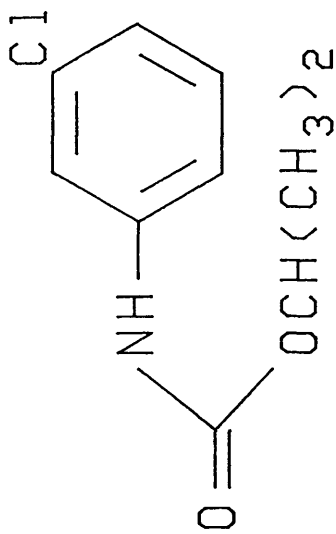
Tecnazene is also used during large scale storage of ware potatoes and the work described in Chapters 5 and 6 was pertinent to this.

In Chapter 5 the results of a study on the effect of tecnazene on the healing of tuber wounds are discussed. This is an important point as early application of a chemical which inhibits this process, such as the other major sprout suppressant chlorpropham, results in water loss from these wounds and, more importantly, facilitates infection by bacterial and fungal pathogens.

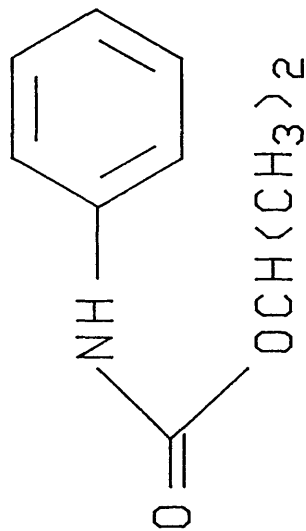
The influence of tecnazene treatment, either alone or in combination with chlorpropham, on the incidence of internal sprouting in stored potatoes was also assessed and is the subject of Chapter 6. This disorder results from growth of sprouts into the tuber tissue rather than out and away from the tuber and can be a major problem in storages containing potatoes destined for processing into crisps. Indeed, it was discussions with technical

personnel from potato processors which led to initiation of this work.

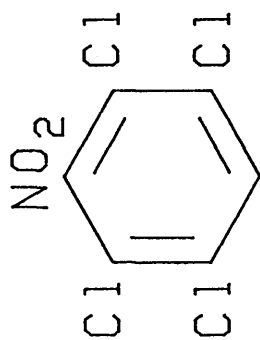
Finally, personal curiosity during observations of the various effects of this rather unique chemical was the spur to the work described in Chapter 4, relating to the mode of action of tecnazene. This was intended to test a theory that gibberellin biosynthesis or site-blocking is the mechanism by which the effects of tecnazene are induced.



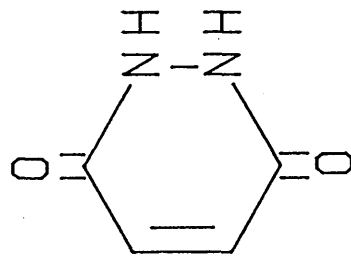
Chlorpropham (CIPC)



Proptham (IPC)



Tecnazene (TCNB)



Maleic Hydrazide (MH)

Fig.1.1 Commercial potato sprout suppressant chemicals.

CHAPTER 2

Tecnazene - Residue analysis and toxicology

2.1. Residue Analysis

2.1.1. Introduction

Analysis of residues which result from the treatment of potato tubers with tecnazene is a subject which has assumed increased importance in light of recent legislation regarding maximum residue levels (MRL's) of pesticides in food - The Pesticides (Maximum Residue Levels in Food) Regulations 1988. Although the document does not specify a UK maximum limit for tecnazene in potatoes, this situation is likely to change within the next few years with a residue limit of around 1 mg/kg a distinct possibility.

While most important in terms of human toxicity of the ingested chemical, tuber residues of tecnazene have also been studied because of their implication in the poor growth of treated seed potatoes. The latter was the basis of the tecnazene residue analysis conducted during the work which will be described.

The analytical options for tecnazene analysis, the method finally chosen, and the method of extraction of the chemical from plant tissue will now be described.

Tecnazene is a polychlorinated aromatic hydrocarbon. It has a melting point of 99°C, a boiling point of 304°C (with decomposition) and a vapour pressure of 0.0024 mBar at 15°C, the latter resulting in appreciable volatility at room temperature (Hartley and Kidd, 1983).

It is only very sparingly soluble in water but is much more soluble in ethanol (up to 4%) and is readily soluble in other organic solvents such as benzene and chloroform.

While colorimetric methods for the analysis of tecnazene have been reported (Auerbach, 1950; Canbach and Zajaczkowska, 1950; Higgins and Toms, 1959), they are unsuitable for the analysis of plant tissue residues as they are non-specific and offer significantly lower sensitivity than can be achieved using other techniques.

The methods best suited to the analysis of tecnazene residues are the chromatographic techniques of gas chromatography (GC) and high performance liquid chromatography (HPLC). Both allow separation of the components of an extract and subsequent detection of those of interest.

HPLC facilitates analysis of compounds not volatile enough, and generally more polar, than those which can be analysed without derivatization by GC. It is also suitable for larger molecules or thermally unstable compounds, and has the added advantage that most of the detectors used are non-destructive.

Bushway et al., (1984) reported an HPLC method for the determination of tecnazene in potatoes. This involved initial extraction of the tecnazene from potato peels or flesh by blending

with acetone, followed by analysis on a C₁₈ column and detection by U.V. absorption at 210 nm. They employed a mobile phase of acetonitrile - methanol - water (35:35:30) at a flow rate of 1.0 cm³/min, resulting in a retention time of approximately 10 minutes. Samples containing tecnazene at a concentration of 1 ppm or higher were directly injected, while samples containing less than 1 ppm tecnazene were partitioned into hexane and cleaned-up using alumina mini-columns.

Average recoveries varied from 86% to 97% with the lower limit of detection reported at 0.08 ppm.

A method for analysis of tecnazene residues by gas-liquid chromatography was described by Dalziel and Duncan (1974). Extraction of the chemical from plant tissue was achieved by blending with ethanol. This was followed by partition into hexane and a wash with 10% sodium carbonate in order to remove ionisable interferences such as fatty acids. The hexane solution was then dried, further cleaned-up using an alumina column and reduced in volume before analysis by gas chromatography with flame ionisation detection. A detection limit of 0.01 ppm was reported.

The latter method did in fact form the basis of the analytical procedure employed throughout this work for the extraction and determination of trace amounts of tecnazene in tuber tissue. Gas chromatography was the preferred technique because although the extraction procedure was more protracted than for HPLC, a choice of gas chromatograph detectors offered potentially greater specificity and sensitivity.

The flame ionization detector is the detector most commonly

used with gas chromatography. It consists of a small hydrogen-air flame burning at a metal jet situated at the end of the column. Organic compounds eluted from the column burn and form ions in the flame which then travel to a collector electrode held at a negative potential. The resulting current is proportional to the concentration of charged species present in the flame and the chemical structure of the eluted molecules. This detector shows high sensitivity to virtually all organic compounds and has good linearity over a wide sample concentration range. It proved to be the most suitable form of detection for all the tecnazene residue analyses conducted during the work which will be described.

Gas chromatography also offered the potential use of more specific detectors such as the electron capture detector (ECD). This mode of detection is highly sensitive to halogenated compounds and as such is often used for the analysis of chlorinated pesticides and herbicides. It is, however, more problematic than flame ionization detection and has a rather smaller linear range (Braithwaite and Smith, 1985).

Gas chromatography can also be combined with other powerful analytical techniques such as mass spectrometry. Although this hyphenated technique was not used in the tecnazene residue analysis which will be reported, it is important in the identification of metabolites of pesticides and was in fact employed in a supervised undergraduate project to identify a tecnazene metabolite. This will be further discussed later.

2.1.2 Extraction method

1. A representative 100 g sample of tuber tissue was taken from a macerate of approximately 8 - 10 tubers.
2. The sample was placed in a Waring electric blender cup along with 100 cm³ of absolute ethanol (James Burroughs Ltd., Witham, Essex) and the mixture homogenised for 1 minute.
3. 100 cm³ of hexane (Rathburn Chemicals Ltd., Walkerburn, Scotland) was then added and the mixture blended for a further 1 minute.
4. The homogenate was filtered under vacuum through Whatman No. 1 filter paper. The blender cup was rinsed with two separate 50 cm³ portions of hexane which were also passed through the filter paper.
5. The filtrate was then quantitatively transferred to a 500 ml separating funnel using two 15 cm³ hexane washes. 100 cm³ of saturated sodium chloride solution was also added in order to increase the ionic nature of the aqueous/ethanol phase and thus encourage greater partition of dissolved tecnazene from the aqueous/ethanol phase to the hexane phase.
6. The hexane layer was collected and any residual water removed using approximately 5 g of anhydrous sodium sulphate (BDH Ltd., Poole, England).
7. After filtering the solution and washing the sodium sulphate residue with 15 cm³ hexane, the combined extract was reduced in volume to approximately 2 cm³ using a rotary evaporator. During this stage the water bath temperature was kept below 35°C in

order to avoid loss of tecnazene.

8. The concentrated extract was transferred to a 2 cm³ volumetric flask and made up to volume before analysis by gas chromatography as described below.

Clean-up

Further sample clean-up, normally only used for samples containing less than 1 mg/kg, was achieved using SEP-PAK silica cartridges (Waters Associates, Milford, Mass., USA). 2 cm³ of hexane were run through each mini-column before 1 cm³ of the concentrated tissue extract was introduced on the column using a 5 cm³ syringe. The column was then leached with a further 5 cm³ of hexane, the first 4 cm³ of which were collected - the use of standard solutions having previously shown this fraction to contain an average of 96% (S.D. 2.2) of the tecnazene applied to the column. Volume reduction to 2 cm³ was again used to concentrate the sample.

2.1.3. Gas Chromatography

Gas chromatograph

All of the results which will be reported were obtained using a PYE PU 4500 gas chromatograph fitted with a flame ionization detector.

Columns

The analyses were carried out using glass columns 1 m x 4 mm (I.D). These 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) - at 5% and 10% loading respectively.

OV17 is a semi-polar phase which, for the same carrier gas flow rate, retains tecnazene for longer than the non-polar OV101 phase.

Using either phase tecnazene was eluted in less than 5 minutes. The injection volume was normally 5 μ l.

Gas chromatograph conditions

Temperatures: Column oven 190°C (isothermal analysis)

Injector 225°C

Detector 250°C

Gas flow rates:

Column carrier gas (nitrogen) 30 cm³/min

Air (to flame ionization detector) 180 cm³/min

Hydrogen (to flame ionization detector) 30 cm³/min

Data collection

Data collection, calibration and integration was achieved using a Shimadzu C-RIB recorder/integrator.

Typical chromatograms for a tecnazene standard and for a tuber tissue extract are shown in Fig. 2.1.

Recovery

Addition of known amounts of tecnazene to samples of tuber tissue which had not been exposed to tecnazene showed the above method to result in recovery, on average, of 89% (S.D. 3.4) of the chemical. All results were corrected for this figure.

2.2. Toxicology

As previously mentioned tecnazene residues are also of importance, indeed increasingly so, in terms of human toxicology - ingestion of trace amounts of the chemical being an almost unavoidable consequence of the consumption of potatoes or potato products (Heikes et al. 1979, Gartrell et al., 1985).

As exposure to a large single dose of the chemical is extremely unlikely it is therefore the chronic effects of the compound which are important i.e. the effects of repeated exposure to small non-lethal doses of the compound.

In a relatively recent report sponsored jointly by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) (Anon, 1983) the data available on the chronic effects of exposure to tecnazene was considered inadequate. Of the work which has been reported Buttle and Dyer (1950) described the effects, in mice and rats, on feeding diets

containing tecnazene at 10, 100 and 1000 times the rate normally applied to potatoes (135 mg/kg). No obvious effects were evident for the diet containing the lowest level, while that with the intermediate level completely inhibited growth and that with the highest level resulted in death, from fatty degeneration of the liver, of 20% of the mice. Tecnazene appeared to be more toxic in rats, with all those fed the highest level diet dead by the end of the 5 week experiment. Abrams et al. (1950) found that the toxicity of tecnazene in pigs is of the same order as for rats.

Buttle and Dyer (1950) observed no ill effects when rats were exposed to tecnazene vapour. Using rabbits they also found that exposure to tecnazene did not result in skin or eye irritation but that it could produce skin discolouration.

Tecnazene was assessed by Searle (1966) as a potential carcinogen. When applied to mice it was found to result in the formation of small tumours, all but one of which were benign.

Mutagenesis testing of tecnazene as part of the National Toxicology programme in the USA yielded no positive results and tecnazene was judged to be non-mutagenic under the conditions used (Yoon et. al., 1985).

From the available data tecnazene is classed as an experimental neoplastigen of moderate to high mammalian toxicity (Sax, 1984).

Although the FAO/WHO considered further toxicological studies on tecnazene desirable, they were able, using data from a study on the related compound quintozone (pentochloronitrobenzene), to recommend an acceptable daily intake (ADI) for man of

0 - 0.01 mg/kg bw.

An important point when discussing tecnazene residues is that the chemical can be metabolised in biological systems. This metabolism forms part of the basic defence mechanisms possessed by all organisms and which are designed to protect them from the detrimental effects of small quantities of various foreign compounds.

This is achieved by either altering the molecular structure of the compound so that the product is less toxic than the original substance, or by increasing the polarity and aqueous solubility of the compound and thus facilitating its excretion from the organism.

An example of tecnazene metabolism in a higher animal was reported by Bray et al., (1953). Dosing rabbits with an aqueous suspension of tecnazene they found that the compound was not readily absorbed from the gut, with an average of 66% of the dose excreted in faeces. The proportion taken-up was, however, greatest when low levels of the chemical were fed - up to 70-78% of a 10 mg dose. For 0.58 g dose 90% of the absorbed chemical was excreted in the urine in the form of metabolites, the most important of which were 2,3,5,6-tetrachloroaniline, a mercapturic acid - N-acetyl-S-(2,3,5,6-tetrachlorophenyl)-L-cysteine and a glucuronide. 4-amino-2,3,5,6-tetrachlorophenol and ethereal sulphate were also found. A negligible amount of the original compound was detected in the urine.

The mercapturic acid is formed as a result of conjugation of tecnazene with glutathione - a tripeptide composed of glutamic acid cysteine and glycine residues - involving glutathione s-aryl

transferase enzymes. If the glycine and glutamic acid are hydrolytically removed, a cysteine derivative of the compound remains. Acetylation of the amino group of the cysteine then frequently occurs so that the excreted material in many higher animals is an N-acetyl cysteine derivative - referred to as a mercapturic acid.

A glucuronide results from conjugation with glucuronic acid from uridine diphosphate glucuronic acid (UDPGA), and involving glucuronyl transferases.

The other metabolites are the products of oxidative, hydrolytic or reductive changes again often involving various enzyme systems.

Several metabolites of tecnazene have also been found in extracts of potatoes which had been treated with tecnazene. Heikes et al. (1979) found the following metabolites.

Tetrachlorobenzene
Trichloronitrobenzene
2,3,5,6-Tetrachloroanisole
Tetrachloroaniline
Tetrachlorothioanisole
Pentachloronitrobenzene
Tetrachloro-p-nitroanisole
Tetrachloro-p-anisidine

Of these, however, trichloronitrobenzene, tetrachlorobenzene and pentachloronitrobenzene were detected in the tecnazene formulation applied to the potatoes.

The disappearance of added tecnazene, and concomitant formation of tetrachloroaniline in soil systems was noted by McGibbon (1984). He found this to be more marked in anaerobic (flooded) soil than aerobic soil, and that addition of ammendments such as glucose or yeast extract increased the rate at which this occurred. Addition of mercuric chloride (an inhibitor of microbial activity) decreased the rate of conversion.

The effect of anaerobic conditions suggests that this conversion could be the result of chemical reduction rather than microbial activity. However, the effects of ammendments, including mercuric chloride, do infer the involvement of micro-organisms. These routes to the formation of the tetrachloroaniline could be operating concurrently.

This type of conversion was also noted in a closely supervised undergraduate project within this department in which the presence of tetrachloroaniline in a soil extract was confirmed by high resolution gas chromatography - mass spectrometry.

Although the metabolites listed form the initial products in the degradation of tecnazene and aid its extraction from living organisms, they are of significance as the toxicity of some of these compounds has been shown to be greater than that of the original compound (Kaiser et al., 1985). Also, for metabolites such as tetrachloroaniline there is the potential for dechlorination and dimerization to form azobenzenes. These are genotoxins and have been reported as metabolites of the other major potato sprout suppressant chlorpropham (Worobey et al., 1987).

Further work on the metabolites of tecnazene would be desirable.

Although maximum residue levels in food have recently been recommended for a range of pesticides in the UK, tecnazene has not been included and is still subject to "good agricultural practice" (GAP) as recommended in the Approved Products for Farmers and Growers handbook.

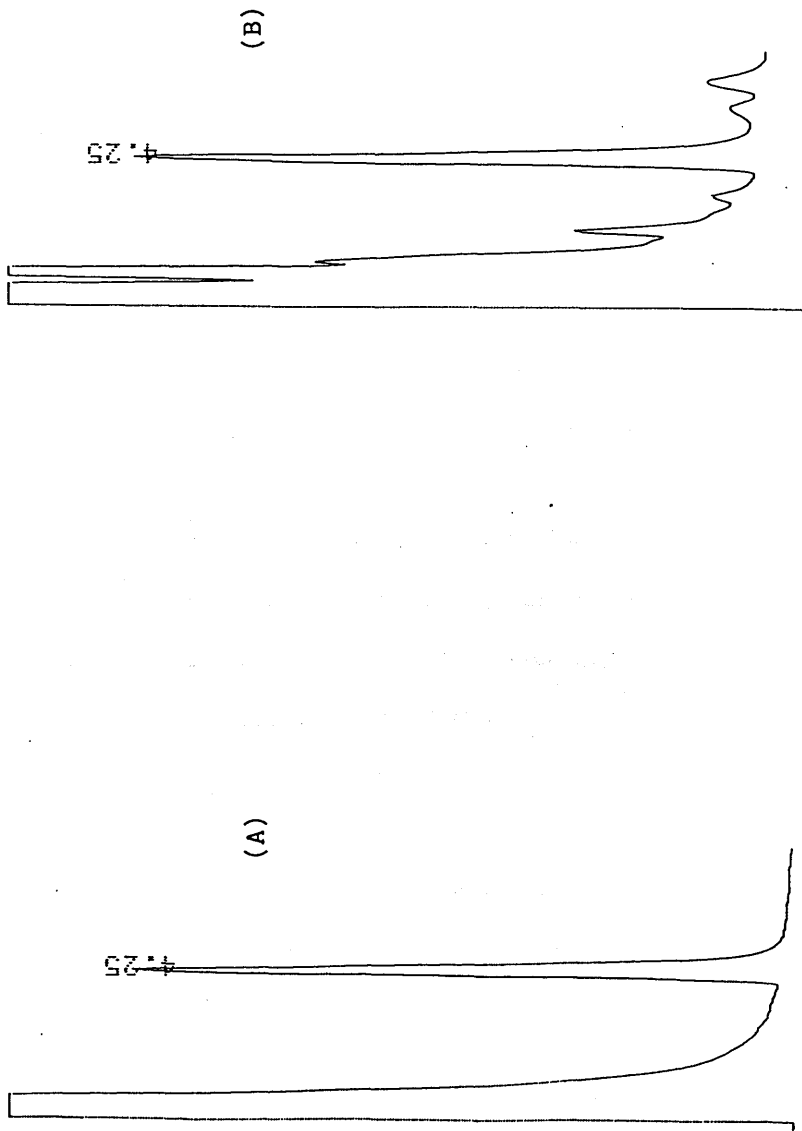


FIG. 2.1. Gas chromatograms of (A) 500 ng of a tecnazene standard and (B) a hexane extract of tuber tissue, both analysed on an OV17 column.

CHAPTER 3

The effect of a range of tecnazene residues on aspects of the subsequent growth of treated seed

3.1. Introduction

The fungicide/sprout suppressant tecnazene, 1,2,4,5-tetrachloro-3-nitrobenzene, is a chemical which is used, although to a lesser extent more recently, on a significant proportion of the stored seed potato crop in Scotland.

Its use in this way has been ostensibly as a fungicide - the Seed Potato Regulations (1984) prohibit the use of sprout suppressant chemicals on potatoes certified as seed - however some control of sprouting has obviously been an important factor, particularly as control of fungal growth may be less than complete at the level of chemical often used.

The major drawback to the use of tecnazene in this way has been its implication in the poor growth of treated seed. Research has indeed been carried out on the effect of tecnazene treatment on the subsequent growth of treated seed and will now be reviewed.

Brown (1947), working with clamped potatoes of two varieties, found that seed treated with tecnazene at 50-80 mg/kg slightly out yielded desprouted controls when planted out, however he suggested that for the purposes of crop production tecnazene suppressed sprouting too much.

In Sweden, Emilsson and Gustafsson (1951) used tecnazene on one variety at 75 mg/kg and 225 mg/kg. At these levels there was a marked decrease in yield (34-36% reduction compared to untreated) when potatoes were treated in January, but not when they were treated in November (5-7% reduction). Tecnazene did not delay development, decrease the number of plants, nor decrease yield if the treated potatoes were green sprouted or aired out sufficiently after treatment but before planting. They recommended that, regardless of variety, dosage of tecnazene should not exceed 12 g per 100 kg of potatoes, nor be applied later than 1st January preceeding spring planting. Adequate airing out before planting required at least 2 weeks.

In a series of trials using clamped tubers Brown and Reavill (1954) showed that treatment with a commercial formulation of tecnazene, applied at normal (135 mg/kg) or double rate, led to a reduction in total yield when compared to untreated, chitted tubers. Reduction in yield was greatest with early harvesting (up to 35%), and was much reduced if harvesting occurred after natural sence (maximum 18%). Results for hand desprouted controls were very similar to this. Treatment at half recommended rate, or treatment midway through the storage season, led to a lower reduction in yield, again dependant upon time of harvest. Yield of treated tubers aired for 7 weeks prior to planting was similar to that from chitted tubers. Tecnazene treated tubers also produced more stems per plant than either chitted or desprouted controls. This was reflected in a higher seed/ware ratio.

Brook and Chesters (1957) treated tubers immediately after lifting with tecnazene (ca. 20 mg/kg), or with the commercial formulation Fusarex (ca. 40 mg/kg). A range of early, medium and late maturing varieties were involved. The marketable yield from the treated seed was in the range 60-120% of that from the controls, although a reduction in yield did occur in the majority of cases. Treatment sometimes led to an increase in the proportion of small tubers.

From work carried out in New Zealand over three seasons, Driver (1961) found that tecnazene treatment at approximately 135 mg/kg, followed by clamp storage for two months prior to planting, led to a delay in emergence, an increase in the proportion of small tubers, and a trend towards a slight reduction in yield (not statistically significant or consistent). A 3 week airing period (under light) led to emergence which was almost as rapid as that for chitted controls, and better than that for controls planted straight from the clamp. A cultivar dependant response to tecnazene treatment is evident from some of the results presented.

In the USA, Murphy and Govan (1967) from trials conducted over two seasons and using cut seed, showed that treatment during December with Fusarex (6% or 20%) at ca. 100 mg of active ingredient per kilogramme of potatoes, followed by storage at 45 °F (7.2°C), neither delayed emergence or reduced yield when compared to either unsprouted or sprouted controls. Application as an aqueous emulsion or as an aerosol also had no significant effect on emergence or yield, although application in the vapour phase as sublimed crystalline material did lead to a significant reduction

in final yield but, surprisingly, did not delay emergence. The amount of active ingredient applied by these methods was unclear. Fusarex application immediately prior to planting had no significant effect on emergence or yield. The greatest effect on emergence or yield during these trials was noted with plants grown from seed tubers which had been desprouted either once or twice.

Murphy et al. (1968) assessed the effect of Fusarex treatment on the subsequent growth of three varieties, over five locations in North Eastern USA and Canada. Tubers were treated with Fusarex (6%) to give an application rate, of ca. 100 mg a.i./kg. Tubers were then stored at 45 °F (7.2°C) until removed for cutting at the end of March. Untreated seed was kept at 38 °F (3.3°C). Desprouted seed was also assessed. The emergence data, despite considerable variation between variety and locations, showed that, in general, plant emergence from treated seed was delayed compared with desprouted and untreated seed. The effect of date of treatment on initial emergence was too erratic between locations and varieties to draw a conclusion. Total emergence appeared to be better, in most cases, when treatment was in January or March, rather than November. Seed treatment with Fusarex decreased yields at two of the locations assessed. At all other locations yields were slightly better when seed had been Fusarex treated. No significant statistical differences in yield occurred between treatments at any of the five test locations when varieties and locations were averaged.

Further work by Murphy et. al. (1969) involved the same cultivars, similar storage conditions and Fusarex treatments, but with the addition of a "Fusarex plus airing" treatment. As in the previous work the results were rather variable. The general trend, despite a few exceptions, was for initial emergence of desprouted and Fusarex treated seed to be delayed when compared to untreated seed. The effect of Fusarex treatment on total emergence was rather erratic - presumably partly attributable to the use of cut seed. When averaged over all locations, yield of November treated seed was significantly lower than for untreated, irrespective of cultivar. January treatment also resulted in reduced yield, although this was statistically significant only for two of the three cultivars. January treatment with two weeks airing prior to cutting led to significant yield reductions for all cultivars. The overall picture, however, is of rather variable effects, depending on growing situation and cultivar.

Seed treatment with a commercial tecnazene dust, at an active ingredient application rate of 100 mg/kg, led to a significant increase in mean emergence time and reduction in yield, as assessed by Dalziel and Duncan (1975). This work involved tubers of the cultivar Golden Wonder, which were treated in October, stored in 50 kg bins at 10°C, and aired for one month prior to planting. In a supplementary exercise, tubers of twelve other varieties were treated at twice the recommended rate at the end of March. These were stored identically, but were not aired before planting. For all cultivars tecnazene treated seed showed a significant delay in emergence when compared to controls. This delay in emergence was

reflected in reduced yield, although it should be noted that this varied between 7% and 40% depending upon cultivar. For all cultivars, including Golden Wonder, tecnazene treatment resulted in a reduction in the size of harvested tubers - this is likely to be a result of an effect on stem numbers.

Most recently, Lindsay and Ruthven (1986) assessed tuber residue levels of tecnazene from treatment until 8 weeks after planting. Treatment was at half, normal and double the recommended rate of 133 mg/kg, with the potatoes stored in a frost-free, unheated building, with no forced ventilation. 8 weeks prior to planting, half of the stored tubers were moved to chitting trays and kept in a well ventilated area. They found that residue levels decreased rapidly and erratically over the first 10 weeks of storage, especially for normal and double dose treatments. Airing treated tubers led to a rapid reduction in residues, again greatest for normal and double treatments - rate of loss increasing with temperature. Tecnazene residues at planting were in the range 5 - 20 mg/kg peel, depending on application rate. All treatments showed some reduction in plant height compared to controls, and again non aired treatments led to smaller plants than comparable aired treatments. Crop harvest was split, with one quarter lifted 15 weeks after planting, and a further quarter allowed to reach maturity 6 weeks later. At first harvest, all treatments produced a greater proportion of small seed, while total yield was reduced only for treatments which had not been aired, and to an extent unlikely to be of commercial significance. The effect of tecnazene treatment on the proportion of small seed was more pronounced by

the second harvest date, but again effect on total yield, although evident for all treatments, was not likely to be commercially significant. All residue analysis was carried on samples of peel, and an attempt was made to relate the relevant values to an interpretation of post-planting tecnazene residues agreed between DAFS, ADAS, the Scottish Colleges of Agriculture and DANI. Further reference will be made to this in the concluding section of this chapter.

From the preceding, the effect of tecnazene treatment on field growth and yield would appear to be rather variable. This variability is probably a result of many factors, the most important of which are likely to be storage regime, conditions at and after planting, cultivar, and time of harvest - these will be expanded upon during the discussion. Other complicating factors include comparison with chitted or desprouted tubers.

The object of this experiment was to observe the effect of a wider range of tecnazene residues than had previously been studied, under more closely controlled storage conditions, and to attempt to relate a residue at planting to an effect on the subsequent growth and yield of treated tubers. The results which will be reported formed the basis of a paper which was presented at the 10th Triennial Conference of the European Association for Potato Research, (Leonard and Duncan, 1987).

3.2 Experimental methods

Super Elite (S.E.) seed stock (35-50 mm) of the cultivars Maris Piper and Desiree was obtained from a seed merchant (J.E. England and Sons, Abernethy, Scotland) during January 1985. Between 28th and 30th January batches of approximately 10 kg were weighed and placed in cardboard boxes for storage, with enough boxes prepared to allow four replicates of seven application rates for each cultivar. Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene, 99%, Aldrich Chemical Co.) was applied on a carrier of 25 g of alumina (alumina grade 0, Spence and Sons, Airdrie, Scotland), at the following levels: 0, 5, 15, 35, 70, 135 and 270 mg/kg - 135 mg/kg being the recommended rate of sprout control. These formulations were prepared by weighing a known amount of alumina into a 500 g bottle and adding sufficient tecnazene to give the required quantity of active ingredient in 25 g of the formation. The jar was then placed in an oven at 80°C, in order to increase the proportion of the chemical in the vapour phase, before being transferred to an end-over-end shaker and shaken overnight. Control treatments received 25 g of alumina containing no tecnazene. Formulations prepared in this way were dusted over the tubers. The boxes were then placed in a cold room, within four independantly randomised blocks and kept at 4°C until planting on 24th April. This was a false situation in that storage at this temperature would prevent any significant sprout growth even in the absence ~~of sprout~~ of sprout suppressant. However, it meant that both treated and untreated tubers showed the same degree of sprout

growth and negated the complicating problem of desprouting control tubers. The boxes were taken from the cold room to the field trial site and immediately prior to planting, a sample of 8-10 tubers was removed from each and stored, frozen, until subsequent residue analysis by the method described in Chapter 2. The remaining tubers were planted in a sandy loam soil at Arkleston Farm, Renfrew, in plots 7 metres long, and comprising three drills - each individual box supplying a single plot. Tubers had 250 mm spacing within drills and drills were 750 mm apart. The plots, like the stored boxes, were individually randomised within blocks, with the single constraint that cultivar must alternate in both directions within the trial (see Fig. 3.1) - this was mainly to facilitate harvesting, one cultivar being red and the other white. The trial was incorporated in a field of ware potatoes and as such received a standard amount of fertiliser, and blight sprays at approximately fortnightly intervals from the beginning of July. The crops was defoliated on 28th September using diquat and harvested on 15th October.

Residues at planting

Residue analysis was carried out on the tuber samples taken at planting. Analysis was by gas chromatography, as described in Chapter 2.

Emergence data

A count of the number of plants emerged was taken at two day intervals from emergence of the first plant until 61 days after planting when four day counts commenced until emergence of all viable plants. This was carried out for all treatments.

Yield data

Harvested tubers were graded over a 45 mm riddel with the fractions thus obtained weighed using a top pan balance. Individual drills within each treatment plot were treated separately and the data obtained presented as a mean yield per drill.

Waterlogging in part of the field led to block 3 being discarded and the results shown are therefore mean values for nine individual replicates - three replicate plots each containing three drills.

Stem counts

Counts of the number of stems per plant were taken between defoliation and harvest. This was achieved by carefully removing the mother tuber from the drill and counting the number of stems attached directly to the tuber. Any daughter tubers uncovered were re-burried.

Because of the laborious and time consuming nature of the assessment, counts were taken for only four of the seven treatments, with only the middle drill of the relevant plots used. This led to a mean figure for each treatment based on approximately 108 individual values.

3.3. Results and discussion

3.3.1. Residues

Table 3.1. shows tecnazene residues, assessed on a whole tuber basis, from samples taken immediately prior to planting.

It is clear that the tuber residue increased with the amount of tecnazene applied, albeit rather erratically - probably a result of the sampling problems posed by the uneven distribution of this volatile chemical. Another point to note, and one which is clearer when the results are represented graphically (Fig. 3.2.), is that for the same application rate Maris Piper tubers contained a consistently higher tecnazene residue than those of the cultivar Desiree. Periderm differences between the cultivars are the most likely explanation for this as the vast majority (approx. 90%) of tecnazene extracted from treated tubers is present in the material which can be removed by thin peeling, and which includes the periderm (Dalziel and Duncan, 1980a). This is probably due to the fairly non-polar, practically water insoluble nature of tecnazene which leads to its association with the hydrophobic material (mainly suberin) impregnating the cells of the periderm.

Differences in thickness rather than composition are implicated as a closely supervised undergraduate project showed that the material extracted by dipping washed tubers in chloroform showed little or no inter-cultivar differences when analysed by gas chromatography, despite good resolution. This opinion is reinforced by the work of Artschwager (1924) which indicted that the thickness of the periderm is indeed usually a varietal feature, although it can be influenced by cultural factors: For example, high levels of nitrogen fertiliser and deep planting produce a thin periderm but phosphate and irrigation produce a thick skin. High soil temperatures can induce the formation of a rough or scaled skin (Gray and Hughes, 1978b).

3.3.2. Emergence characteristics

Table 3.2. and 3.3. show the effect of the various treatments on emergence time.

Mean emergence time (MET) represents the mean time, in days, by which all tubers had emerged (produced a viable above-ground shoot).

T75% is the mean time taken for 75% of the planted seed to emerge and is a useful figure in that it discounts tubers which are abnormally slow to emerge and which may have a disproportionate effect on MET.

As treatment rate increased there was an increase in T75%, with the delay becoming significant from the 35 mg/kg treatment for Maris piper and from the 15 mg/kg treatment for Desiree. The mean emergence time mirrored this increase in T75%, however it was not statistically significant for Desiree at any treatment level.

Total emergence was reduced at the higher treatment levels for Maris Piper but unaffected for Desiree. The fact that Maris Piper tubers emergence more slowly than Desiree over all treatments, and as such were exposed for a longer period of time to possible pathogen infection and/or multiplication, could explain this, i.e. the quicker a tuber-borne sprout emerges, the quicker it becomes independent of the mother tuber, and the less susceptible it is to rotting of the mother tuber.

Emergence profiles give a broader picture of the effect of tecnazene treatment on emergence (Figs. 3.3-3.16). Each profile is a plot of the number of tubers emerged since the previous count (interval emergence), against the number of days since planting. They show a normal distribution for controls and lower rates of tecnazene application. However, as rate of application increases, and with it the residue at planting, the distribution shifts to the right and at the higher rates tends to become truncated and skewed, with marked tailing. This effect is more evident for the cultivar Maris Piper than for Desiree.

3.3.3. Yield

The effect of tecnazene treatment on total yield varied with cultivar (Tables 3.4, 3.5). For the cultivar Maris Piper all treatments led to a reduction in yield which was greatest at the highest treatment level. Treatment at the 5 mg/kg level appeared to lead to a relatively large yield reduction, however this figure was thought to be erroneous as it was not a reflection of a delay in emergence and was not part of the trend evident in the other results. For Desiree treatment at 5, 15 and 35 mg/kg led to a slight increase in yield before declining at the higher treatment levels. This initial increase was partly a result of tecnazene treatment leading to an increase in the yield of small (<45 mm) tubers, an effect which was noted for both cultivars and which will be further discussed in section 3.3.4.

A problem noted when the yield data was analysed more closely was that a large variation had occurred between the individual drill within some plots. When the total yield from individual drills was calculated it became clear that two drills from the six drill wide strip showed a considerably lower yield than the other four (Fig. 3.17, 3.18). The explanation for this seemed to be tractor wheel damage, which can lead to foliage damage and soil compaction so that yields from damaged rows are decreased by up to 30%. Loss of crop depends mainly on the proportion of rows damaged and varies greatly with soil type and season (Hide and Lapwood, 1978).

In this particular field trial, very favourable growing conditions during the early part of the season had led to rapid early growth which, together with the prevailing westerly wind, meant that the plants in drills 1 and 3 (Fig. 3.19) had "lodged" between drills 1 and 2, 3 and 4 respectively by the time of the first blight spray and as a result their foliage damaged. This obviously led to a reduction in yield for two of the three drills in each of the affected plots but was not thought to have greatly influenced the overall trend, particularly as the yield from drills not directly affected (drills 2 and 5) taken separately (Tables 3.6, 3.7) showed a similar pattern to that for yield from all drills

Brown and Reavill (1954) showed that the time of harvest influences ~~and~~ ^{the} yield difference between tecnazene treated and untreated seed. This is likely to have been a contributing factor to the variation in the effect of tecnazene treatment on total yield observed in the literature cited, and could be explained either by differences in the physiological age of the planted seed, or by differences in the point at which harvest date (or defoliation date) crosses the yield development profile.

The former would be the result of an effect similar to that noted when the growth of chitted seed is compared to that of non-chitted seed. Chitted seed emerges faster than non-chitted seed and initiates tuber formation at a lower leaf area index. This results in earlier bulking of the chitted seed, and leads to a higher yield from this seed if harvesting (or defoliation) is carried out before natural senescence of the crop. However this

effect is reversed if natural senescence is allowed to occur as the non-chitted seed initiates tubers at a higher leaf area index, and so allows bulking at a higher rate and for a longer period of time than for the chitted seed, leading to the crossover effect evident in Figure 3.20 (Wurr, 1978).

This effect might occur when non-chitted tecnazene treated seed is compared to chitted control tubers, although it is likely to be to a lesser extent, and to be influenced by the length of emergence delay shown by the treated seed. This theory assumes that delayed emergence as a result of tecnazene treatment does not lead to a reduced leaf area - perhaps a dubious assumption considering the effect of tecnazene treatment on plant height noted by Lindsay and Ruthven (1986).

Seed which had sprouted and been desprouted prior to planting would be physiologically older than treated seed showing no sprouting and the effect described above might therefore also be expected, indeed may be compounded. This would be accompanied by a loss of sprouting vigour in the desprouted seed, possibly leading to a delay in emergence compared to chitted or non-sprouted controls, which could in turn further minimise or nullify any reduction in yield as a result of tecnazene treatment. Evidence for this type of effect is provided by the results observed for desprouted seed by Brown and Reavill (1954), and Murphy and Goven (1967).

The assumption made above, regarding the effect of tecnazene treatment on leaf area, is also required in the theory which could account for harvest date influencing any difference in yield

between tecnazene treated and untreated seed of the same physiological age. If the final leaf area is unaffected by tecnazene treatment then the yield development profile of treated seed will eventually, after natural senescence, reach the same maximum as for untreated seed. This means that the date of harvest, or defoliation if practised would influence any yield difference as follows (see Fig. 3.21): Early harvest (1) would result in the largest yield difference between treated and untreated seed as the profiles are separate and parallel. Harvesting at a later date (2), when the profiles are starting to converge, would result in a smaller difference, while taking this theory to its extreme, harvesting after senescence of both treated and untreated crops (3) would result in no yield difference. This final scenario is very unlikely under Scottish conditions as early frost would probably lead to haulm death before natural senescence. Also, under these climatic conditions harvesting after natural senescence is not economically viable because the colder, wetter conditions lead to an increased likelihood of tuber damage, disease infection and thus to poor storage. Another important point to note is that in the production of seed potatoes the seed/ware ratio is normally too low by the time natural senescence has occurred.

3.3.4. Stem numbers

The increase in yield of small tubers, or in the proportion of small tubers comprising total yield, for tecnazene treatments in this trial has been noted by several other authors (Brown and

Reavill, 1954; Brook and Chesters, 1957; Driver, 1961; Dalziel and Duncan, 1975; Lindsay and Ruthven, 1986).

This effect is thought to be a result of tecnazene treatment influencing the number of stems produced by each plant. In order to verify this stem counts were taken for four treatments during the period between defoliation and harvest. The method of assessment (Section 3.2.) involved counting only those stems which would be classed as mainstems using the EAPR physiology group definition, and the results are shown in Tables 3.8 and 3.9.

It is accepted that counts taken after defoliation may be prone to underestimation as competition can lead to stems dying and disappearing before crop maturity (Allen, 1978). However, for this work relative differences are more important than absolute values, and would still be likely to show even assuming the unlikely event of a significant underestimation of stem numbers.

It is clear from the results that tecnazene treatment led to an increase in the mean number of mainstems per plant, despite the fact that storage at 4°C may itself be expected to result in a higher number of stems per plant than storage at a higher temperature (Bodlaender, 1987). This increase in stem numbers is reflected in a higher proportion of small tubers in the total yield than for the controls.

It is also interesting to note that this increase in the proportion of smaller tubers is also evident for the lowest level tecnazene treatments, for which stem numbers were not assessed. If this is indeed also an effect of an increase in the number of mainstems per plant it may suggest the possibility of using low

levels of tecnazene, which would have little or no effect on total yield, to manipulate yield distribution.

3.3.5. Correlation of total yield with mean emergence time and with residue at planting

Fig. 3.22 shows a plot of total yield against the mean emergence time. The best-fit line is shown for each cultivar as is a correlation coefficient. For each cultivar a good correlation obviously exists between the mean emergence time and the total yield, with decreasing total yield as the mean emergence time increases.

This procedure was also carried through for the total yield and tecnazene residue at planting (Fig. 3.23). There is a good correlation between these parameters for Maris Piper, however it is much poorer for Desiree. This is likely to be due to the problems involved in obtaining a representative sample of tubers treated with this volatile chemical, as it tends to redistribute within the storage container. The best-fit line shows decreasing yield with increasing residue at planting.

3.4. Conclusion

As previously mentioned it seems clear, from comparison of all relevant work, including the current study, that the effect of tecnazene treatment on the yield from seed can be rather variable.

This can be explained by the influence of four major factors:

- (1) Storage conditions
- (2) Climatic conditions at and for the first few weeks after planting
- (3) Time of harvest
- (4) Cultivar

Cultivar effects are unpredictable, and while it might be possible to assess them it is unlikely to be practically viable. The effect of time of harvest has already been discussed. The remaining factors, (1) and (2), both depend on the fact that tecnazene has a growth retardant effect rather than being phytotoxic - c.f. chlorpropham, the other major sprout suppressant chemical, which is a mitotic poison and kills active meristematic tissue - and that the effect of tecnazene is reversible i.e. normal sprout growth resumes in the absence of the chemical (Brown and Reavill, 1954).

Storage conditions, including chitting conditions, are important as they affect the rate of loss of the applied chemical - high temperatures and/or a high rate of ventilation will hasten loss of the chemical, while lower temperatures and limited ventilation will have the opposite effect (Dalziel, 1978).

Climatic conditions at and just after planting are important as they are likely to affect the growth of sprouts on treated tubers; both directly by the influence of soil temperature on the

rate of growth, and indirectly by affecting the rate of loss of tecnazene from the tubers. It is this latter effect which is most important as the former would apply to both treated and untreated tubers. This means that under warm conditions at planting and assuming adequate moisture and nutrients, any gap in emergence time between treated and untreated seed will be narrowed. Conversely any gap is likely to be widened under unfavourable (cold) conditions. All of this assumes that the main effect of tecnazene treatment on yield is a result of delayed emergence and a consequently shorter growing season, which considering the correlation between the two, would appear to be a justifiable statement.

The above, along with the inherent problems in sampling treated tubers, means that a definitive effect cannot be attributed to a specific residue at planting. However, residue at planting can be a useful guide to possible effects on subsequent field growth of treated seed when assessed in a broader context. As such the work would lead to a general concurrence with the conclusion of Dalziel (1978), in that it would be desirable to have residues of less than 1-1.5 mg/kg in tecnazene treated tubers prior to planting. As the level of chemical often used may be around 70 mg/kg, often less but rarely much higher (personal discussion with growers), airing of tubers at raised temperatures (above 10°C) for a period of weeks, using light to control sprouting, will almost always be a necessity if the levels quoted are to be achieved (Dalziel and Duncan, 1980b, suggest a minimum of six weeks airing at 12°C).

If these guidelines are followed little or no effect on growth or yield would be expected, indeed for seed producers a beneficial increase in the proportion of small tubers would be likely. This increase, which was evident at all treatment levels in this trial, would appear to be a result of the increase in the average number of mainstems noted for the 35, 70 and 135 mg/kg treatments when compared to controls. As previously mentioned this might indicate the potential use of low level tecnazene treatments to influence stem numbers and hence yield distribution. Further work would, however, be required to assess the minimum period of exposure to tecnazene necessary to produce this effect, as this would obviously be a function of both time of application and conditions of storage.

The results presented would not be in conflict with those of Lindsay and Ruthven (1986) who found some yield reductions, unlikely to be of commercial significance, from treated seed with peel residues in the range 5-20 mg/kg at planting (it is assumed that if represented on a whole tuber basis these figures would be lower by a factor of approximately ten).

Their findings would call into question the wisdom of the advisory services (D.A.F.S., A.D.A.S., The Scottish Colleges of Agrigulture and D.A.N.I.) in attempting to predict the likely effect on crop performance of a residue obtained 6-8 weeks after planting (Table 3.10) as they indicate that the loss of tecnazene from planted tubers is such that residue differences apparent at planting would not be noticeable 6-8 weeks after planting.

Tecnazene Residues in Whole Tubers at Planting

Tecnazene applied (mg/kg)	Residue at planting (mg/kg ± S.E) - Maris Piper	Residue at planting (mg/kg ± S.E) - Desiree
0	0.06 ± 0.01	0.03 ± 0.01
5	1.40 ± 0.15	0.89 ± 0.24
15	1.89 ± 0.07	0.91 ± 0.17
35	1.90 ± 0.34	1.39 ± 0.13
70	3.25 ± 0.35	2.62 ± 0.42
135	3.60 ± 0.68	2.12 ± 0.23
270	5.35 ± 0.41	3.75 ± 0.29

TABLE 3.1

Effect of Tecnazene Residues on Emergence of Treated Seed

MARIS PIPER

Tecnazene Applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	T 75% (days)	M.E.T. (days)	Total Emergence (%)
0	0.06 \pm 0.01	40.3	50.7	99.4
5	1.40 \pm 0.15	40.7	50.5	99.7
15	1.89 \pm 0.07	42.5	54.3	98.1
35	1.90 \pm 0.34	47.0*	59.8*	97.5
70	3.25 \pm 0.35	50.0*	59.5*	93.1
135	3.60 \pm 0.68	47.5*	61.8*	93.8
270	5.35 \pm 0.41	51.7*	63.5*	93.5
Tukey LSD _{0.05}		3.6	7.5	

TABLE 3.2

DESIREE

Tecnazene Applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	T 75% (days)	M.E.T. (days)	Total Emergence (%)
0	0.03 \pm 0.01	35.3	43.0	100
5	0.89 \pm 0.24	36.2	42.5	100
15	0.91 \pm 0.17	38.0*	43.0	99.6
35	1.39 \pm 0.13	39.5*	43.7	100
70	2.62 \pm 0.42	39.7*	46.7	100
135	2.12 \pm 0.23	41.7*	47.7	100
270	3.75 \pm 0.29	42.0*	49.7	100
Tukey LSD _{0.05}		2.3	7.5	

TABLE 3.3

* significantly different from control at 5% level

Effect of Tecnazene Residues on Yield from Treated Seed

MARIS PIPER

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg ± S.E)	Yield >45 mm (kg/drill)	Yield <45 mm (kg/drill)	Total Yield (kg/drill)
0	0.06 ± 0.01	19.7 ± 1.7	4.3 ± 0.3	24.0 ± 1.7
5	1.40 ± 0.15	13.3 ± 1.7	5.5 ± 0.6	18.8 ± 1.5
15	1.89 ± 0.07	15.4 ± 1.9	5.5 ± 0.5	20.9 ± 1.7
35	1.90 ± 0.34	15.7 ± 1.7	4.7 ± 0.3	20.4 ± 1.8
70	3.25 ± 0.35	15.2 ± 1.7	4.8 ± 0.4	20.0 ± 1.7
135	3.60 ± 0.68	15.1 ± 1.8	4.4 ± 0.5	19.5 ± 1.7
270	5.35 ± 0.41	14.0 ± 1.6	3.8 ± 0.4	17.8 ± 1.7
Tukey LSD _{0.05} 7.5 LSD _{0.05} 1.9 LSD _{0.05} 7.4				

TABLE 3.4.

DESIREE

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg ± S.E)	Yield >45 mm (kg/drill)	Yield <45 mm (kg/drill)	Total Yield (kg/drill)
0	0.03 ± 0.01	15.8 ± 1.6	3.0 ± 0.2	18.8 ± 1.6
5	0.89 ± 0.24	16.6 ± 1.7	4.3 ± 0.2	20.9 ± 1.8
15	0.91 ± 0.17	16.1 ± 1.3	4.5 ± 0.4	20.6 ± 1.3
35	1.39 ± 0.13	16.3 ± 1.6	4.3 ± 0.3	20.6 ± 1.4
70	2.62 ± 0.42	14.0 ± 1.3	4.0 ± 0.2	18.0 ± 1.3
135	2.12 ± 0.23	12.9 ± 1.6	4.2 ± 0.3	17.1 ± 1.8
270	3.75 ± 0.29	13.6 ± 1.4	3.7 ± 0.4	17.3 ± 1.7
Tukey LSD _{0.05} 6.6 LSD _{0.05} 1.4 LSD _{0.05} 6.9				

TABLE 3.5

$$\text{Tukey LSD}_\alpha = t_{\alpha/2, a(n-1)} \sqrt{\frac{2\text{MSe}}{n}}$$

YIELD DATA (Drills 2 and 5 taken separately)

MARIS PIPER

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	Yield >45 mm (kg/drill \pm S.E)	Yield <45 mm (kg/drill \pm S.E)	Total Yield (kg/drill \pm S.E)
0	0.06 \pm 0.01	20.4 \pm 0.6	3.9 \pm 0.5	24.3 \pm 1.1
5	1.40 \pm 0.15	19.3 \pm 2.3	4.1 \pm 0.6	23.4 \pm 2.5
15	1.89 \pm 0.07	19.8 \pm 0.9	5.3 \pm 0.4	25.0 \pm 1.2
35	1.90 \pm 0.34	14.8 \pm 0.5	3.7 \pm 0.3	18.6 \pm 0.6
70	3.25 \pm 0.35	17.5 \pm 1.4	5.0 \pm 0.6	22.5 \pm 1.5
135	3.60 \pm 0.68	15.9 \pm 1.6	4.4 \pm 0.6	20.4 \pm 2.2
270	5.35 \pm 0.41	15.0 \pm 2.0	3.0 \pm 1.0	18.0 \pm 3.0
Tukey LSD _{0.05} 7.2 LSD _{0.05} 2.9 LSD _{0.05} 9.2				

TABLE 3.6

DESIREE

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	Yield >45 mm (kg/drill)	Yield <45 mm (kg/drill)	Total Yield (kg/drill)
0	0.03 \pm 0.01	18.6 \pm 0.1	3.0 \pm 0.2	21.6 \pm 0.3
5	0.89 \pm 0.24	18.0 \pm 1.8	4.2 \pm 0.4	22.2 \pm 1.6
15	0.91 \pm 0.17	15.8 \pm 0.8	3.2 \pm 0.2	19.0 \pm 1.0
35	1.39 \pm 0.13	17.4 \pm 0.6	3.9 \pm 0.2	21.3 \pm 0.5
70	2.62 \pm 0.42	15.5 \pm 0.6	3.3 \pm 0.4	18.8 \pm 0.7
135	2.12 \pm 0.23	17.1 \pm 0.8	4.8 \pm 0.7	21.9 \pm 1.3
270	3.75 \pm 0.29	10.5 \pm 3.1	2.8 \pm 0.9	13.3 \pm 4.0
Tukey LSD _{0.05} 7.1 LSD _{0.05} 2.4 LSD _{0.05} 8.7				

TABLE 3.7

Effect of Tecnazene Residues on Number of Stems and Yield Distribution

MARIS PIPER

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	Stem No. (\pm S.E)	Proportion of Tubers <45 mm (as % age of total yield)
0	0.06 \pm 0.01	6.2 \pm 0.2	17.9
5	1.40 \pm 0.15	-	29.3
15	1.89 \pm 0.07	-	26.3
35	1.90 \pm 0.34	7.4 \pm 0.2	23.0
70	3.25 \pm 0.35	7.7 \pm 0.2	24.0
135	3.60 \pm 0.68	8.0 \pm 0.3	22.6
270	5.35 \pm 0.41	-	21.3

TABLE 3.8

DESIREE

Tecnazene applied (mg/kg)	Residue at Planting (mg/kg \pm S.E)	Stem No. (\pm S.E)	Proportion of Tubers <45 mm (as % age of total yield)
0	0.03 \pm 0.01	3.5 \pm 0.1	16.0
5	0.89 \pm 0.24	-	20.6
15	0.91 \pm 0.17	-	21.8
35	1.39 \pm 0.13	4.3 \pm 0.1	20.9
70	2.62 \pm 0.42	4.2 \pm 0.1	22.2
135	2.12 \pm 0.23	4.5 \pm 0.2	24.6
270	3.75 \pm 0.29	-	21.4

TABLE 3.9

Advisory Services Interpretation of Tecnazene Residues in Samples
 6-8 Weeks after Planting and Effect on Growth

Residue Level (mg/kg) in peel	Effect on Growth
Less than 0.5	No noticeable effect
Greater than 0.5 - 3.5	Delay in emergence may be seen but probably not sufficient to cause yield loss
Greater than 3.5 - 10.0	Progressive increase in delayed emergence and increased likelihood of effect on yield
Greater than 10.0	Probable effect on crop stand (some non-emergence) in increasing effect on yield reduction

TABLE 3.10

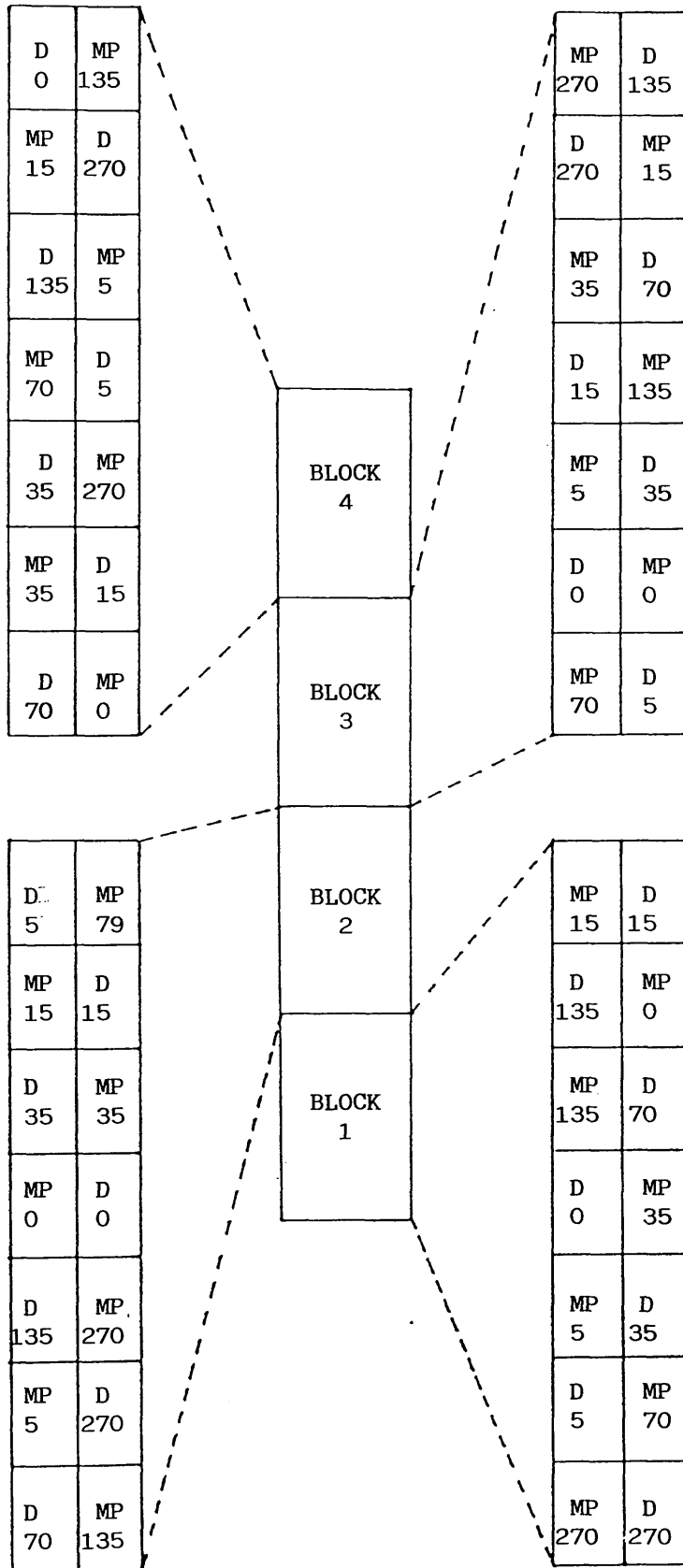
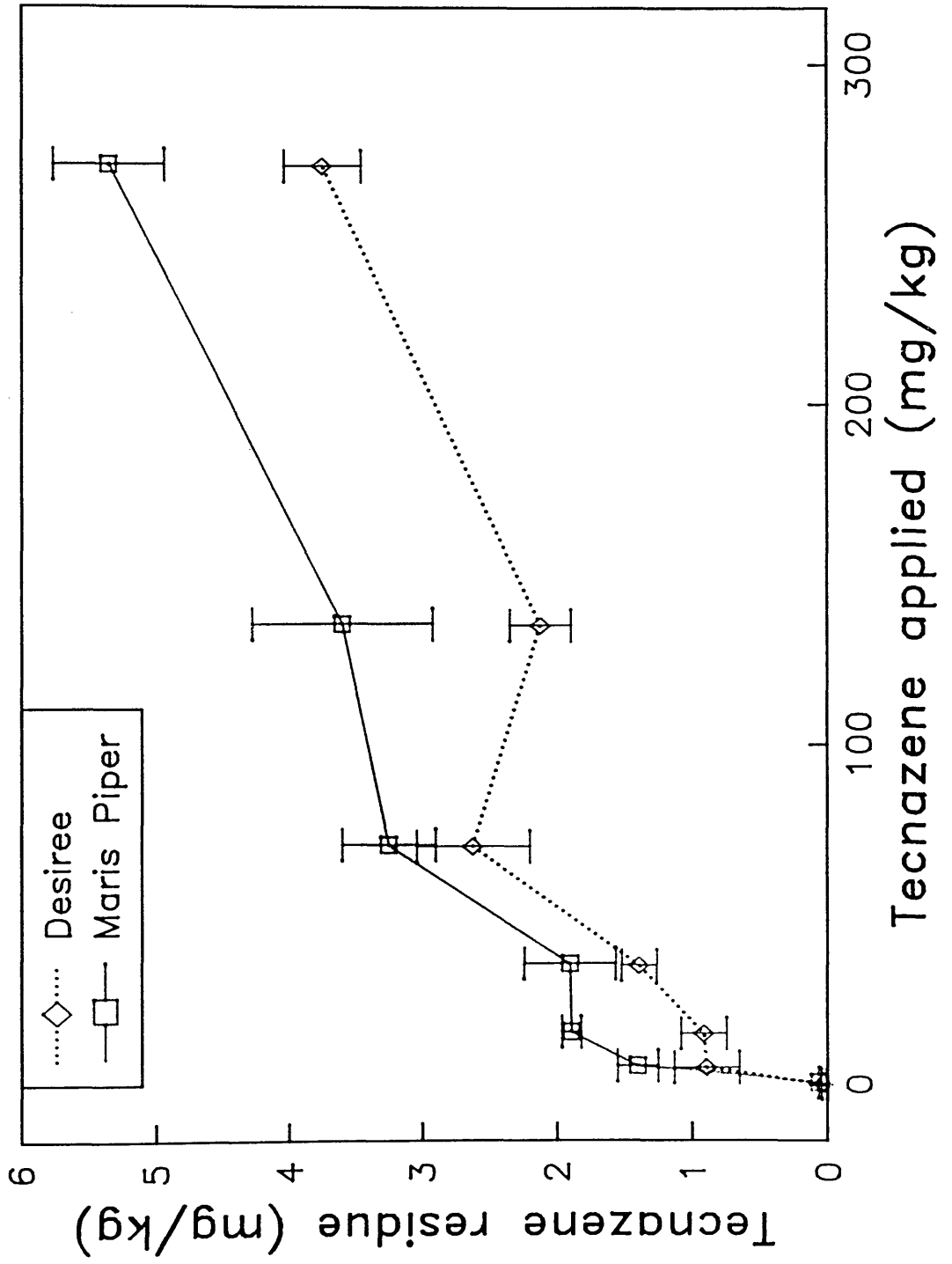


FIG. 3.1. Field trial plot plan.

Fig.3.2 Tecnazene residues in tubers at planting.



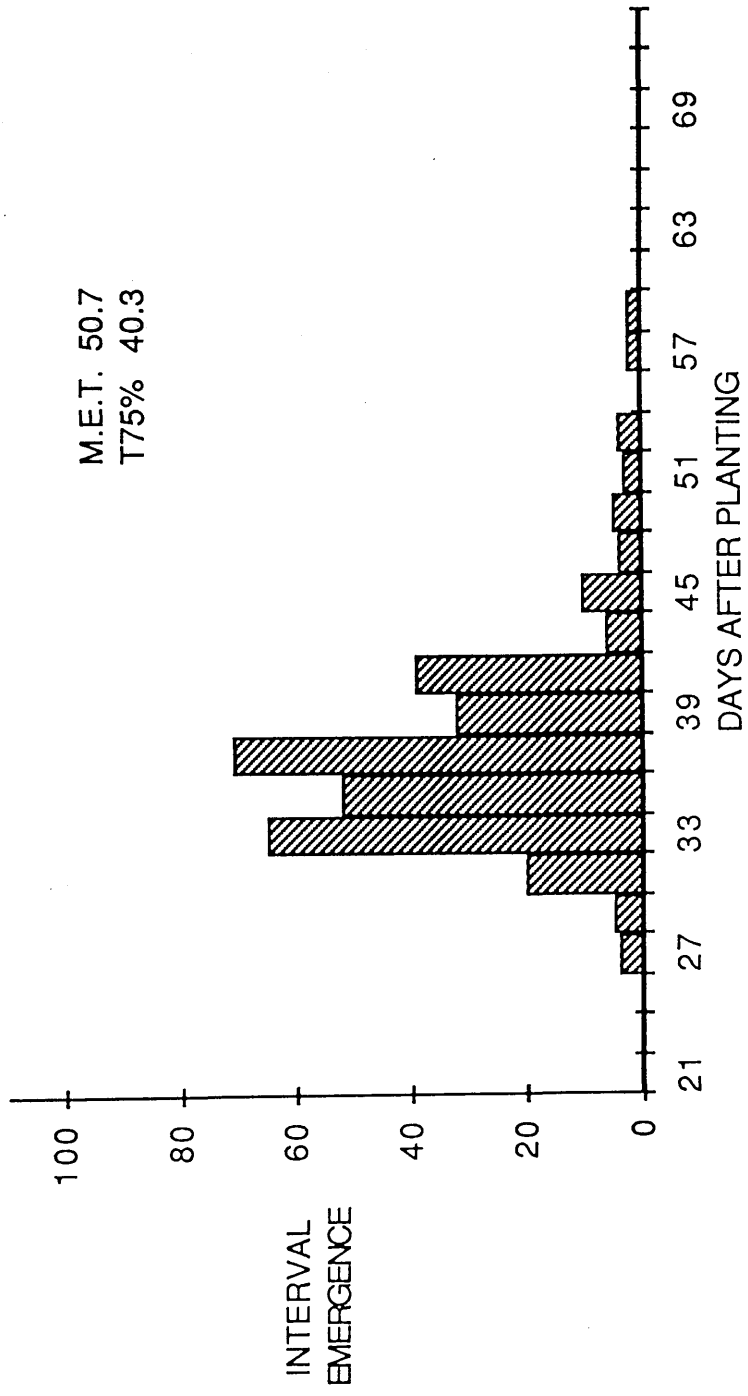


Fig.3.3.Emergence profile for Maris Piper seed treated with tecnazene at 0mg/kg

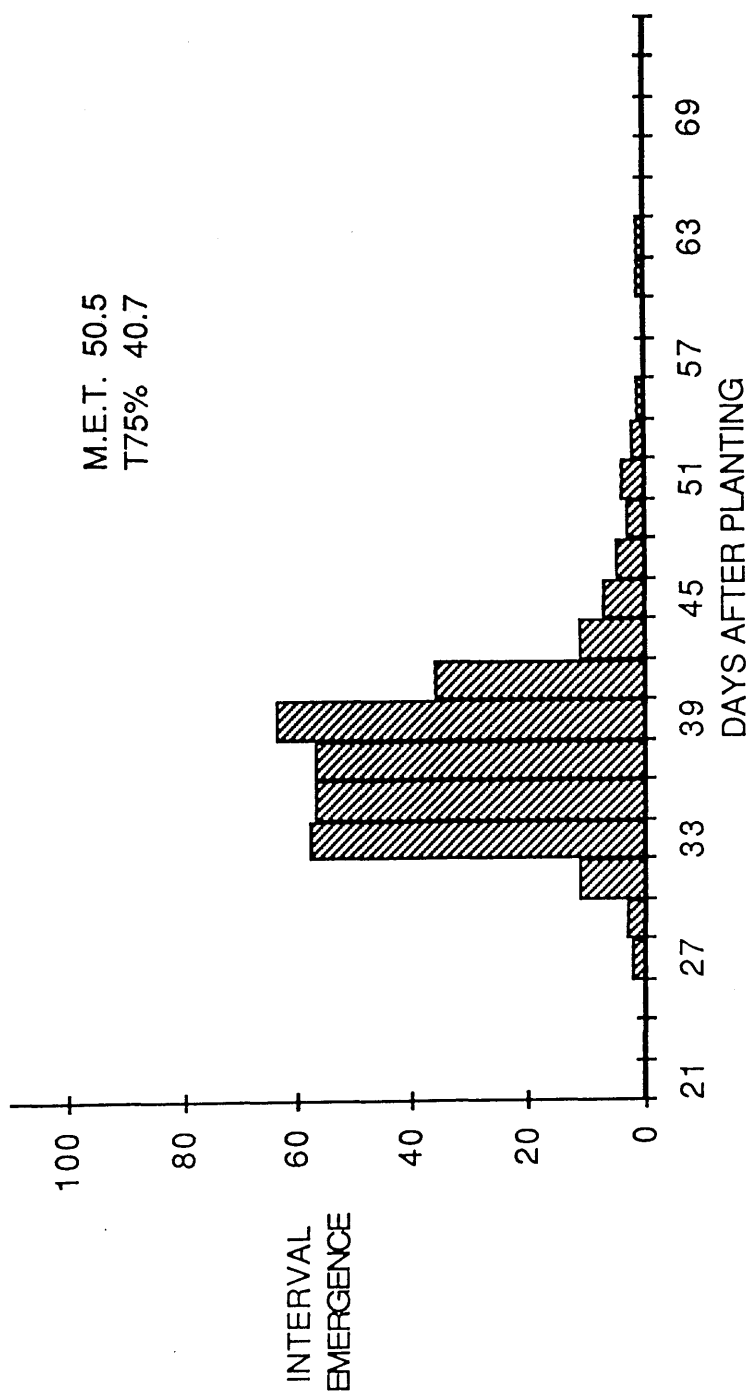


Fig.3.4. Emergence profile for Maris Piper seed treated with tecnazene at 5mg/kg

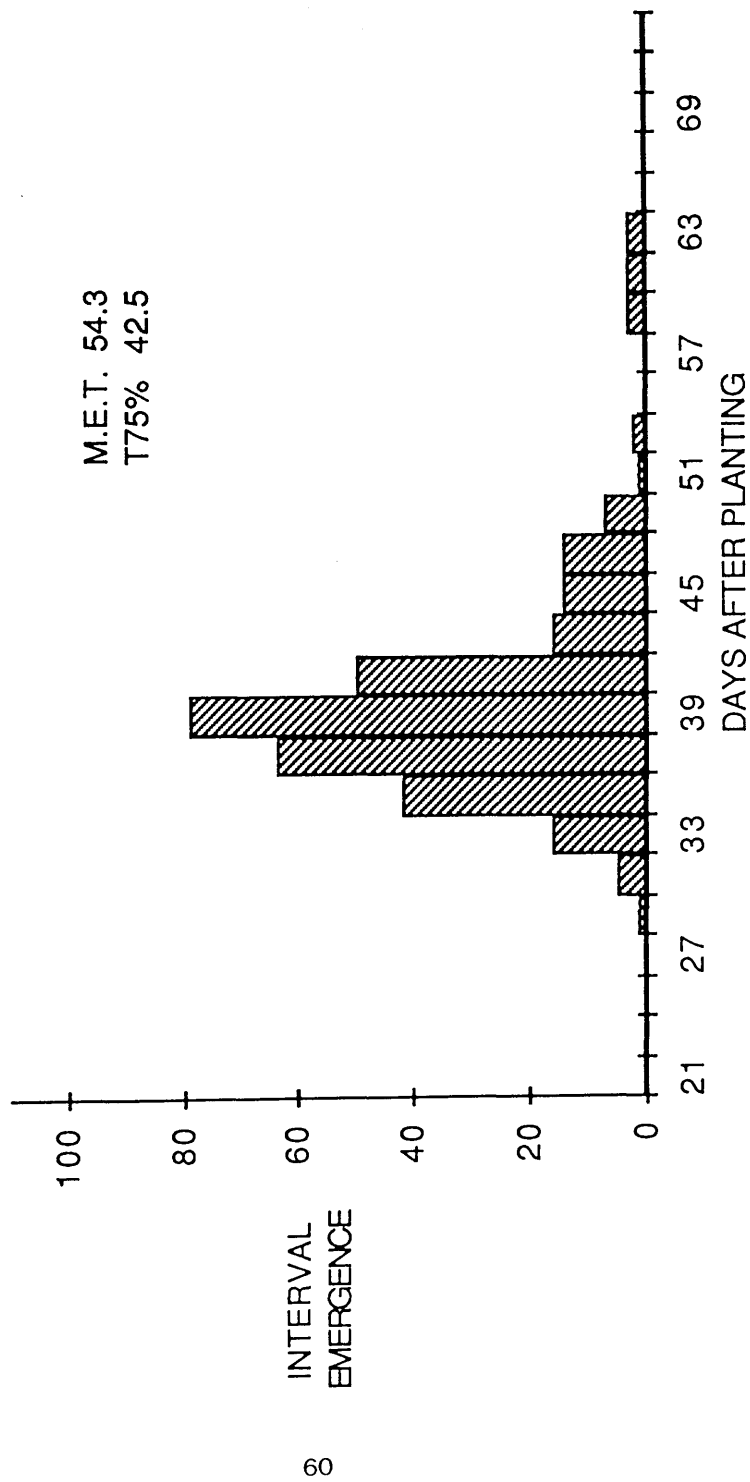


Fig.3.5.Emergence profile for Maris Piper seed treated with tecnazene at 15mg/kg

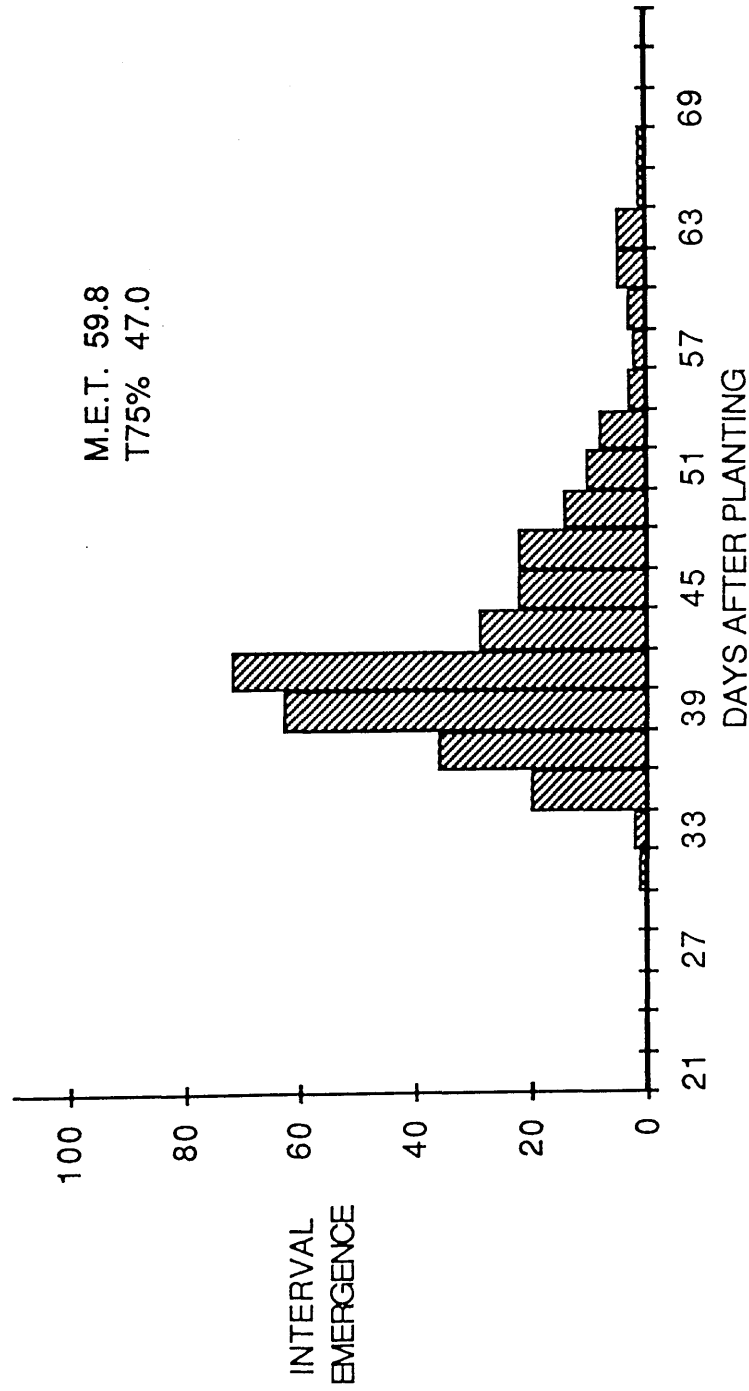


Fig.3.6.Emergence profile for Maris Piper seed treated with tecnazene at 35mg/kg

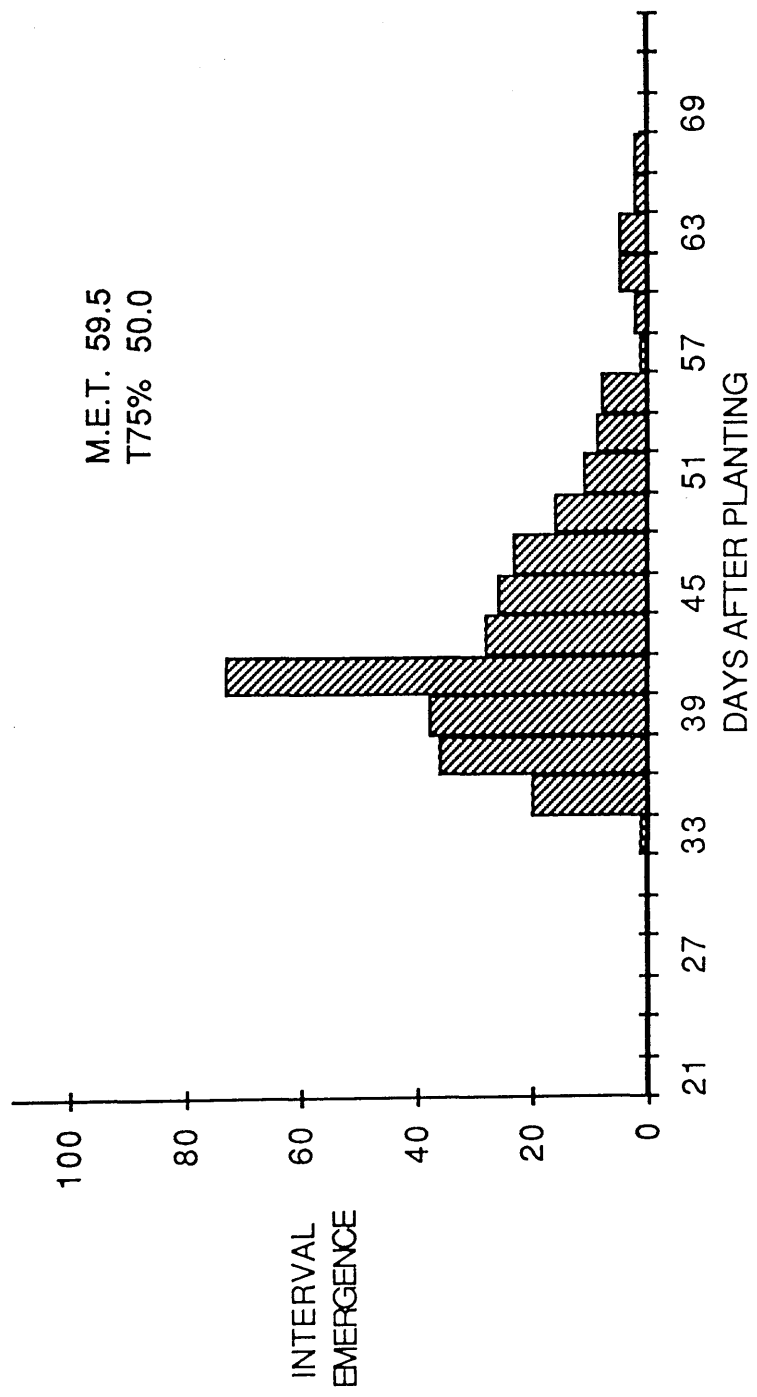


Fig.3.7.Emergence profile for Maris Piper seed treated with tecnazene at 70mg/kg

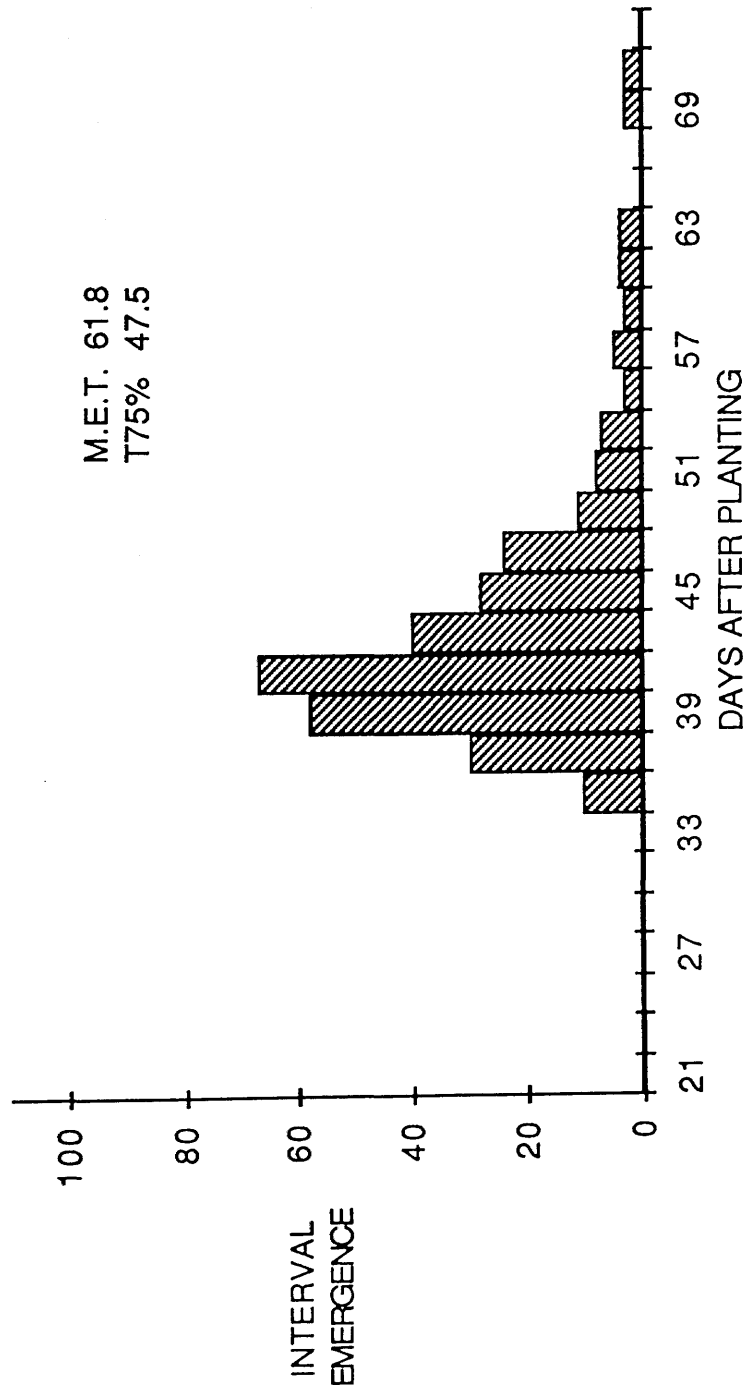


Fig.3.8.Emergence profile for Maris Piper seed treated with tecnazene at 135mg/kg

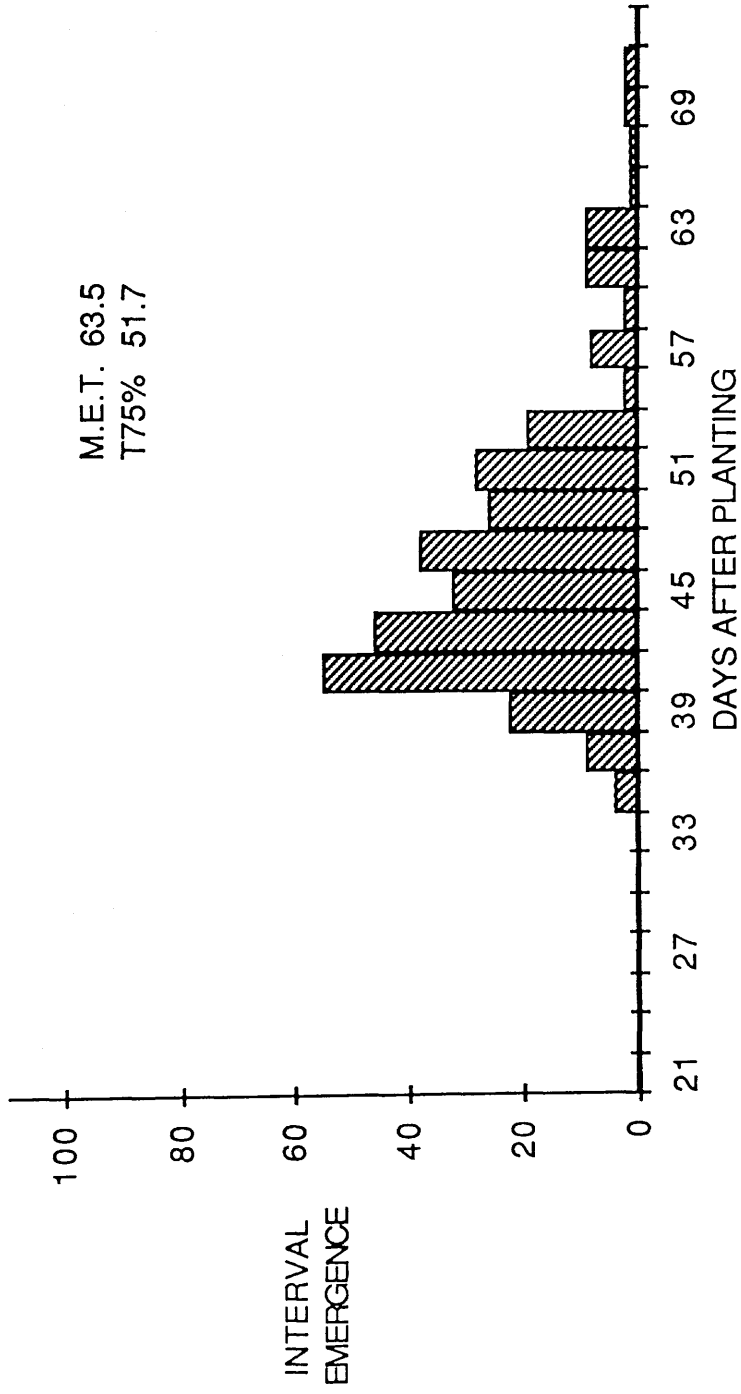


Fig.3.9.Emergence profile for Maris Piper seed treated with tecnazene at 270mg/kg

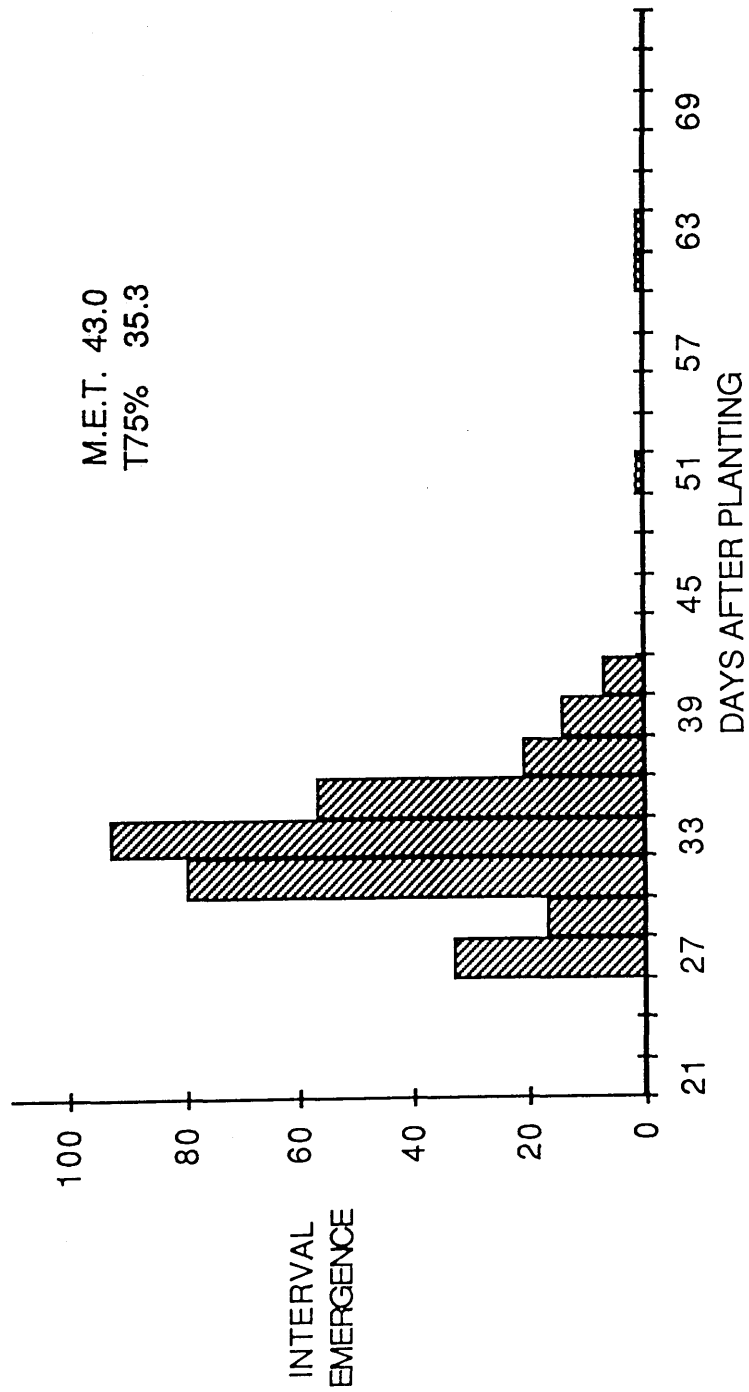


Fig.3.10.Emergence profile for Desiree seed treated with tecnazene at 0mg/kg

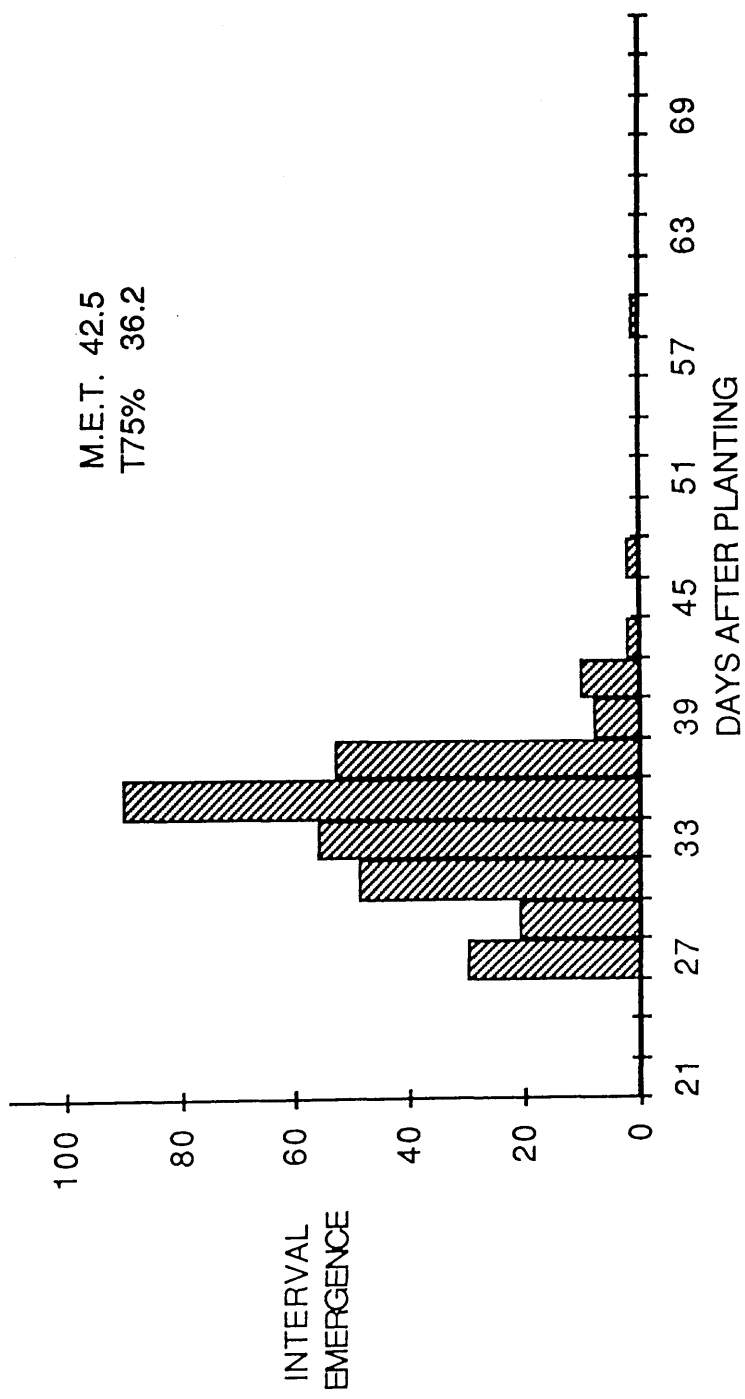


Fig.3.11.Emergence profile for Desiree seed treated with tecnazene at 5mg/kg

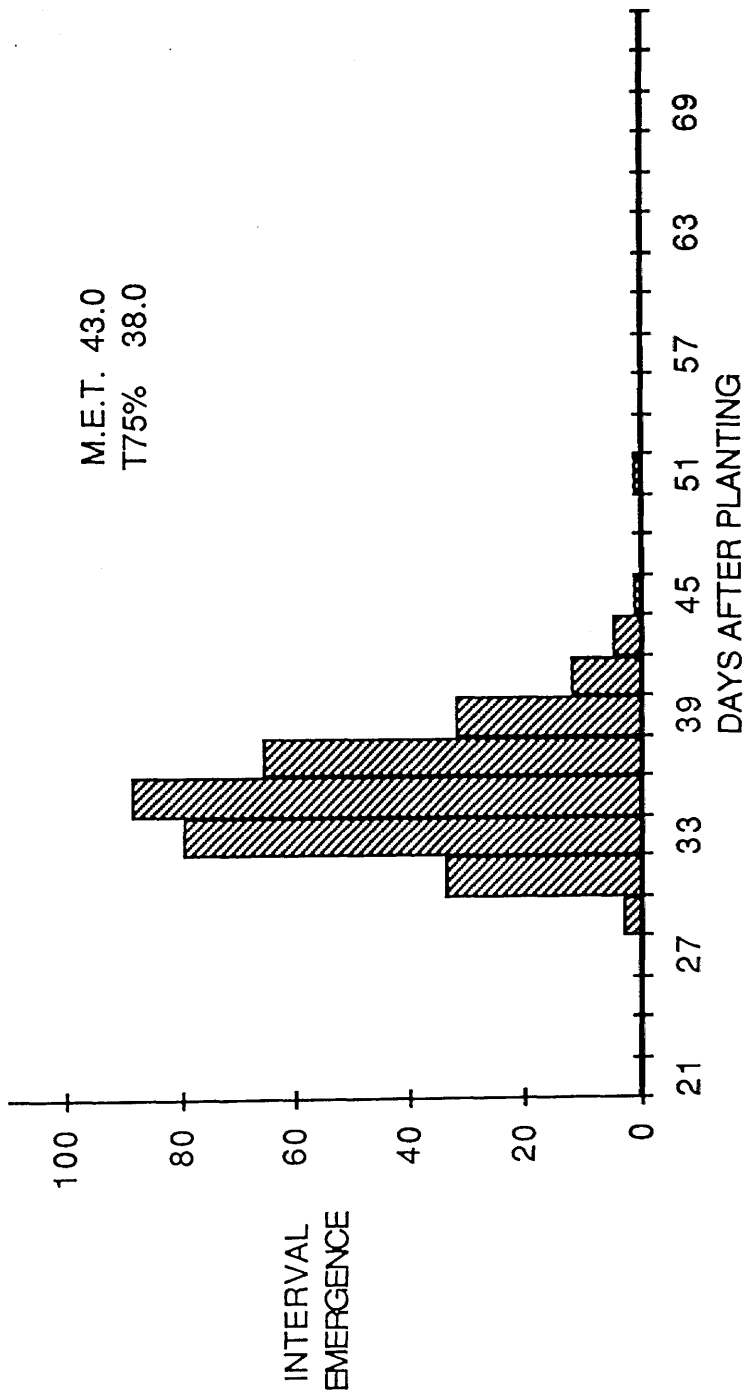


Fig.3.12.Emergence profile for Desiree seed treated with tecnazene at 15mg/kg

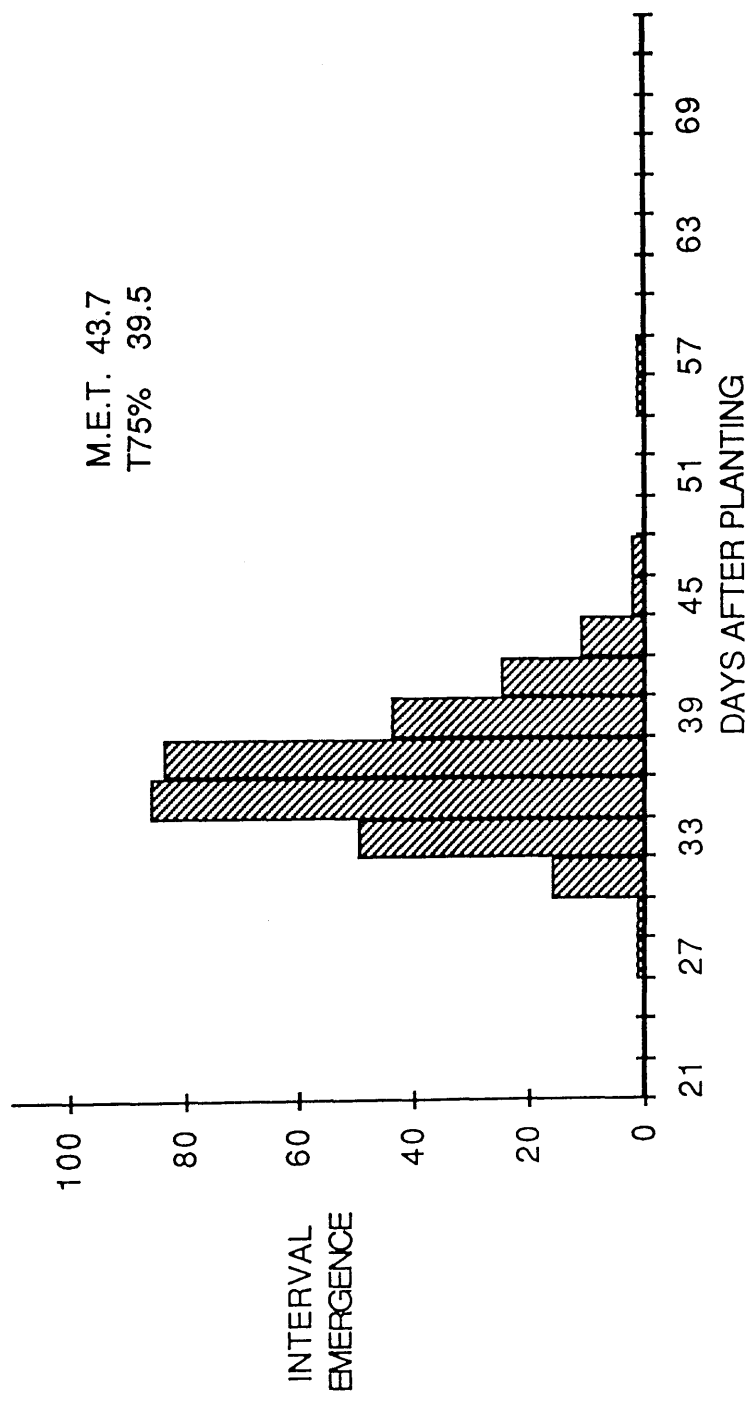


Fig.3.13.Emergence profile for Desiree seed treated with tecnazene at 35mg/kg

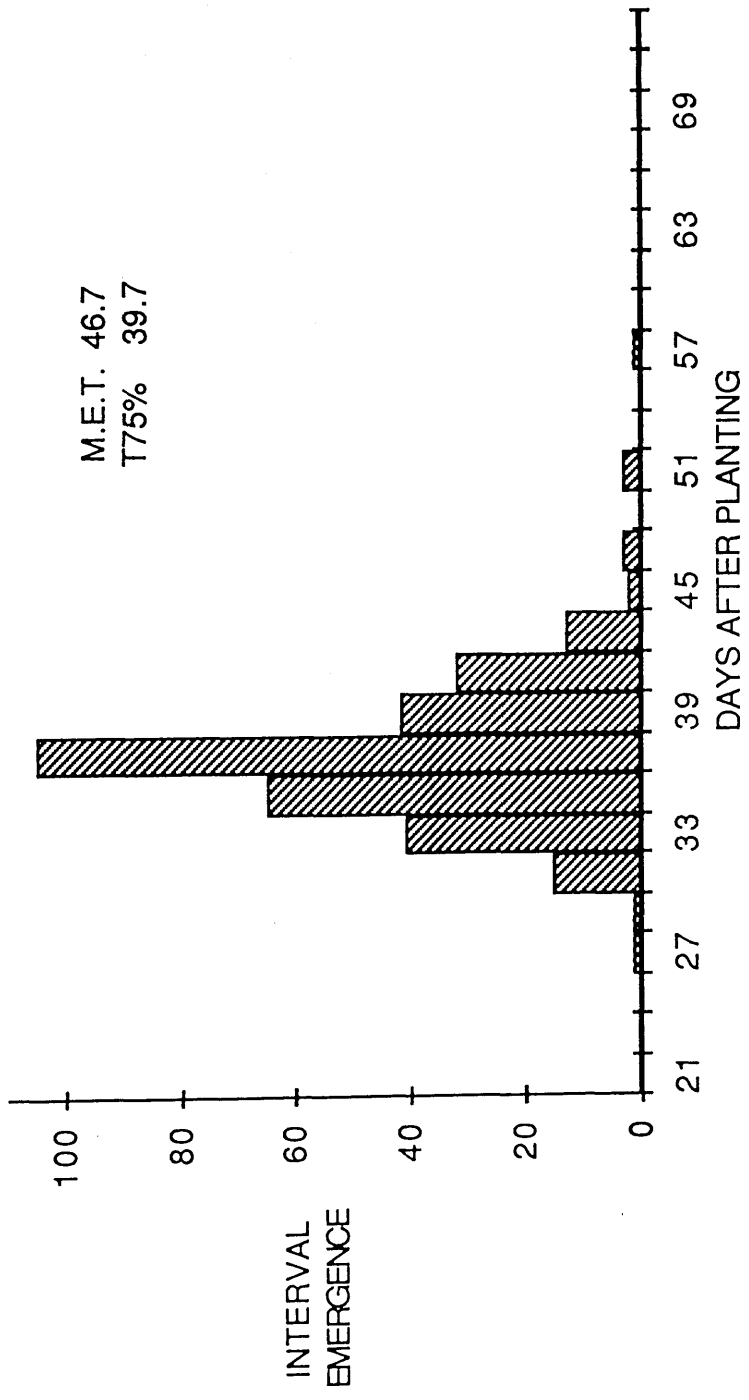


Fig.3.14.Emergence profile for Desiree seed treated with tecnazene at 70mg/kg

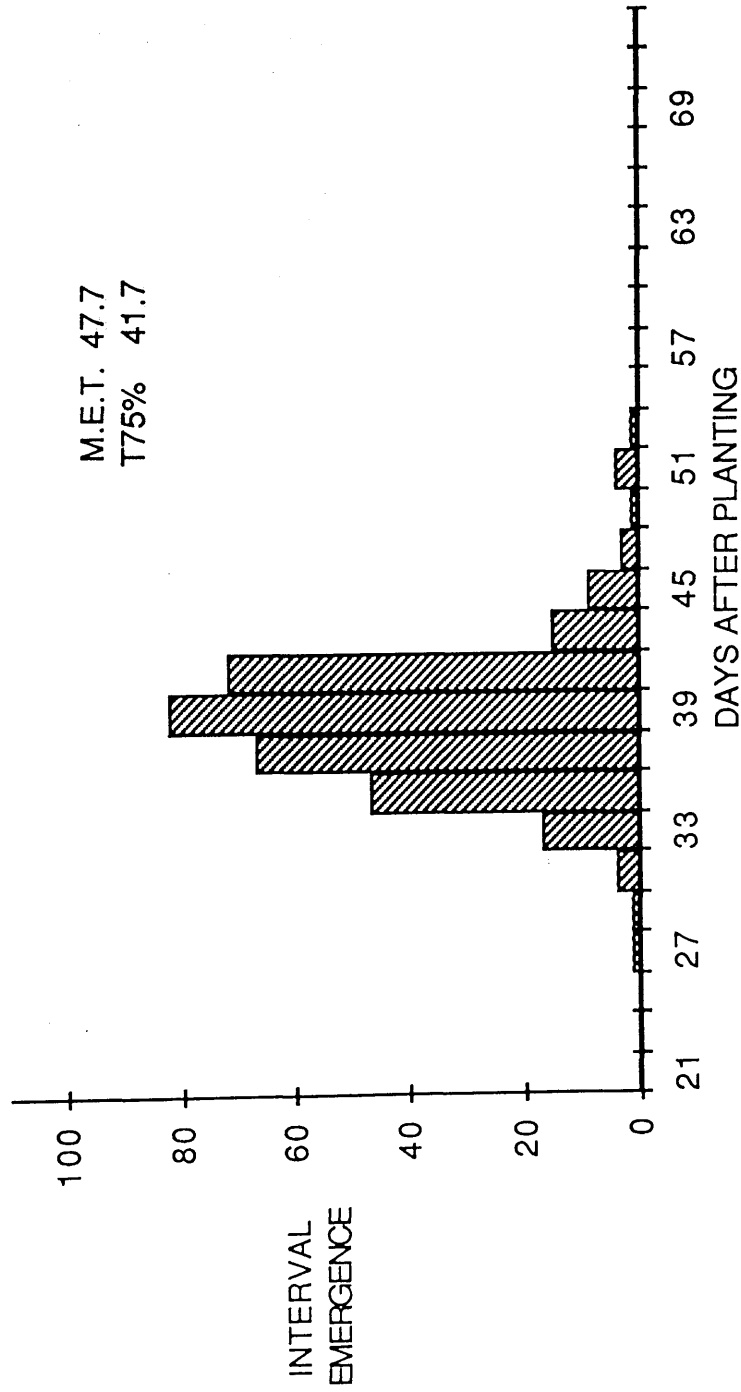


Fig.3.15.Emergence profile for Desiree seed treated with tecnazene at 135mg/kg

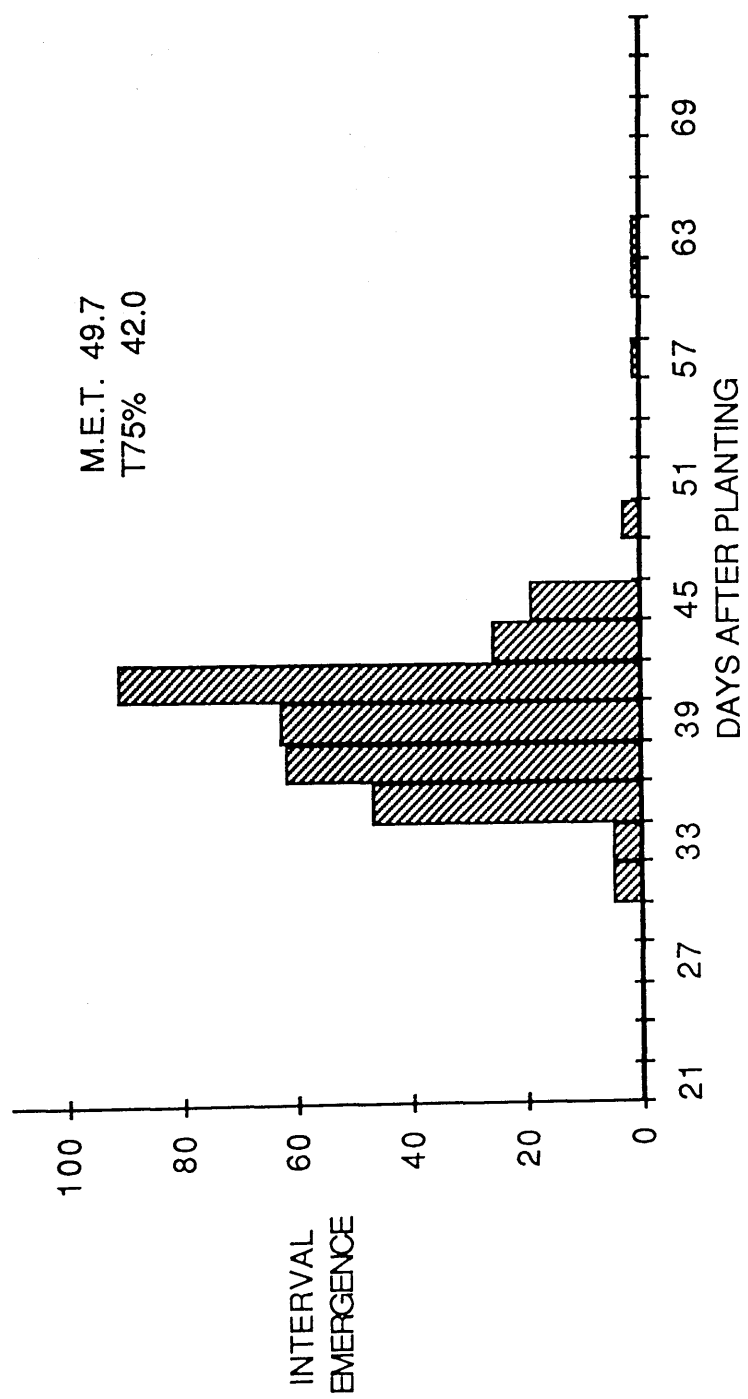


Fig.3.16.Emergence profile for Desiree seed treated with tecnazene at 270mg/kg

Fig. 3.17 Total Yield Per Drill – M.Piper

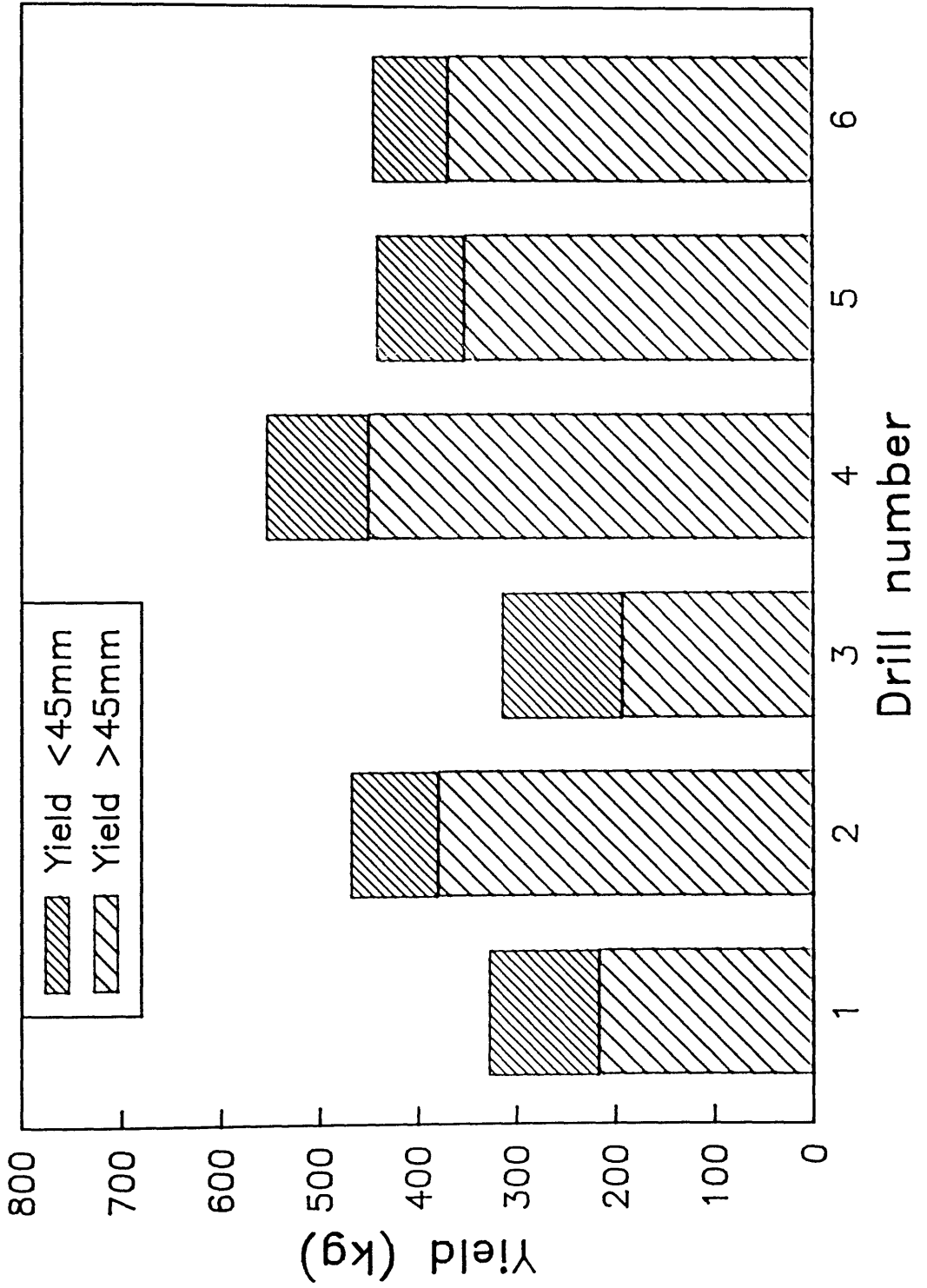
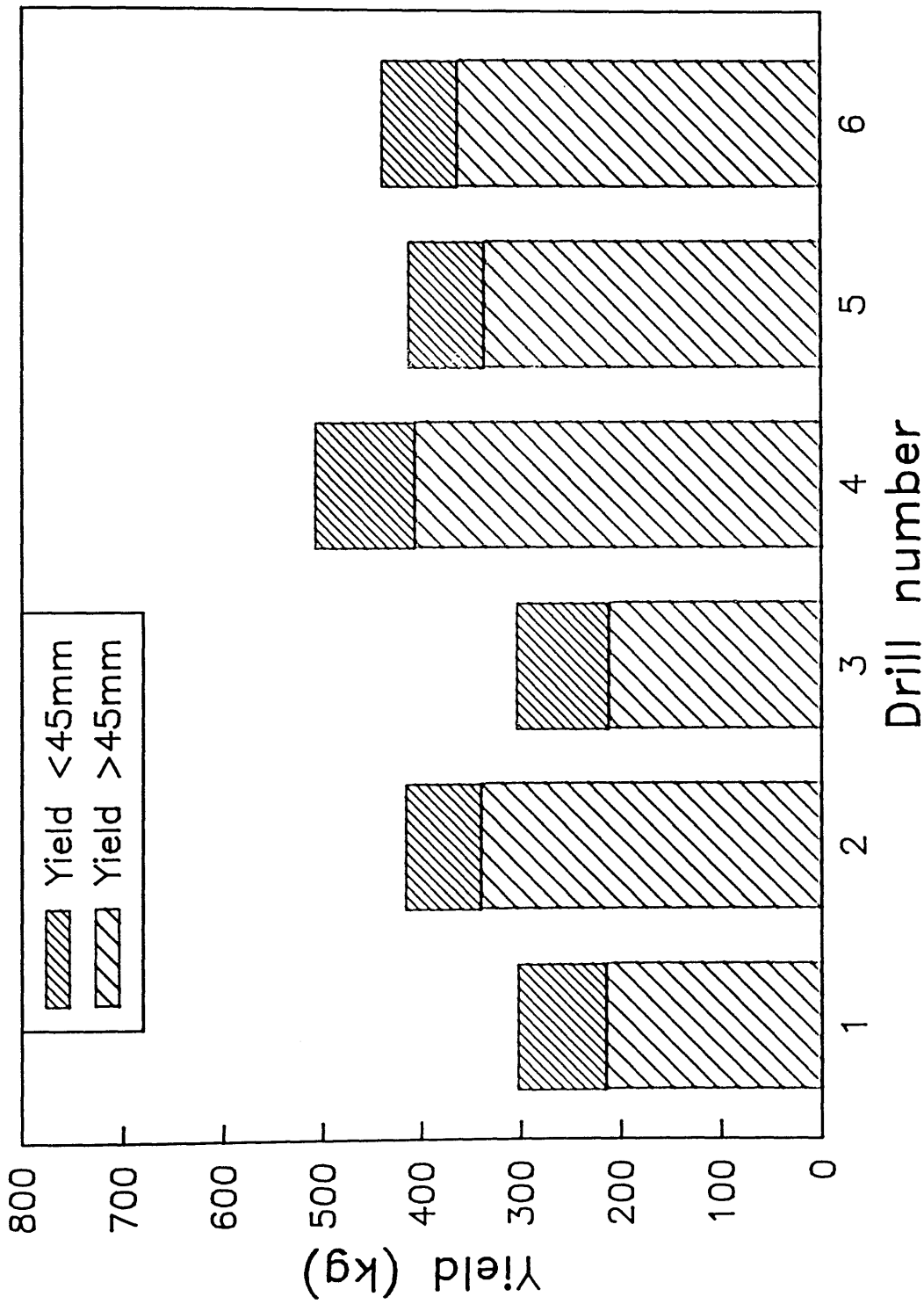


Fig. 3.18 Total Yield Per Drill – Desiree



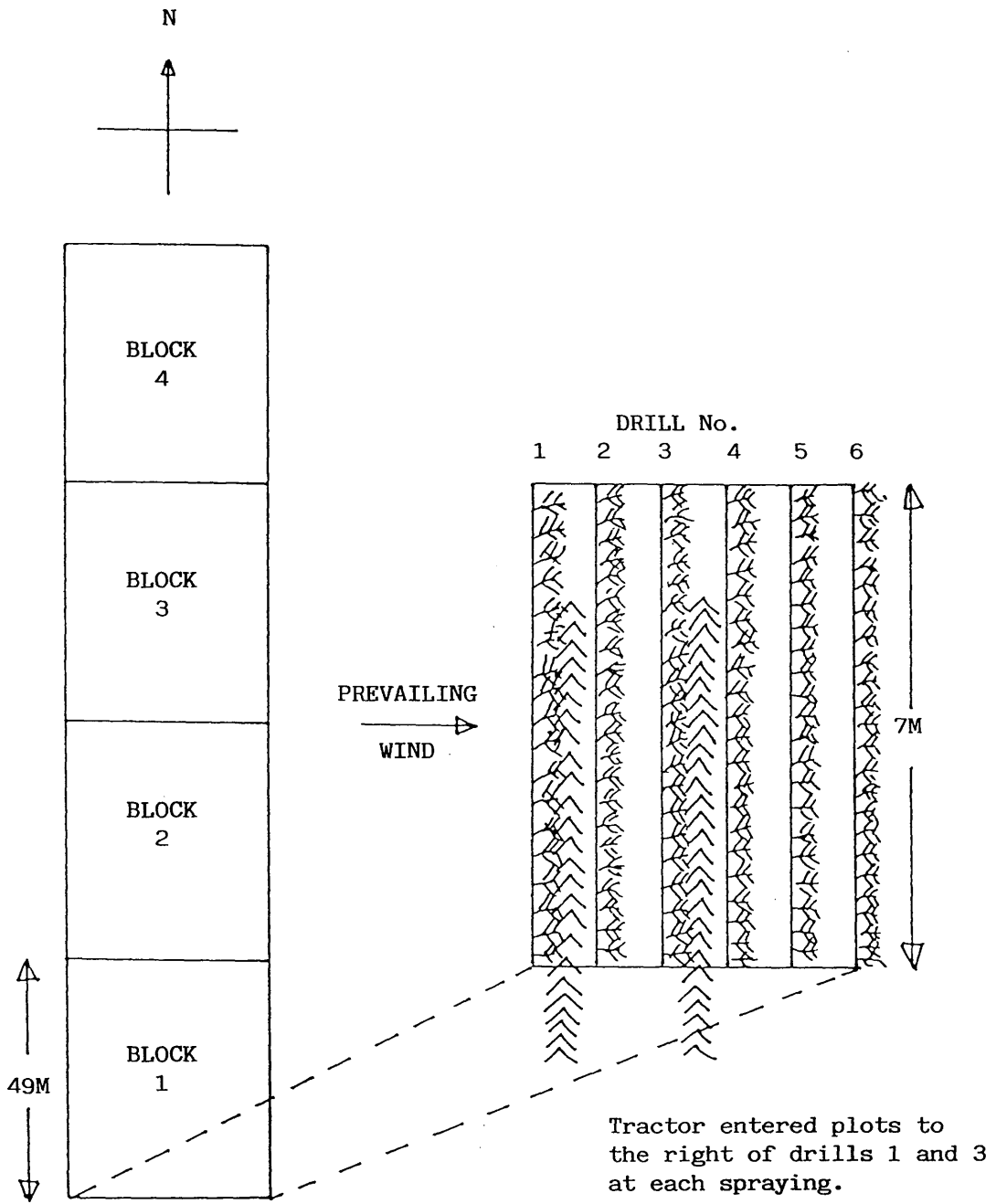


FIG. 3.19. Geographical situation of field trial and pattern of spraying operations.

Fig. 3.20 Effect of physiological age on the pattern of leaf and tuber development.

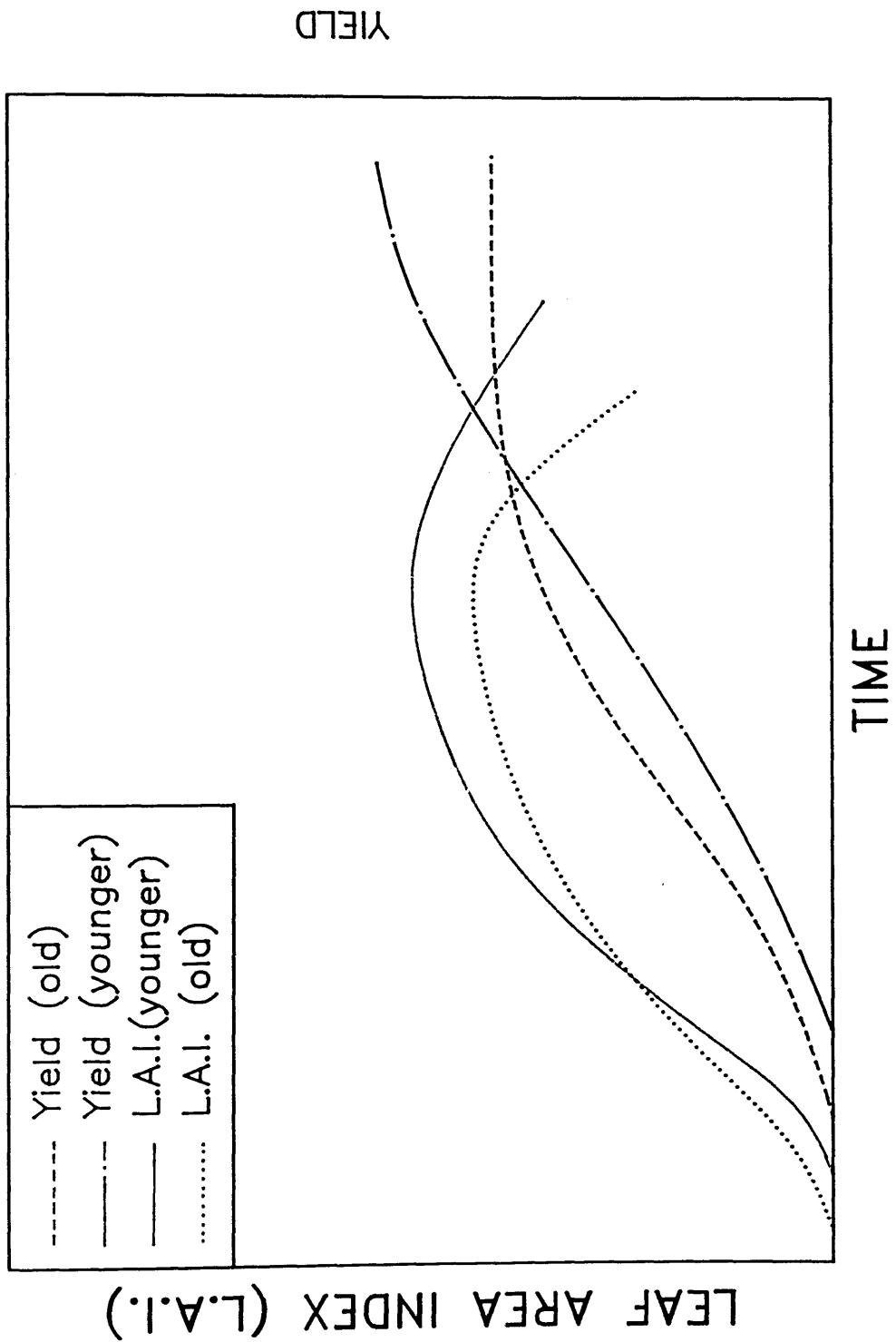


Fig. 3.21 Theoretical yield development profiles for tecnazene-treated and untreated seed.

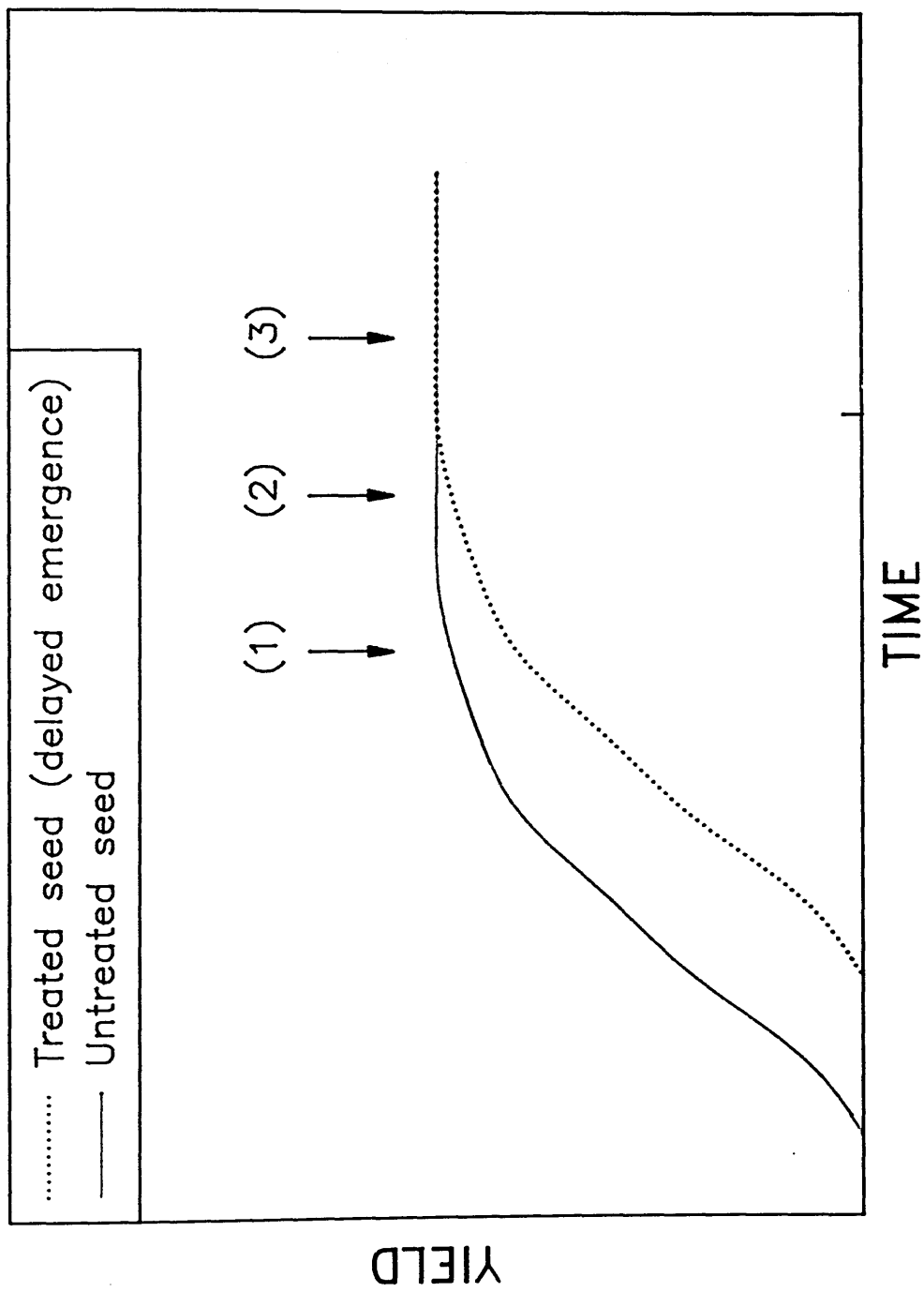


Fig. 3.22 The effect of mean emergence time (M.E.T.) on total yield

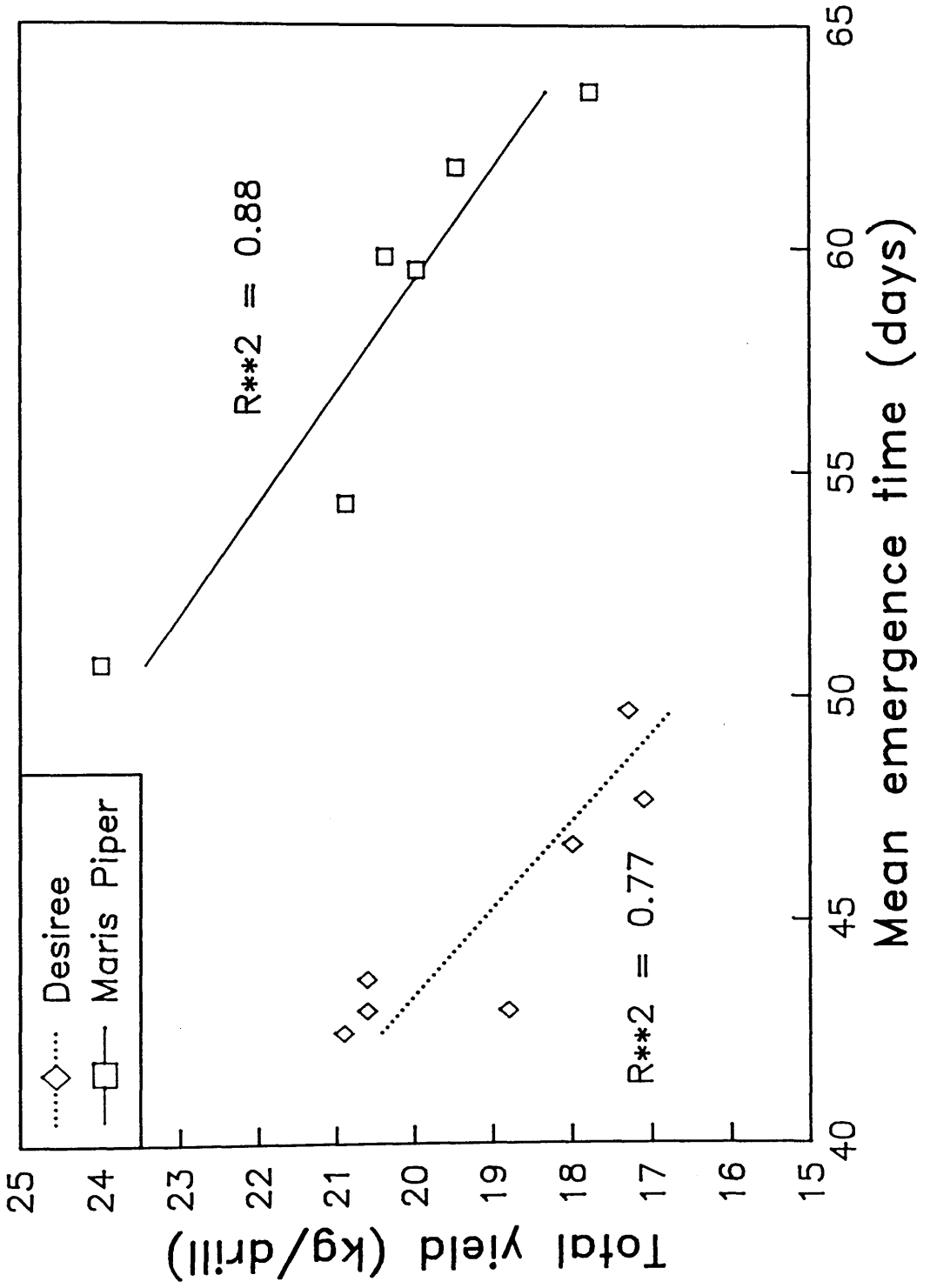
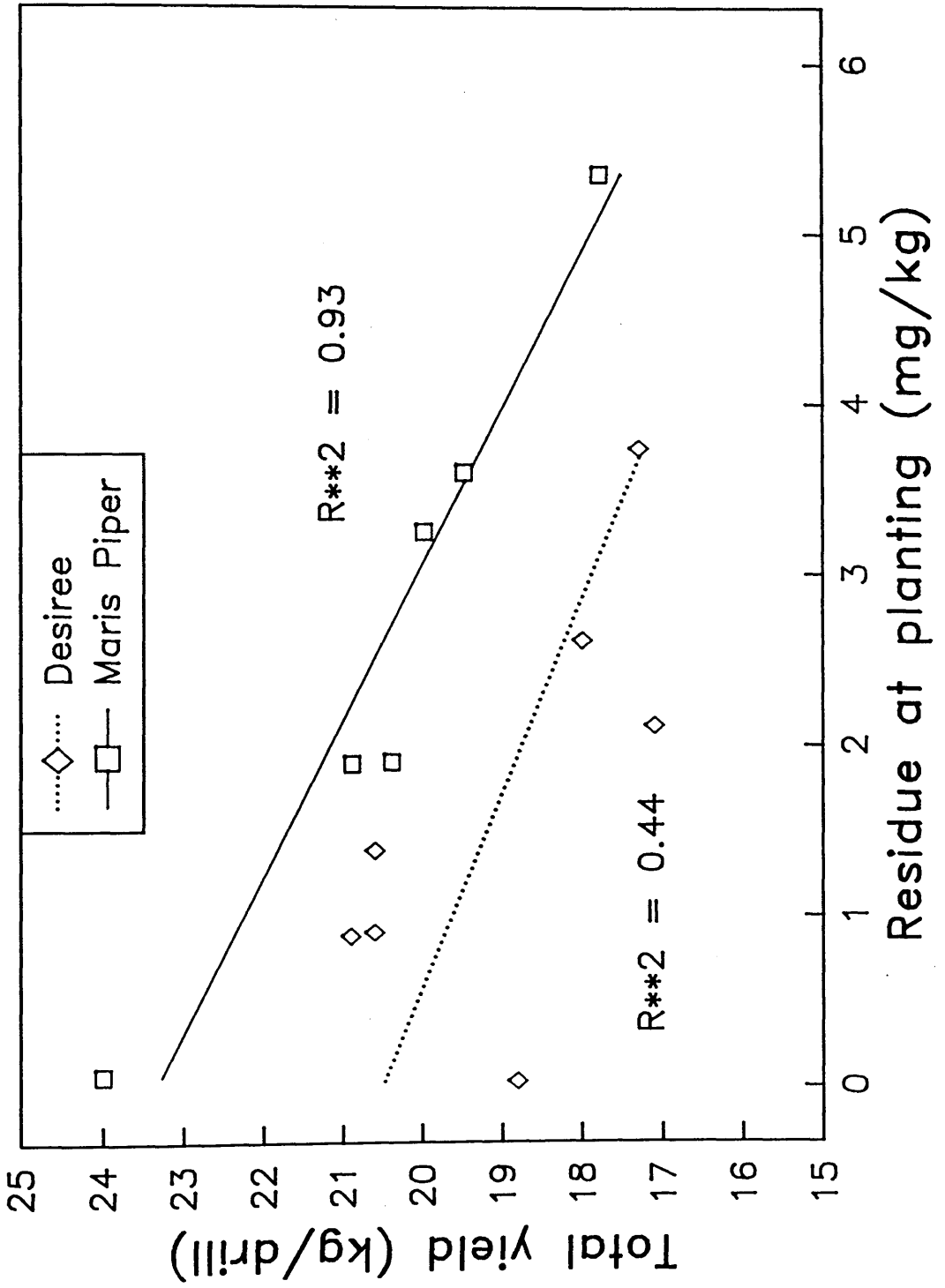


Fig. 3.23 The effect of tecnazene residues at planting on total yield



CHAPTER 4

Mode of Action of Tecnazene

4.1. Introduction

Although there has been speculation by some authors (e.g. Driver, 1961) the precise mode of action of the fungicide/sprout suppressant tecnazene is not yet clear.

The experimental work incorporated in this chapter was carried out in an attempt to elucidate the mode of action of tecnazene, and was based on a theory designed to explain the observed morphological effects of tecnazene on potato sprouts, tuber tissue and fungi. Literature relating to cross-resistance between tecnazene and members of other groups of fungicides was also assessed and will form part of the discussion.

The effect of tecnazene on treated potato tubers and on fungi show it to be an intriguing chemical in that:

1. Its action on potato sprouts is growth regulatory rather than being phytotoxic and is reversible (Brown and Reavill, 1954) - the converse being true of the other major sprout suppressant chemical chlorpropham.
2. Wound healing of damaged potato tubers would appear to be unaffected - again very different from chlorpropham. (Leonard et al, 1986).

3. Apical sprout dominance is lost at the eyes of treated tubers. (e.g Driver, 1961; also personal observations).
4. As an antifungal agent tecnazene is fungistatic rather than fungitoxic (Reavill, 1954).
5. In screening tests for sprout suppressant activity involving cress seedlings tecnazene appeared to inhibit shoot growth much more markedly than root growth (personal communication with I.M.G. Boyd).

Much of this evidence would be accommodated if tecnazene was to interfere with plant growth hormone balance. Obviously effects such as that on apical dominance could be explained in simplistic terms by inhibition of auxin synthesis or blocking of the site of action. However, gibberellin biosynthesis inhibition or site blocking might provide a better overall explanation of the effects noted, despite a far from complete understanding of the way in which they themselves operate.

The gibberellins (GAs) are a large family of diterpene acids. They were originally isolated as metabolites of the fungus *Fusarium monoliforme*, the imperfect stage of *Gibberella fujikuroi*, and were shown to cause a wide range of growth responses when applied to intact plants. The GAs are now known to be of widespread, and probably universal, occurrence in higher plants where they are generally accepted to function as hormones. Currently 62 individual GAs are known. Of these, 25 have been isolated from the

fungus *Gibberella fujikuroi*, 51 from higher plants and 14 are common to both sources. The structure of GA₃, common to both plants and fungi, is shown in Fig. 4.1. Many other fungi have been examined for GA production but only recently has a fungus other than *G. fujikuroi* been shown to produce GA's - the ascomycete *Sphaceloma manihoticola* produces GA₄ (Rademacher and Graebe, 1979).

One of the most striking effects of gibberellins on higher plants and their seedlings (including potato sprouts) is the rapid elongation of the internodal regions of stems (Krishnamoorthy, 1981a). Conversely compounds which interfere with gibberellin biosynthesis or action e.g. paclobutrazole etc. inhibit internode elongation resulting in a stunted plant.

It is thought that gibberellins influence growth by influencing both cell division and elongation. Cell division is affected in two ways. In the subapical region of both rosette and caulescent plants GA₃ increases the size of the meristematic region and also increases the proportion of cells which are undergoing division (Loy, 1977). This can be explained by an effect on the cell cycle - the cycle of changes concerned with replication during the life span of a cell, from the mitosis which gives its origin to the following mitosis that divides it into daughter cells. On the basis of DNA replication the cycle is divided into the G₁ phase, following mitosis, when the diploid quantity of DNA is present; then the S phase, of a few hours, during which the quantity of DNA doubles (with replication of the chromosomes); then the G₂ phase when the tetraploid quantity of DNA is present; and finally the M or mitotic phase. Jacquard (1968) proposed that one of the effects

of GA₃ is to promote the onset of DNA synthesis in cells which are arrested in the G₁ phase of the cell cycle. This is supported by the data of Liu and Loy (1976) on the effect of GA₃ on the cell cycle of watermelon seedlings and could explain the fact that tecnazene controls sprouting of dormant tubers - in effect increasing the length of the dormant period - while, although there is a slight reduction in rate of growth, control of sprout growth on tubers which have broken dormancy is largely ineffective (Brown and Reavill, 1954). That is to say, when applied to dormant tubers tecnazene, by inhibiting gibberellin biosynthesis or action, may prevent or slow down the attainment, by cells in the meristematic region, of the capacity for mitosis - a necessary prerequisite for sustained growth. A slowing of the rate of cell elongation would also be expected, and could account for the slight reduction in rate of growth of active sprouts treated with tecnazene.

The very limited nature of this effect of tecnazene on elongation of growing sprouts could be explained in terms of solubility since tecnazene, as will be described shortly, is only very sparingly soluble in aqueous solutions (<1 µg/cm³). On the assumption that to elicit an effect tecnazene must penetrate the plant tissue to the extra-cellular matrix, if not the cytoplasm, then it must be active at this concentration. However, because of the hydrophobic nature of tecnazene this solubilisation and penetration of the aqueous based medium bathing, and encapsulated by, cell walls is likely to be a relatively slow process.

When dormant tubers are treated with tecnazene adequate time will be available for this process to occur at the meristamatic region of the undeveloped sprouts - a statement which is unlikely to be true of tubers which possessed developing sprouts at time of treatment i.e. the cells in the meristem are dividing and extending (growing) too quickly to allow the concentration of tecnazene within the plant tissue to build up to a level at which marked sprout growth inhibition would take place.

The apparent lack of influence of tecnazene on the wound healing process in damaged tuber tissue might be another result of an anti-gibberellin effect in that the effect of GAs would appear to be a function of the state of differentiation of the cells in question. Unlike cytokinins, which promote cell division in cultured cells, GAs do not have a pronounced effect on cell numbers in suspension cultured plants. GA₃ does not promote cell division in cultured cells whose capacity to synthesise their own GAs has been prevented either by a single gene mutation or by chemical inhibitors of GA₃ biosynthesis (Rappaport, 1980) probably because these cells, if grown in the absence of plant growth hormones, are poorly differentiated. The same would be true of cortical and medullary potato tissue i.e. gibberellins are likely to play little or no role in wound healing and their inhibition, or site blocking, is therefore, likely to have little or no effect on the process.

From existing evidence the influence of tecnazene on apical dominance is unlikely to be explained by a direct effect on gibberellin levels, but could be a secondary effect resulting from consecutive inhibition of growth of the existing sprout or sprouts

at that eye - an explanation which assumes that the mechanism of correlative inhibition (apical dominance) involves competition for available nutrients or growth factors.

The effect of tecnazene on various fungi was studied by Reavill (1954) who found that it strongly retarded germination but did not stop it, and that complete germination was obtained in all cases. She also observed that treated spores produced stout, stumpy germ tubes which branched earlier, and were thicker than the controls. The effect of tecnazene on linear growth varied considerably from fungus to fungus, with treated colonies regaining normal growth rates ca. 3 days after removal from the tecnazene vapour.

Although these effects of tecnazene on fungi are not yet clearly understood and may not be fully explained by the mode of action theory outlined earlier, it should be noted that gibberellins have been shown to influence the growth and development of a number of species of fungi. In yeast, for example, GA can promote sporulation as well as growth (Kamisaka, Masuda and Yanagishima, 1967).

Finally, another observation which could be explained in terms of an anti-gibberellin effect is that in sprout suppressant activity screens involving cress seedlings, tecnazene, while inhibiting shoot growth, appeared to have no effect on root growth. Although gibberellins are produced in roots they exhibit little or no effect there (Krishnamoorthy, 1981b).

The objective of the experimental work which will now be described was to assess possible anti-gibberellin effects using

simple bioassays, and was based on the theory mentioned earlier that although tecnazene is only very sparingly soluble in aqueous solution, the level to which it is soluble is effective, i.e. the tecnazene which is active in sprout suppression is that which penetrates the area at the dormant eye where cells with potential for division and elongation are present.

The initial part of the work involved assessment of the solubility of tecnazene in water, as a figure for this was not available in any of the literature surveyed, e.g. The Pesticide Manual (1987) describes tecnazene as being "practically insoluble in water". Once this data was obtained very dilute solutions of tecnazene were prepared and used in bean seedling and oat coleoptile bioassays - both commonly used plant growth hormone bioassays.

4.2. Materials and Methods

4.2.1. Solubility of tecnazene in water

A saturated aqueous solution of tecnazene was prepared by first adding 0.1 g tecnazene to ca. 3 litres of water in a 5 litre volumetric flask. Gentle heating with agitation was then applied to the mixture using a magnetic stirrer/heater over a period of 5 hours. The solution was then left overnight in a constant temperature room at 20°C, after which a sample of 100 cm³ of the solution was removed and filtered, initially under gravity through

Whatman No. 1 filter paper, then through a 20 micron filter (Millipore). The filtered sample was divided into two 50 ml aliquots and to each was added 50 cm³ of hexane, followed by 25 cm³ of saturated NaCl solution. Solvent and solution were further mixed in a separating funnel prior to separation of the phases and re-extraction of the aqueous phase with a further 50 ml of hexane. The hexane extracts were then bulked, dried over anhydrous sodium sulphate (BDH Ltd., Poole, Dorset) and filtered into a 500 cm³ round bottomed flask. The extract was reduced in volume to 2 cm³ on a rotary evaporator and analysed for tecnazene by GLC.

This resulted in a mean figure for aqueous solubility at 20°C of 0.9 ppm.

4.2.2. Bean Seedling Bioassays

BATCH 1 The initial set of beans was grown in the absence of any chemicals in order to assess the natural variation in growth for the system employed. Dwarf beans of the variety Canadian Wonder were grown in trays of expanded mica with 24 beans per tray, planted at a depth of ca. 2 cm. Water was supplied as required via a reservoir held in a tray below that containing the mica plus beans.

The trays were placed in a growth room at 25°C under long-day conditions (16 hours light followed by eight hours dark).

After 10 days, 12 beans of even growth were taken from each tray, transplanted into 4 inch pots - again containing expanded mica - and returned to their original base tray. Water was supplied as before and the trays returned to the growth room for a further five days before removal of the pots in order that measurements of the seedlings could be taken. As gibberellins and their inhibitors are known to affect both internode and leaf stem length on bean plants (Krishnamoorthy 1981a), these were the parameters which were noted. Also measured was the distance from the roots to the cotyledons and from the cotyledons to the first internode.

The results from this preliminary work are shown in Table 4.1.

BATCH 2 This batch of beans was germinated using the method described above, with the exception that transplanting into pots was carried out 8 days after planting. The potted beans were then grown on for a further 7 days in either water, a 0.5 ppm tecnazene solution or a 0.5 ppm solution of paclobutrazole - the common name for (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol. This compound, kindly supplied by the ICI Plant Growth Regulation section, is known to act by inhibiting the biosynthesis of gibberellins (Lever et al., 1982) and as such was employed for comparison with the other treatments.

The concentration at which these compounds were used was based on results from the initial section of this work which indicated the solubility of tecnazene at 20°C to be around 1 ppm.

On removal of the beans from their pots, the following parameters were measured: length of longest rootlet, distance between roots and cotyledons, between cotyledons and first node, between first node and second node and the total leaf stem length. The results are shown in Table 4.2 and Plate 4.1.

BATCH 3 In this case a solution was prepared which, it was assumed, would be saturated with tecnazene - enough having been added to create a 1 ppm solution if all the tecnazene was to dissolve. Water and paclobutrazol at 1 ppm were again used. The beans had been germinated as above, transplanted after eight days and grown on in the above solutions for 7 days.

Measurements were taken as before and the results are presented in Table 4.3.

BATCH 4 Germination of the beans was as above although in this case transplanting was not carried out until 10 days after planting.

Although exposure to dilute tecnazene solutions reduced overall growth in the above trials, it was felt that perhaps not all of the tecnazene had dissolved. In an attempt to increase the amount of tecnazene in solution, and presumably the resultant uptake, the compound was initially dissolved in ethanol (in which it is 4% w/v soluble) to give a 0.5 mg/cm^3 solution. 5 cm^3 of this was then added to 5 litres of water to give 0.5 ppm tecnazene in

0.1% ethanol. A small amount of ethanol in aqueous solution is itself likely to influence growth and for this reason a 0.1% ethanol solution was also used, as was water alone. The beans were grown in these solutions for a further 15 days - significantly longer than before - in the hope that any differences in growth rate would be amplified.

Leaf stem lengths were not noted for this or subsequent batches. The total weight of shoot tissue was noted for this batch but not for subsequent batches.

The results are shown in Table 4.4. and Plate 4.3.

BATCH 5 With germination as above, the beans in this case were grown on in either a 0.5 ppm aqueous tecnazene solution, 0.5 ppm tecnazene in 10^{-5} molar GA_3 , or water alone - the objective being to show that any growth inhibition as a result of tecnazene treatment could be overcome by addition of GA_3 . The GA_3 concentration was chosen in an attempt to avoid excessive growth of the GA_3 treated plants. Despite this the GA_3 treated beans did have to be removed from the growth room and measured at an earlier stage than the other plants, as indicated in the results (Table 4.5).

A rather unusual feature was noted for this set of beans in that those grown in solutions containing tecnazene possessed distinctly brown coloured roots on removal from their pots at the end of the experiment (Plate 4.2). No obvious reason for this was apparent, the only difference from previous batches being that the

post transplantation period of growth, and therefore the period of exposure to tecnazene was longer than before - again in an attempt to maximise any differences between the treatments.

BATCH 6 Until this stage beans had only been exposed to tecnazene solutions after germination and an initial period of growth in water alone - the purpose being to allow differentiation between an effect on germination and an effect on growth.

In this case, however, beans in two of the six trays involved were germinated in a saturated aqueous tecnazene solution. Treatment with tecnazene was continued after transplanting these beans, and initiated for beans in two of the remaining trays. Beans from the third pair of trays continued to be supplied with water alone and thus acted as controls.

Results are shown in Table 4.6. The roots of those beans grown on in tecnazene solution were again discoloured although, rather strangely, those which had also been germinated in tecnazene solution seemed less coloured than those germinated in water then treated with tecnazene.

A set of oat coleoptile bioassays were also carried out to assess possible effects of dilute tecnazene solutions on their growth. The procedure was as follows: Oats of the variety Maris Tabard were soaked for two hours in a beaker of water, laid out on damp tissue paper in trays and exposed to light in a growth room for eight hours in order to suppress growth of the first internode. The oats had been placed in the trays with the embryos pointing in

the same direction and after the period of exposure to light they were covered with foil and laid at an angle of about 40° with the embryos pointing downwards - this procedure is designed to lead to the growth of straight coleoptiles as a result of the gravitropic effect. After six days incubation at 25°C, coleoptile sections 10 mm long, from 3 mm behind the coleoptile tip, were cut and placed in petri dishes in a solution of 1 mg/litre MnSO_4 - a preventative measure against Mn deficiency during further growth of the coleoptile sections. After three hours the sections were removed and placed in petri dishes containing 25 cm³ of either citrate - phosphate buffer pH5/2% sucrose, or this solution saturated with tecnazene. Eight petri dishes containing six coleoptile sections were prepared for each treatment and stored, dark, in a growth room at 25°C for 5 days. The sections were removed from the dishes, measured, and the results tabulated (Table 4.7).

4.3. Discussion

The results of the initial experimental batch (Table 4.1) were taken as being indicative of the natural variation evident for beans grown by the method employed. The variation, as measured by the standard deviation, was considered to be of a level which would permit continuation of the experiment.

Experimental batches 2 and 3 (see Tables 4.2, 4.3) indicated that tecnazene solution can inhibit the growth of bean plants to which they have been applied, and that this effect appeared to be

influenced by the concentration of tecnazene in solution - a 1 ppm solution resulting in greater inhibition than a 0.5 ppm solution - and by the period of exposure to the given solutions.

Inhibition was most evident when the growth of the youngest (uppermost) regions of the plants, as measured by internodal distance, was compared to that for the controls. A similar pattern of inhibition was also noted for the gibberellin biosynthesis inhibitor employed for comparison in these batches, although in both cases its inhibitory effect greatly exceeded that achieved by tecnazene solutions - a result which could perhaps be explained by the fact that the solubility of paclobutrazole in aqueous solution is much greater than that of tecnazene.

With Batch 4 an attempt was made to ensure the dissolution in water of all added tecnazene by first dissolving the required amount in absolute ethanol, in which it is up to 4% soluble. As previously mentioned it was accepted that even a small amount of ethanol could itself inhibit growth and for this reason an ethanol control was included in the experiment.

This resulted in greater growth inhibition by the tecnazene solution than had been previously obtained (Table 4.4, Plate 4.3), even when the effect of the ethanol alone is taken into account. It should, however, be noted that the post-transplantation growth period was several days longer than in the preceding experiments - a deliberate attempt to optimise any differences between the respective treatments. It would seem that this had been achieved, albeit at the cost of greater variation in growth of the individual plants.

The fifth experimental batch was designed again with the solubilisation problem in mind. In this case enough tecnazene was added for creation of a saturated solution. Two such solutions were prepared, and to one was added enough GA₃ to give a concentration of 10⁻⁵ M. This level is sub-optimal for GA₃ induced growth and was chosen in the hope that potentially excessive growth of treated plants would be avoided. This was achieved to some extent, although it was still necessary to remove and measure the TCNB/GA₃ treated plants earlier than those treated with TCNB alone. The results showed that inhibition as a result of growth in a tecnazene solution, evident for those plants treated with tecnazene alone, could be overcome by addition of exogenous GA₃.

The final set of beans was used to assess the effect, when compared to controls and treatment after germination, of germination in a solution saturated with tecnazene. While inhibition of growth was indeed observed for beans germinated and subsequently grown on in a saturated tecnazene solution, it was rather surprisingly not as great as that observed for plants germinated in water alone before subsequent growth in a similar tecnazene solution.

The root discolouration noted for both this and the preceding set of beans was an effect not observed for any of the previous experimental batches, which only differed in that they were exposed to tecnazene for a shorter period of time. The discolouration did not appear to be a result of necrosis as the roots appeared otherwise similar to those of the controls. This effect is as yet unexplained, although it may be of interest that the foliar applied

sprout suppressant maleic hydrazide, an inhibitor of mitosis (Corbett et al., 1984b) results in a similar discolouration when applied to grass as a growth retardant.

The results of the oat coleptile bioassays showed a slight reduction in the growth of those sections exposed to tecnazene when compared to controls (Table 4.7). As mentioned previously this particular bioassay is fairly specific for anti-auxin activity, however compounds which inhibit gibberellin biosynthesis or action would still be expected to show an effect. The degree of effect shown by tecnazene in this case might be limited by its rate of penetration into the tissue - again related to its aqueous solubility.

In conclusion, when taken in an overall context the results of the bean seedling bioassays indicate that a solution containing dissolved tecnazene, albeit at concentrations around 1 part per million or less, can indeed result in a reduction in the rate of elongation of the internodal regions of the bean plants. This inhibition can be overcome by application of exogenous GA₃, and while this might provide more evidence for an anti-gibberellin mode of action for tecnazene, it cannot exclude all other mechanisms.

Rather interestingly an effect similar to that exhibited by the treated bean plants was reported by Buchenauer and Grossman (1977) following treatment, via soil, of tomato and cotton, seedlings with the systemic fungicide Triadimefon, 1-(4-chlorophenoxy)-3,3-dimethyl-1(1,2,4-triazol-1-yl)-2-butanone. Observed effects included retardation of the elongation of the upper internodes of shoots of tomato and cotton plants - complete

reversal of which could, however, be achieved by exogenously applied gibberellic acid (GA₃). Seed treatment of wheat and barley with this chemical resulted in reduced growth of coleoptiles, primary leaves and roots; effects which were not completely counteracted by the addition of GA₃. Also of note is the fact that triadimefon strongly reduced the synthesis of gibberellin-like substances in *Fusarium moniliforme*, and inhibited the development of haustoria of *Erysiphe graminis* f.sp. *hordei*. Studies on its mode of action revealed that triadimefon blocked ergosterol biosynthesis in *Ustilage avenae*.

Often cross resistance between different groups of fungicides can be a useful indicator of the mode of action of one or other of the groups involved. In the case of the aromatic hydrocarbon fungicides, of which tecnazene is a member, cross resistance has in fact been noted with the dicarboximide fungicides e.g. vinclozolin, iprodione and procymidone (Lyr and Casperson, 1982; Leroux and Gredt, 1982). This information is, however, of limited benefit as the mode of action of the dicarboximides is also yet to be clarified, although it does mean that information available about the dicarboximides may be of relevance with regard to the aromatic hydrocarbons, and vice-versa.

This would include the findings of Buchenauer (1976) that vinclozolin caused free fatty acid accumulation in treated sporidia of *Ustilage avenae*, while triglyceride synthesis was inhibited.

This work also indicated that there were no apparent differences between control and treated sporidia in the incorporation of ^{14}C acetate into fractions of phospholipids, sterols and sterol esters - the results showing no decrease in the ergosterol level between treated and untreated sporidia.

Georgopoulos et. al. (1979) noted that the dicarboximides stimulate somatic segregation in diploid strains of *Aspergillus nidulans*, indicating interference in nuclear processes. This effect was also observed for tecnazene, albeit when applied at relatively high concentrations, leading the author to state that the fungitoxicity of the hydrocarbons is a result of their effect on the chromosomes, and possibly the mitotic spindle.

Most recently Lyr and Edlich (1986) stated that aromatic hydrocarbon fungicides and dicarboximide fungicides induce a lipid peroxidation in sensitive fungi, the cause of which is assumed to be a specific interaction of the fungicides with flavin containing enzymes - a cytochrome C reductase appearing to have a key role. In *Mucor mucedo* all but two of the ergosterol biosynthesis inhibitor fungicides investigated also induced, at high concentrations, a lipid peroxidation well correlated with a growth inhibition. Although the author states that no cross resistance exists in *M. mucedo* between ergosterol biosynthesis inhibitors and aromatic hydrocarbon or dicarboximide fungicides, the results would indicate some common effects on fungi.

While further work, probably at the cell constituent level, will be required to clarify the mechanism of action of tecnazene it seems apparent that some of its effects, if not the primary mode of

action, are similar to those of the ergosterol biosynthesis inhibitors and that action by an anti-gibberellin effect cannot be ruled out. Radiolabelled tecnazene could be useful in determining the site of action of the compound.

BATCH 1

Period of germination plus early growth: 10 days

Period of post transplantational growth: 5 days

	CONTROL 1	CONTROL 2
	Mean (S.D)	Mean (S.D)
Root to Cotyledons	117 (11.4)	120 (12.1)
Cotyledons to First Node	47 (10.4)	48 (6.7)
First Node to Second Node	39 (16.7)	35 (16.3)
Total Leaf Stem Length	51 (11.7)	49 (10.8)
Total Distance between Root and Uppermost Node	203	203

All figures are mm. and are mean values of 12 replicates.

TABLE 4.1.

BATCH 2

Period of germination plus early growth: 8 days

Period of post transplantation growth: 7 days

	CONTROL		0.5 ppm TECNAZENE		0.5 ppm PP333	
	Mean (S.D)	Mean (S.D)	Mean (S.D)	% Inhibition	Mean (S.D)	% Inhibition
Root to Cotyledons	106 (9.3)	105 (11.3)	105 (11.3)	1.1	105 (15.8)	1.2
Cotyledons to First Node	52 (8.2)	48 (8.9)	48 (8.9)	7.3	38 (7.9)	26.4
First Node to Second Node	60 (20.1)	53 (19.3)	53 (19.3)	12.4	3 (1.6)	94.5
Total Leaf Stem Length	63 (9.1)	61 (8.0)	61 (8.0)	3.0	30 (4.3)	51.5
Total Distance between Root and Uppermost Node	218	206	206	5.5	146	33.0

All figures are mm. and are mean values of 12 replicates.

TABLE 4.2.

BATCH 3

Period of germination plus early growth: 8 days

Period of post transplantation growth: 7 days

	CONTROL		1.0 ppm TECNAZENE		1.0 ppm PP333	
	Mean (S.D)		Mean (S.D)	% Inhibition	Mean (S.D)	% Inhibition
Root to Cotyledons	125 (11.6)		115 (12.5)	7.9	122 (16.9)	2.2
Cotyledons to First Node	61 (7.3)		56 (8.1)	8.9	50 (7.7)	17.4
First Node to Second Node	94 (24.9)		74 (23.2)	20.3	6 (2.7)	93.5
Total Leaf Stem Length	64 (7.7)		66 (8.3)	-2.5	36 (5.1)	44.8
Total Distance between Root and Uppermost Node	280		245	12.5	178	36.4

All figures are mm. and are mean values of 12 replicates.

TABLE 4.3.

BATCH 4

Period of germination plus early growth: 10 days

Period of post-transplantation growth: 15 days

	CONTROL		0.1% EtOH		0.5 ppm TCNB in 0.1% EtOH	
	Mean (S.D)		Mean (S.D)	% Inhibition	Mean (S.D)	% Inhibition
Root to Cotyledons	81 (8.4)		89 (11.8)	-8.8	86 (13.1)	-5.9
Cotyledons to First Node	34 (3.2)		36 (9.8)	-5.6	34 (5.1)	-1.5
First Node to Second Node	45 (12.6)		35 (11.9)	22.4	30 (10.0)	32.3
Second Node to Third Node	54 (8.4)		42 (7.9)	22.8	32 (10.6)	40.6
Third Node to Fourth Node	56 (23.0)		46 (28.4)	18.3	23 (22.1)	58.1
Total Wt Shoot Tissue (g)	4.13 (0.70)		3.70 (0.56)	10.3	3.43 (0.60)	16.8
Total Distance Between Root and Uppermost Node	270		248	8.1	205	24.1

All figures are mm. and are mean values of 12 replicates.

TABLE 4.4.

BATCH 5

Period of germination plus early growth: 7 days

Period of post-transplantational growth control and TCNB treated: 15 days
 TCNB plus GA₃: 7 days

	CONTROL		TCNB SOLN. (SATD.)		TCNB + GA ₃ (10 ⁻⁵ M)	
	Mean (S.D)		Mean (S.D)	% Inhibition	Mean (S.D)	% Inhibition
Root to Cotyledons	112 (12.4)		109 (12.0)	2.7	109 (11.0)	2.7
Cotyledons to First Node	33 (7.5)		39 (9.1)	-18.2	56 (4.9)	-69.7
First Node to Second Node	48 (16.1)		50 (17.6)	-4.2	137 (23.8)	-185.4
Second Node to Third Node	57 (11.7)		67 (11.1)	-17.5	148 (34.5)	-159.6
Third Node to Fourth Node	109 (18.9)		88 (32.5)	19.3	116 (52.4)	-6.4
Forth Node to Fifth Node	99 (52.6)		49 (51.4)	50.5	-	-
Total Distance Between Root and Uppermost Node	359		353	1.7		

All figures are mm. and are mean values of 12 replicates.

TABLE 4.5.

BATCH 6

Period of germination plus early growth: 10 days

Period of post transplantational growth: 14 days

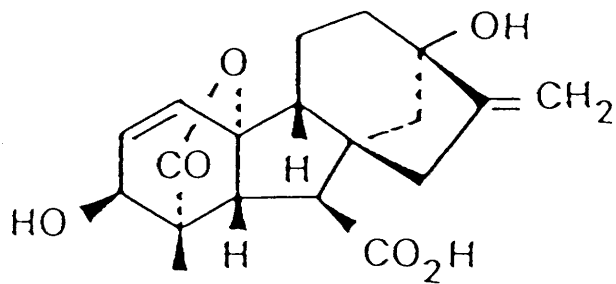
	CONTROL		SATD. TCNB SOLN. AFTER GERM.		SATD. TCNB SOLN. THROUGHOUT	
	Mean (S.D)	Mean (S.D)	Mean (S.D)	% Inhibition	Mean (S.D)	% Inhibition
Root to Cotyledons	104 (11.8)	106 (11.2)	111 (9.6)	-1.9	111 (9.6)	-6.7
Cotyledons to First Node	34 (5.8)	37 (5.8)	34 (7.0)	-8.8	34 (7.0)	-
First Node to Second Node	48 (19.7)	50 (13.9)	41 (18.1)	-4.2	41 (18.1)	14.6
Second Node to Third Node	59 (18.0)	50 (12.5)	49 (18.2)	15.3	49 (18.2)	16.9
Third Node to Fourth Node	108 (32.2)	70 (23.5)	90 (28.1)	35.2	90 (28.1)	16.7
Fourth Node to Fifth Node	97 (49.6)	22 (17.7)	62 (50.6)	77.3	62 (50.6)	36.1
Total Distance Between Root and Uppermost Node	450	335	387	25.6	387	14.0

All figures are mm. and are mean values of 12 replicates.

TABLE 4.6.

	Mean Length of Coleptile Section (mm)	Standard Deviation
Citrate/Phosphate Buffer pH 5/2% Sucrose	11.8	1.5
Tecnazene Saturated Citrate/Phosphate Buffer pH/5 2% Sucrose	11.2	1.4

TABLE 4.7



GA₃

Fig. 4.1. Structure of GA₃

Plate 4.1. Effect of tecnazene and PP333 on internodal elongation in bean seedlings.



Plate 4.2. Discolouration resulting from application of tecnazene solution via roots.





Plate 4.3. Effect of tecnazene in 0.1% ethanol on internodal elongation in bean seedlings.

CHAPTER 5

The Effect of Tecnazene on the Healing of Tuber Wounds

5.1. Introduction

Damage to a proportion of potato tubers during harvest and subsequent handling is an undesirable but almost inevitable consequence of commercial production techniques. This damage often results in the skin of the tuber being broken (wounding). Wounding leads to water loss from the tuber, resulting in a poorer final product and, more importantly, facilitates the access of fungal and bacterial pathogens into the tuber.

Chemicals, such as fungicides or sprout suppressants, applied post harvest to a crop with the aim of maintaining tuber quality throughout the storage season, may in fact have a detrimental effect should any of the chemicals employed interfere with the process of wound healing.

While all the relevant work (Audia et al., 1962; Reeve et al., 1963; McGee, 1984) has shown the sprout suppressant chlorpropham (isopropyl N-(3-chlorophenyl carbamate) to inhibit wound healing, there have been conflicting reports regarding the effect of tecnazene - Cunningham, (1953) noting inhibition of wound healing resulting from tecnazene treatment but McGee (1984) observing no adverse effect.

The objective of the work which will be outlined in this section was to clarify the effect of tecnazene on the healing of wounds using a technique employed extensively by McGee, and differing only in the method of application of the chemical. However, before further discussion of the methods used it would be beneficial to consider the current state of knowledge regarding the process of wound healing, and to note relevant published literature.

The initial response to the wounding of potato tuber tissue is a rapid increase in the rate of protein and nucleic acid synthesis in the cells immediately below the cut surface (Sato et al., 1978; Borchert and McChesney, 1973; Sadava and Crispeels, 1978). This is accompanied by membrane degradation and is followed by the deposition of a thin superficial layer of suberin - a polymer thought to be composed of a phenolic matrix to which aliphatic components are attached (Cottle and Kolattukudy, 1982). Later a number of subsurface layers of cells divide, their cell walls become impregnated with suberin and waxes and the cells outside them die and collapse to leave a wound periderm similar to the natural skin of the potato (Priestly and Woffenden, 1923; Artschwager, 1927).

Surface suberization tends to occur over a period of hours after wounding, with cell division leading to periderm formation taking place over a period of weeks (McGee 1984).

Several factors influence the rate of the wound healing process. These include temperature, relative humidity, oxygen/carbon dioxide concentration, and cultivar. The rate of

healing increases with temperature reaching a maximum at around 20 - 25°C, but declining at temperatures greater than this (Walker and Wade, 1978; Thomas, 1982). High relative humidity (>80%) normally favours wound healing (Artschwager, 1927) although very high relative humidities may result in proliferation, with the cells at the wound surface expanding (Lange et al., 1970). The oxygen to carbon dioxide ratio also has an effect on the rate of suberin development and periderm formation. A lowering of the oxygen concentration can slow the process (Lipton, 1967), as can an increase in the carbon dioxide concentration relative to normal atmospheric conditions (Wigginton, 1974). The rate of wound healing has been observed to vary with cultivar by several authors (Priestly and Woffenden, 1923; Artschwager, 1927; Smith and Smart, 1959; Wigginton, 1974; Ali et al., 1975; McGee 1984).

Procedures for Studying the wound Healing Process in Potato Tubers

Until relatively recently studies on the wound healing process normally involved histological methods, in which stained sections of fixed tissue were analysed by light microscopy. These methods are rather protracted, and are likely to include a large element of subjectivity. The stains employed also tend to be non-specific.

However, methods have since been developed which involve assessment of the rate of water loss for a given tuber surface area. These are based on the principle that as wound healing progresses, the rate of diffusion of water through the tissue

decreases. Burton (1978c) has indeed shown that the rate of water loss from a tuber is increased dramatically if the periderm is removed, while Kollatukudy and Dean (1974) have observed a direct correlation between the development of resistance to water loss in potato tuber discs and the rate of suberisation of the periderm layer.

An analogy can be drawn between studies of this sort and those on transpiration from leaves, since in both cases the movement of water through plant tissue is involved. As such, these studies must involve, as for transpiration studies, cognisance of the difference between "external" and "internal" resistance to water loss.

The "external" resistance to water loss can be defined as a combination of those factors which limit the diffusion of water vapour away from a surface, whether it be a leaf surface or the surface of a potato tuber. These factors include temperature, relative humidity, and wind speed. "Internal" resistance refers to the resistance of the tissue in question to the outward movement of water. In the case of leaf surface this would be influenced by the cuticle, and by the degree of opening of the stomata, while for the cut surface of a potato tuber this will be influenced, as previously described, by the extent of suberisation and periderm formation.

The method for assessment of wound healing which was employed in the work which will be presented was that developed by Jarvis and Duncan (1979), and was based on assessment of water loss, as described above. The method involved weighing potato tuber discs

of known dimensions, prior, and subsequent, to exposure of the discs to a stream of air - a practice designed to minimise external resistance and thus reduce analysis time.

5.2. Materials and Methods

Tubers of the cultivar Record were used in this work. These were received shortly after harvest and stored at 8°C for up to four weeks prior to use.

Discs of tuber tissue 11 mm in diameter and 4 mm deep were prepared. This involved an initial surface sterilisation step of flaming the tubers three times with ethanol. Tuber cores 11 mm in diameter were then taken using a No. 7 cork borer, and sliced into the appropriately sized discs using a piece of equipment consisting of 10 metal blades bolted together at 4 mm intervals.

Discs from several tubers were pooled, mixed and six placed on the inverted lid of a petri dish. The base of the dish, containing a thin layer of water agar, was then replaced. The purpose of the agar was to maintain high relative humidity within the dish, which, as mentioned previously, encourages wound healing. Agar was prepared by autoclaving a 1.2% (w/v) solution of Agar Technical (Oxoid Agar No. 3) for 2 hours. While still warm this was then transferred to sterile plastic petri-dishes. As for tuber disc cutting, agar plate preparation was conducted under aseptic conditions in an inoculating room.

Enough discs were prepared for analysis of four replicates of each treatment on eight analysis dates. (Initially, then 3, 6, 9,

12, 15, 18 and 21 days after preparation). Extra discs were also prepared as replacements in the event of disease development in any of the dishes. Fresh tuber discs were also cut on each analysis date.

Treatments involved vapour phase application of either tecnazene or chlorpropham. This was achieved by placing a discrete reservoir of crystalline chemical (100 mg), contained in a plastic holder, at the centre of each dish. Control plates were also prepared, to which no chemical was added. Chlorpropham was included in these studies as a reference as all previous work had shown it to have an inhibitory effect on wound healing.

The plates for each treatment were stored in separate cardboard boxes in a room in which the temperature was maintained at approximately 24°C.

Resistance to water loss was determined from weight loss of the discs after exposure to an airstream of known temperature for a set period of time.

The six potato discs were removed from each treatment dish and placed in a pre-weighed, clean, dry petri dish. The dish was again weighed, after which the lid was removed and the discs exposed for 90 seconds to a stream of air generated by an electric hair dryer clamped 30 cm above the discs. This preceded further weighing of the dish before and after each of three subsequent 20 second exposures to the airstream.

The initial 90 second exposure covers the period over which disc water loss is not linear (Jarvis and Duncan, 1979), while the weighing differences between the 20 second exposures provide three

replicate values for rate of water loss.

Analysis of the discs took place in the same room in which they were incubated. The airstream temperature and relative humidity were noted on each analysis date. Airstream temperature varied within the range $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and relative humidity in the range 34-42%.

This procedure was conducted for both freshly prepared discs and for aged discs. The results obtained were incorporated into the following equations.

$$r_{\text{ext.}} = d (1 - \text{r.h.})/E_0$$

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

$r_{\text{ext.}}$ - total external resistance to water loss.

$r_{\text{int.}}$ - total internal resistance to water loss.

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

E_0 - rate of water loss per unit area of fresh discs.

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

r.h. - relative humidity.

Two sets of discs were prepared and analysed as described above.

The results for Set 1 are shown in Tables 5.1.-5.3. and Figure 5.1, and for Set 2 in Table 5.4.-5.6 and Figure 5.2.

For both sets of discs it appears evident that exposure to chlorpropham had an adverse effect on wound healing, as measured by rate of water loss, when compared to either tecnazene treatment or the control. In both cases no consistent differences in the development of resistance to water loss was noted between tecnazene treated and control discs.

The results are broadly in agreement with those of McGee (1984). His work involved the same method of assessment of the rate of wound healing, and differed only in the method of application of the chemicals. For his method the chemicals were first dissolved in acetone to give a solution of known concentration. 5 μ l of the appropriate solution was then injected into a 50 μ l droplet of sterile, distilled water which had been previously pipetted onto the surface of each disc. The main problems associated with this method of application include the fact that both water and small amounts of organic solvent can have a deleterious effect on the wound healing process and, perhaps more importantly, that when tecnazene is applied as described above its low aqueous solubility can result in its precipitation from solution during injection into the water droplet - a phenomenon reported by McGee himself. While controls can, to a certain extent, account for the effect of various solvents, the influence of the latter problem on the healing of cut tissue is not known. This method of application is also rather unrealistic considering that in a commercial situation tecnazene is applied on a dust carrier and relies on vapour phase distribution for activity.

Another difference between this work and that of McGee was

that his period of assessment was nine days, while that for the results reported here was 21 days. In both cases analysis was conducted at 3 day intervals. The longer period of assessment for the work discussed in this chapter was chosen as it was considered that it would result in a more complete picture of the effect of the various treatments on the wound healing process, especially considering the variation sometimes observed when using this method, and the fact that the process is far from complete 9 days after wounding. However, even over 9 days McGee found that tecnazene, applied to discs at 10 ppm, 100 ppm and 200 ppm, had no deleterious effect on the development of internal resistance to water loss. By the final assessment date it appeared, in fact, to have increased the internal resistance of the treated tubers when compared to the controls.

These results were reinforced when histological assessment of suberization and periderm development was conducted on the same discs 9 days after they were cut. By this method of assessment untreated discs showed the strongest degree of periderm development although the effective control, water plus acetone, exhibited less periderm development than all three tecnazene treatments.

McGee (1984) also found that storage, under commercial conditions, of tubers initially treated with tecnazene resulted in periderm development which was better than that for chlorpropham treated tubers, but not as strong as that for untreated tubers. However, an important point noted by McGee was that the facility in which the tecnazene tubers were stored, had in previous seasons been used to store chlorpropham treated tubers. This can result in

carry-over of chlorpropham in concrete and wooden boxes (Boyd and Duncan 1986) at a level which might have an inhibiting effect on wound healing.

The adverse effect of chlorpropham on the wound healing which was observed in the results presented was also noted for McGee's disc experiments at all but the lowest (1 ppm) level. This low level effect could not be explained and was not evident in the results of histological examination of both tuber discs and commercially stored cut tubers.

Work by Jarvis (Leonard et al., 1986) also found that chlorpropham treatment rendered potato tuber discs more susceptible to infection by gangrene (*Phoma exigua* var. *foveata*) when inoculated around 4 - 6 days after wounding, a phenomenon indicative of inhibited periderm formation. As mentioned in the introduction other authors have also found chlorpropham to exhibit a negative effect on wound periderm development.

5.3. Discussion

The results observed for the work comprising this chapter, although of relevance to all with an interest in potato storage, is of particular importance in large scale storage of potatoes destined for crisping.

This market requires potatoes which are low in reducing sugars and as such necessitates storage of tubers at relatively high temperatures (around 8°C). Sprout growth at this temperature would be excessive if it was not controlled by chemical means i.e.

by use of the sprout suppressants chlorpropham and tecnazene.

The use of chlorpropham is most widespread because it is relatively cheap, and because when applied as a thermal fog re-application is fairly simple. However, its drawbacks include its effect on the wound healing process which, even allowing a curing period prior to initial application, can lead to a greater level of natural pathogenic infection than for tubers given an initial treatment of tecnazene (McGee 1984). This procedure of applying tecnazene during store filling, and chlorpropham thereafter, is one which has recently become more widespread for commercial potato storage. It has been based on the belief that tecnazene has little effect on wound healing and will thus control sprouting, will permit wound healing, and delay the first application of chlorpropham. It also has the added benefit of providing some control of certain fungal species (Reavill, 1954).

The delay in the application of chlorpropham which tecnazene facilitates is desirable in order to minimise problems with "blemish" a condition caused by an abnormal form of skinspot (*Polyscytalum pustulans*) which results in lesions larger and deeper than those associated with skinspot. These symptoms are evident for tubers treated with either propham (isopropyl N-phenyl-carbamate) (Ives, 1955), or chlorpropham (French, 1976).

The practice of initially treating tubers with tecnazene results in delayed development of this condition, and of other conditions caused by pathogenic infection (McGee, 1984). This procedure is supported by the results presented in this chapter. It is also suggested that the first application of chlorpropham

could be further delayed if the initial period of "curing" - storage at an elevated temperature with restricted ventilation - was modified.

This practice was originally devised by Malcolmson and Gray (1968) to encourage rapid wound healing and avoid severe gangrene infection. However, the temperature normally employed (ca. 15°C) has the disadvantage of resulting in a more rapid loss of tecnazene, by volatilization, than would occur at a lower temperature. Sprouting therefore occurs earlier, and with it the need for chlorpropham application. Development of this situation could be delayed, and the goal of periderm formation still achieved, if the curing temperature was reduced.

Control (untreated) discs - SET 1

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.26	0.01
3	0.34	0.08
6	0.48	0.23
9	0.98	0.74
12	1.07	0.82
15	1.31	1.05
18	1.76	1.53
21	1.77	1.52

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.1.

Tecnazene treated discs - SET 1

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.25	0.00
3	0.34	0.08
6	0.58	0.33
9	1.01	0.77
12	1.14	0.89
15	1.08	0.82
18	1.20	0.97
21	1.69	1.44

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.2.

Chlorpropham treated discs - SET 1

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.25	0.00
3	0.32	0.06
6	0.40	0.15
9	0.54	0.30
12	0.72	0.47
15	1.02	0.76
18	0.80	0.56
21	1.23	0.98

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.3.

Control (untreated) discs - SET 2

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.25	0.00
3	0.32	0.07
6	0.64	0.40
9	0.71	0.49
12	1.25	1.01
15	1.17	0.94
18	1.36	1.13
21	1.55	1.32

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.4

Tecnazene treated discs - SET 2

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.25	0.00
3	0.31	0.06
6	0.75	0.51
9	1.17	0.95
12	1.00	0.76
15	1.25	1.02
18	2.11	1.88
21	2.01	1.78

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.5.

Chlorpropham treated discs - SET 2

DAY	TOTAL RESISTANCE TO WATER LOSS	INTERNAL RESISTANCE TO WATER LOSS
0	0.25	0.00
3	0.30	0.05
6	0.42	0.18
9	0.71	0.49
12	0.77	0.53
15	0.77	0.53
18	1.04	0.81
21	0.98	0.75

Units for all figures: $\text{mg}^{-1} \text{cm}^{-2} \text{s}$

TABLE 5.6.

Figure 5.1. Development of resistance to water loss in aged tuber discs.

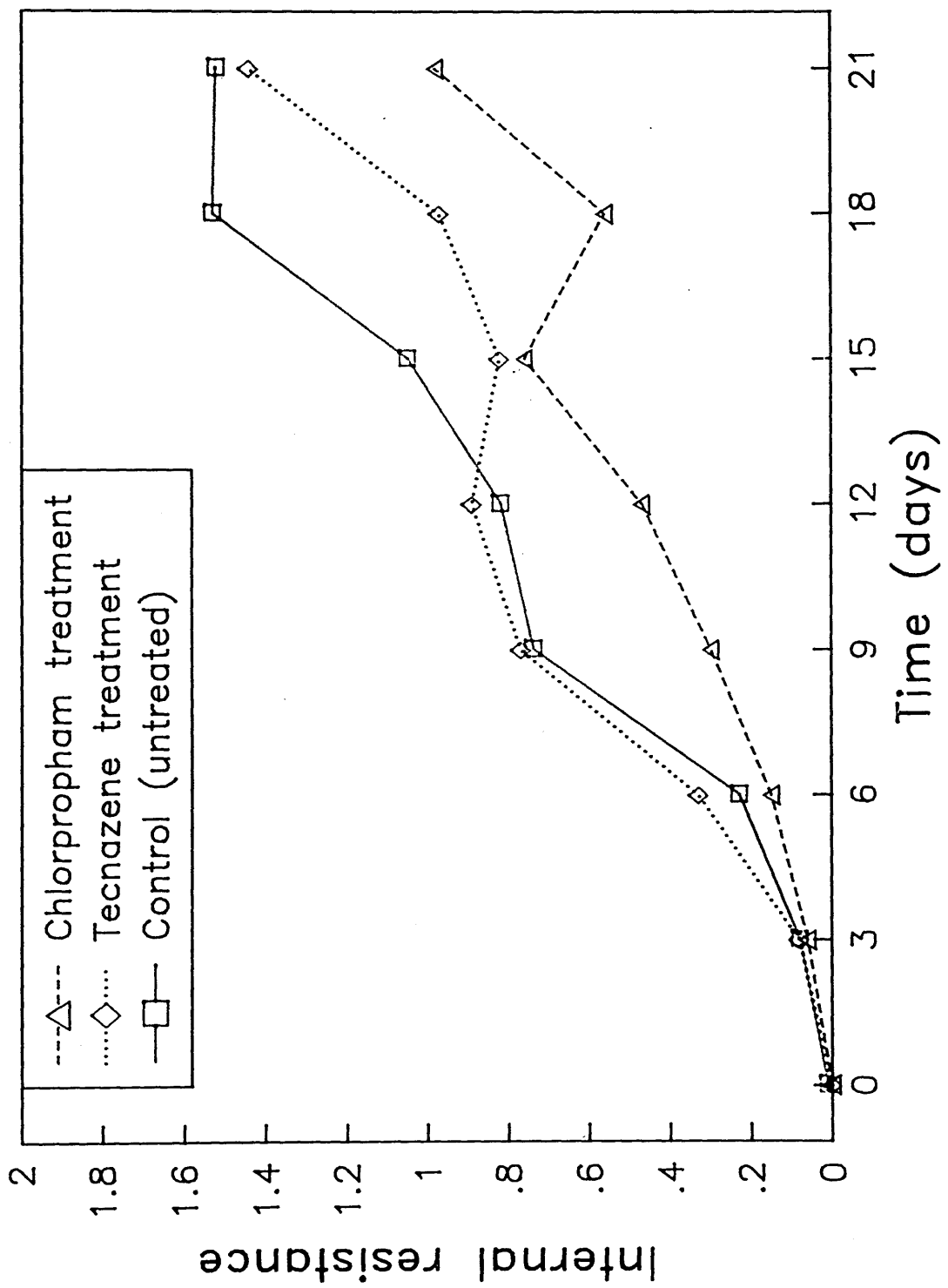
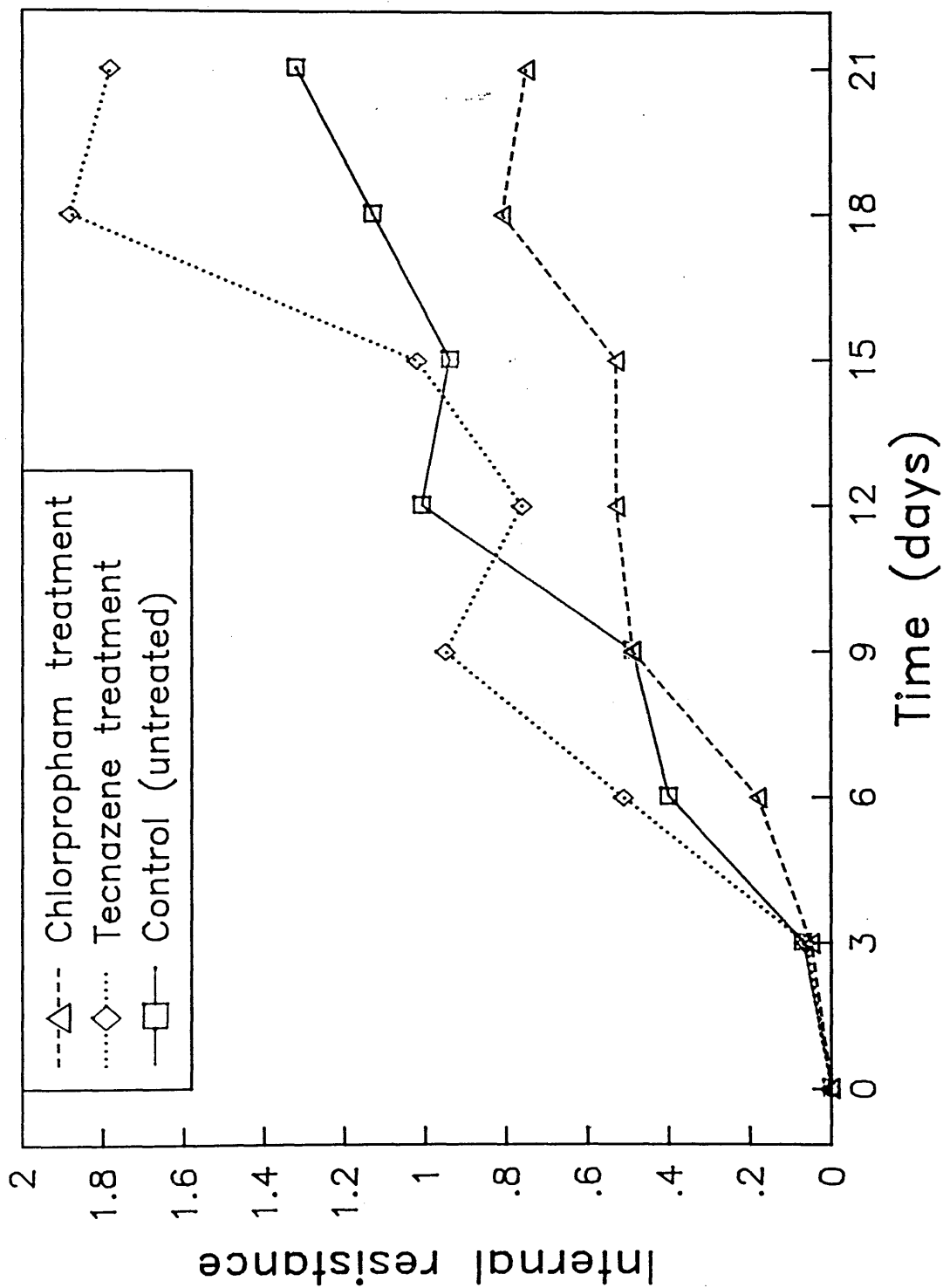


Figure 5.2. Development of resistance to water loss in aged tuber discs.



CHAPTER 6

The Effect of Sprout Suppressant Chemicals on Internal Sprouting of Potato Tubers

6.1. Introduction

The phenomenon of internal sprouting (Plates 6.1, 6.2) occurs when one or more of the sprouts from the eye of a tuber grow through the tissue of that or an adjacent tuber, rather than up and away from it.

It is a disorder which has long been known (Muller, 1846; Gager, 1912; Stewart, 1918), but towards which little research had been directed prior to it becoming a problem of commercial significance during the 1960-61 storage season in the United States of America. This prompted work by several groups and resulted in a number of publications during subsequent years, which provided a better understanding of the factors which may predispose tubers to this condition.

These could be summarised as follows:

1. The degree of Tuber sprouting.

This, and the level of internal sprouting, can be influenced by:

- (i) Climatic conditions during the growing season.
 - (ii) Storage temperature.
 - (iii) Use of sprout suppressant(s).
2. Pressure caused by adjacent tubers or walls.

3. The pattern of sprouting.
4. The level of ventilation.
5. Cultivar.

The degree of tuber sprouting must be considered a factor as complete control of sprouting will prevent internal sprouting (Hruschka et al., 1965; Ewing et al., 1968). Sawyer and Dallyn (1964) stated that the environmental conditions which are most conducive to external sprouting are the same conditions which give the greatest susceptibility to internal sprouting - a finding based on the fact that their check (control) tubers showed more internal sprouting than any of those treated with sprout suppressants at a variety of levels. However, Hruschka et al, (1965) also experimenting with sprout suppressant treatments, and using similar storage conditions, found that untreated tubers showed profuse external sprout growth but practically no internal sprouts. Irrespective of these conflicting results, the previous statement, that the degree of tuber sprouting is a factor in internal sprout development, is still valid and will be further discussed later.

Climatic conditions during the growing season and at harvest will influence internal sprouting as a result of their effect on the physiological age of the harvested tubers. This is because physiologically older tubers appear more prone to the disorder than those less mature (Sawyer and Dallyn, 1964). However, an important point noted by the above authors is that regardless of the maturity of the crop, internal sprouting can be triggered by storage environment in a relatively short period - presumably partly because storage conditions can also affect physiological age.

Storage temperature would also appear to be an important factor in the development of internal sprouts. Hruschka et al. (1965) found that most external and internal sprouts developed at about 65°F (18°C), and that few sprouts developed when stored below 55°F (13°C). These results are, however, complicated by the fact that some of the stored tubers had been treated with the sprout suppressant chemical chlorpropham.

It should already be evident that the factors which have so far been mentioned are inter-related i.e. storage temperature and physiological age can both affect the degree of sprouting. This will continue to be the case during this review.

Sprout suppressant chemicals, mainly chlorpropham, have been assessed with regard to their effect on internal sprout development. Sawyer (1961) reviewed the problem in relation to chlorpropham as a result of the unusual amount encountered in the USA during the storage season 1960-61, but found no relationship between the use of any sprout suppressants and the incidence of internal sprouting. Neither could any mention of internal sprouting be found in the many research reports concerning the development of chlorpropham. Further work by Sawyer and Dallyn (1964) involving chlorpropham treatment at several levels, but also including a tecnazene treatment, showed that, in general, as the dosage of chlorpropham was increased, the amount of internal sprouting decreased. Check (control) treatments had a higher level of internal sprouting than all but one of the chlorpropham treatments, although the difference at this level was not statistically significant. Tecnazene treated tubers exhibited

internal sprouting at a slightly lower level than that observed for check tubers at both of the storage temperatures used (60-63°F and 70°F). At the higher temperature the level was greater than that noted for any of the chlorpropham treatments. However, at the lower temperature two of the chlorpropham treatments did exceed the tecnazene treatment in terms of the observed level of internal sprouting. It was not clear from this experiment whether or not chlorpropham and tecnazene treated tubers were stored in a separate environment from the check tubers. This is an important point as exposure to trace levels of these volatile chemicals has been cited as a possible cause of internal sprouting (Hruschka et al 1965). In an assessment of the effect of low marginal doses of chlorpropham Sawyer and Dallyn (1964) found that checks stored in a room with no sprout inhibitor treatments, and no previous history of inhibitors, had the most internal sprouting. Checks buried in the sprout inhibitor treated pile had less internal sprouting than isolated checks but more internal sprouting than any of the inhibitor treated potatoes. Hruschka and Heinze (1967) employing dip treatments of chlorpropham found that the highest level of internal sprouting occurred in samples dipped in 500 ppm chlorpropham emulsion. At higher concentrations all growth including inward growth of sprouts was reduced. Less internal sprouting was also observed at lower concentrations - presumably because increased sprout elongation telescoped more lateral buds out of the range where they would grow back into the tuber. Ewing et al. (1968) found that the incidence of internal sprouting decreased with increasing rate of chlorpropham. All tubers in this

case were stored in a common environment and as such there were no control tubers which had not been exposed to chlorpropham.

Pressure caused by adjacent tubers or walls has also been cited as a factor in the development of internal sprouts (Davis, 1961). This was verified by the results of a series of experiments conducted by Ewing et al. (1968) in which weights were used to simulate pile pressure. They found that, irrespective of the presence of chlorpropham, pile pressure during storage increased internal sprouting. They also found that internal sprouts exhibited negative geotropism and that whether the parent tuber or an adjacent tuber was penetrated depended mainly on whether the developing sprout happened to be on the upper or lower part of the tuber.

The pattern of sprouting at the eye of a tuber would also appeared to influence the level of internal sprouting. In particular, the development of a dense cluster of sprouts would seem to predispose internal sprout growth. This was first mentioned by Davis (1961) who stated that ingrown (internal) sprouts originate at eyes which have partially developed external sprouts, the growing points of which, in the instances referred to, were either moribund or dead. He further commented that the more dense the cluster of sprouts at a given eye, and the more the necrosis is restricted to the apices, the more probable is the occurrence of one or more ingrown sprouts. These arise mainly from the axillary buds on the partially developed external sprouts. Hruschka et al (1965) also noted that chlorpropham in concentrations below that necessary to prevent sprouting may at

certain levels alter the normal course of sprout growth and cause an increase in internal sprouts. This would result from the cessation of growth at the terminal points of external sprouts, and in turn to the redirection of growth potential to lateral growth or bud growth at points near the tuber. When properly situated these buds may enlarge and sprout into the tuber thus causing internal sprouts.

The association between rosette external sprouts and internal sprouts was again observed by Ewing et al (1968), although they did emphasize that the correlation was not absolute, with occasional penetration of other tubers occurring even in potatoes with long, normal sprouts. Wien and Smith (1969) have further demonstrated that increases in degree of rosette sprouting increase internal sprouting and have provided evidence that, in the absence of chemical inhibitors or other volatiles, localised calcium deficiency in the meristematic region of growing sprouts may be a factor in the production of rosette sprouts.

The level of ventilation would appear to indirectly effect the degree of internal sprouting as a result of its influence on the other factors involved. Sawyer and Dallyn (1964) noted that when sprout inhibitors are involved, and air movement tends to dilute the effect of the inhibitor, internal sprouting may be accentuated by increases in air movement. However, if air movement results in low temperatures, and humidities considerably lower than those which produce considerable root hairs, increasing the air movement may decrease internal sprouting - based on the finding that internal sprouting tended to increase rapidly as the level of

humidity was increased to the point where root hairs developed profusely in the storage environment (external sprouting was also accentuated at this humidity).

Significant differences have also been observed in the level of internal sprouting between different cultivars stored under the same conditions (Hruschka et al. 1965; Sawyer and Dallyn, 1964).

It is clear from the preceding that the occurrence of internal sprouting, and the degree to which it is exhibited, can be influenced by one or more of the factors mentioned. The use of sprout suppressant chemicals is obviously an important point, although the results of the work to date have been inconclusive in terms of whether treated tubers show more internal sprouting than those which have not been exposed to any chemicals.

The work which will be described in this section relates to the influence of sprout suppressants on internal sprouting in tubers stored under temperature conditions normal for potatoes destined for crisp production in Britain i.e. at around 8°C, with ventilation as required to maintain this temperature. It is linked to the increasing commercial significance of losses as a result of internal sprouting, and to the perception among some of those in the industry (personal discussions with technical personnel) that the relatively recently introduced practice of initial tecnazene treatment before subsequent chlorpropham application has exacerbated the problem.

The experimental work therefore involved single and multiple applications of tecnazene or chlorpropham and combination of the two chemicals, although always with tecnazene applied first. Built

into this system was application of these chemicals after sprout growth of approximately 1 cm or 2 cm, and manual sprout tip removal on untreated tubers when sprouted to the same degree. This was designed to produce dense sprout clusters and will be fully discussed later.

6.2. Materials and Methods

Tubers of the cultivar Record were employed - this being by far the most predominant of those used in the crisp production industry. These were received on October 22nd 1986, shortly after harvest. Batches of approximately 10 kg were then placed in individual cardboard boxes with loose fitting lids. Enough boxes were prepared to give four replicates of each of the following:

Treatment No.	Description
1	Control (no treatment)
2	Chlorpropham (10 ppm) initially
3	Chlorpropham (10 ppm) initially then after sprout growth of approx. 1 cm.
4	Chlorpropham (10 ppm) initially then after sprout growth of approx. 2 cm.
5	Tecnazene initially
6	Tecnazene initially then after sprout growth of approx. 1cm.
7	Tecnazene initially then after sprout growth of approx. 2 cm.
8	Tecnazene initially then chlorpropham after sprout growth of approx. 1 cm.
9	Tecnazene initially then chlorpropham after sprout growth of approx. 2 cm.
10	Manual de-tipping after sprout growth of approx. 1 cm.
11.	Manual de-tipping after sprout growth of approx. 2 cm.

Both sprout suppressant chemicals were applied on an alumina carrier. Tecnazene was applied at 135 ppm. Chlorpropham was applied at 10 ppm for all but the final application, which was at 5 ppm.

All boxes were then stored in a controlled temperature room at 8°C until March 23rd 1987. At this stage those tubers which had been treated with chlorpropham, either alone or in combination, were showing such slow sprout growth that it was decided to remove them to ambient storage (ca.15°C) to encourage sprouting and, if likely, internal sprouting. By April 22nd the control (untreated) tubers had sprouted excessively and were removed and individually assessed for internal sprout development.

The remaining tubers, both at 8°C and at ambient, were held until the beginning of June when, between the 3rd and 7th, individual tubers were assessed for internal sprouting. This involved slicing each tuber along the axes of the sprouting eye(s).

A chronological list of events is shown over:

Date	Treatment No.	Action
22/1/86	All	Tubers boxed
22/10/86	2,3,4, 5,6,7,8,9	Chlorpropham applied at 10 ppm Tecnazene applied at 135 ppm
2/12/86	3,8 6	Chlorpropham applied at 10 ppm Tecnazene applied at 135 ppm
6/1/87	10,11	Sprouts manually de-tipped
29/1/87	7 9 10	Tecnazene applied at 135 ppm Chlorpropham applied at 10 ppm Sprouts manually de-tipped
16/2/87	11	Sprouts manually de-tipped
26/2/87	10	Sprouts manually de-tipped
23/3/87	10	Sprouts manually de-tipped
26/3/87	2,3,4,8,9	Moved from 8°C storage to ca. 15°C storage
24/3/87	11	Sprouts manually de-tipped
17/4/87	10	Sprouts manually de-tipped
22/4/88	1	Removed from storage and assessed for internal sprouting
24/4/88	2,3,4,8,9	Chlorpropham treatment at 5 ppm
18/5/88	10	Sprouts manually de-tipped
3-7/6/88	2,3,4,5,6,7,8,9 10,11	Removed from storage and tubers assessed for internal sprouting

6.3. Results and Discussion

The results of the storage experiment which has just been described are shown below:

Treatment No.	Total No. of tubers assessed	Chemical(s) applied	No. of Tubers showing internal sprouting	Internal sprouting as a percentage of the tubers assessed
1	240	None	0	0
2	245	Chlorpropham	1	0.4
3	243	Chlorpropham	0	0
4	258	Chlorpropham	0	0
5	244	Tecnazene	3	1.2
6	246	Tecnazene	3	1.2
7	239	Tecnazene	4	1.7
8	244	Tecnazene/ Chlorpropham	2	0.8
9	252	Tecnazene/ Chlorpropham	6	2.4
10	243	None	0	0
11	251	None	0	0

Although the overall level of internal sprouting was relatively low, a pattern did seem apparent in that all but one of the tubers which exhibited internal sprout growth had been treated with tecnazene, either alone or preceding chlorpropham application. This could be explained in terms of the sprouting pattern evident on tecnazene treated tubers. The chemical appears to overcome apical dominance at the sprouting eye resulting in development of a dense cluster of sprouts (Plate 6.3). This type of sprout growth could, as described in the introduction, orientate new growth, whether as axillary buds of existing sprouts or as new sprouts, downwards and into the tuber as opposed to out and away from it.

This theory, which was the basis of the work undertaken, would appear to be supported by the sprouting pattern on those tubers possessing internal sprouts (see Plates 6.4 - 6.8) This type of pattern has also been noted on commercially stored tubers showing internal sprouting and which had been treated with both tecnazene and chlorpropham (Plate 6.9)

It was visual assessment of these tubers, reference to the work of Davis (1961), Hruschka et al. (1965) and Wien and Smith (1969), plus a knowledge of the effect of tecnazene and chlorpropham on sprout growth, which led to a theory that an initial tecnazene application followed by chlorpropham treatment could, under certain conditions, lead to an internal sprouting problem. This might occur in areas where the tecnazene has not been able to fully control sprouting either through poor distribution of the chemical or loss by ventilation. The result would be sprouting, and development of sprout clusters. Subsequent

chlorpropham application would kill the growing apices of these sprouts but might not penetrate a dense sprout cluster to prevent regrowth of new sprouts or axillary buds of existing sprouts, either of which, if orientated downwards into the tuber by the sprout cluster, could result in internal sprout growth. Loss of applied chlorpropham through volatilization could lead to the same sort of re-growth.

From the results shown it seems that tecnazene can also have this effect even when applied alone. However the low levels of internal sprouting noted, and the fact that all chlorpropham and tecnazene/chlorpropham treatments were stored at a higher temperature for approximately 2 months, makes direct quantitative comparison of the treatments impossible.

While it is accepted that storage temperature plays a role in internal sprout development, it appears unlikely that this is the only explanation for the internal sprouting noted at the higher storage temperature. It would seem to be more than coincidence that all but one of those ambient stored tubers possessing internal sprouts had been treated with tecnazene.

The level of internal sprouting noted when tecnazene was applied alone was lower than that noted by Sawyer and Dallyn (1964), but of the same order as that noted by Ewing et al (1968). In both the above cases the check (untreated) tubers showed more internal sprouting as did the lower level chlorpropham applications.

It was perhaps surprising, in the light of previous work, that the incidence of internal sprouting in the tubers treated solely with chlorpropham was not higher. This might have been expected if the chlorpropham treatments had resulted in complete control of sprouting. However the transfer of these treatments to higher temperature storage did result in sprout growth, but obviously not in the development of internal sprouts.

Admittedly the degree of sprout growth was to a certain extent dependant on the position of the tubers within the box - greatest sprouting occurring around the edges where chemical has been lost as a result of volatilization, and least sprouting in the centre of the boxes, presumably because of a higher concentration of chemical. This pattern was, however, noted in all boxes treated with chemical(s), although sprouting was, in general, less evident in those boxes treated only with chlorpropham and this could be the reason for the absence of significant internal sprouting.

The chlorpropham results would be in conflict with much of the American work which found that this chemical, when applied at a level which would permit some sprout growth, did result in significant internal sprouting (Sawyer and Dallyn, 1964; Hruschka et al. 1965). In some cases this was greater than that noted for the controls, but in others less.

The control (untreated) tubers in this work showed no internal sprouting. This was despite the fact that they were stored in the same room as the treated tubers and as such could have been exposed to low levels of the chemicals used in the experiment - a factor which has been noted in previous work to

influence the sprouting pattern and thus result in internal sprouting (Hruschka et. al, 1965).

Manual de-tipping of the sprouts of untreated tubers did not result in any internal sprouting. This had been based on work by Wien and Smith (1969) who had shown that this type of treatment can lead to development of a dense cluster of sprouts and to the growth of internal sprouts. However, the sprout clusters which developed from this experimental work did not appear dense enough to cause internal sprouting, probably because the lower storage temperature (8°C) resulted in a significantly slower rate of sprout growth, and less frequent de-tipping than that by Wien and Smith (60°F).

This difference in storage temperature must be part of the explanation for the overall level of internal sprouting noted in this work being considerably lower than that noted in much of the American work. Another factor is probably the inter-cultivar variations noted previously - to which differences in sprouting vigour and depth of eyes probably contribute.

In conclusion, the work described in this chapter does indicate that tecnazene, either applied alone or in advance of chlorpropham application, can result in internal sprouting at a level greater than that for untreated tubers or tubers treated only with chlorpropham.

Plate 6.1. Internal sprouting in the cultivar Record.



Plate 6.2. Internal sprouting in the cultivar Record.





Plate 6.3. Sprout clusters on tecnazene treated tubers.

Plate 6.4. Internal sprouting in a tuber treated with tecnazene and chlorpropham in the laboratory.



Plate 6.5. Internal sprouting in a tuber treated with tecnazene and chlorpropham in the laboratory.



Plate 6.6. Internal sprouting in a tuber treated with tecnazene and chlorpropham in the laboratory.



Plate 6.7. Internal sprouting in a tuber treated with tecnazene and chlorpropham in the laboratory.

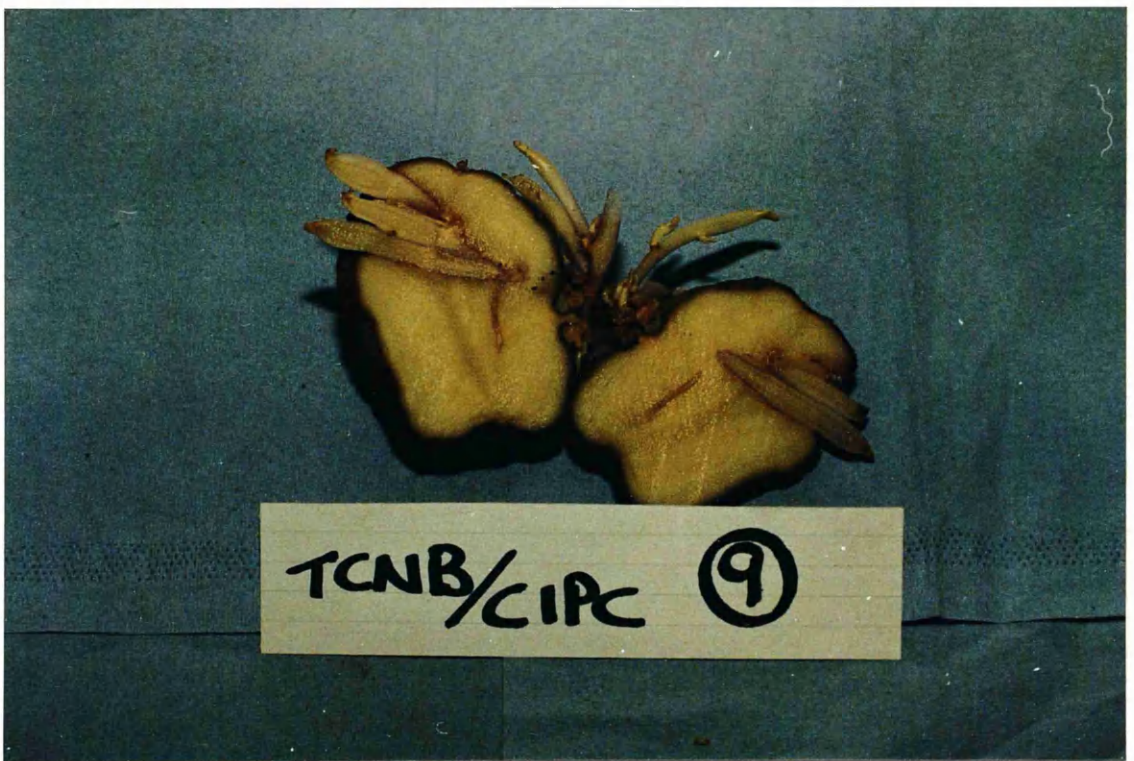


Plate 6.8. Internal sprouting in tubers treated with tecnazene in the laboratory.

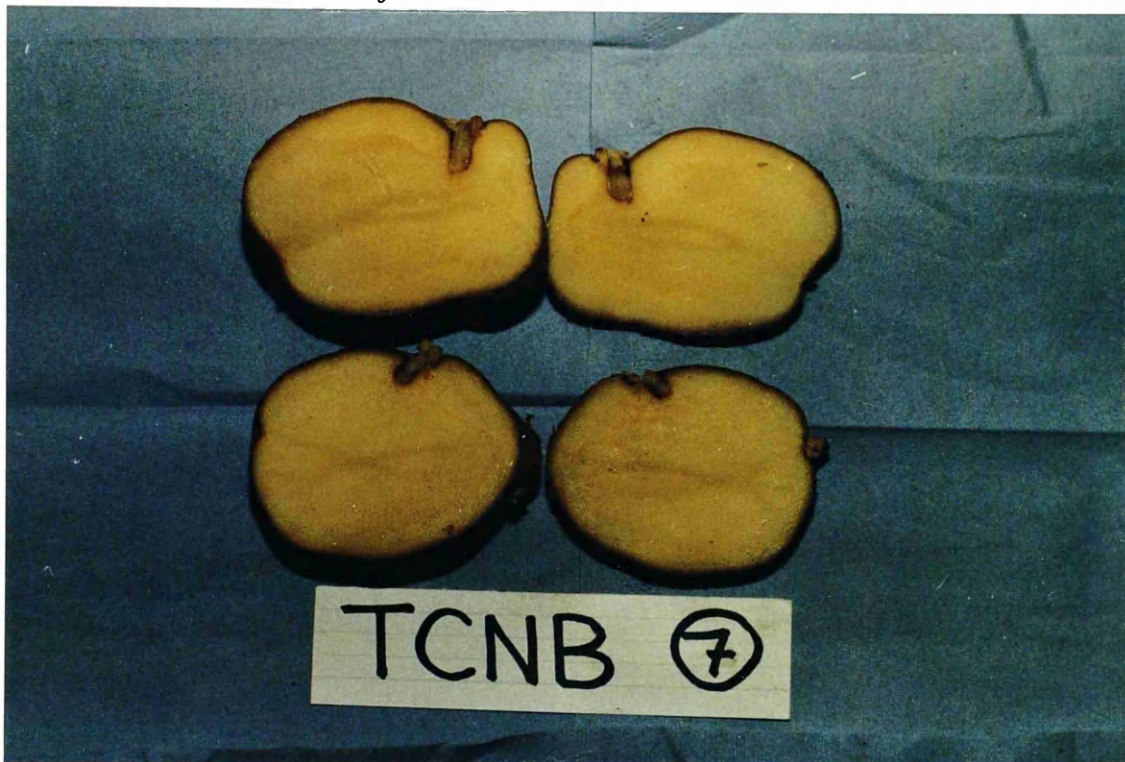


Plate 6.9. Internal sprouting in a tuber from a commercial potato store in which tecnazene and chlorpropham had been applied.



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