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POTATO SPROUT SUPPRESSANTS

WITH PARTICULAR REFERENCE

TO TECNAZENE.

JOHN DALZIEL

Thesis presented
for the Degree of
Doctor of Philosophy,
October 1978.

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ACKNOWLEDGEMENTS

I am extremely grateful to Dr.H.J.Duncan for his supervision of the work described in this thesis. His advice and encouragement throughout the project was greatly appreciated.

I am also indebted to the academic and technical staff, and to the research students of the Agricultural Chemistry Section not only for their general assistance and interest but particularly for their help with the field experiments where they endured many hours of back-breaking work, often in adverse climatic conditions.

The co-operation of Mr.J. Beveridge during the design and running of these field experiments is also gratefully acknowledged, as is the assistance of Mr.T.C.Aitchison with statistics and computing.

The financial support of the Department of Agriculture and Fisheries for Scotland in the form of a post-graduate studentship from 1971-1973 is also acknowledged.

To my wife, Anna, special thanks are due not only for her patience and encouragement throughout the preparation of this thesis but also for her efficiency in typing a rather difficult manuscript.

SUMMARY

This thesis examines the problem of potato sprouting and its control with chemical sprout suppressants. Emphasis is placed on 1) the analysis of tecnazene residues and their implications 2) the effect of tecnazene on subsequent seed performance and 3) the effects of sprout suppressant chemicals in general on glucose, fructose and sucrose levels during storage.

The causes and extent of the problem of potato sprouting were assessed, alternative methods of sprout control reviewed, and the requirement for an adequate means of chemical sprout suppression confirmed.

To highlight problems associated with sprout suppressants, compounds which have been suggested but are not currently used were reviewed. Major disadvantages were ineffectiveness, inconvenient application methods, the occurrence of tuber abnormalities, inhibition of wound healing, high phytotoxicity, unsuitability for use on seed and high toxicity.

In light of this information the currently used compounds, maleic hydrazide, chlorpropham, propham and tecnazene were critically examined and problems for practical investigation identified.

The use of maleic hydrazide was investigated experimentally and it was found to be extremely difficult to apply in Scottish climatic conditions. Its effect on sprouting and weight loss was inadequate to permit long term storage, although observations indicated that it may be of more value for medium term storage. Application in mid-August at 1.5-2.5kg ha^{-1} is suggested.

A method for the analysis of tecnazene residues was developed. It involves extraction with hexane, clean-up on alumina and gas chromatography. Recovery was 91.6% $\pm$ 2.8. The minimum detectable amounts were 10μg kg^{-1} potatoes when using flame ionisation detectors and 1ng kg^{-1} with electron capture detection.

Using this method, laboratory treated and commercial samples were found to contain 2-8mg kg^{-1} after washing in running water. Peeling reduced residues by 85-95%, but tecnazene was detectable

even in the centre of tubers. Washed, peeled and boiled tubers contained 0.1-1.0mg kg^{-1}.

Crisps made from treated potatoes contained 0.25-0.6mg kg^{-1}. During processing the frying oil was contaminated and subsequent untreated batches contained small amounts of tecnazene.

The effect of storage conditions on tecnazene residue levels was investigated and a statistical model constructed to describe the process of tecnazene loss. Results indicate that allowing air to circulate freely around tubers considerably accelerates the loss of tecnazene, especially at higher temperatures. Ventilation for 5-8 weeks at $12^{o}C$ is required to produce residue levels lower than 1mg kg^{-1}.

Storage bins which had contained tecnazene treated material, or were adjacent to such bins were heavily contaminated with tecnazene after standing empty for 6 months.

In light of these findings the effect of tecnazene on flavour, and the metabolism, toxicology and non-biological degradation of tecnazene are discussed.

The influence of tecnazene on the subsequent field performance of treated seed was studied over 4 growing seasons. Models were constructed to describe the effects of storage environment and residues on emergence, yield and size distribution. Based on these models and for an "average" cultivar, residues of 0.0, 0.25, 0.50, 0.75, 1.0 and 1.5mg kg^{-1} produced delays in emergence of 0,2, 4,6,8 and 13 days respectively. Subsequent reductions in total yield were 0,1,3,8,14 and 31%, and the increases in the seed-sized fraction were 0,27,31,33,34 and 37% respectively. To minimise these effects seed must be stored at $12^{o}C$ for 5-8 weeks before planting. Sprout growth under these conditions is unmanageable unless illumination is employed. It is suggested that the effects of tecnazene on size distribution could be exploited by seed producers and the canning industry. It is recommended that regardless of use tecnazene treated seed should always be pre-sprouted.

Gas chromatographic analysis of soluble sugars in potatoes was investigated in some depth and existing methods modified. The resultant method was used to examine effects of tecnazene, chlor-propham and a new sprout suppressant, dimethylnaphthalene, on

glucose, fructose and sucrose levels in tubers during storage at 10^{o}C. All 3 chemicals prevented the accumulation of excessive amounts of the 3 sugars during 6 months storage. The magnitude of this effect is likely to be of significance to the processing industry.

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CHAPTER 1

INTRODUCTION

1.1. THE POTATO CROP AND ITS UTILISATION

Potatoes have long been regarded as a staple item in the British diet, and despite fluctuations in price and supply there is a consistent demand in Britain for over 5 million tonnes per annum. The potato is therefore an extremely important crop to British agriculture, and in 1977 British farmers grew approximately 200,000ha of potatoes of which 35,000ha were in Scotland (Anon, 1977). Underlying these figures are certain trends which are relevant to this thesis.

Over the years the number of farmers who grow potatoes has gradually declined. In 1962 there were 70,000 producers registered with the Potato Marketing Board, but 10 years later, this figure had dropped to 40,000. The quantity of potatoes produced, however, has remained remarkably constant. There is, therefore, a trend in the industry towards increased specialisation and larger production units (Rennie, 1973).

The decline in the number of producers has been accompanied by a slowly falling acreage and very much improved yields. The average yields in 1955, 1965, 1971 and 1977 were 17, 24, 27 and 30tha^{-1} respectively (Rennie, 1973; Anon, 1977).

There have also been dramatic changes in the utilisation of the potato crop, especially during the last decade. In 1966 the fresh market accounted for some 93% of ware production, but by 1976 this figure had declined to approximately 80%. This substantial reduction is due to the increasing consumption of the rapidly

expanding processing industry and is a trend which shows every indication of continuing.

Of that portion of the 1974 crop which was processed (more recent figures show somewhat anomolous trends due to climatic factors), 44% was used for crisp manufacture, 36% for chips, 16% for dehydrated products and about 4% for canning (Anon, 1975b). The growth in frozen chip production has been particularly rapid.

The seed potato industry is of great importance to Scotland. Of the 35,000ha of potatoes which are currently grown in Scotland, approximately 20,000ha or 60% are grown for seed. As with the British ware situation, the acreage grown in Scotland for seed has changed substantially in recent years. In 1955 there were 32,000ha grown for seed. By 1964 this figure had increased to 35,000ha but then dropped sharply until in 1971 it reached 20,000ha where it has apparently stabilised (Arbuckle, 1973; Anon, 1977).

Of the current acreage about $\frac{1}{3}$ is grown by about 100 merchant-growers and the remainder by about 3,500 individual producers, many of whom rent land on neighbouring farms in order to create economically viable units (Arbuckle, 1973). Based on these figures the average individual producer grows only 4ha.

1.2. TRENDS IN POTATO STORAGE

The past 25 years has seen substantial changes in the techniques of potato storage. Outdoor clamp storage has almost disappeared and the vast majority of potatoes are now stored in buildings many of which have been specially designed for this purpose. Bulk storage is by far the most popular technique, although bin and box storage are now becoming more common. In Scotland storage of seed in 0.5 or 1.0tonne boxes is now widespread especially amongst the merchant-growers, as numerous cultivars can be conveniently handled in the same store by this method.

The reasons for adopting a particular storage technique are complex and include not only the quality of the end-product of storage with which the bulk of this thesis is concerned, but the often over-riding considerations of economics, ease of handling and even current fashion.

The changing pattern of utilisation has also led to modifications in storage systems to meet the requirements of the processing industry. The economics of processing are such that processing plants must be kept running throughout the year. Because of this requirement for continuity of supply and the fact that it is not economically (and on occasions not technically) feasible to use first earlies for processing, maincrop potatoes must be stored from harvest until the following July when it usually becomes possible to purchase the new year's crop.

Sprouting as will be seen in later Chapters, has always been a problem during the storage of potatoes. With the advent of well-insulated potato stores, coupled with the demands of the fresh market for high quality potatoes late in the storage season, and the quality criteria and extended periods of storage associated with the processing industry, some form of sprout suppression is essential.

Seed, of course, is almost always stored until early Spring and the problem of unwanted sprout growth has always existed. Again, however, the problem may be magnified by the higher storage temperatures occasionally necessitated by other considerations.

1.3. THESIS OBJECTIVE

In light of the trends in utilisation and storage which have been outlined above it is obvious that the prevention of sprouting during storage is assuming increasing importance and growers, merchants and processors are turning to chemicals to solve this problem. The implications of this trend demand closer scutiny.

It is, therefore, the main objective of this project to examine the benefits and problems associated with the use of these sprout suppressant chemicals. Their use on both seed and ware will be considered in terms of their efficiency, safety and their effects on quality. Because of the importance of seed potato production to Scottish agriculture, particular emphasis will be placed on tecnazene which is widely used as a sprout suppressant on crops, at least part of which is destined for use as seed.

As a first step towards achieving this objective the information available in the literature on several aspects of potato sprouting

and its control was reviewed as follows.

1) The factors which influence the sprouting of tubers and the resultant losses were examined to assess the causes of the problem and its magnitude.

2) Methods of sprout control, including environmental control, were reviewed to determine whether in fact the use of chemicals is necessary.

3) In order to highlight the problems which can arise from the use of chemical sprout suppressants, various compounds which have been suggested but were never, or are no longer, used commercially were examined.

4) In the light of this information a survey was then made of the available information on the currently used compounds. Areas giving cause for concern and which required practical investigation were then identified.

These surveys constitute Chapters 2 and 3 of this thesis.

As a result of these investigations and after consultation with various individuals closely associated with the potato industry experiments were undertaken to provide information on the following topics.

1) The use of maleic hydrazide, a chemical which is widely used in the U.S.A., under Scottish conditions.

2) The levels of residues resulting from the use of tecnazene.

3) The effect of cooking and processing on tecnazene residues.

4) The effect of storage conditions on tecnazene residues.

5) The effect of tecnazene on the performance of seed potatoes in terms of emergence, yield and size distribution.

6) The effect of tecnazene and other sprout suppressants on the soluble sugar content of tubers.

These practical investigations are described in Chapters 4,5 and 6 with the exception of the maleic hydrazide experiment which has more conveniently been placed in Chapter 3.

CHAPTER 2

POTATO SPROUTING

2.1. INTRODUCTION

It is the aim of this Chapter to examine the various factors which influence the sprouting of potato tubers during storage and to assess the commercial significance of the resultant losses.

As some excellent review articles are available on certain aspects of potato sprouting these will be cited where appropriate thus allowing more expansive discussion of areas where no such material is available. The apparent balance of this treatment may therefore diverge from the relative importance of the various topics. However, in the interests of brevity this approach is considered justified.

The degree of sprouting shown by a given sample of tubers at a given point in time is controlled by 1) the time at which sprouting commenced and 2) the rate of subsequent sprout growth, both of which are dependent on genetic and environmental influences. The former also involves the complex concept of dormancy which is briefly discussed below.

It should be noted that in the light of the evidence described this differentiation between the dormant period and the growth period is somewhat artificial, although apparently certain factors may affect one but not the other.

2.2. CONCEPTS OF DORMANCY

There has been considerable discussion in the literature as to the precise definition of tuber dormancy. As pointed out by Rappaport and Wolf (1969) such disagreement is understandable because it is difficult to characterise either induction or termination in arrested growth in unextended stems by a distinct morphological change such as occurs during conversion from a vegetative to a reproductive form as in the case of true seeds.

One view advocated by Burton (1963) is that the dormant period is that time when the buds are not growing for whatever reason. His concept considers the tuber as being in a state of constant change with the environment intimately affecting internal processes. Burton suggests that the most logical starting point for the dormant period is the cessation of stolon elongation and the commencement of tuber formation. The dormant period is terminated by the commencement of extension growth of the sprout.

Emilsson (1949), however, distinguishes between "rest" and "dormant" periods, the rest being a specialised form of dormancy in which buds fail to elongate even under environmental conditions suitable for growth. The rest period is sometimes known as internal dormancy. The dormant period according to Emilsson is that time when buds fail to grow because environmental conditions, particularly temperature, are unfavourable. This form of dormancy is known as external dormancy.

The terminology of Emilsson has been adopted by many authors probably because its differentiation between internal and external factors makes it more convenient to use.

Davidson (1958) has expressed the view that the potato tuber has no rest period and that the appearance of a sprout is not an indication that dormancy has been broken but merely the first visible indication of growth which has been progressing for some time and can be examined microscopically.

Whilst there is no disagreement about Davidson's experimental findings it is nevertheless convenient to have some term to cover that period when no external growth is apparent, and a term such as "tuber quiesence" or "apparent dormancy" might be preferable to

distinguish this state from true dormancy such as is found in seeds.

Of the accepted terminologies, Burton's concept of tuber dormancy appears to be more realistic than the rather artificial "rest" period of Emilsson, and is substantiated by a considerable amount of experimental evidence which has been reviewed at length by Burton himself (Burton, 1957; 1963; 1966).

In much of the published work the dormant period has been measured from the date of harvest which as Burton (1957) indicates is a purely arbitrary date if this occurs after the death of the haulm, and suggests that the time of tuber initiation is a much more satisfactory starting point, since, in many instances the dormant period may be over before harvest.

A further advantage of adopting tuber initiation as a starting point can be found in the results of Emilsson (1949) and Burton (1963) both of whom found that tubers harvested when immature had a longer rest period than those harvested in more mature condition. They also found that if plants were defoliated on different dates and tubers left in the ground and therefore under similar environmental conditions until a harvest date common to all treatments, then irrespective of the date of defoliation all samples started to sprout at approximately the same time. At the very least this indicates that the dormant period is substantially determined at a very early stage in the development of the tuber, although, of course it is then subject to modification by numerous environmental factors which will be discussed later.

For the purposes of the present discussion, therefore, the terminology of Burton will be adopted.

2.3. THE MECHANISM OF DORMANCY

Over the years dormancy has been studied in relation to numerous factors in an attempt to find a satisfactory explanation of the phenomenon and to account for its onset and termination. However before discussing these points it is perhaps worth noting the warning given by Burton (1966).

"We must not expect to find some one substance, variation in the concentration of which will lead to dormancy or growth. Rather we

should expect to find a complex balance of reactions, each of them subject to promotion or inhibition, the culmination of which is growth. The inhibition of any one of these reactions could lead to dormancy ...."

1) The supply of soluble carbohydrates

The earliest attempt to explain potato dormancy was by Müller-Thurgau (1882) who suggested that dormancy resulted from a deficiency of soluble carbohydrates. This has been discounted by Burton (1957) on the basis that levels of sugars in potatoes during dormancy are at variance with this theory (Appleman and Millar, 1926).

2) Oxygen, carbon dioxide and respiration

Various theories on the role of oxygen, carbon dioxide and respiration in controlling dormancy have been proposed. For example Thornton (1939) suggested that the dormant period was terminated by restriction of the oxygen supply due to progressive suberisation of the periderm in the ageing tuber. The concentration of CO_2 and various terminal oxidase systems have also been considered in possible controlling mechanisms (Burton, 1952a; 1958c; Mapson and Burton, 1962). All of these possibilities have however been discussed at length elsewhere (Burton, 1950; 1957; 1963; 1966; Bruinsma, 1962; Emilsson and Lindblom, 1963) and have been discounted.

3) Glutathione

Compounds containing sulphydryl groups have been implicated in cell division for many years (Hammett, 1929). Miller (1931) suggested that the amounts of such compounds, particularly glutathione, might be a controlling influence in the onset and termination of dormancy. Emilsson (1949) noted that the concentration of glutathione rises during the dormant period and reviewed the existing evidence on the role of glutathione in sprouting. More recent findings indicate that much more work is required before definite conclusions can be drawn (Emilsson and Lindblom, 1963; Ashford and Levitt, 1965; Burton, 1968a). The role of glutathione will be further discussed in Chapter 4 in relation to the metabolism of tecnazene.

4) Volatiles

It has been shown by Burton (1952b; 1952c) and others that potato tubers produce volatile metabolites which if allowed to accumulate will prevent sprouting. Many of these compounds have since been identified but their role in controlling dormancy remains unexplained (Burton, 1968; Burton and Meigh, 1971; Meigh et al, 1973).

5) Growth regulants

The role of growth regulants in controlling plant development has been extensively investigated and numerous texts and review articles on this subject are now available (eg. Shantz, 1966; Wain, 1968; Wilkins, 1969). The role of these compounds in controlling dormancy has also been reviewed (Wareing and Saunders, 1971), and of particular relevance to the present discussion is a review on dormancy in tubers by Rappaport and Wolf (1969). Comprehensive reviews of the chemistry and biochemistry of auxins (Ray, 1974), gibberellins (MacMillan, 1974), cytokinins (Helgeson, 1968; Skoog and Armstrong, 1970; Leonard, 1974), abscisic acid (Cornforth et al, 1965; Milborrow, 1974a; 1974b) and ethylene (Yang, 1974) have been published.

As this subject is so well documented it is considered that no useful purpose would be served by presenting a detailed discussion in this thesis. Suffice to say that of all the growth regulants, gibberellins and abscisic acid are considered at present to be more deeply implicated in the dormancy of potato tubers and its termination. Indolyl acetic acid is considered to play an important role in controlling apical dominance.

2.4. FACTORS WHICH INFLUENCE DORMANCY AND SPROUTING

1) Cultivar

The duration of dormancy, for a given cultivar, may vary from year to year. Nevertheless it has been observed in experiments on numerous cultivars that consistent responses are produced over several generations indicating that whilst many factors may interact

to influence dormancy there is undoubtedly a considerable genetic influence (Emilsson, 1949; Burton, 1957). Once a tuber has commenced sprouting the subsequent rate of sprout growth is also cultivar dependent (Burton, 1957).

In view of the commonly held belief that early cultivars are more prone to sprouting it is of interest to note that Emilsson (1949) found no correlation between maturity class and the length of the dormant period. Burton (1966) points out, however, that once dormancy has broken the subsequent rate of sprout growth in early cultivars is often greater.

In the experiments of Burton (1963) with 11 British cultivars stored at 10°C the average dormant period ranged from 11 weeks (cultivar King Edward) to 25 weeks (cultivar Ulster Prince) after tuber initiation, which for most cultivars was around the beginning of June. In practice this implies that most British cultivars stored at 10°C have broken dormancy by 1 January and many long before this time.

2) Pre-harvest factors

As mentioned above the dormant period for a particular cultivar will vary from season to season. Generally cold wet seasons result in a longer dormant period than do warm dry ones which tend to encourage premature senescence and hence affect the physiological age of the tuber (Burton, 1963).

Similarly other factors such as the origin of the seed from which the crop has been grown have been shown to have effects which can probably be accounted for by physiological age (Emilsson, 1949; Burton, 1963).

The influences of maturity and defoliation were discussed previously and require no further expansion.

3) Temperature

The most important post-harvest factor which influences dormancy and sprouting is the storage temperature and its effects have been well investigated (Barker,1937; Schippers, 1956).

It has been shown that although different cultivars respond to varying degrees to the influence of temperature the general nature of the response is common to all.

When tubers are stored at uniform temperatures then the dormant period is in general shortened by higher temperatures in the range 4 -21^{o}C (Wright and Peacock; 1934).

The more extensive work of Schippers (1956), in which he investigated 40 cultivars stored at various temperatures, has shown that on average increasing the temperature from 10^{o}C to 20^{o}C shortened the post-harvest dormant period by approximately 20%, whilst lowering the temperature from 10^{o}C to 5^{o}C increased the post-harvest dormant period by about 70%. Lowering the temperature by a further 2^{o}C to 3^{o}C almost doubled the figure.

As far as sprout growth is concerned it has been shown that growth is very slow below 5^{o}C to such an extent that in some cultivars it may go unnoticed. Over the range 5^{o}C to 15^{o}C each increment of 1^{o}C in storage temperature produces a greater increase in the rate of sprout growth. Above 15^{o}C each increment has a diminishing effect although still significant up to the optimum temperature for sprout growth which is 25^{o}C (Barker, 1937; Burton, 1957).

It should be noted however that the rate of sprout growth even under constant temperature storage is influenced by previous storage history. Generally a period of storage at cold temperatures (below 7^{o}C) will result in an increased rate of sprout growth, when transferred to favourable conditions for sprouting, as compared with that produced under similar favourable conditions but with no cold treatment.

It has also been found that if tubers are stored at a temperature at which little or no growth occurs, and samples are transferred at intervals to more favourable conditions then the rate of sprout growth of successive samples will increase to a maximum then decrease (Schippers, 1956; Krijthe, 1962) This has been interpreted as indicating that there exists in the tuber a potential for growth or sprouting capacity which varies with time.

When tubers are stored from harvest under favourable conditions for sprout growth and samples manually desprouted at intervals then a similar optimum sprouting capacity is observed in the rate of re-growth. In this case, however, the reduction in growth potential, late in the season, is partly due to loss of vigour caused by tuber exhaustion because of the large proportion of the tubers resources lost during the initial sprouting process (Krijthe, 1962).

At high storage temperatures the period of maximal sprouting capacity of some cultivars may have passed to such an extent that emergence in the field may be adversely affected (Burton, 1963).

The effects of temperature will be further discussed in Chapter 3 where the possibilities of controlling sprouting, by modifications to the storage environment, are considered.

4) Light

It is a common observation that potato sprouts grown in the light are much shorter and stronger than those produced under normal dark storage conditions. Wassink et al, (1950) showed that this inhibition could be induced by exposure to blue, violet, red and infra-red wavelenghs. The effects of yellow and green light are less dramatic. Wassink et al (1950) and Dinkel (1963) found that illumination of mature tubers retards the break of dormancy (see also Davidson, 1958). Light is, of course, widely used commercially during pre-sprouting of seed to produce sprouts which will withstand mechanical damage during planting.

5) Humidity

It has been reported that storage at high humidities will shorten the dormant period (Thornton, 1939; Emilsson, 1949). Davidson (1958) on the other hand found that humidity had very little effect on dormancy. Burton (1973) suggests that some of the earlier findings on this subject were not due to humidity at all. He suggests that the 100% R.H. conditions used in these early experiments were such that they produced a film of moisture on the tuber surface which would reduce the oxygen content of tubers. This as will be explained below would have an effect on dormancy (Burton, 1968a; Burton and Wigginton, 1970).

More recent results indicate that tubers stored for long periods show marginally lower sprouting at 95% R.H. than at 85% R.H. (Sparks, 1973b).

Once sprout growth has commenced humidity appears to affect the form it takes in that adventitous roots may be formed under very humid conditions whereas branching may be increased under drier conditions (Burton, 1966).

6) Oxygen

The level of oxygen in the storage atmosphere has a pronounced effect on both dormancy and sprout growth.

The dormant period may be shortened by storage in atmospheres with a modified oxygen content. The concentration at which the greatest number of buds commence growth at 10 - 20°C is about 2 - 4% early in the storage season but rises to 14 - 23% by early summer (Burton, 1968a).

Similarly the optimal oxygen concentration for sprout growth varies from 4 - 5% to 17 - 20% throughout the storage season. Low concentrations of oxygen which earlier in the season will stimulate growth will in fact inhibit growth later in the season (Thornton, 1939; Burton, 1958c; Burton, 1968a).

7) Carbon dioxide

Although carbon dioxide has no apparent effect on dormancy (Burton, 1963) it does have a considerable effect on sprout growth, which is stimulated by increasing the CO_2 in the atmosphere to an optimal value of 2 - 4%. Above this point sprouting is normally retarded (Burton, 1958c).

Temporary storage at very high CO_2 levels (10 - 60%) for a few days will stimulate subsequent sprouting.

8) Ventilation

The effects of ventilation on sprouting are complex because the rate of ventilation and the duration of the ventilating periods will affect numerous other factors such as humidity and the atmospheric concentration of carbon dioxide and oxygen, whose effects have already been considered. High rates of ventilation will also prevent the accumulation of natural sprout suppressing volatiles (Burton, 1952c; Burton and Meigh, 1971; Meigh et al, 1973)

Sparks (1973a, 1973b, 1978) has reported that the net result of increasing the rate of ventilation, at least during long term storage, is a marked reduction in sprouting. This tends to suggest that in practice the accumulation of CO_2 may be a very important factor in potato storage. It should also be noted however, that he found intermittent ventilation caused less sprouting than continuous ventilation.

It is somewhat difficult to reconcile these findings with the generally held belief that ventilation increases sprouting. This was first recorded by Butler (1919) and according to Burton (1966) and others is a matter of common observation which he accounts for by the removal of volatile growth inhibiting substances which would otherwise accumulate in the storage atmosphere (Burton, 1952b; 1952c).

One factor which might help explain this apparent disagreement is that the temperature of potato tubers is usually marginally higher than the ambient temperature of the store, and increased evaporative losses at high ventilation rates (Sparks, 1978) could result in more effective cooling due to withdrawal of a greater amount of latent heat. Therefore tubers subject to high ventilation rates may attain lower temperatures for at least a proportion of the time. Sprouting under these conditions might well be reduced as found by Burton (1969) in experiments with intermittent refrigeration.

2.5. LOSSES DUE TO SPROUTING

Sprouting is rarely a serious problem during short term storage, except where potatoes are stored at high temperatures for processing. However, as was mentioned above most British cultivars have broken dormancy by early January and if tubers are to be stored beyond this period then losses due to sprouting are likely to result. In the case of tubers destined for processing, storage at higher temperatures means that these losses assume economic significance very early in the storage season.

Sprouting results in different types of loss which for convenience can be divided in to 5 categories, these being 1) weight losses due to increased evaporation, 2) losses associated with the transfer of weight from a valuable tuber to a useless sprout, 3) loss in visual and compositional quality, 4) loss in seed quality and 5) losses due to difficulties in grading and handling.

The relative importance of these five categories in economic terms is, of course, dependent upon the ultimate fate of the particular sample in question. It should also be noted that losses in each of

the categories are extremely variable and very much dependent upon individual storage regimes. Any figures quoted below should, therefore, be treated only as an approximate guide.

1) Evaporative losses

Evaporation of water from the potato tuber follows the same general pattern as evaporation from a water surface, except that it occurs at a very much slower rate because of the barrier provided by the periderm (Burton, 1973).

The rate of evaporation is most conveniently expressed as weight of water lost per cm^2 of tuber surface per hour per millibar vapour pressure deficit (mg cm^{-2} hr^{-1} mb^{-1}) (1 millibar = 100Pa (pascals)).

Immature tubers show very high rates of water loss soon after initiation and prior to the formation of the periderm. Less immature tubers at the stage when they might be marketed as earlies have lower but still very fast rates of evaporation of the order of 0.1-0.8mg cm^{-2} hr^{-1} mb^{-1}. Even mature tubers show relatively high rates of loss when harvested (0.03mg cm^{-2} hr^{-1} mb^{-1}) but these levels drop very quickly after the first few weeks of storage to about 0.01mg cm^{-2} hr^{-1} mb^{-1}, and this low level is maintained until sprouting commences when the rate of evaporation rises dramatically and may even attain 1mg cm^{-2} hr^{-1} mb^{-1} under extreme conditions (Burton, 1973).

The reason for this dramatic increase is of course the relatively high permeability of the epidermal layer of the sprout compared with the periderm of the tuber.

Even a relatively low amount of sprouting can increase evaporative losses dramatically. It has been shown that the weight loss from a sample containing 1% by weight of sprouts was 0.19% of the original weight stored per week per millibar vapour pressure deficit at a ventilation rate of 50m^3 t^{-1} hr^{-1}. This compares with 0.13% $week^{-1}$ mb^{-1} at the same ventilation rate in the case of non-sprouting tubers and 0.31% $week^{-1}$ mb^{-1} when the sample contained 4% by weight of sprouts. When one considers that the average water vapour deficit for eastern England is approximately 2.3mb in April and 2.7mb in May and June then the resultant weight losses are of great economic significance (all figures recalculated from Burton, 1966).

2) The weight of sprouts

The weight of sprouts at grading can amount to a significant proportion of the total weight of tubers stored.

Drew and Deasy (1943) using experimental clamps found that sprout growth by May ranged from 2.5% of the original weight stored for the cultivar Arran Banner to 3.7% for the cultivar Kerr's Pink. In a subsequent storage season losses by June ranged from 3.7% for the cultivar Up to Date to 8.7% for Kerr's Pink.

According to Wilson et al (1962) losses from tubers stored in buildings are less than those stored in clamps, although shrinkage may be considerably greater. By placing small samples in commercial stores they found that the average weight of sprouts ranged from 0.2% in stores emptied between December and February to 1.4% in stores emptied after February. None of their samples were placed at the peripheries of the stores where they report that sprouting was more pronounced than in the central areas.

Twiss and Jones (1965), during a survey of wastage in bulk-stored maincrop potatoes in Great Britain, recorded losses due to the weight of sprouts ranging from 0 to 5.4%. It is interesting to note that the highest recorded loss was once again with the cultivar Kerr's Pink stored for only 117 days under Scottish conditions.

More recently Sparks (1978) reported that losses due to the weight of sprouts amounted to 3.4% after 8 months storage when only natural convective ventilation was used but as little as 1.4% when high rates of airflow were employed ($18-75 m^3 t^{-1} h^{-1}$)

3) Loss in visual and compositional quality

Although the losses in compositional quality described below are associated with sprouting this does not necessarily imply that if sprouting is prevented then these losses will not occur. It is, however, likely that they may at least be reduced.

a) visual quality

The loss in visual quality due to the sprouting of potatoes is not usually due to the presence of sprouts, as these are almost invariably removed by pre-marketing grading procedures, but is usually due to shrinkage produced by excessive evaporation. In less

extreme cases such losses can be detected only by the "spongy" feel of tubers which have sprouted.

b) soluble sugar content

As the effects of sprouting on soluble sugar levels will be considered in some detail in Chapter 6 suffice to say for the present that sprouting causes a significant reduction in processing quality for both fried and dehydrated products because it is usually accompanied by a marked increase in reducing sugar levels.

c) dry matter content

The stored potato tuber is continually respiring and in so doing its carbohydrate reserves are slowly diminished.

The effects of this process on weight loss are not particularly serious, averaging a loss of 0.08% of the original weight stored per month during most of the storage season, but almost doubling to 0.15% per month when sprouting is well advanced.

The reason for this comparatively small weight loss is that water is produced during respiration partially balancing the loss due to expended carbohydrate.

The effect on dry matter is much more serious amounting to a loss of 0.8% of the original dry matter stored per month during most of the storage season and a very significant 1.5% per month when freely sprouting (Burton, 1966).

Thus during storage there is a considerable drop in the energy content and processing quality of the tubers. This loss is almost doubled if tubers are allowed to sprout.

d) "minor" nutrients

Burton (1966) has reviewed the changes in levels of other nutrients which occur throughout the storage period. In general, as might be expected, long periods of storage and sprouting lead to a gradual decline in nutritive quality. This is especially obvious with respect to ascorbic acid and the B vitamins.

Arslanov et al (1971) has reported that ascorbic acid losses can be reduced by preventing sprouting with tecnazene.

4) Loss in seed quality

Pre-sprouting or chitting of seed potatoes under controlled conditions is, of course, often carried out with a view to maximising subsequent yield and some aspects of this type of

sprouting will be considered in the next Chapter.

Unwanted sprout growth can, however, be a serious problem resulting in losses due to evaporation and the weight of sprouts as discussed for ware. These are even more important to the seed grower because of the higher value of the crop.

As these unwanted sprouts are usually removed by the grading operation prior to sale this can lead to further loss - the loss of seed vigour. The importance of this loss will depend on the capacity of the seed to produce new sprouts and this, as previously discussed, is very dependent on the cultivar and on the physiological age of the tubers when the original sprouts were lost. Generally, however, the yielding capacity of desprouted seed is below that of seed planted with the original sprouts intact (Murphy et al, 1968; 1969).

Another factor affected by the loss of original sprout growth is apical dominance. Generally the apical bud (at the rose end of the tuber) will sprout first and suppress the sprouting of other buds.(Burton, 1966). If the source of this suppression (the apical sprout) is removed then the other buds will freely sprout. Thus size distribution in the subsequent crop will be affected by increased stem numbers.

5) Grading and handling difficulties

Stores in which tubers have sprouted take longer to empty and grading time is increased markedly.

Sparks (1978) found that a batch containing 3.8% sprouts took 43 min t^{-1} to grade whereas batches containing 2.7% and 1.4% took 28 and 16 min t^{-1} respectively. The implications of these findings are of considerable commercial significance. Disposal of large quantities of sprouts can also present problems.

Summary

Sprouting is not a serious problem in short term storage except where potatoes are stored at high temperatures.

During long term storage, however, when the various losses are summed the total loss caused by sprouting can be considerable. The loss of saleable weight alone due to increased evaporation and sprout growth can amount to a sizeable percentage of the crop. The losses in quality are more difficult to quantify but undoubtedly are of great significance.

CHAPTER 3

THE CONTROL OF SPROUTING

A wide variety of methods have been suggested for the control of potato sprouting. These methods can be conveniently divided into three categories as follows:- 1) environmental methods, 2) methods involving irradiation and 3) chemical methods.

As this thesis is concerned primarily with chemical sprout suppression the other methods are not considered in great depth. Sufficient material will, however, be presented to indicate the main advantages and limitations of control by environmental manipulation and irradiation and why chemical control is considered necessary. Whilst discussing the environmental control of sprouting some general aspects of potato storage are presented as these are considered essential background to subsequent experimental work.

As the Scottish potato industry is orientated towards seed production particular emphasis has been paid to this aspect.

3.1. THE ENVIRONMENTAL CONTROL OF SPROUTING

3.1.1. Introduction

The economics of potato storage at the present time, and in the forseeable future are such that very sophisticated controlled environments such as those used for banana storage are not a practical proposition. Therefore it is usual in potato storage to modify only those environmental parameters which are relatively easy to manipulate and which will produce greatest beneficial effects within

strict economic constraints.

In practice this means that only modifications to temperature, ventilation rate and perhaps humidity are considered commercially feasible, and even in these cases great care has to be taken to ensure that the benefits from their control are worthwhile in financial terms. To minimise capital investment and subsequent running costs most commercial stores, therefore, have varying degrees of partial control of these factors rather than the precise control such as might be encountered in a laboratory growth chamber.

The current discussion will therefore be confined to these environmental factors which are easily controlled within the economic constraints of agriculture at the present time and the forseeable future.

Before moving on to discuss these factors one possibility for the more distant future which should at least be mentioned is controlled atmosphere storage. Some of the effects of modified atmospheres on sprouting were discussed in the previous Chapter and Burton (1974b) has discussed the biophysical principles underlying this type of storage. Secondary effects on the tuber have also been investigated (Harkett, 1971; Burton, 1974a).

From the effects of temperature, ventilation and humidity on sprouting discussed in Chapter 2 it would seem at first sight a reasonably simple task to devise a storage environment where sprouting is minimised or suppressed completely. This could be achieved for example by simply storing the tubers at 2-4^{o}C where sprouting can be suppressed for an almost indefinite period. Sprouting, however, is not the only factor which must be considered when devising the optimum storage environment.

Modifications to the environment also affect other factors such as rotting, ware quality and the physiological state of seed, and these effects must be taken into account when assessing the extent to which the environment may be modified to control sprouting.

It is therefore the intention below to briefly assess the effects of environment (in the limited sense described above) on factors which are likely to influence the choice of storage conditions in commercial situations.

Taking these effects and the information on sprouting in Chapter 2 into account the optimal storage environments for different types

of potato will be described and the degree of sprout control which can be expected will be assessed.

1) Overheating

Recent trends in potato storage towards very much larger bulk stores have meant that the problem of overheating now assumes much greater importance in the selection of environmental conditions than it did in the past.

Tubers in store are continually respiring and in so doing they liberate large quantities of heat amounting to 8-12k cal per tonne per hour for healthy mature tubers at normal storage temperatures. Immediately after harvest this figure may be greater by a factor of 4 (Burton, 1966).

This heat raises the temperature of the tubers above that of their surroundings. When air is allowed to circulate freely around the tubers this heat is rapidly dissipated, but when tubers are stored in bulk, air movement is restricted and the temperature of the tubers and the air between them will rise. With no forced ventilation this warm air will rise at a rate dependent upon the difference between its temperature and that of the ambient air above the stack.

As the temperature of the stack increases then the difference in density between the air in the stack and the ambient increases, thus both the amount of heat removed by unit volume of air and the flow rate increases i.e. the efficiency of cooling increases.

Although there is a marginal increase in the respiration rate (and hence the heat produced) as the temperature rises this is swamped by the increased efficiency of cooling and eventually a state of thermodynamic equilibrium is reached i.e. the stack will maintain a constant temperature.

Burton (1966) has shown that this equilibrium temperature can be calculated from the dimensions of the stack and various physical constants.

If heat production is very high (eg. with very immature tubers) or the temperature of the ambient air is high, or if air flow is obstructed (eg. excessive amounts of soil or by the dimensions of the stack) then before the equilibrium state has been reached the potatoes can rise to a temperature of greater than 15-20^{o}C where increased respiration produces vast amounts of heat which cannot be

compensated by the increased convective cooling. In this situation the whole mass overheats and total loss may ensue.

The physical dimensions of the potato stack which can be ventilated by natural convection is therefore limited. The critical dimensions of stacks above which overheating is likely may be calculated from the mathematical expressions given by Burton (1966).

It is therefore essential in modern bulk stores where potatoes are stored at depths of 3 to 7m that some form of supplementary ventilation with ambient or artificially cooled air is applied. Rates of discontinuous and continuous ventilation have been discussed at length by Burton (1966).

2) Water loss

Excessive shrinkage caused by water loss is a more serious problem in modern stores because of the extra ventilation which is used for the reasons given above. As water loss has already been discussed in Chapter 2 when discussing the losses due to sprouting it is sufficient to state for the purposes of the present discussion that if water loss is to be minimised then low storage temperatures, high humidity and low ventilation rates are desirable. This subject has been reviewed by Burton (1966) and Rastowski (1978).

3) Chilling injury

Storage at very low temperatures below $-1^{o}C$, for prolonged periods usually results in freezing of the tubers. However, storage at temperatures which are low but not sufficiently low to freeze the tuber may result in damage which is usually referred to as chilling injury or low temperature breakdown.

The symptoms are dependent on cultivar, duration of storage and temperature but are usually brown or black patches on the skin and discolouration in the flesh varying from reddish-brown to dark brown (Richardson and Philips, 1949). Subsequent emergence can be reduced or in severe cases completely inhibited. Of particular interest to the present discussion is the finding that such effects may be produced at temperatures as high as $2^{o}C$ (Richardson and Philips, 1949; Burton, 1973).

4) Wound healing

Inevitably when potatoes are harvested a percentage sustain damage to the periderm which can lead to increased respiration and water loss and allow easy access to pathogens. The environment in which tubers are stored has a marked influence on the rate at which these wounds heal and because major losses can result the encouragement of wound healing usually takes priority over other storage considerations during the first few weeks of the storage period.

Wound healing involves two processes. The first is the deposition, at the damaged surface, of a complex waxy material whose composition has been described by Brieskorn and Binnermann (1972). This initial process is known as suberisation and it is followed by the formation of wound periderm. Storage temperature is closely related to the rate at which these processes occur.

In the most quoted work on the subject Artschwager (1927) has shown that in the two cultivars examined, superficial cells at the cut surface become suberised, in humid conditions, after 1 day at 21^{o}C, 2 days at 15^{o}C, 3 days at 10^{o}C, 5 to 8 days at 5^{o}C, and over 8 days at 2.5^{o}C. Formation of wound periderm commenced 1 day after suberisation at 15^{o}C, 1-3 days at 10^{o}C, 5 days at 7^{o}C and had not commenced by the end of the experimental period when tubers were stored at 5^{o}C and below.

Humidity is also reported by Artschwager (1927) to have an effect on wound healing. Dry conditions adversely affect suberisation and especially periderm formation. He also reported that saturated atmospheres will inhibit wound healing. However, as previously discussed, Burton (1973) has questioned much of the early work on high humidity conditions because of water film formation with consequent anaerobiosis (Burton and Wigginton, 1970).

Under British conditions ventilation with outside air will normally result in a reduction in the humidity inside the store and hence adversely affect wound healing.

Wound healing is then a major consideration in the earlier part of the storage season necessitating storage at relatively high temperatures (greater than 10^{o}C and preferably 15^{o}C) and at relatively high humidity for 2-3 weeks after harvest. These

requirements are further complicated in large commercial stores by the fact that they may be filled over a period of several weeks. The 2-3 week period when applied to the last potatoes to be loaded into store may mean that the first loads have been stored at 15°C for as much as 6 weeks. The implications of these observations on sprouting are of great commercial significance.

5) Fungal and bacterial rotting

Compared with other stored products such as cereals, potatoes are extremely susceptible to damage by micro-organisms. The rotting caused by fungi and bacteria results in greater storage losses than any other single factor (Twiss and Jones, 1965).

Extensive reviews of storage diseases are available (Burton,1966; Boyd, 1972) and so the present discussion will be confined to a brief outline of the effects of environment on pathogen development with a view to examining the extent to which the environment may be modified to control sprouting.

The most important environmental factor in the development of rots, and in some cases their subsequent spread, is temperature. In general increasing temperature will promote the growth of fungi and bacteria on a culture medium until an optimum is reached, after which growth slows down fairly rapidly until a point is reached where the organism is killed. However, when the organism is growing on potato tissue the shape of this growth curve may be substantially altered due to the effects of temperature on the wound healing process and other natural defence mechanisms. It is therefore necessary to distinguish between the optimum growth conditions for the fungus or bacterium in culture and the optimum conditions for the development of rots.

Dry rot besides being a disease of great commercial significance is as typical (or at least as typical as any) in its response to environment, as many of the fungal diseases of potatoes and should serve as an example.

The optimum temperature for the development of dry rot caused by Fusarium solani var. avenaceum is 20-25°C whilst that caused by Fusarium solani var. caeruleum is 15°C. Development of rots caused by either fungus is very much slower at lower temperatures and is

almost inhibited completely at 5°C.(Burton, 1966).

High humidity as was discussed above promotes wound cork formation which can hinder initial fungal invasion. If, however, invasion occurs then high humidity can in fact encourage development of subsequent rotting.

Most fungal pathogens show a similar response to temperature and humidity preferring reasonably warm temperatures (>10°C) and high relative humidity for optimal growth. Minimisation of disease therefore demands storage at fairly low temperatures (<10°C).

There is, however, one exception which is extremely important to Scottish agriculture and that is gangrene which is caused by various Phoma species. The temperature optimum for the development of Phoma rots is in the range 0-5°C. Storage at higher temperatures such as those providing optimum conditions for other fungi may in fact arrest the development of gangrenes. Incidence of the disease is greatest when wet conditions prevail near the end of the growing season and if a period of relatively cold storage ensues, then losses can amount to a sizeable percentage of the stored crop. Storage at temperatures at which sprouting is totally inhibited (2-4°C) can therefore lead to very serious losses particularily in Scotland (Burton, 1966).

Overall minimisation of fungal rots therefore demands a temperature of between 5 and 10°C. If gangarene is not likely to be a problem then the lower of these two extremes is preferable. Where gangarene is a problem then the higher figure is preferred, although if this is not possible 7°C will at least reduce the problem.

Recent developments in the field of chemical control of tuber rots may lead to reduction in the importance of certain fungal rots in the selection of optimal storage environments. The use of 2-aminobutane as a fumigant for the control of gangrene, skin-spot and silver scurf as reported by Graham and his co-workers is already widespread in Scotland.(Graham and Hamilton, 1970; Graham et al, 1973; 1975). Thiabendazole and benomyl are also used commercially for the control of gangrenes and other tuber diseases (Murdoch and Wood, 1972; Tisdale and Lord, 1973; Copeland and Logan, 1975; Logan et al, 1975; Boyd, 1975).

Bacterial rots are also a major problem in potato storage. Bacterial soft rots have a higher optimal development temperature than fungal rots being around 25-30°C. They can, however, develop slowly even at 5°C. Bacterial soft rots do not normally develop unless tubers are covered with a water film in which case their development is almost inevitable. In modern commercial stores the main source of such water films is condensation which drops onto the surface layers of tubers due to inadequate roof insulation or inadequate absorbant coverings. Where high humidity conditions are employed the risk of such condensation is greater as almost any cold surface is likely to produce condensation.

Blackleg is another major bacterial pathogen on British crops and it exhibits a similar response to temperature.

Overall minimisation of bacterial rots demands storage at low temperatures and the absence of surface moisture, the latter probably being of greater importance.

Satisfactory chemical control of bacterial rotting has not yet been demonstrated in practice although research is being conducted in this field (Janiak, 1972).

6) Compositional quality

Some of the effects of environment on compositional quality were briefly mentioned in Chapter 2 and as stated there, these will be discussed at length in Chapter 6. For the purposes of the present discussion it is sufficient to say that storage at low temperatures (say 5°C) will preserve nutritional quality but destroy processing quality due to the accumulation of excessive amounts of reducing sugars. Storage at medium temperatures (say 10°C) will prevent cold temperature accumulation of sugars but may hasten a second sweetening process known as senescent sweetening. Thus for potatoes destined for early processing 10°C may be ideal whilst for those to be processed later in the storage season a temperature of 8°C may be preferable.

7) Physiological age and apical dominance

Storage temperature has a profound effect on the physiological age of seed tubers and in turn physiological age affects numerous aspects of subsequent field performance including the rate of

emergence, the rate of haulm growth, the number of main stems per tuber planted, the time of tuber initiation, the ratio of haulm weight to tuber weight, total yield and size distribution within the yield (van der Zaag, 1973).

In general the number of main stems per plant increases with the physiological age of seed. The rate of both sprout and haulm growth initially increases with advancing physiological age but then decreases. The ratio of haulm weight to tuber weight and the number of days between emergence and tuber initiation decreases with physiological age (van der Zaag, 1973).

Seed tubers for early production should have an advanced physiological age at planting to produce early bulking whereas tubers which are planted for maincrop ware production should be slightly younger thus allowing them to achieve more of their genetic potential before senescence.

In seed production manipulation of the physiological age could be exploited to a greater extent to permit earlier defoliation and hence reduce the risk of viral disease, and allow easier harvesting.

The production of advanced physiological age material demands pre-sprouting and controlled conditions to ensure sprouts which are resistant to mechanical damage during planting.

Storage at low temperatures ($4^{o}C$) is now practised by some large commercial organisations and this may result in minimisation of weight loss and sprouting and hence maximise profits for these organisations. The buyer, however, may well be receiving seed which is physiologically too young for the purposes intended.

Manipulation of the storage environment may also affect the apical dominance of seed tubers. If a small number of stems is required (thus reducing tuber numbers and increasing size) then tubers may be stored at $15^{o}C$ till sprouts begin to develop then cooled to $4^{o}C$ to prevent excessive sprout growth. If on the other hand an increase in tuber numbers is desired (eg. for canning or for seed) then seed may be stored at $4^{o}C$ (after wound healing) until a few weeks before planting and then pre-sprouted under illuminated conditions at $15^{o}C$ (Burton, 1966). It is interesting to note that these very different results may be produced from seed which has a similar physiological age if expressed in "day ^{o}C". Other aspects

of apical dominance have been reviewed by Goodwin (1963).

Thus the effects of temperature on apical dominance have also to be taken into account when selecting the optimal storage environment for seed potatoes.

3.1.2. Optimal storage environments and resultant sprouting

As explained above numerous factors have to be taken into account when selecting the optimal storage environment for a given batch of potatoes. The resultant conditions are therefore a compromise which must be made on the basis of economic considerations. For example a seed grower is unlikely to gain any short term financial advantage in supplying seed in optimal physiological condition from which only the purchaser will profit. His prime consideration is therefore in the short term to sell the maximum weight of healthy seed.

,In any particular situation the optimal storage environment will depend on the design of the store, the condition of the potatoes and their ultimate fate. Nevertheless several general situations exist and these are described below.

1) For all potatoes a wound healing period of at least 2 weeks is required during which the temperature should be greater than $10^{o}C$ (preferably $15^{o}C$) and the humidity should be as high as possible (preferably 95% R.H.). Some ventilation will be necessary to prevent overheating due to the high respiration rate of freshly harvested tubers. These conditions must apply from the time the last tubers are loaded into the store. Unfortunately this means that the physiological age of some tubers in large stores may have advanced to such an extent that dormancy may be broken before the end of the wound healing period.

2) For ware potatoes destined for the fresh market successful storage demands a temperature of $7^{o}C$ although if rotting (not gangrene) is probable then storage at $4^{o}C$ might be preferable. In this case reducing sugars will accumulate but this should be of little consequence to their market value provided they are not stored for lengthy periods. Where gangrene is a problem a temperature of at least $7^{o}C$ is required. Relative humidity should be maintained at as high a level as possible although where no

forced ventilation is used slightly drier air is permissible. Ventilation should be the minimum required to prevent overheating and CO_2 accumulation. Under these conditions sprouting is unlikely to be a problem until January or February. Storage beyond this time at 7^oC is likely to require further measures to control sprouting.

3) For ware potatoes destined for processing a higher storage temperature is required. Temperatures of 8^oC for frozen chips and dehydrated products and 10^oC for crisps are common. At these temperatures water loss can be a serious problem unless attention is paid to both ventilation and humidity. Storage at either temperature will invariably result in excessive sprouting, especially since many of these stores are very large and as explained earlier some tubers may have received an excessively long wound healing period. Storage beyond January at these temperatures is not possible without some form of non-environmental sprout suppression.

4) For seed potatoes storage at low temperature (around 4^oC), high humidity and low ventilation rate will produce maximum recovery of stored material except where gangrene is a problem when the storage temperature should be around 7^oC. It is therefore quite possible in many instances to control the sprouting of seed potatoes using environmental manipulation only. Such storage will of course, produce physiologically young seed and apical dominance is likely to have been destroyed. Because gangarene is such a problem in Scotland and because Scottish seed tends to be physiologically too young for many purchasers storage at slightly higher temperatures around $6-7^oC$ might be a better general recommendation. Under these conditions, however, weight loss and sprouting may become a problem with late delivery seed.

A further problem in the case of seed production is the factor of scale. Much of the seed grown in Scotland is produced by growers whose scale of operations cannot justify the type of controlled environment storage facilities which are used by large scale processors. In many instances these producers have little control over temperature and during mild winters sprouting can be a very serious problem.

3.1.3. Conclusion

Sprouting is only one of many factors to be considered in a commercial storage regime and the possibilities of achieving complete control of sprouting by environmental manipulation are extremely limited. In the case of tubers destined for processing they appear in fact to be non-existent. The ever increasing importance of this sector of the market makes some form of sprout control other than environmental manipulation absolutely essential. The processing industry as we know it today simply could not exist without a satisfactory means of suppressing sprout growth.

3.2. THE CONTROL OF SPROUTING BY IRRADIATION

3.2.1. General

Over the years irradiation has been extensively investigated as a technique to inhibit the sprouting of potatoes, and a considerable volume of literature has accumulated particularly with respect to its secondary effects on potato quality.

Excellent sprout inhibition may be achieved by exposing tubers to 5-20k rad of gamma radiation from a ^{60}Co source (Sparrow and Christensen, 1954; Burton and Hannan, 1957)(100rad = 1gray). Typical dose rates appear to be the order of 50k rad h^{-1}. The complete dose can therefore be administered in a few minutes. X-rays are also potent sprout inhibitors (Hagberg and Nylsom, 1954).

Pätzold and Weiss (1957) found that tubers irradiated with 270 to 900rad of gamma rays from a ^{60}Co source retarded sprouting and reduced weight loss, but very much higher doses of the order of 7krad were required for lasting effects. The effectiveness of the treatment appears to be independent of the size of the tubers but the treatment was less effective with deep layers of tubers. In general irradiated tubers lost less water, starch, dry matter, and ascorbic acid during subsequent storage. When tubers were planted out 6 months after treatment those which had received less than 900rad outyielded controls. Those which received up to 5rad germinated, but at levels greater than 6krad no buds developed.

From a general appraisal of the literature the most commonly recommended dose for complete control of sprouting is 10krad.

Irradiation appears, then, on preliminary examination to offer excellent control of sprout growth, and Sawyer (1967) has reported that pilot scale commercial application has been investigated in Canada. Irradiation has, however, several major disadvantages and these are discussed below.

3.2.2. Secondary effects

Irradiation has other effects on the tuber besides the prevention of sprouting. Brownell et al (1956) reported that a dose of approximately 14krad will completely inhibit periderm formation. Although smaller doses may minimise this detrimental influence it is nevertheless a very serious problem as any commercial plant for irradiation would necessarily involve the passage of tubers through an irradiation chamber probably on some sort of conveyor belt. Thus to treat tubers they must be handled and this will inevitably produce a certain amount of damage. This problem can be minimised to a certain extent by irradiating tubers after several weeks storage when they are less likely to sustain damage, rather than immediately after harvest. The inhiition of wound healing by irradiation has been shown by Sawyer (1956) to lead to a substantial increase in rotting during subsequent storage. Sandret (1970), however, reports that 10krad of either gamma or X radiation, whilst, completely inhibiting sprouting, produced no increase in susceptibility to bacterial and fungal infection over that found in chemically treated tubers.

There is a considerable volume of literature which suggests that irradiation can lead to higher levels of blackspot and after-cooking blackening (eg. Sawyer, 1956; Skou, 1966; Pätzold, 1974).

The effect of irradiation on sugar levels has also been well investigated (Burton and Hannan, 1957; Burton et al, 1959). It has been shown that senescent sweetening is hastened by irradiation, and that in the period immediately after exposure, tubers irradiated with 10krad show a marked increase in sucrose concentration which reaches a maximum after 5 days but then falls over the ensuing 3 weeks. There is also an immediate increase in the reducing sugar content which despite a steady decline remains at levels above those

of untreated controls (Burton, 1975). Much of the American research on this subject indicates that these effects have only a limited effect on processing quality.(Sawyer, 1956; 1967; Smith, 1968). Pätzold (1974) found no significant difference between sugar levels in irradiated and chemically treated tubers, although his results suggest slightly higher levels in the irradiated samples.

Further aspects of these main effects and numerous other biochemical changes associated with the irradiation of potato tubers have been discussed or reviewed by Skou (1966), Sawyer (1967), Smith (1968), Sharma (1973) and Burton (1975).

3.2.3. Safety

The use of radiation whether it originates from nuclear sources (gamma radiation) or electronic sources (X-radiation) demands stringent safety precautions. Whilst the doses used to irradiate potato tubers (usually 10krad) are often referred to in literature as "modest", it should be borne in mind that the International Commission on Radiological Protection recommend that the maximum occupational exposure to which personnel may be subjected is 5rems per annum. As both gamma and X-rays have a relative biological effectiveness of unity this implies a maximum exposure of 5rads per annum. The cost of handling sources and the shielding required comply with these rules means that if ever adopted, irradiation could only be used in very large installations.

A further factor which is not often discussed in the literature is the toxicology of treated tubers. Recent Russian research work has shown that dominant lethal mutation occurred in spermatozoids, spermatids and spermatocytes of mice receiving oral doses of an alcoholic extract of tubers which had been exposed to the generally recommended dose of 10krad of gamma radiation (Kopylov et al, 1972). In light of these findings the advisability of irradiating tubers for human consumption requires further investigation.

3.2.4. Conclusion

The general conclusion which may be drawn from the published work is that at high dose rates the detrimental effects of radiation are serious, but by minimising doses, and irradiating at the correct time with the minimum handling damage, the use of gamma or X-radiation

could provide an alternative means of sprout suppression to the chemicals currently used should they become unavailable. With our present state of knowledge, however, irradiation cannot compete with chemical treatment whether the assessment is on the basis of quality, cost or safety.

3.3. CHEMICAL CONTROL OF SPROUTING

Introduction

The disadvantages of environmental control and irradiation as instruments of sprout suppression have been discussed above. The only alternative available at present is chemical sprout suppression, and growers merchants, and processors are turning in increasing numbers to this method to provide efficient and economical control of sprout growth.

Chemical sprout suppressants are with a few exceptions applied during the storage period. The most successful of these chemicals are active in the vapour phase, although they may actually be applied as solids, liquids or gases. Generally the active compound is diluted in a medium suitable for the application method used. Some are dispersed in an inert filler (e.g. Kaolin) and dusted onto the tubers as they are placed in storage. Others are partially vapourised to form a "fog" or "mist" which diffuses amongst the tubers. The vapour may also be blown through the tubers using a suitable ducting system to ensure even distribution.

Chemicals used for sprout suppression

Many chemicals have been suggested as inhibitors of potato sprouting yet relatively few are used commercially. Although this thesis concentrates on those which are in commercial use, a survey of those which have been used commercially and now superceeded by others and even those which have never reached commerce is considered to be of value, in that many of the disadvantages of our present chemicals are highlighted in their predecessors.

Most sprout suppressant chemicals have applications in other fields of agriculture and horticulture and their sprout suppressant properties discovered after their commercial introduction for other uses. Some were by-products of the huge

research effort in the plant growth regulator field. Very few sprout suppressant chemicals have been designed specifically for sprout suppression.

A few of the chemicals may be used on seed tubers, but most inhibit sprouting so efficiently that subsequent germination is temporarily or permanently impaired.

Early research on some of these compounds has been reviewed by Perlasca (1956).

3.3.1. POTATO SPROUT SUPPRESSANTS WHICH ARE NOT CURRENTLY USED

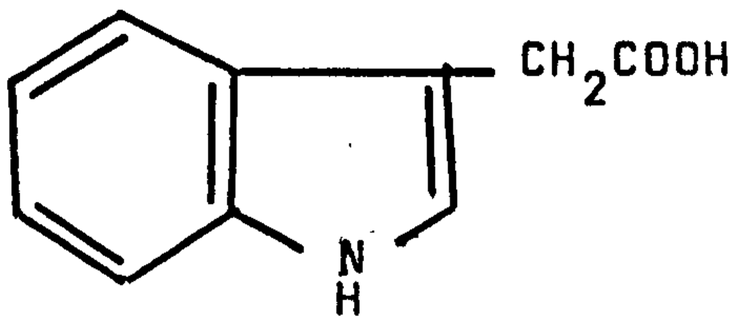
Discussed under this heading are a) chemicals which have been suggested as sprout suppressants but have proved of little or no commercial value, b) chemicals which have been suggested but which have been used commercially but which have now been superceeded by other chemicals.

It should be noted that these compounds are not described in chronological order of their discovery, but an attempt has been made to classify them on the basis of their chemical structures and properties. Post-harvest application should be assumed unless otherwise stated.

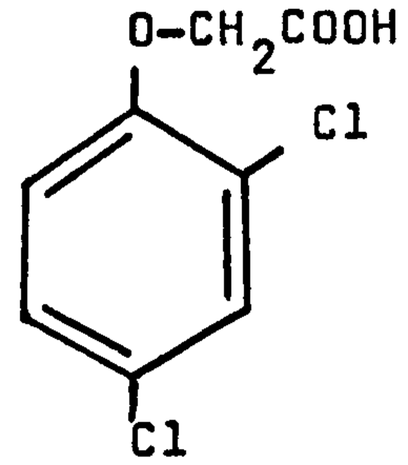
1. INDOLE ACETIC ACID

The elucidation of the structure of the auxin, indole acetic acid (3-indolylacetic acid, IAA)(see fig. 3.3.1.) stimulated research into possible agricultural applications. IAA was shown to suppress the sprouting of explanted eyes of potato tubers when applied as the sodium salt (Guthrie, 1938). Smith et al (1947) reported that spray application of IAA to a growing crop at a concentration of 150mg dm^{-3}, applied at 500-1000dm^3 ha^{-1} did not affect plants, yields, specific gravity or reducing sugar content of tubers, but after 3 months storage the harvested tubers showed significantly less sprouting than controls. This treatment, however, was not practical since to obtain adequate sprout suppression very

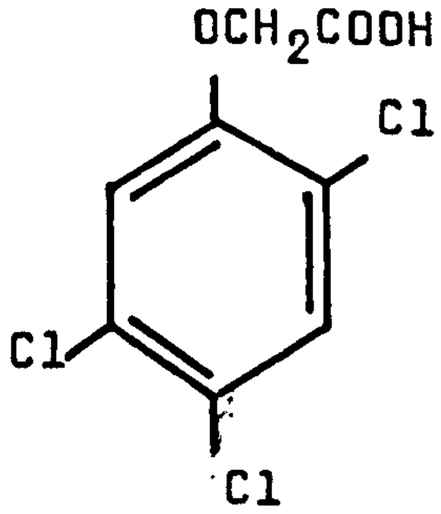
Figure 3.3.1. Sprout suppressant compounds



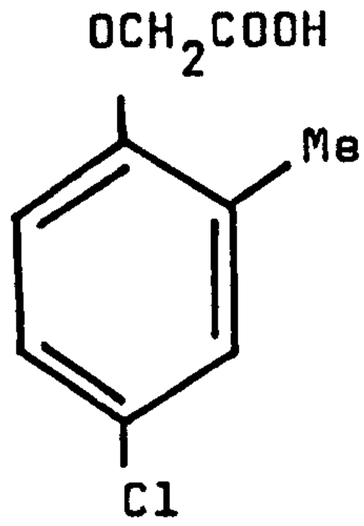
I.A.A.



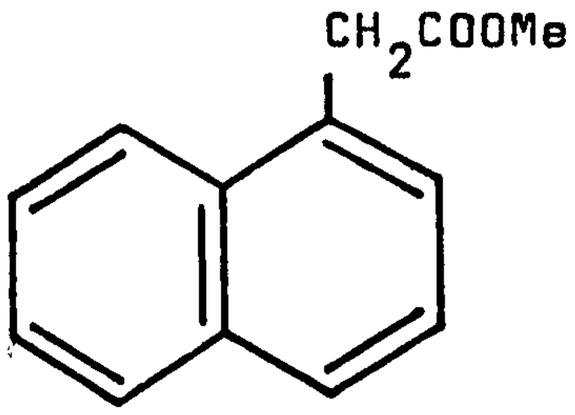
2,4-D



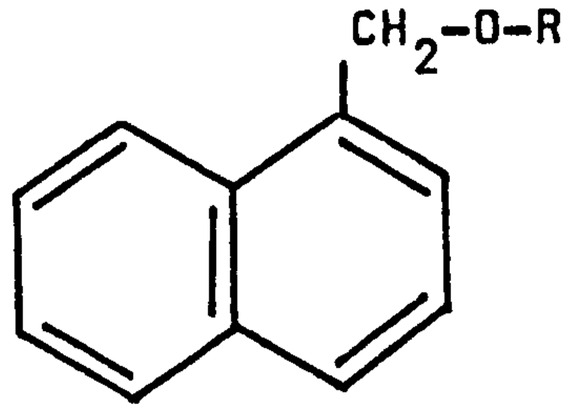
2,4,5-T



MCPA



MENA



ALKOXYMETHYL NAPHTHALENE

large amounts of IAA must be introduced into the tuber tissue and more efficient alternatives were available.

2. PHENOXY COMPOUNDS

Research into the biological properties of compounds with structural similarities to IAA eventually led to the introduction of the phenoxyalkanoic acid herbicides shortly after World War II. Their discovery is described by Fletcher (1974). Several of the phenoxyacetic acetic acid herbicides have been suggested as potato sprout suppressants.

i) 2,4-D

2,4-dichlorophenoxyacetic acid (2,4-D) (see fig. 3.3.1.) has been used as its sodium salt with limited success at high concentration only (Ulrich and Ildis, 1948). At low concentration it actually stimulates sprout growth. Kenneth (1945) reported that the methyl ester of 2,4-D although effective as a suppressant caused rapid breakdown of tubers and noted abormal growth around the eyes. Smith et al (1947) reported significant sprout control after foliar applications of the sodium salt of 2,4-D, however complete control would have necessiated use of much higher concentrations.

ii) 2,4,5-T

Smith et al (1949) found that 2,4,5-trichlorophenoxyacetic acid(2,4,5-T) (see fig. 3.3.1.) applied as the sodium salt in a spray containing 50-300$\mu g\ cm^{-3}$ and as a dust containing the isopropyl ester significantly reduced sprouting, but in a later experiment Ellison (1952) obtained poor sprout suppression when using the methyl ester of 2,4,5-T on a larger scale. Ellison and Smith (1949) also applied 2,4,5-T to the foliage of the growing plant prior to flowering and produced significant reductions in sprouting of the subsequently harvested crop. However, Driver (1957) using a similar foliar spray noticed a significant increase in cracking of the tubers. Wood and Ennis (1949) noted that tubers from plants sprayed with the butyl ester of 2,4,5-T

were more susceptable to common scab, and that fewer layers of wound cork were produced. 2,4,5-T would now be disqualified as a sprout suppressant because of its toxicology (Drill and Hirayzka, 1953) and because of the impurity 2,3,7,8-tetrachlorodibenzo-p-dioxin which it always contains. This compound is extremely toxic and can produce teratogenic effects at the parts per million level (Courtney et al, 1970; but see also Hartley, 1975).

iii) MCPA

2-methyl-4-chlorophenoxyacetic acid (MCPA) (see fig. 3.3.1.) has also been tested as a suppressant and found to be effective at fairly high concentrations when applied as the methyl or ethyl ester in a dust formulation (N.V. Aagrunol Chemiscke Fabriek, 1962). 2-methyl-4-chlorophenoxybutanoic acid (MCPB) was found to be ineffective at all concentrations tested.

iv) PHENOXY ALKYL HALIDES

Phenoxy alkyl halides and various other phenoxy alkane derivatives have been patented as potato sprout suppressants after small scale experiments (N.V.Aagrunol Chemiscke Fabriek, 1960) e.g. 1-(2-methyl-4-chlorophenoxy)-3-chloropropane.

3. NAPHTHALENE DERIVATIVES

i) ESTERS OF NAPHTHALENEACETIC ACID (INCL. MENA)

Research into the physiological activity of IAA analogues also led to the discovery of the herbicidal properties of naphthaleneacetic acid. This work eventually led to the introduction of the methyl ester of α naphthalene acetic acid (see fig. 3.3.1.)(MENA) as a commercial potato sprout suppressant.

MENA can be applied in a variety of ways, the most common of which is as dust containing about 2% of the active ingredient (Denny, 1945; Marth and Schultz, 1950; Edwards, 1949; Ellison, 1952; Emilsson et al, 1955). The application rate is usually in the range 0.125-1.2g/10kg^{-1} of potatoes, of active ingredient. MENA has also been applied on impregnated paper strips but is less effective by this method (Daines and

Campbell; 1946, Ulrich and Ildis, 1948), and during marketing by incorporation in washing water, and as a wax emulsion (Findlen, 1955). It can also be introduced as a vapour into the potato store (Sawyer and Dallyn, 1957).

After application the chemical actually penetrates the potato tuber to a depth of at least 10mm (Vadimov and Shtenberg, 1953) where it appears to concentrate in the meristematic tissue (Rakitin, 1955). Using a biological assay it has been estimated that residues from MENA treatment amount to no more than 5mg kg^{-1} in the whole tuber and 0.1mg kg in peeled cooked tissue (Denny, 1942).

Studies with ^{14}C labelled MENA have shown that it is readily decarboxylated, especially at higher temperatures (Rakitin et al, 1957). The ease with which it is metabolised could partially account for the very high application rates required for autumn application (0.12g kg^{-1}) whereas smaller amounts (0.09-0.12g kg^{-1}) will suffice for spring application (Emilsson et al, 1951). Volatilization, however, is generally accepted as being the more important factor in explaining these differences.

MENA is not suitable for application to seed tubers as it appears to irreversibly delay emergence, reduce the number of stems per plant, reduce the stand of plants, and reduce the yield significantly (Pujals et al, 1947; Rakitin and Troyan, 1949; Gandarillas and Nylund, 1949). These effects can be reduced by a preplanting treatment with ethylene chlorohydrin.

The low toxicity of MENA does make it suitable, however, for the treatment of ware, (Sporn and Penciu, 1959) and it has been widely used on a commercial basis in the U.S.A., South America, Australia, New Zealand, Europe and Russia. MENA does not affect ascorbic acid or dry matter contents of stored tubers (Driver, 1957) and its effect upon sugar levels is negligible (Denny et al, 1942).

The major disadvantage of MENA is that it inhibits wound cork formation allowing disease organisms to penetrate e.g. dry rot caused by Fusarium solani var. caeruleum is increased by treatment with MENA. (Cunningham, 1953;

Ellison and Cunningham, 1953). The problem eventually led to the discontinuation of the commercial use of MENA as any advantage gained in successful suppression of sprouting was being lost through increased fungal and bacterial problems (Wagner et al 1952).

Another problem reported by Denny (1942) was the production of malformations on the tuber surface which he states to be due to abnormal growth of underlying cells. These lumps measured 5-30mm in diameter and were composed of much firmer tissue than other portions of the tuber. The frequency of such malformations in his 1942 experiments was from 5-10% of all treated tubers although in previous experiments he had observed no abnormalities whatsoever.

The ethyl ester of αnaphthaleneacetic acid has also been used as a sprout suppressant, and as both the methyl and ethyl esters are metabolised to the same parent compound its properties are very similar. The ethyl ester is, of course, slightly less volatile (Yabuta and Tamari, 1942).

ii) ALKOXYMETHYL NAPHTHALENES

The 1- alkoxymethyl naphthalenes (see fig. 3.3.1.) were investigated in detail (Kruyt and Veldstra, 1951) using talc formulations of a homologous series of these ethers. It was discovered that methoxy and ethoxy derivatives suppressed sprouting at an application rate of 60mg kg^{-1} of tubers, but as the alkyl chain lengthens the inhibition decreases. They also described an oleate coacervation procedure which they suggest might be of value in the pre-selection of potential chemical sprout suppressants.

The methoxy derivative has also been investigated by other workers. Emilsson et al (1951) showed that 120mg kg^{-1} was effective for short term storage but that more was required when tubers were stored for longer periods. Rakitin (1955) tested over 100 compounds for sprout suppressing ability and noted that 1-naphthylmethyl ether was amongst the 5 most active compounds tested.

iii) CHLORONAPHTHALENE

Alpha chloronaphthalene (see fig. 3.3.2.) has been tested as a sprout suppressant by various workers (Emilsson et al, 1949; Edwards, 1952). Findlen (1955) showed however that although extremely effective at 100mg kg^{-1}, it caused injury to the tubers by killing the buds completely, and causing necrotic lesions at the lenticels.

iv) NAPHTHOXY COMPOUNDS

Naphthoxy alkyl halides have been patented for use as potato sprout suppressants (N.V.Aagrunol Chemische Fabriek, 1960). A formulation of 4% active ingredient on Fullers earth is suggested.

v) DIMETHYL NAPHTHALENES

1,4-dimethylnaphthalene and 1,6-dimethylnaphthalene (see fig. 3.3.2.) were identified as natural components of potato peel and evaluated as sprout suppressants by Meigh et al (1973) who found them to have activity comparable with that of isopropyl-(N-3-chlorophenyl)-carbamate, the most widely used commercial suppressant at the present time. These and related compounds are at present under investigation in this laboratory and preliminary (unpublished) results indicate they hold considerable promise for the future.

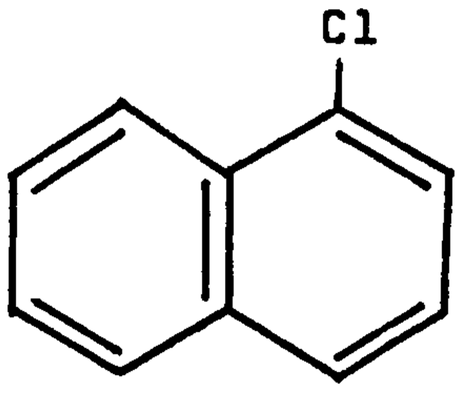
4. ALIPHATIC ALCOHOLS AND ETHERS

i) ALCOHOLS (INCLUDING NONANOL)

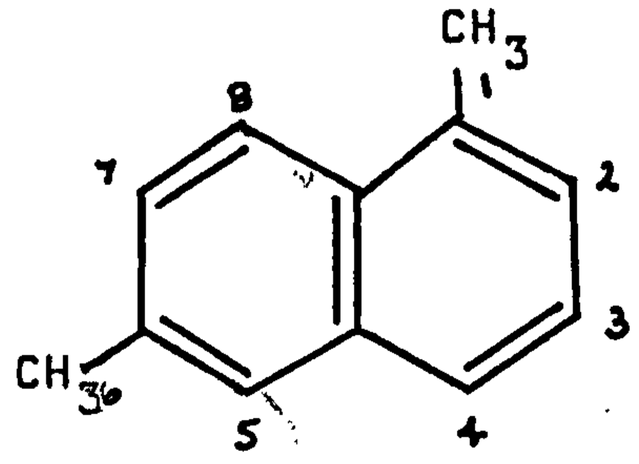
Ethanol vapour was described as retarding sprout growth by Huelin (1933). Burton (1956) showed that the vapour of n amyl alcohol was a very effective sprout inhibitor. He then screened a series of alcohols for sprout suppressant properties and demonstrated that nonyl alcohol (3,5,5-trimethyl-hexan-1-ol)(see fig. 3.3.2.) had considerable promise for commercial use (Burton, 1956; 1958a; 1958b).

It was eventually introduced under the trade name "Nonanol" which was applied as a vapour, either continuously or intermittently as desired, by vaporizing the chemical into

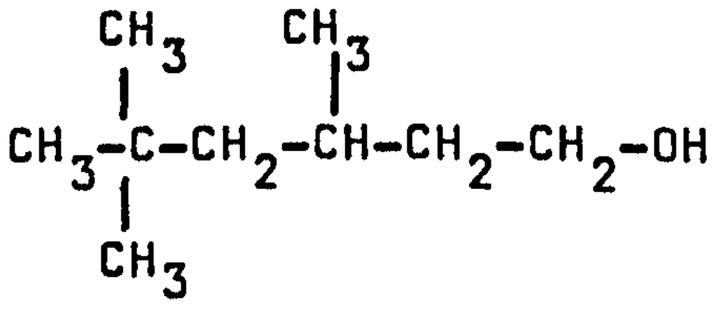
Figure 3.3.2. Sprout suppressant compounds



∝ CHLORONAPHTHALENE

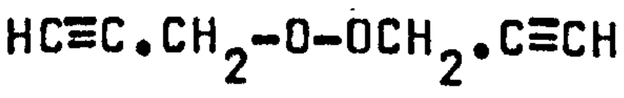


1,6 DIMETHYLNAPHTHALENE

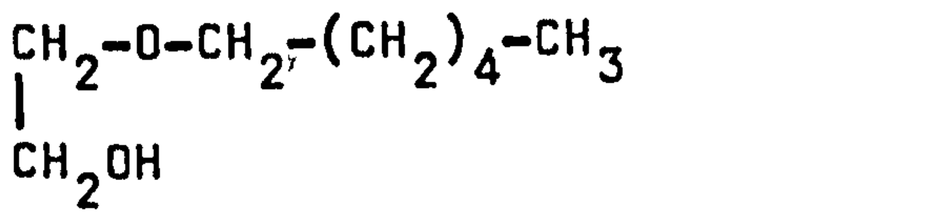


NONANOL

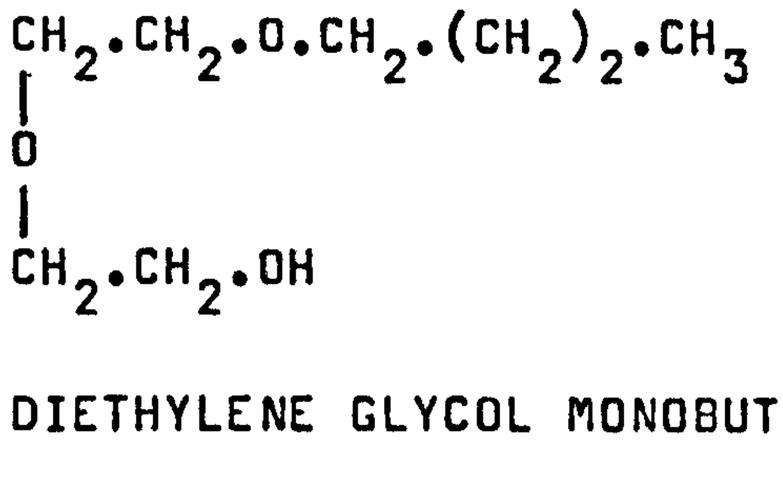
PROPARGYL ALCOHOL



DIPROPARGYL



ETHYLENE GLYCOL MONOHEXYLETHER



DIETHYLENE GLYCOL MONOBUTYLETHER

the ventilation system of the potato store. This was achieved by allowing the alcohol to drip onto a hot plate at the fan intake. It was found that concentrations of 60-100µg dm^{-3} in air would completely inhibit sprouting (Burton,1966; Meigh et al, 1973). Nonanol cannot be applied by conventional means i.e. on a solid carrier or as a "fog", as this would produce concentrations which would prove toxic to the tubers (Burton,1966).

Factors influencing the uptake of the chemical by the tubers are described by Currah and Meigh (1968), the most important of these being the applied concentration and humidity.

Nonanol has, however, several disadvantages, the most important of which is due to its mode of action. Nonanol acts by blackening and killing the developing sprout. In suitable conditions the dead sprout can provide an entry into the tuber for pathogenic micro-organisms (Meigh,1969). Sawyer (1967) noted that alcohols could cause lenticel pitting when applied at high concentrations, also providing access to pathogens. These factors can greatly increase rotting of tubers during storage and eventually led to the discontinuation of the use of nonanol as a sprout suppressant.

Nonanol also has a strong smell which can be unpleasant when handling recently treated tubers.

A gas chromatographic method for the analysis of nonanol in atmospheric samples has been described by Meigh and Currah (1966) and residues may be determined by the colorimetric method of van Vliet and Schreimer (1960).

Propargyl alcohol (2-propynol)(see fig. 3.3.2.) has been patented as a sprout suppressant (Hessel,1962), but Meigh (1969) has shown that it is less effective than nonanol. Meigh also indicates that in general the sprout suppressing activity of a low molecular weight alcohol is enhanced when there is an ethylenic or acetylenic bond in the α, β position with respect to the hydroxyl group.

ii) ETHERS

Dipropargyl ether (see fig. 3.3.2.) has been suggested as a sprout suppressant (Hessel, 1961). It is claimed to be more effective than amyl or nonyl alcohols.

Various glycol monoethers and their derivatives have also been proposed (Kasikhin et al 1966). However their claims have not been fully substantiated by Meigh's experiments (1969). Ethylene glycol monohexylether (see fig. 3.3.2.) did however give adequate suppression in one experiment at a concentration of 76µl dm^{-3} in air and diethylene glycol monobutylether (see fig. 3.3.2.) gave some suppression at 54µg dm^{-3}. At lower concentrations however these compounds apparently stimulate sprouting.

5. ALIPHATIC HALOGEN COMPOUNDS

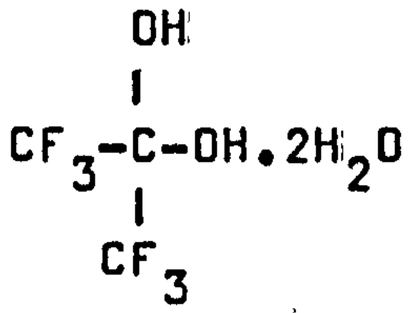
i) HALOGENATED KETONES

Halogenated ketones were shown to be efficient sprout suppressants when tubers were dipped in a 1% solution for 1 min. (Batchelor, 1968). They have also been used as haulm killers and it has been noted that when used as such they are translocated to the tuber and will inhibit sprouting during subsequent storage (Batchelor, 1969). One of these compounds, hexafluoroacetone trihydrate (1,1,1,3,3,3- hexafluoropropane- 2,2- diol dihydrate) (see fig. 3.3.3.)(also known as GC - 7887) has been tested by Sawyer and Dallyn (1966) and gave good sprout control when applied at 5kg ha^{-1} as a haulm killer. This material could well be worth further investigation.

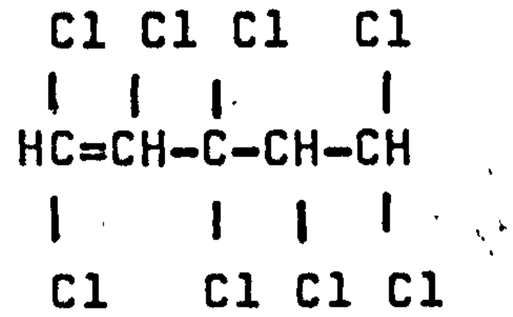
ii) HALOGENATED FATTY ACIDS

Halogenated fatty acids are well known herbicides e.g. trichloroacetic acid (TCA) and 2,2-dichloropropanoic acid (Dalapon) whose effects have been widely studied (Ashton and Crafts, 1973). Dettweiler (1958) has shown that such acids or their esters with alcohols containing at least three carbon atoms will prevent potatoes from sprouting when applied in the gaseous phase.

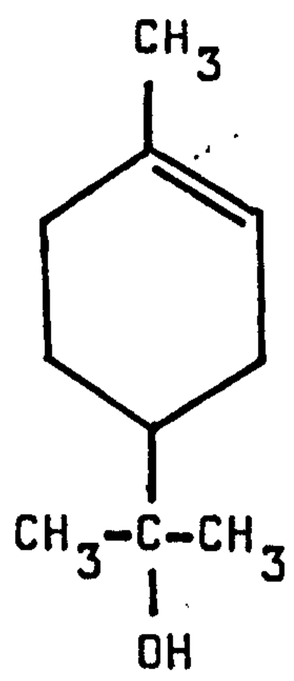
Figure 3.3.3. Sprout suppressant compounds



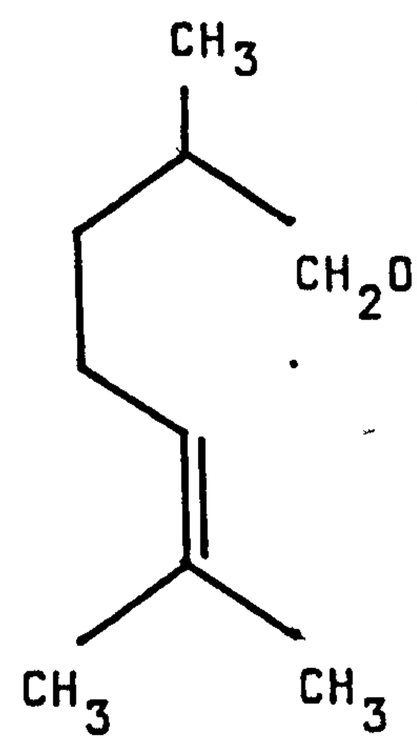
HEXAFLUOROACETONE TRIHYDRATE



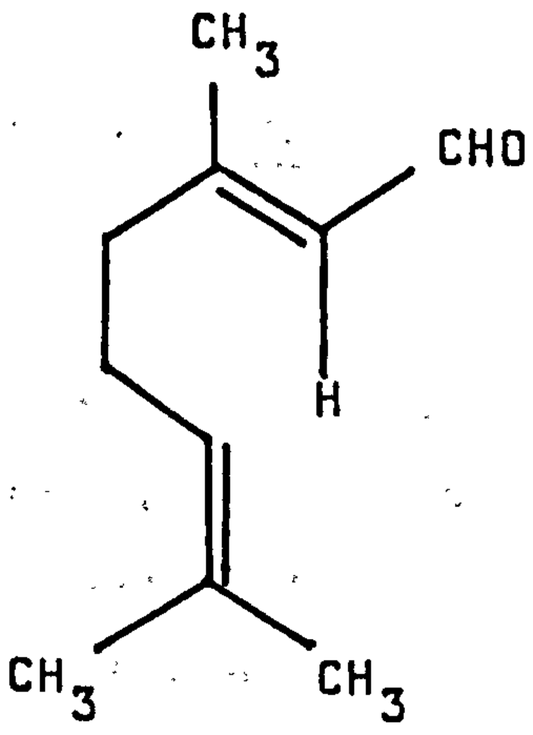
1,1,2,3,3,4,5,5 OCTACHLORO-1-PENTENE



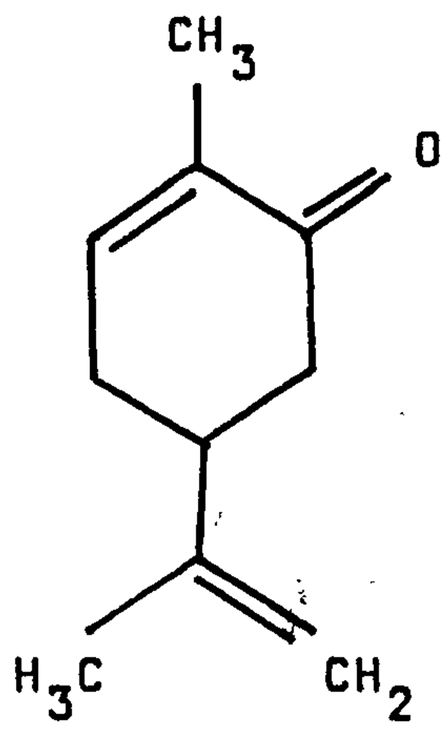
α TERPINEOL



CITRONELLOL



CITRAL



CARVONE

As halogenated acids are known to be translocated (both symplastically and apoplastically) it might be advantageous to test these compounds as translocated suppressants. On the other hand their mode of action might cause wound healing problems.

iii) HALOGENATED ALKENES

Halogenated alkenes have also been shown to have sprout suppressant properties. 1,1,2,3,3,4,5,5- octachloro - 1 - pentene (see fig. 3.3.3.) appears to be active at relatively low concentrations (20 - 160mg kg^{-1} when applied as a dust)(Koopman, 1954). This compound is also claimed to have the considerable advantage that the inhibition is reversible, and after an airing period the tubers will regain their ability to germinate, hence it could find application in the treatment of tubers intended for seed. It should be noted however that such claims have been made for a wide variety of chemicals and unsubstantiated by later research under commercial conditions.

iv) ALKYL HALIDES

The vapour of methyl bromide (bromomethane) is a well known fumigant with insecticidal, fungicidal and herbicidal properties. It has been shown to inhibit the germination of potato tubers (Procedes Agricole Moderne S.A., 1951). The toxic properties of alkyl halides however, make their application hazardous for unskilled personnel even when chloropicrin (trichloronitromethane) is added as a warning gas (Martin, 1972).

6. TERPENOID COMPOUNDS

Chemically all terpenoids can be considered to be derived from a basic branched 5 - carbon unit, the isopentane unit, and are classified according to the number of units present in the molecule. A hemiterpene contains 5 carbon atoms, a monoterpene - 10, a sesquiterpene - 15, a diterpene - 20 and a triterpene - 30. Probably more individual terpenes exist than any other group of natural products (Goodwin and Mercer, 1972).

Some terpenoid compounds are well known inhibitors of plant growth, and their importance as allelopathic compounds has long been recognised (Rice, 1974). A number of plant growth substances are also terpenes e.g. abscisic acid. It is not surprising, therefore, that a wide variety of these compounds have been investigated as potential sprout suppressants.

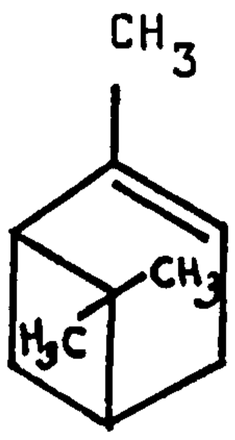
i) ALPHA TERPINEOL

The monoterpene, ∝ terpineol (1-methyl-4-isopropyl-cyclohex-1-en-8-ol)(see fig. 3.3.3.) has been the most widely investigated of these compounds. Emilsson et al (1949) found that ∝ terpineol was more efficient than MENA as a sprout inhibitor. Edwards (1952) observed poor inhibition however, even at fairly high application rates, and noted a decrease in the percentage of marketable tubers. Findlen (1955) applied ∝ terpineol in an aqueous wax emulsion at 100-200mg kg^{-1} and noted a reduction in sprouting, but less than that produced by MENA. Meigh (1969) tested a wide range of volatile organic compounds amongst which was ∝ terpineol which he found to be an effective suppressant at high concentrations only (125µg dm^{-3} of air). Overall it appears that ∝ terpineol is effective at high application rates which would be commercially unacceptable.

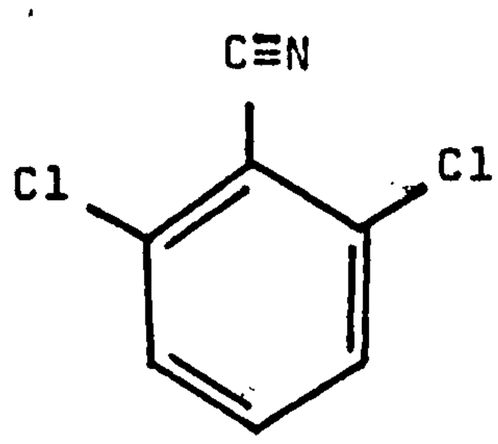
ii) OTHER TERPENOIDS

Other terpenoid compounds screened by Meigh (1969) using a continuous application technique which showed some potential include citronellol (3,7-dimethyloct-8-enol-1), citral (3,7-dimethyl-octa-2,7-dienal-1), and carvone (2-methyl-5-isopropenyl-cyclohex-2-en-1-one)(see fig. 3.3.3.). These compounds have been further tested in this laboratory and failed to show any promise using a single application on an adsorbant under more commercially orientated experimental conditions (Beveridge et al, 1978). Other terpenoid compounds including ∝ pinene (see fig. 3.3.4.) are now being tested. However in general it would appear that for most terpenoid compounds unacceptably high application rates are required.

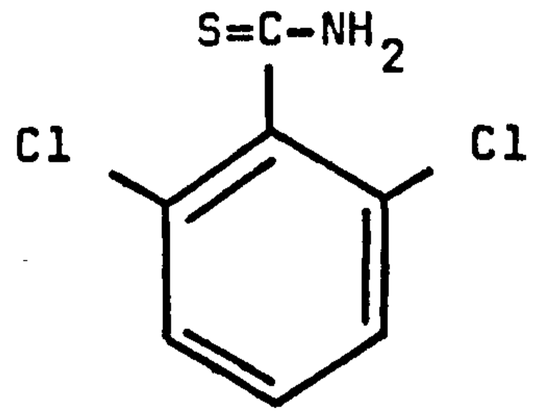
Figure 3.3.4. Sprout suppressant compounds



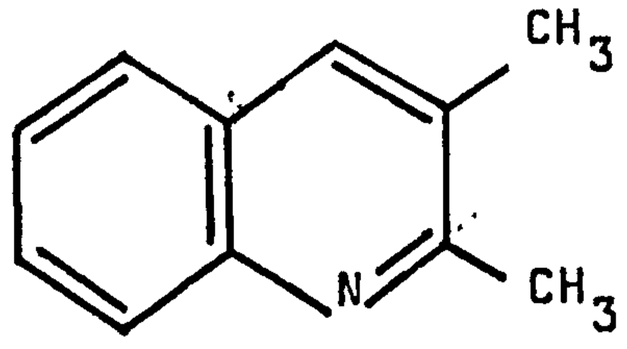
∝ PINENE



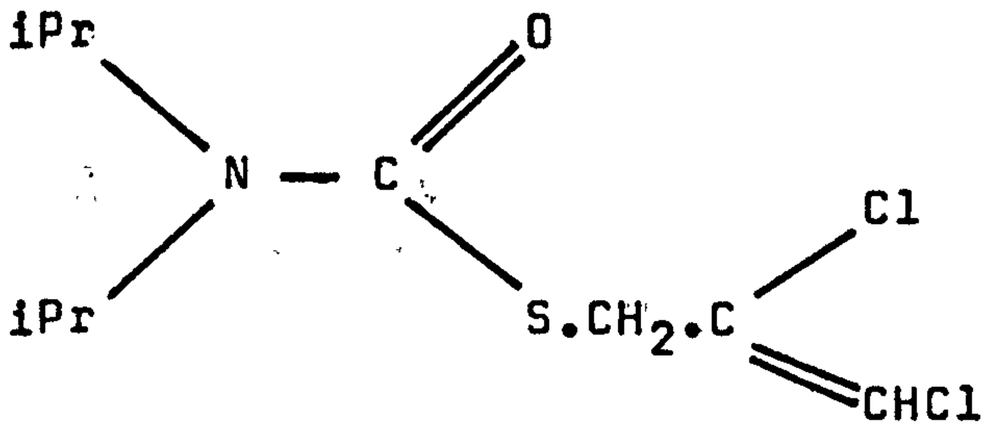
DICHLOBENIL



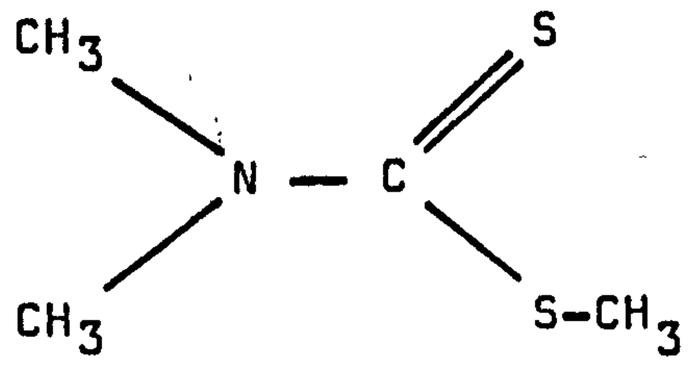
CHLORTHIAMID



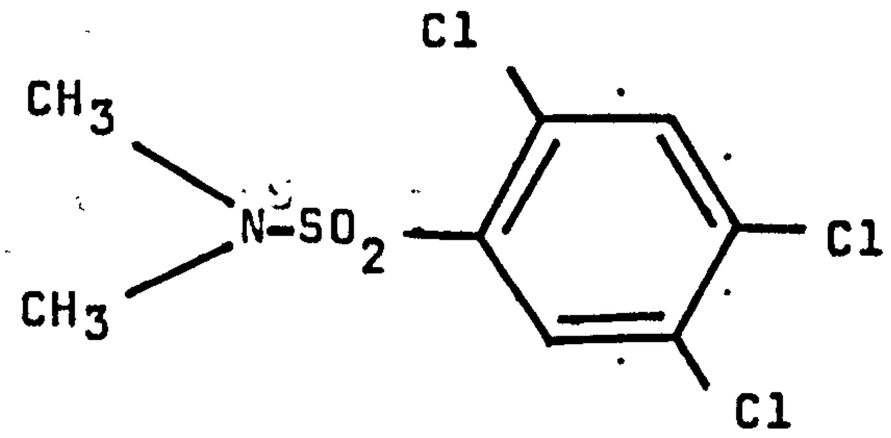
2,3-DIMETHYLQUINOLINE



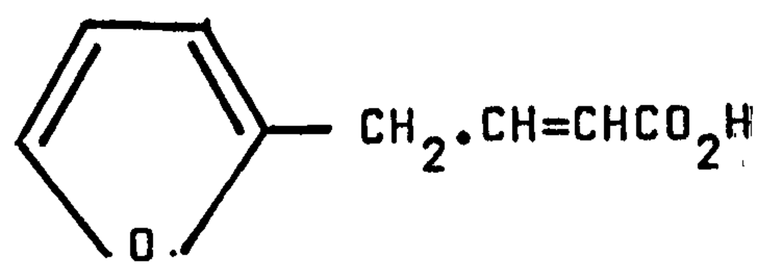
DI-ALLATE



METHYL DIMETHYLDITHIOCARBAMATE



N,N DIMETHYL-2,4,5-TRICHLORO-
BENZENE SULPHONAMIDE



2-FURFURYLACRYLIC ACID

7. MISCELLANEOUS ORGANIC COMPOUNDS

i) DICHLOBENIL

Dichlobenil (2,6-dichlorobenzonitrile) (see fig. 3.3.4.) is a well known soil acting and aquatic herbicide which has been used as a sprout suppressant (Sawyer, 1967; van Vliet and Hertog, 1966). It is still approved in the U.K. as a suitable chemical "to kill shoots on potato clamps" (Anonymous, 1976). It is a powerful inhibitor of actively dividing meristems (Martin, 1972) and would therefore presumably inhibit wound periderm formation as well.

ii) CHLORTHIAMID

Chlorthiamid (2,6-dichlorothiobenzamide) (see fig. 3.3.4.) is a herbicide introduced by Shell Research Ltd. in 1963 as a total herbicide. In suitable conditions it is slowly converted to dichlobenil (Martin, 1972). It is marketed as a 7.5% granular formulation called "Prefix" and receives a similar approval to dichlobenil. Neither should be used in the vicinity of seed potatoes. Their use is best restricted to destroying potatoes on old clamp sites and in dumps.

iii) 2,4-DINITROTOLUENE

2,4-dinitrotoluene produced sprout control comparable to that obtained by MENA in the experiments of Emilsson et al (1955) but no other references to its use as a suppressant were located during the course of this survey.

iv) QUINOLINES

2,4-dimethylquinoline, 2,3-dimethylquinoline (see fig. 3.3.4.), 2-chloroquinoline, 6-chloroquinoline, and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline have been shown to control potato sprouting on an experimental scale. Denny (1954) demonstrated that 0.5-1.0g of 2,3-dimethylquinoline per kg of tubers almost completely inhibited sprouting. This application rate would render commercial use of these materials impracticable.

v) DI - ALLATE

Di - allate (S - 2,3 - dichloroallyl NN - di - isopropylthiolcarbamate) (see fig.3.3.4.) is marketed as a herbicide for the control of blackgrass and wild oats. (Martin, 1972). It is relatively volatile and this is a major limitation in its use as a herbicide as it must be incorporated into the soil immediately after application. This property has been turned to advantage in the field of sprout suppression (Sawyer, 1967), although it is less effective than nonanol (Meigh, 1969). Being a mitotic inhibitor, however, might lead to problems with respect to wound healing.

vi) DITHIOCARBAMATES

Compounds of the general formula R (R)- NCSSR" where R and R' are H or alkyl groups and R" is Me, Et, Pr, Ph, etc. have been proposed as suppressants. (Czyzewski et al, 1952). The example of methyldimethyldithiocarbamate (see fig. 3.3.4.) is given but it would appear to be active only at relatively high concentrations.

vii) ARENESULPHONAMIDES

Arenesulphonamides with the general formula $ASO_2N(R)R'$ in which A is aryl (with or without halogen, alkyl, or nitro substitution), R is H or alkyl, and R' is alkyl, have been patented as suppressants. (Nultsch and Jumar, 1960). A 1 - 5% dust formulation of NN dimethyl - 2,4,5 - trichlorobenzene sulphonamide (see fig. 3.3.4.) is said to give complete control for 6 months at $15^{o}C$.

viii) FURFURYL ACRYLIC ACID

2 - furfuryl acrylic acid (see fig. 3.3.4.) has been shown to be an effective suppressant but has a powerful aroma which renders treated tubers unacceptable for ware use (Yabuta and Tamari, 1942).

ix) N - DIMETHYLAMINO - MALEAMIC ACID

N - dimethylamino - maleamic acid (see fig. 3.3.5.) is closely related to succinamic acid (see fig. 3.3.5.) a known growth regulating compound. Sprays of N - dimethylamino - maleamic acid have been found to retard the growth of a variety of crops. It is readily translocated, relatively non - phytotoxic and has a long residual action. Applied as a foliar spray at approximately 9kg ha^{-1} in 150dm^3 ha^{-1} it has been found to suppress potato sprout growth in subsequently harvested tubers. Unfortunately the very early application time used produced adverse effects upon yield. Potato tubers will sprout again after a period of suppression. (Riddell et al, 1962). This compound could be worthy of further investigation.

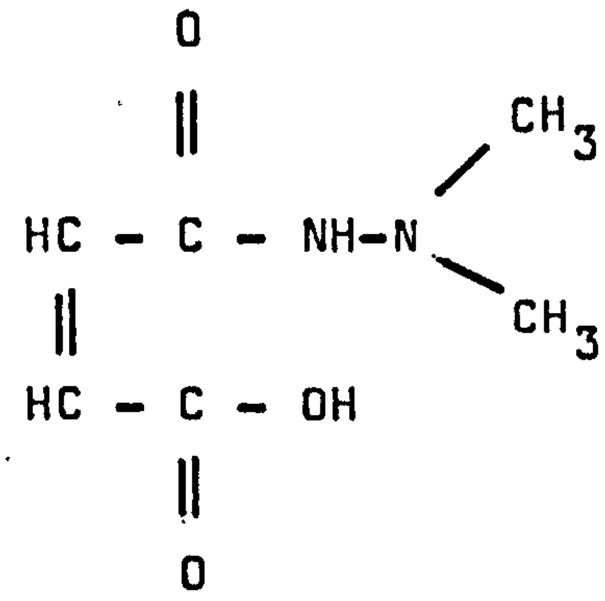
8. GENERAL CONCLUSIONS

Of the multitude of chemicals described above only nonanol and MENA can be said to have had any degree of commercial success and even they are no longer used. However the survey has shown that the criteria for a successful suppressant are numerous and varied. The major problems associated with the use of the sprout suppressants described in the preceeding pages are summarised below. (not necessarily in order of importance).

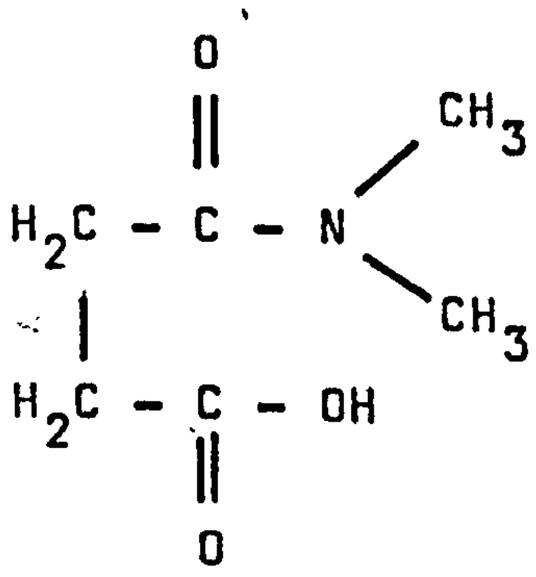
i) INEFFECTIVENESS

Obviously a good chemical sprout suppressant must suppress sprouting effectively. Many of the compounds discussed produced significantly lower levels of sprouting than untreated controls, but this does not imply adequate performance. An efficient suppressant must be able to completely suppress sprouting for the period desired (at least 2 months) at acceptable and economical application rates - usually at 10 - 100mg kg^{-1} if applied to the tubers and at 1 - 5kg ha^{-1} if applied as a pre-harvest treatment.

Figure 3.3.5. Sprout suppressant compounds



N DIMETHYLAMINO - MALEAMIC ACID



SUCCINAMIC ACID

ii) APPLICATION METHODS

Although a wide variety of application techniques have been used in experimental work, only a few are applicable to commercial situations. The application technique should produce reproducible results and cause minimum modification to normal cultural and storage practices.

a) post - harvest application

Dust applications are only really practical if they are carried out as the potatoes are being placed in store and hence are suitable for chemicals which demand a several week curing period although later dust applications have been proved technically possible. To produce even effects the chemical must be volatile.

Vapour applications generally involve specialized equipment. Burton has shown that continuous vapour applications are possible in commercial situations, but must by their nature involve more supervision and management.

Fog applications again involve specialised equipment but are more suitable for treatment of potatoes already in store and hence for the chemicals which inhibit wound healing.

Spray applications have not been successful because of the increased rotting produced by high volumes of water. This factor could now, of course, be overcome by the recently developed ultra-low volume "mist" applicators developed for use with fungicides.

b) pre-harvest application

Pre-harvest application can involve foliar sprays or soil application of a chemical either in granular or spray form. Generally however (as discussed later under "Maleic hydrazide")the effects of such applications can be variable and very much dependent upon climatic conditions. Early treatments can affect subsequent growth and yield. Late sprays can produce incomplete suppression. Haulm killing sprout

suppression treatments have been reported but not used in practical situations. All soil applications tried so far have been unsuccessful.

iii) UNDESIRABLE PHYSIOLOGICAL EFFECTS

a) sprout stimulation

Many of the compounds tested although providing adequate inhibition at high application rates appear to stimulate sprouting at low concentrations. This has proved a serious problem with auxin - related compounds, as would be predicted from their postulated mechanism of action. It should be noted that once applied the initial concentration decays due to volatilisation and metabolism and hence low concentrations will eventually prevail.

b) abnormalities

Various abnormalities in tuber anatomy have been noted after treatment with some suppressants, such as abnormal growth round the eyes (2,4-D), abnormal growths on the tuber surface (MENA)and increased cracking of tubers (2,4,5-T).

c) inhibition of wound healing

Inhibition of both periderm and suberin development has been observed with some compounds (2,4,5-T,MENA). This can lead to increased fungal and bacterial infection.

d) high phytotoxicity

Some compounds used as suppressants have shown a high toxicity to potato tissue causing necrosis of the more delicate areas. Although attempts have been made to minimise this by judicious choice of application rates and methods it has still proved a major problem. If the young buds are killed outright and decay follows then this provides access to micro-organisms andincreases rotting (e.g. nonanol). Necrosis at lenticels has also been observed with similar results. A good sprout suppressant should therefore suppress the growth of young buds without killing them.

iv) EFFECTS ON PROCESSING CHARACTERISTICS

Although somewhat controversial the effects of the sprout suppressants described above on such parameters as sugar levels appears minimal. Information of this nature however, was limited to the more successful compounds.

v) EFFECTS ON THE SUBSEQUENT GROWTH OF SEED

None of the chemicals above have been proven suitable for use on seed potatoes. Most in fact are unsuitable. It has proved difficult if not impossible to find concentrations which will produce adequate suppression in storage and yet allow seed to germinate normally on planting.

vi) RESIDUES AND TOXICOLOGY

Many of the investigations described above were carried out in a period when residue analysis techniques were in their infancy and toxicological legislation less demanding than it is now, so the information available is limited. Nevertheless some general points are apparent. Concentrations of suppressants used are almost invariably high - of the order of 10 - 300mg kg^{-1} . In most cases a proportion of the active chemical actually penetrates the tuber although the depth to which it penetrates varies. This implies that if a suppressant is used it must inevitably be consumed if the potatoes are eaten. Peeling reduces residue levels. Application methods depending upon translocation from the foliage probably result in higher residue levels in the consumable parts of the tuber. For these reasons and because of the importance of the potato in the human diet in temperate regions sprout suppressant chemicals should be non-toxic and easily metabolised. Chemicals used must also be free of toxic impurities. (see 2,4,5-T).

Levels of residues which have been reported for the above compounds indicate that the use of sprout suppressants results in residues of the order of 0.1 - 10mg kg^{-1}.

It was also noted that some compounds (e.g. alkyl halides) have proved toxic to those applying the chemical.

vii) OTHER UNDESIRABLE PROPERTIES

a) inflammability

Highly inflammable compounds create hazards for those applying the chemical and cause a fire risk. Many of the chemicals suggested as suppressants have low flash points. Also worthy of note is the fact that solutions of the chemical suggested for "fogging" have often been in inflammable solvents.

b) high volatility

Although volatility is desirable in post-harvest applied chemicals, very high volatility can lead to excessive ventilation losses and render a compound uneconomic.

3.3.2. SPROUT SUPPRESSANTS IN CURRENT COMMERCIAL USE

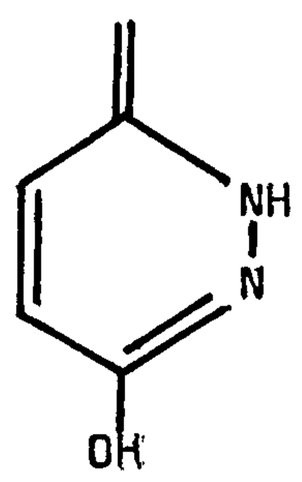
Although the quantity of sprout suppressants used is increasing, the variety of chemicals available is decreasing. There are at present only 4 chemicals which are widely used. These are, maleic hydrazide, chlorpropham, propham and tecnazene (see fig. 3.3.6.).

Maleic hydrazide is applied as a pre-harvest foliar spray and is widely used in the U.S.A., but not in Europe.

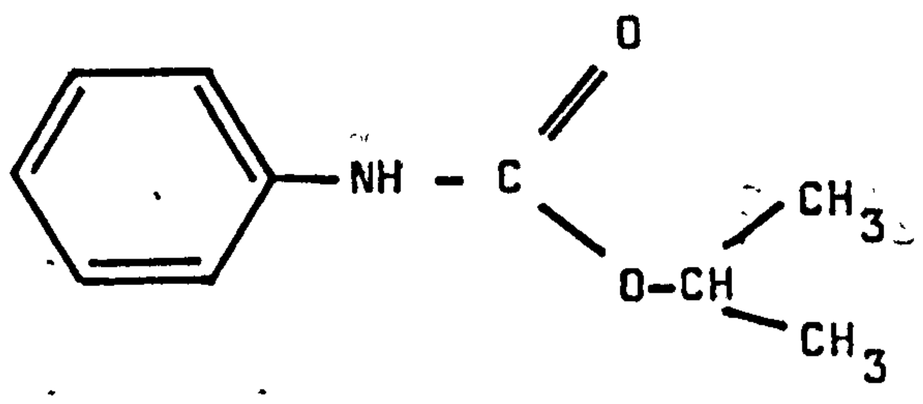
Chlorpropham and propham are closely related compounds widely used in the major European potato growing areas and, the U.S.A.

Tecnazene is the only commercially available sprout suppressant that may be used with any degree of confidence on seed potatoes. It is therefore widely used in Scotland. These compounds are more fully discussed below.

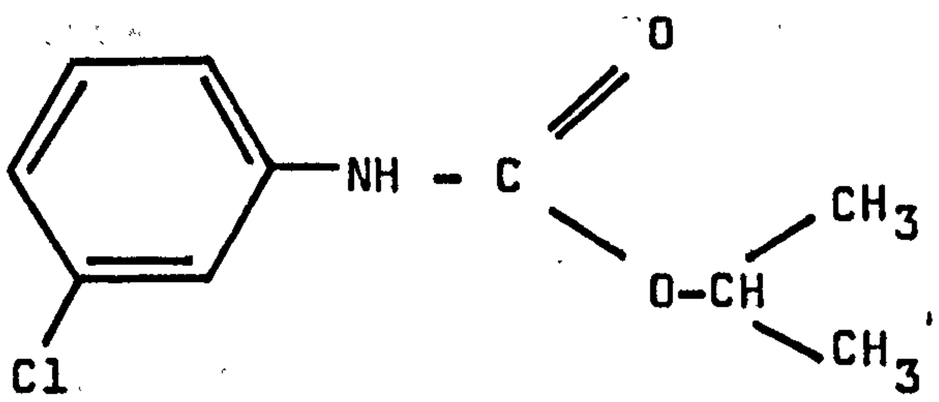
Figure 3.3.6. Currently used sprout suppressant chemicals



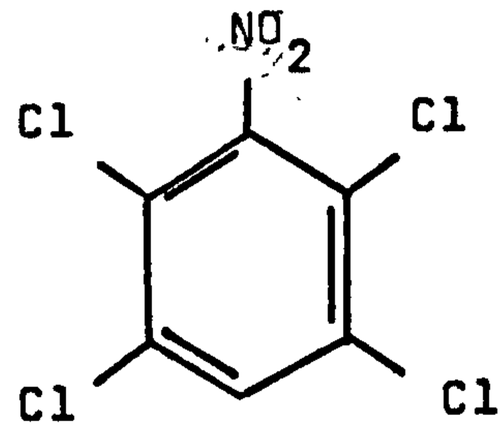
MALEIC HYDRAZIDE



PROPHAM



CHLORPROPHAM



TECNAZENE

3.3.2.1. MALEIC HYDRAZIDE

a) GENERAL

Maleic hydrazide is the trivial name for 6 - hydroxy - 3 - (2H) - pyridazinone. It is sometimes referred to by the initials MH. Its growth regulant properties were first described by Schoene and Hoffmann, (1949). It is a white non-volatile crystalline powder of mp.296^{o}C. Its solubility at 25^{o}C is 0.6% w/w in water, 0.1% w/w in ethanol. Maleic hydrazide behaves as a mono-basic acid forming water soluble alkali metal and amine salts. The diethanolamine salt is very soluble (70% w/w) in water and is the most common formulation of maleic hydrazide, although the sodium salt is also used (Amatsu and Karazaua, 1956).

It is translocated in plants and inhibits cell division but not extension and is used for the retardation of the growth of grass, hedges and trees, for the prevention of sucker development in tobacco and as a potato sprout suppressant (Martin, 1972).

Early experiments showed that maleic hydrazide was ineffective as a sprout suppressant when used as a dip, but when tooth picks soaked in a 2500μg cm^{-3} solution were inserted into tubers inhibition was observed (Marshall and Smith, 1951).

Foliar application has been widely investigated although it should be noted that almost all of these experiments were conducted in the U.S.A. The importance of correct timing of application was demonstrated by Kennedy and Smith (1951 and 1953) who obtained satisfactory sprout control using a 1000μg/cm^{-3} solution at 1300 dm^{3}ha^{-1} (1.7 kg ha^{-1}), applied 6 weeks before harvest. Earlier applications caused injury to foliage, produced many small unmarketable tubers, and reduced yields. Higher application rates increased the effectiveness of the treatment but also increased the undesirable side-effects. Later applications on senescing foliage have been shown to be ineffective (Paterson et al, 1952; Rao and Wittwer, 1955). Similar results have been reported by numerous other researchers, and these have been

reviewed by Perlasca (1956). Various workers have suggested that the ideal application time is best judged by the stage of flowering and "blossom fall" application is generally accepted as most satisfactory. At this stage tuber set is complete and the foliage shows no signs of senescence (Sawyer and Dallyn, 1958).

b) Abnormalities

Tuber abnormalities have been noted after use of high rates of maleic hydrazide. Tuber skins may become rutted and furrowed and in certain cases growth cracks have been observed after application (Poapst and Genier, 1970). This problem is only of commercial significance when the spray is badly applied and some plants receive duplicate applications, as the normal commercial dosages 1.5 - 3 kg ha^{-1} are below the level required to produce such abnormalities.

c) Processing characteristics

There is apparent disagreement over the effect of maleic hydrazide on the sugar content of tubers and ether important processing parameters. Kennedy and Smith (1953) observed no effect on specific gravity, after peeling blackening, reducing sugar content and crisp colour. Zaehringer et al (1966) found no effect on sucrose accumulation. Gooding and Hubbard (1956) found no effect on sucrose or reducing sugar levels. Other workers noted lower reducing sugar levels in treated tubers (Paterson et al, 1952; Salunkle et al, 1953). Some of these apparent discrepancies have been explained by more complex theories (Payne and Fults, 1955; Moll, 1973), which maintain that in general, early and optimally treated tubers show lower reducing sugar contents at harvest than do controls, and late treated tubers show increased reducing sugar content. Moll (1973) also maintains that in general maleic hydrazide prevents sucrose accumulation. These effects would appear to be cultivar dependent and long term storage fluctuations are variable. It is however agreed that the effect of maleic hydrazide on sugar levels is small enough to be of little commercial significanee.

d) Residues and uptake

Chemical residue determination methods for maleic hydrazide are somewhat cumbersome, involving reduction and hydrolysis, distillation of the resultant hydrazine which is then estimated colorimetrically (Lane, 1965) or preparation of the trimethylsilyl derivative which is determined by gas-chromatography (Haeberer et al, 1974). Despite this fact several workers have conducted detailed investigations into the levels of maleic hydrazide absorbed by the potato and hence the levels required to produce physiological effects.

Franklin and Longhead (1964) using a foliar spray of 2500 $\mu g\ cm^{-3}$, 3 weeks past full bloom, showed that sufficient uptake and translocation had occured in 24 hours to produce a tuber residue of 6 $mg\ kg^{-1}$ which was enough to adequately inhibit sprouting during 6 months storage at $10^{o}C$. After 48 hours the level was 17 $mg\ kg^{-1}$ rising to a maximum of 36 $mg\ kg^{-1}$ after 7 days. 2 - 4 hours after treatment the tubers contained 4 $mg\ kg^{-1}$ which in fact stimulated sprouting. Approximately 60 hours were required for 50% uptake of the applied chemical. It has been shown however that the rate of uptake is dependant on a variety of environmental and other factors. Humidity in particular appears to have a dramatic effect, 50% absorption requiring 128 hours at low humidity and only 2 hours at high humidity (Crafts, 1961). Poapst and Genier (1970) showed that levels $>$ 50 $mg\ kg^{-1}$ were required to produce the tuber abnormalities discussed above.

e) Mode of action

The precise mode of action of maleic hydrazide has not been thoroughly elucidated although many of its biochemical and physiological effects have been reported. Most of this research has been carried out on plants other than the potato.

Cytological studies showed cessation of mitosis of the broad-bean (Vicia fabae) with breakage of chromosomes. A concentration of 0.0005 M will stop mitosis and concentrations of 0.0001 M and greater will produce chromosome breakage

(Darlington and McLeish, 1951). It is worth noting that the lower level would be approximately equivalent to 10 mg kg^{-1} in a potato tuber, which is of the same order as that observed in practice to produce substantial sprout suppression. It should be noted however that such breakages were not observed in all plant species tested at these concentrations.

Further studies have however failed to pin-point the precise site of action but it is known that maleic hydrazide does not appear to act as a base analogue or as a sulphydryl inhibitor. Other effects have been reviewed by Crafts (1961) and Ashton and Crafts (1973), who indicate that maleic hydrazide is generally accepted to inhibit cell division but not cell elongation.

f) Seed

Maleic hydrazide destroys apical dominance of both tubers and individual sprouts, and although treated tubers develop a large number of active eyes, true sprouts do not develop. Maleic hydrazide cannot therefore be used as a sprout suppressant on crops intended for seed (Paterson et al, 1952; Kennedy and Smith, 1953). Rakitin (1973) reported similar findings but noted that the effects of maleic hydrazide could be overcome with gibberellins, thiourea, and other sprouting stimulants.

g) Toxicology

Darlington and McLeish (1951) concluded their paper on the cytological effects of maleic hydrazide in plants with the statement:

"Since nearly all chromosome - breaking agents have so far proved to be cancer - producing as well, we hope that the agricultural use of this agent will not be encouraged before suitable tests are made."

This statement stimulated research into the toxicology of maleic hydrazide and various workers produced conflicting results over which controversy still rages.

Barnes in a paper with 8 others (1957), including some leading names in the field of toxicology, reported that

after extensive testing they found that the oral LD_{50} value for rats was 6950 mg kg^{-1} for the sodium salt and 2340 mg kg^{-1} for the diethanolamine salt; that maleic hydrazide was non-irritant and no toxic effect was observed in two year feeding tests on rats with a diet containing 5% of the sodium salt; that it is non-carcinogenic, and that it has no effect on mitosis in isolated mammalian tissue even at a concentration of 0.001 M. They did in fact observe tumours in early experiments but failed to reproduce them in somewhat modified subsequent tests, and concluded that they were due to natural causes.

The U.S. Government subsequently established a tolerance of 50 mg kg^{-1} for residues of maleic hydrazide in potatoes and 150 mg kg^{-1} in potato crisps. (Anon, 1960a)

Maleic hydrazide was subsequently shown to be mutagenic to Bacillus megatherium (Northrop, 1963) and to Drosophila melanogaster (Nasrat, 1965).

In 1965 Dickens and Jones (1965) reported that maleic hydrazide, when injected in arachis oil into rats at doses of 2 mg / week, produced tumours in 3 of the test animals after 84, 95 and 105 weeks. They used free maleic hydrazide unlike Barnes et al who used the sodium and diethanolamine salts but conclude that this is unlikely to explain the difference in their findings.

Epstein et al (1967) using intraperitoneal injections of the free acid produced a highly significant increase in the incidence of hepatomas (liver tumours). In a paper in which they are very critical of the conclusions of Barnes et al (1957), Epstein et al conclude that maleic hydrazide is a weak carcinogen and suggest that tolerances of residues should be reduced or it should be banned because of its widespread use.

However the technique used in these experiments has been questioned. Grasso and Goldberg (1966) maintain that repeated subcutaneous injections can produce sarcoma at the point of injection not necessarily due to the chemical under test. The experiments of both Dickens and Jones (1965) and Epstein et al (1967) have been criticised in that they bear

little relation to the ways in which the human body comes into contact with maleic hydrazide i.e. through ingesting treated potatoes or through skin contamination during application.(Barnes, 1976) In this article Barnes maintains that his own methods (see Barnes et al, 1957) are the more valid estimate of toxic hazards.

Recent Russian research has shown that some toxic effects on the liver, blood and nervous system can be produced with low levels of maleic hydrazide ingestion (1.5mg of the diethanolamine salt per kg of body weight) (Mukhorina, 1973). It was noted in these experiments that much higher doses of the sodium salt (7mg kg^{-1}) failed to produce any effect.

It should be noted that maleic hydrazide does not receive approval for the suppression of potato sprouting in the UK although it is approved for similar use on onions. (Anon., 1976)

h) Summary

Maleic hydrazide is unique amongst the commercially used sprout suppressants in that it is the only one which can be applied, indeed must be applied via the foliage. Inherent in this observation are its principal advantages and disadvantages.

Maleic hydrazide is undoubtedly an extremely efficient chemical which may be applied using standard spraying equipment - it may even be mixed with other chemicals (Sawyer 1967). It penetrates the leaf readily and translocates well to the desired site of action. This however introduces another field operation into a potato growing program, an operation which requires reliable weather conditions for predictable results in a season which is notoriously unreliable.

Its use also demands that marketing decisions must be made before harvest, and once applied the resultant treated material is of no value as seed. Often decisions on whether to retain the seed fraction for planting are not made until well through the storage season when price levels and availability are determined.

The fact that maleic hydrazide is evenly distributed

throughout the tuber has toxicological implications. Other suppressants are generally concentrated in the outer layers of the tubers and the bulk of the residue is removed during preparation. Levels of maleic hydrazide ingested by humans are consequently much higher - an observation all the more pertinent in view of toxicological studies.

Despite these obvious disadvantages it was considered during the early stages of the work described in this thesis that it would be of value to investigate the possibilities of using maleic hydrazide under Scottish conditions.

3.3.2.2. AN INVESTIGATION INTO THE USE OF MALEIC HYDRAZIDE UNDER SCOTTISH CONDITIONS

Introduction

The experiment described below was designed to investigate the use of maleic hydrazide as a sprout suppressant under typical Scottish conditions. The factors which were considered to be of prime importance in this experiment were:

1) Optimum application rate
2) Optimum timing of application (see below)
3) Effect on yield and size distribution within the total yield
4) The effectiveness of maleic hydrazide in suppressing sprouting during long term storage.

Experimental

a) Materials

Certified seed potatoes of the cultivars Maris Piper and Golden Wonder were purchased locally. The seed was not pre-sprouted.

As the diethanolamine salt of maleic hydrazide has consistently been shown to give better performance in the field than the sodium salt this formulation was used (Smith et al, 1959). A 200g dm^{-3} solution of this salt was prepared by dissolving equimolar quantities of maleic hydrazide and diethanolamine in deionised water. For field application this solution was dispensed into the sprayer as required.

b) Planting and Husbandry

The experimental plot was situated at the Plant Pathology Dept., West of Scotland Agricultural College, Auchincruive, near Ayr, and was approximately 0.15ha of sandy loam soil. The plot had received a heavy application of FYM and was uniformly treated with 1200kg ha^{-1} of compound potato fertilizer immediately before planting. Tubers were planted by hand on 22 April 1974 at 300mm spacing in drills 700mm apart. Each plot consisted of 3 drills each 4m long. The experimental area was proected by two guard drills.

Weed control was achieved by applying 3kg ha^{-1} linuron and 2kg ha^{-1} paraquat at 10% emergence using a 4 nozzle knapsack sprayer fitted with a pressure gauge to ensure even application. Blight was

prevented by spraying with captafol at 14 day intervals during August.

c) Experimental design

The experimental design consisted of 4 independently randomised blocks each containing all 16 treatments. The treatments consisted of 2 cultivars x 4 maleic hydrazide application rates (including control) x 2 application times in all possible combinations. However, due to adverse weather conditions during August one of the timings had to be abandoned i.e. the two application times were identical giving 8 replicates for each remaining treatment.

d) Maleic hydrazide application

It was planned to apply maleic hydrazide to one set of plots in mid-August and to the other 2 weeks later. As the weather prevented the first treatment maleic hydrazide was applied to all plots on 2 September. The applications were chosen after consulting the literature and were 0.0, 0.625, 1.56 and 3.13kg ha^{-1} applied in 625dm^3 ha^{-1} of water i.e. the spray concentrations were 0.0, 1.0, 2.5 and 5.0mg cm^{-3}. All figures apply to maleic hydrazide and not to the diethanolamine salt. Application was made using the knapsack sprayer described above.

Unfortunately there was some precipitation within 24h of application but as the highest dose rate produced visual symptoms within a few days it must be assumed that a substantial proportion of the dose had been taken up by the foliage.

e) Harvesting, grading and storage

The crop was harvested on 4 October. The yield from each plot (3 drills x 4m long) was bulked and transported to the laboratory where it was graded and the yields of large (>42mm) and small(<42mm) tubers were recorded. After approximately 2 weeks storage at 15-20 C, 10kg of the small fraction from each plot were boxed and stored at 10^{o}C in a constant temperature room for 9 months.

The effect of the various treatments on the amount of sprouting and on relative weight loss was then assessed by examining a sample of 20 tubers.

Results

The mean plot yield and size distribution figures were calculated from the 8 replicate treatments and these are reported in table 3.3.1. Similarly the figures for sprouting and weight loss after 9 months storage were calculated and are reported in table 3.3.2.

The data from all seven observed and calculated variables (i.e. yield of tubers >42mm, yield of tubers <42mm, total yield, % of tubers > 42mm in the total yield, % by weight of sprouts in a 20 tuber sample, number of sprouts per tuber, and the weight of a twenty tuber sample with sprouts removed) were then subjected to analyses of variance, and from the resultant mean squares, F statistics were calculated for all sources of variation including block number and all possible interactions. As block number was shown to have a non-significant effect, this factor and its interactions were dropped from the analyses and the mean squares figures for these sources of variation added to the residual mean squares figures and F-statistics recalculated. This provides a more severe test as by including too many factors in an analysis of variance, the residual mean squares figure can become so small that non-significant factors may in fact be shown to be significant. The final F-statistics are shown in table 3.3.3.

Discussion

The difficulties experienced in the application of maleic hydrazide sprays demonstrates the first major problem in its use under Scottish conditions. The weather during August 1974 was by no means unusual for Scotland and similar problems would have been experienced, for example, had the experiment been conducted during 1978. It is almost impossible to guarantee a dry period even within plus or minus one week of the optimum application period. As this factor is critical (Kennedy and Smith, 1951; 1953) this is likely in itself to prevent the use of maleic hydrazide under Scottish conditions, unless uptake can be accelerated by the use of additives such as surfactants and humectants so that a smaller weather "window" is required.

As can be seen from tables 3.3.1. and 3.3.3. the application of maleic hydrazide had no significant effect on yield or size

Table 3.3.1. The effect of pre-harvest applications of maleic hydrazide on yield and size distribution (means of 8 replicate plots)

Cultivar	Application rate (kg ha^{-1})	Yield of large tubers* (kg)	Yield of small tubers** (kg)	Total yield (kg)	% large tubers
Maris Piper	0.0	30.3	11.1	41.4	73.2
	0.625	30.0	8.7	38.7	77.5
	1.56	30.8	8.1	38.9	79.1
	3.16	31.9	10.0	41.9	76.1
Golden Wonder	0.0	16.5	8.3	24.8	66.5
	0.625	17.3	8.1	25.4	68.1
	1.56	15.8	8.5	24.3	65.0
	3.16	16.2	7.8	24.0	67.4

* Yield of tubers > 42mm ** Yield of tubers < 42mm

Table 3.3.2. The effect of pre-harvest applications of maleic hydrazide on sprouting and weight loss during subsequent storage (mean of 8 replicates)

Cultivar	Application rate	% Weight of sprouts in 20 tuber sample	Number of sprouts per tuber	Weight of 20* tuber sample (kg)
Maris Piper	0.0	16.8	4.15	1.07
	0.625	14.7	4.36	1.09
	1.56	12.3	4.83	1.10
	3.13	11.0	5.49	1.22
Golden Wonder	0.0	19.2	1.86	0.66
	0.625	11.5	4.02	1.11
	1.56	9.2	3.72	1.12
	3.13	8.7	4.33	1.25

* Weight of 20 tuber sample with sprouts removed

Table 3.3.3. F statistics from analyses of variance on the effects of pre-harvest applications of maleic hydrazide on yield, size distribution and sprouting.

Variable	F statistic from each source of variation		
	Cultivar	Application rate	Interaction
Yield of large tubers (>42mm)	99.0**	0.58	0.58
Yield of small tubers (<42mm)	3.97	2.03	2.29
Total yield	91.4**	1.06	1.66
% Large tubers	48.2**	1.22	0.82
% Weight of sprouts in 20 tuber sample	6.30*	9.55**	3.10*
Number of sprouts per tuber	11.3**	6.8**	9.71**
Weight of 20 tuber sample (no sprouts)	0.77	8.43**	5.62**
Degrees of freedom [a]	1	3	3

a Total degrees of freedom = 63, within replicates = 56

* Significant at the 5% level, F(1,56) = 4.00, F(3,56) = 2.76

** Significant at the 1% level, F(1,56) = 7.08, F(3,56) = 4.13

distribution. Almost all of the experimental variation can be accounted for by the single factor of cultivar. As visual symptoms of the highest application rate (3.13kg ha^{-1}) were apparent in the form of yellowing and premature senescence it can only be assumed that tuber formation was well advanced by the application date. Whilst grading the crop no increase in tuber abnormalities was observed even at the higher application rate where they might have been expected (Poapst and Genier, 1970).

During storage it was observed that maleic hydrazide treated samples sprouted later than did the controls but by the end of the very long storage period, differences in sprout growth were diminishing. Nevertheless as can be seen from tables 3.3.2. and 3.3.3. the weight of sprouts was significantly lower in the treated samples. The effect on the cultivar Golden Wonder was marginally greater than on Maris Piper, a fact substantiated by the significant interactive term in the analysis of variance results. As might be expected cultivar oo its own had a significant effect on the weight of sprouts in the sample. However, for commercial purposes the degree of sprout control after 9 months storage was totally inadequate, even when maleic hydrazide was applied at the highest rate.

Treatment had also a significant effect on the number of sprouts per tuber - especially with the cultivar Golden Wonder. Analysis of variance on this variable indicates that cultivar, treatment and interactive effects were all highly significant. These findings are in agreement with those of Paterson et al (1952) and Kennedy and Smith (1953)..

Applications of maleic hydrazide also considerably reduced weight loss - especially in the case of Golden Wonder. Both treatment and interactive terms are highly significant although the main effect of cultivar is not.

Conclusion

The experiment demonstrated that the application of maleic hydrazide in Scotland is difficult because of climatic conditions. The effects of maleic hydrazide on sprout control and weight loss were inadequate to permit long term storage, although observations indicated that sprouting was retarded and this might allow its use

for medium term storage. It is unfortunate that an earlier application was not possible as it is likely on the basis of results from this experiment and from the literature that mid-August is probably the optimum time to apply this chemical under Scottish conditions so that the maximum degree of sprout control is obtained with no effect on yield. As the highest application rate used visually damaged foliage the optimum application rate probably lies in the range 1.5-2.5kg ha^{-1}.

3.3.2.3. CHLORPROPHAM

a) General

Chlorpropham is the name approved by the International Urganisation for Standardisation (ISO) and the British Standard Institution (BSI) for isopropyl-N-(3 chlorophenyl) carbamate (see fig. 3.6) also known as m-chlorocarbanilate. The Weed Science Society of America (WSSA) have in the past used the initials CIPC to describe this chemical, and it is by this name it is most commonly known in the potato industry. During the course of this thesis it will be referred to as chlorpropham.

It is synthesised by the reaction of m-chloroaniline with isopropyl chloroformate or of isopropanol with m-chlorophenyl isocyanate. Its solubility in water is 89mg dm^{-3} at $25^{o}C$, it is moderately soluble in petroleum oils, and soluble in lower alcohols aromatic hydrocarbons and most organic solvents.(Martin, 1972) Chlorpropham melts at $41^{o}C$ and is stable below $100^{o}C$ - its stability above this point is somewhat dependent upon conditions (see later). It is slowly hydrolysed in acid or alkali (Kearney and Kaufman, 1969).

Chlorpropham is a mitotic poison and was commercially introduced as a herbicide in 1951 (Witman and Newton, 1951). Marth and Schultz (1950) first reported its use experimentally as a potato sprout inhibitor in 1950, after experiments using a 1% dust. There are now a wide variety of commercial formulations available in the U.K., the most common being fogable liquids (eg. B.L.500 (Wheatley Chemical Co.), Mirvale 50AC (Ciba-Geigy), Taterpex 80 (Mirfield Agricultural Chemicals), and granules (eg. Bygran (Wheatley Chemical Co.). It is normally applied at rates of 10-30g of active chemical per 1000kg of potatoes although many processors use higher rates. It is now the most widely used sprout suppressant in Europe, U.S.A., Australia and many other countries.

Some of the advantages of chlorpropham as a sprout suppressant were obvious from the initial experiments of Marth and Schultz (1950,1952). Chlorpropham was found to be a "very

potent sprout inhibitor," indeed it is the most potent of the chemicals commercially available at present (Van Vliet and Sparenberg, 1970; Meigh et al, 1973). Only 10-20mg kg^{-1} of potatoes or 10-20µg dm^{-3} in air is required to completely suppress the sprouting of potatoes.

Marth and Schultz also noted its high volatility and suggest this fact might be useful in reducing residues after treatment, although rendering it useless for foliar application. This volatility was later exploited by Sawyer and Dallyn (1956) who applied chlorpropham successfully as a vapour. This is now the most common application method and involves the atomisation of a solution of chlorpropham in a non-inflammable solvent (eg. dichloromethane) by means of special equipment (eg. "Swing fog") and the distribution of the fog produced using a recirculating ventilating system (where available) to ensure even application. The active chemical condenses on the surface of the potato where it slowly volatilises to maintain an inhibitory concentration of chlorpropham in the atmosphere. (Van Vliet and Sparenberg, 1970) Depending on the dosage rate, ventilation regime and storage temperature, a single application will inhibit sprouting from 3-12 weeks (Van Vliet and Schriemer, 1963).

Other application methods dependent on the volatility of chlorpropham have been suggested. Van Vliet and Sparenberg (1970) soaked filter paper in a chlorpropham solution, packed the filter papers into open ended plastic cartridges, and placed these in the ventilating ducts of the store. This approach reduced residue levels in tubers yet maintained adequate suppression. Mc Connell (1962) showed that sprouting could be inhibited by storing potatoes in chlorpropham impregnated containers. He showed that 0.01% chlorpropham in paper sacks will retard sprouting. Danielson (1959) reported on the rate of release of chlorpropham from granular carriers.

b) Wound healing

Several problems have been encountered in the use of chlorpropham. It is known to inhibit the natural wound healing process which takes place after tuber damage, and so allows access

to pathogenic organisms, with consequent disease losses. Craft and Audia (1959) reported that chlorpropham had little effect on suberin development, but reduced wound periderm development. They also noted that it reduced the accumulation of o-dihydroxy-phenols in response to wounding, which might partially account for the increased susceptibility to pathogenic organisms (Kosuge, 1969).

In later experiments (Audia et al, 1962) it was shown that in freshly cut tuber slices dipped in various concentrations of chlorpropham, that periderm formation was retarded by 25µg cm^{-3} and completely inhibited by 50µg cm^{-3} . 1250µg cm^{-3} were required to inhibit suberin formation. These observations were correlated with susceptability to Erwinia carotovara infection. Reeve et al (1963) showed complete inhibition of wound periderm formation on whole tubers in the presence of only 1mg kg^{-1} based on tuber weight. They noted only slight inhibition of sprouting, and no inhibition of suberisation at this concentration. They also noted that chlorpropham apparently inhibits Fusarium solani inoculates.

The practical implications of these observations are extremely important. Adequate time (2-3 weeks) must elapse after harvest to allow wound healing to take place, before chlorpropham may be applied. It is fortunate therefore that chlorpropham is so easily applied as a vapour to a filled potato store.

c) Internal sprouting

Chlorpropham is also thought to encourage internal sprouting, although this point was the subject of much controversy during the early 1960 s.

Sawyer (1961) and Sawyer and Dallyn (1964) maintained that environmental factors had more influence on the occurence of this physiological phenomenon than does chlorpropham. Others have shown an increase in the incidence of internal sprouting when chlorpropham is used at levels sufficient to only partially retard normal sprouting (Hruschka et al, 1965; Ewing et al, 1968). These later workers also noted that higher rates of application eliminated the problem.

d) Processing characteristics

The effects of sprout suppressants on reducing and non-reducing sugar levels in stored potatoes are complex and will be further discussed in Chapter 6. Chlorpropham is no exception and, apparently conflicting reports on its effects have been published, although some consensus is beginning to appear.

Zaehringer et al (1966) found chlorpropham had no effect upon sugar levels. Baijal and Van Vliet (1966) reported that chlorpham slightly reduced sugar accumulation on storage but had no effect upon the distribution pattern. Schippers (1975) showed that chlorpropham treated tubers had not significantly different sugar levels from maleic hydrazide treated batches. He did not however include an untreated sample in his otherwise elaborate experiments Van Vliet and Schriemer (1963) found that the levels of both hexose and sucrose were higher after 6 months storage at $10^{o}C$ of tubers (CV. Bintje) treated with chlorpropham compound with an untreated control. Isherwood and Burton (1975) in experiments at $20^{o}C$ (CV. Home Guard) found increased sucrose levels but no increase in glucose or fructose levels, but also noted similar findings with hand-desprouted samples. Moll (1968) suggests that the effects of chlorpropham are temperature dependent. At $7^{o}C$ and $12^{o}C$ he found decreased monosaccharide and sucrose levels but at $20^{o}C$ noted sucrose accumulation was stimulated by chlorpropham.

The effects of chlorpropham on sugar levels are obviously complex and require further investigation. There does however appear to be agreement in the more recently published work that chlorpropham stimulates sucrose accumulations during the period when normal senescent sweetening might be expected to occur. Perhaps some quantification of the concept of "physiological age" of the tuber might lead to better understanding of these effects.

e) Residues and uptake

Quantitative estimation of chlorpropham residues has presented problems because of its chemical and physical properties, and so levels which have been published should be interpreted with caution.

The properties of chlorpropham which have presented most problems are firstly its high resistance to acid and base hydrolysis (Kearney and Kaufman, 1969) and its high volatility (Newman and Downing, 1958). The latter factor can cause problems during concentration stages, especially under vacuum.

Gard and Rudd (1953) described an analytical method which involved extraction with dichloromethane, concentration, hydrolysis with 1:1 sulphuric acid, steam distillation and measurement of the resultant 3-chloroaniline colourimetrically using a hypochlorite-phenol method which produces a blue colour absorbing at 645nm. The lower limit of detection was 0.05mg kg^{-1} and the recovery 89%. They experienced problems with the hydrolysis step and the method was insufficiently sensitive to determine any residue due to field application of chlorpropham as a herbicide (Gard and Reynolds,1957). The introduction of a celite clean-up procedure however improved the method (Gard et al,1959). Using these techniques the residue of chlorpropham resulting from treatment of potato tubers for sprout suppression was 0.4mg kg^{-1}. Peeling reduced this residue and crisps prepared from these tubers gave residues of less than 0.05mg kg^{-1} (Gard,1959). Various other methods based on similar principles have been reviewed by Van Vliet and Hertog (1966).

More recent analysis methods tend to avoid the hydrolysis step and hence involve determination of the intact molecule.

Fergusson et al (1963) reported an infra-red analysis using the 1110 and1210cm^{-1} absorption peaks of chlorpropham.

Vogel and Deshusses (1965) used gas chromatography (GC) for the final determination after an initial "dry" extraction method involving masceration of potato tissue with anhydrous sodium sulphate and extraction of the resultant powder with chloroform in a Soxhlet unit.

It should be noted that gas chromatography of chlorpropham is not without its difficulties. Degradation can occur at high temperatures (230^{o}C)(Romagnoli and Bailey,1966). Such problems can be overcome by the preparation of trimethylsilyl derivatives, but this involves an extra step (Fischbein and

Zielinski,1965).

Van Vliet and Hertog (1966) described a method involving steam distillation as an extraction technique but preferred extraction with dichloromethane: both involved final determination by gas chromatography. The lower limit of detection was 0.3mg kg^{-1}. Using these techniques they discovered that no residues of chlorpropham were present below a depth of about 8mm from the tuber surface. Using the latter method Van Vliet and Sparenberg (1970) estimated that tubers treated with 10mg kg^{-1} of propham and chlorpropham in a 1% dust formulation, contained combined residues approximately one month after treatment of 3.6mg kg^{-1} in unpeeled tubers and 1.7mg kg^{-1} in peeled tubers. These levels fell during subsequent storage for 5-6 months to 0.7 and 0.3mg kg^{-1} respectively. Similar values were obtained for "fog" applications. They suggest that residue levels can be lowered by utilizing the cartridge application method described above.

Sensitivity of gas chromatographic methods can normally be improved by incorporating a clean-up procedure prior to injection. Cerny and Blumenthal (1972) reported such a clean-up on alumina of a hexane extract, which increased the sensitivity when using a thermionic detector to 0.02mg kg^{-1}. Similarly Onley and Yip (1971) reported that clean-up of an ethanol extract on magnesium oxide produced an extract suitable for chromatography on thermionic, electron capture and flame photometric detectors with a detection limit below 0.01mg kg^{-1}.

Using carbon-14 labelled chlorpropham (isopropyl(1,$3^{-14}C$)-N-(3-chlorophenyl) carbamate) Steinbeiss et al (1972) found that after "fog" treatment with 10mg kg^{-1} the residue after 5 months storage was 0.55mg kg^{-1} in peeled tubers. They also noted that peel residues appear to be unrelated to ventilation intensity and unexpectedly residues in peeled tubers increased with ventilation intensity.

With ^{36}Cl labelled chlorpropham Jumar et al (1968) found that treatment at commercial rates gave residues of 0.2mg kg^{-1} in cooked peeled potatoes.

Absorption studies in other plant species indicate that (true) seeds absorb substantial quantities of chlorpropham when

applied as a vapour. Neither killing the seeds with methyl bromide or the presence of enzyme inhibitors affected the absorption so it was concluded that the absorption was a physical accumulation process (Ashton and Crafts, 1973).

Translocation studies (Prendeville et al, 1968) indicate that once absorbed by the roots of various plant species chlorpropham may undergo apoplastic but not symplastic distribution.

f) Mode of action

The mode of action of the carbamate herbicides has been comprehensively reviewed by Crafts (1961), Ashton and Crafts (1973), Buechel (1972) and so is only briefly discussed below. It should be pointed out that in these three reviews there is no reference to their use as potato sprout suppressants.

The carbamates are well known mitotic poisons, causing chromosome abnormalities at very low levels. However it is generally believed that the effects of these compounds are probably due to interference with numerous biochemical systems within the plant, due to hydrogen bonding between the carbonyl oxygen of the carbamate and imino hydrogens of peptide nitrogens on proteins, or vice versa. These theories are substantiated by the fact that replacement of the carbonyl oxygen with a sulphur (which may not form hydrogen bonds) or replacement of the imino hydrogen (or ortho substitution of the ring causing steric hindrance) respectively will eliminate activity. It has also been suggested that chlorpropham is more active than propham because of increased liposolubility, or the chlorine in chlor-propham may participate in hydrogen bond formation or that it might serve to control the spacial configuration and hence orientate the molecule with respect to a particular site of action. Both compounds will inhibit oxidative phosphorylation, RNA synthesis and protein synthesis, and inhibit the Hill reaction, although the latter observation is of little relevance to sprout inhibition.

g) Seed

When chlorpropham is used at concentrations high enough to produce satisfactory sprout suppression it will permanently impair the performance of treated seed. It must never therefore be used on seed or on ungraded material of which a proportion may be required for seed.

Chlorpropham is as has been emphasised already a highly volatile compound and occasionally accidental contamination has occurred, because the vapour has drifted from treated ware to seed stock in the vicinity. Steinbeiss et al (1972) reported that when treated potatoes were stored near untreated ones they found a residue of 0.65mg kg^{-1} in the untreated material. Generally it is accepted that 10mg kg^{-1} are required to completely inhibit sprouting, but Reeve et al (1963) noticed a slight inhibition at 1mg kg^{-1}. Such inhibition would produce delayed emergence.

During 1974, the Agricultural Development and Advisory Service (ADAS) took samples from planted crops where sprouting and emergence was absent or poor and subsequent analysis of peelings from these samples indicated that only 2 out of 40 were not contaminated with chlorpropham. (Incidentally only 5 were not contaminated with tecnazene - see Chapter 5) Levels of chlorpropham in these samples ranged from 0.64 to 80mg kg^{-1}. During 1975 similar samples produced levels ranging from 0.6 to 2.0mg kg^{-1} (Golightly and Hawkins, 1975). The origin of this contamination was not always obvious. On one farm a residue of 1.1mg kg^{-1} was detected after storage in a store where no chlorpropham had been used during the previous two storage seasons.

Extreme care must therefore be exercised in the use of chlorpropham.

h) Toxicology

As the toxicology of the carbamate sprout suppressants is a subject of some complexity, controversy, and of considerable consequence to the potato industry world-wide, it will be reviewed

in considerable depth. However before commencing the following points should be made.

Firstly feeding experiments must always be interpreted with caution as they are often carried out with highly purified material which is hardly ever in the same state as it would be consumed in practise.

Secondly metabolic studies which have been conducted have been concerned primarly with the use of these compounds as herbicides and not as sprout suppressants. The potato store is an almost unique biological environment. It contains soil, bacteria, fungi and potatoes in a complex relationship so extrapolations from more simple systems must again be interpreted with caution. Nevertheless, in the absence of truly relevant data some conclusions can and must be drawn.

Chlorpropham and propham are readily absorbed by experimental animals. Rats given a single oral dose of the chemical (10-20mg kg^{-1}) excrete 90% in the urine within 48 hours. (Van Esch & Kroes, 1972) Using ^{36}Cl labelled chlorpropham Jumar et al (1968) showed that given orally to mice it was rapidly excreted, 67% of the label being found in the urine and 33% in the faeces. This rapid excretion results in a relatively low acute toxicity towards mammals. The acute oral LD_{50} for rats is 5000-7000mg kg^{-1}. No toxic effects were observed when applied to shaved rabbits for 24 hours or when it was fed at dietary levels of 2000mg kg^{-1} for two years to rats. (Larson et al, 1960) A U.S. Federal Food and Drug Administration tolerance of 50mg kg^{-1} was subsequently established for residues in potatoes after sprout inhibiting treatment. (Anon,1960b)

However urethanes (esters of carbamic acid, H_2NCOOH) have been the subject of considerable interest to toxicologists, and chlorpropham is a substituted urethane. Urethane itself (ethyl carbamate) and ethyl urethane (ethyl-N-ethyl carbamate) are proven mutagens (Northrop, 1963). Urethane is an active carcinogen in mice (Barnes, 1976) and Van Esch et al (1958) was able to demonstrate that several related compounds could cause skin cancer in mice which had been exposed to the co-carcinogen croton oil. Chlorpropham however was much less active than

urethane in this respect. Jumar et al (1968) found that mice maintained on a diet containing the equivalent of 750mg kg^{-1} body weight per day, for two years did not have a higher incidence of tumours than did untreated mice. In more recent experiments van Esch and Kroes (1972) found long-term exposure to either chlorpropham or propham in the diet or by subcutaneous injection produced no indication of carcinogenesis.

Despite these negative findings, however, it is interesting to note that the Dutch tolerance for of chlorpropham plus propham has remained at the extremely low level of 0.5mg kg^{-1}.

Another factor which is causing concern amongst toxicologists is the production of possible toxic metabolites from agricultural chemicals. The toxicology and residual life of these metabolites is often completely unknown. Such biotransformations have been extensively investigated in the case of carbamates in animals, plants and soils. Little information is available, however, on the extent to which these changes occur in a potato store, or on their toxicological implications. Because of the great importance of this subject, not only to the continuing use of chlorpropham and propham but also to later discussion on the metabolism and toxicology of tecnazene, it was considered essential that it should be briefly discussed.

There are 3 primary biotransformations which are of relevance to chlorpropham, these being hydrolysis, hydroxylation, and conjugate formation and although they are discussed independently below, all three may be occurring at the same time.

1) Hydrolysis

Hydrolysis may be either ester hydrolysis (catalysed by esterase enzymes) or amide hydrolysis (catalysed by amidase enzymes). The mechanisms of both have been discussed by Herret (1969). Regardless of which route is followed the primary products from the hydrolysis of chlorpropham are identical these being 3-chloroaniline, isopropanol and carbon dioxide. The non-biological equivalents of these processes are of little consequence under normal environmental conditions (Hance, 1967).

It has been clearly demonstrated that microbial enzymes will catalyse these processes in the soil, especially under warm, moist

conditions with an adequate supply of nutrients (Kaufmann, 1967; Herret, 1969). Their occurrence in the potato store must therefore be considered.

It has also been shown that fungi may hydrolyse carbamate herbicides (Kaufmann and Blake, 1973, but see also Lanzilotta and Pramer, 1970). Hydrolysis in higher plants appears to be species dependent (Still and Mansager, 1969; Herret, 1969), and one report indicates that potato tissue does not metabolise chlorpropham at all Jumar et al, 1968). This is however unlikely, although the process may be very slow.

The eventual fate of carbamate derived anilines has also been the subject of much investigation. In the soil it has been shown that peroxidase enzymes of fungal or bacterial origin will catalyse the condensation of 2 molecules of substituted aniline to form substituted azobenzenes (Bartha and Pramer, 1967; 1970; Kearney et al, 1969; Matsunka, 1971; Bordeleau and Bartha, 1972a; 1972b). Thus in the case of chlorpropham, hydrolysis yields 3-chloroaniline from which 3,3'- dichloroazobenzene may be produced. After this initial stage higher polymers may be formed. In the case of propham no azobenzene has been detected as polymerisation takes place immediately (Bordeleau and Bartha, 1972c). Mechanisms for these processes have been postulated (Bordeleau et al, 1972; Kaufmann, 1975; Bartha, 1975).

The toxicological implications of azobenzene formation are little understood, although it is known that they are persistent (Chisaka and Kearney, 1970; Kearney et al, 1970; Bartha, 1971). None of the herbicide derived chloroazobenzenes have been shown to be carcinogenic although azobenzenes are eg. butter yellow (4,4' dimethylamino-azobenzene)(Weisburgher and Weisburgher, 1966). However they do cause a skin condition known as chloracne in employees of herbicide manufacturing plants (where their formation is also a problem), and Poland et al (1976) has compared them to the well known acnegens encountered in the production of 2,4,5-T (2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran) and to the polychlorinated biphenyls. Poland et al (1976) have shown that the ability of compounds to elicit acne can be correlated with their induction of hepatic aryl hydrocarbon hydroxylase in chicken embryo, and on this basis have surveyed the potential acnegenic activity of

numerous azobenzenes and azoxybenzenes. They found that although 4,4'-dichloroazobenzene showed some activity, 3,3'-dichloroazobenzene was inactive at the levels tested (Poland, 1977).

Should this similarity of properties of azobenzenes to dioxins extend to the well known teratogenic effects this would have most serious consequences.

The possibility of mixed azobenzene formation also exists by condensation of anilines from different sources (Bartha, 1969). In the case of potatoes this could well be linuron or monolinuron as these compounds are often used as herbicides.

Anilines derived from herbicides have also been shown to form diazoaminobenzenes or triazenes in soil (Plimmer et al, 1970). The toxicology of these compounds is also unknown.

Other fates of herbicide derived anilines include the formation of conjugates - mainly glucosides (Still, 1967; Herret, 1969; Yih et al, 1968; Matsunka, 1971), and incorporation into lignin complexes (Yih et al, 1968).

2) Hydroxylation

There are three types of hydroxylation of relevance to a discussion of phenylcarbamate metabolism these being, aryl hydroxylation, N-hydroxylation, and alkyl hydroxylation (Herret,1969). Aryl hydroxylation appears to be of considerable importance, at least in plants (Still and Mansager, 1973). N-hydroxylations of carbamates have been clearly demonstrated in animals (Irving, 1964) but James and Prendeville (1969) failed to isolate N-hydroxylated products from plant tissue. As N-hydroxy ethylcarbamate has been shown to cause chromosome damage in plant cells these metabolites are certainly worthy of further investigation. Alkyl hydroxylated products from chlorpropham metabolism in plants have been found but their toxicology is unknown (Prendeville et al, 1968; James and Prendeville, 1969). Hydroxylated metabolites may, of course, undergo subsequent conjugation.

3) Conjugate formation

Conjugates are formed as a result of reactions between foreign substances and naturally occurring compounds such as glucose, alanine, and aspartic acid. Often, however, it is necessary for the foreign

substance to undergo hydroxylation first. In the case of chlorpropham James and Prendeville (1969) found that in 3 out of the 4 plant species tested β glucosides were formed. The biological activity of many of these conjugated products is unknown although it is accepted that in general they result in detoxification.

One final factor which should be mentioned is that one chemical may have a significant effect on the rate of degradation of another. This effect may be synergistic or antagonistic. For example it is known that the insecticide carbaryl (1-naphthyl methylcarbamate) which is often used on potatoes can dramatically increase the persistence of chlorpropham in soils by competitive inhibition of the degradative microbial enzymes (Kearney and Kaufmann, 1969; Kaufmann, 1970).

To conclude this discussion on the toxicology of the carbamates it would appear that if the philosophy of "innocent until proven guilty" is adopted then the carbamates must be considered safe. The metabolism of this group of compounds is so diverse that it may be many years before the full implications of their use is apparent. The potential production of substituted azobenzenes is of particular interest. It is also worth noting that current toxicological testing and residue estimation procedures do not take into account any metabolites which may be produced before potatoes are consumed.

i) SUMMARY

Chlorpropham is extremely effective and the most successful chemical which has been used as a sprout suppressant. It is extremely potent requiring low dosages to be effective. Wound healing and internal sprouting problems associated with its use can be overcome by suitable management techniques. It appears to have little significant effect on important processing characteristics. Inevitably treated tubers contain residues after treatment, but with appropriate management these can be kept to a minimum. However, should the current Dutch tolerance level of 0.5mg kg^{-1} become E.E.C. legislation this would have considerable implications for users in this country - particularly the processing industry where multiple applications are at present commonly used.

Two areas which give cause for concern however,are contamination of seed stocks and toxicology. The former has occurred frequently but should be reduced by better education of users. The latter point is likely to be the subject of controversy for some years until the metabolism of chlorpropham in the potato store, and the toxicology of the resultant metabolites have been fully investigated.

3.3.2.4. PROPHAM

a) General

Propham is the ISO, BSI and WSSA approved name for isopropyl-N-phenylcarbamate (see figure 3.5), also known as isopropyl carbanilate. WSSA once used the initials IPC and it is by this name it is most commonly known in the potato industry. It has also been referred to as IPPC (Perlasca, 1956). Its herbicidal properties were first reported by Templeman and Sexton, (1945, 1946) who selected it from a series of arylurethanes. Like chlorpropham it is a mitotic poison.

It is synthesised by the reaction of aniline and isopropyl-chloroformate in the presence of sodium hydroxide. It is a white crystalline solid m.p. 87°C, whose solubility in water at 25°C has been reported at 32, 100, and 250mg dm^{-3} (Martin, 1972). It is soluble in most organic solvents. It is stable below 100°C and is slowly hydrolysed by acid and alkali. It is more volatile than chlor-propham (Ashton and Crafts, 1973).

Rhodes et al (1950) first reported the use of propham as a potato sprout suppressant. They used a dust formulation and showed propham to be a more active sprout suppressant than MENA. Like chlorpropham it is now available as a variety of formulations, but unlike chlorpropham it is now seldom used on its own. In the U.K. it is available in a mixture with chlorpropham as a fogable formulation known as Pommetrol M (Fletcher Ltd.) and it is imported, again as a chlorpropham mixture as Luxan (Elst-Overbetuwe) Anti Spruit SC.

Propham is an extremely potent sprout inhibitor producing good results at 7-100g per 1000kg of potatoes, dependent upon conditions and storage period (Rhodes et al, 1950; Emilsson et al, 1951a; Edwards, 1952; Dettweiler, 1952; Ciferri , 1953; Emilsson et al, 1955; Driver, 1957). It is however slightly less active than chlorpropham hence it has largely been superseded.

b) Wound healing and internal sprouting

Like chlorpropham, propham will inhibit wound healing,

allowing easier access to micro-organisms. Increased severity of skin spot lesions (Ives, 1955) and silver scurf (Castello Branco, 1957) have been reported. It is therefore inadvisable to apply the inhibitor at harvest. A period of 2-3 weeks should first be allowed before applying the inhibitor to the stored potatoes as a "fog" or as suggested by Ophuis (1956) by blowing a dust formulation through the ventilating system, which then deposits on the surface of the tubers.

During the course of this survey no reference was found which indicated any relationship between propham and internal sprouting, although Sawyer (1967) maintains that"effective treatments containing external sprouts will also contain internal sprouts".

c) Processing characteristics

The effect of propham on the processing characteristics of potato tubers has not been widely investigated. Results which have been reported are mainly of a qualitative rather than quantitative nature.

It has been reported that propham used at rates adequate to produce good sprout control (27mg kg^{-1}) produced no undesirable effect on cooking quality or appearance (Edwards,1952; Downie, 1952), nor does it influence ascorbic acid or dry matter levels (Driver, 1957).

More recent work has indicated that like chlorpropham the accumulation of sugars caused by sprouting can be reduced by using propham. (Baijal and Van Vliet, 1966) Neither chemical appears to affect the distribution of sugars within the tubers. However it should be borne in mind that the most recent research on chlorpropham indicates that these effects are complex. (see 3.3.2.3d)

Wilson and Harris (1954) found that no detectable off-flavour resulted from the use of propham.

d) Residues and uptake

Methods of analysis for propham residues are similar to those for chlorpropham, and because of the close similarity between the compounds similar problems are likely to be encountered (see 3.3.2.3c). Some methods may be used for the simultaneous

estimation of both.

Early methods depended on extraction, hydrolysis, steam distillation of the resultant aniline and its colorimetric estimation by the hypochlorite-phenol method (Bissinger and Fredenberg, 1951) or using p-phenylenediamine as a coupling reagent (Merz and Kammerer, 1953). Using the latter Nultsch (1959a) found that tubers treated with 100mg kg^{-1} as a 5% dust contained 0.6mg kg^{-1} when analysed 1 year later. They failed to find any residue in peeled tubers even when using 5kg samples.

More recent methods generally involve gas chromatography of the intact molecule (Vogel and Deshusses, 1965; Van Vliet and Hertog, 1966; Cerny and Blumenthal, 1972)(see section 3.3.2.3e). Using gas chromatography Vogel and Deshusses found residues of 1.8-5.4mg kg^{-1} in various samples tested.

Van Vliet and Sparenberg (1970) have investigated the effect of application method on subsequent residues, however their experiments were conducted using a mixture of chlorpropham and propham and unfortunately they only published combined residues figures (i.e. chlorpropham plus propham)which have already been discussed (see 3.3.2.3e).

Numerous workers have studied the relative volatilities of the phenylcarbamate herbicides. These studies have shown that propham will undoubtedly volatilise more quickly than chlorpropham from inert surfaces such as glass slides and filter paper (Newman and Downing, 1958). However, it has been demonstrated that in soil studies adsorptive capacity has a major influence on this volatilisation behaviour, but that propham is still more volatile than chlorpropham (Rose, 1951; Parochetti and Warren,1966). Other experiments have shown that propham and chlorpropham actually show very similar dissipation patterns in soil but because chlorpropham is inherently more toxic it appears to have more prolonged effects (Parochetti and Warren, 1968)

Although soil adhering to potato tubers in store will play a role in the rate of volatilisation it would appear reasonably safe to extrapolate from these results that propham will be lost more quickly from the tuber surface. With increasingly demanding

legislation on residue levels this factor might lead to a revival in the use of propham, especially in short term storage programmes, or at least to an increase in the number of formulations containing both chemicals.

e) Mode of action

For a general discussion of the mode of action of the carbamates - see 3.3.2.3f. Investigations into the mode of action of propham when used specifically as a potato sprout suppressant have met with little success (Dettwieler, 1952; Nultsch, 1958; 1959b; 1960).

f) Seed

Although Rakitin and Troyan (1949) observed that tubers treated at a rate of 25-50mg kg^{-1} grew normally after 30-40 days open air chitting, the more generally accepted view is that propham is unsuitable for the treatment of seed (Mattingley and Downie, 1953; Nultsch, 1960). Concentrations of propham that produce satisfactory sprout inhibition during storage irreversibly impair seed performance. Very low dosages of propham can in fact stimulate sprouting (Dettweiler, 1952).

No reports of accidental contamination of seed by propham were discovered during the preparation of this survey. (see section on chlorpropham 3.3.2.3g)

g) Toxicology

The toxicology of propham has already been discussed in the previous section (3.3.2.3h), but see also Holder and Ryan (1968) for mammalian metabolic studies.

h) Summary

Propham is a very effective sprout suppressant which has been commercially accepted. Its properties are, as might be expected, very similar to those of chlorpropham. Its major disadvantage in use is its inhibition of wound periderm formation, but this can be largely overcome by delaying application. It appears to have little significant effect upon processing characteristics.

Increasingly demanding residue legislation might lead to

an increase in its popularity should it be proven that its rate of loss from the tuber surface is substantially more rapid than chlorpropham. Further investigations into the relative depth of penetration of the two compounds into the potato tuber would also be interesting.

Observations upon the toxicology of the two compounds indicate that propham is certainly no worse than chlorpropham. In fact early indications are that the toxicity of propham metabolites might be lower than those of chlorpropham.

3.3.2.5. TECNAZENE

The following discussion on tecnazene and its properties takes a somewhat different form to that employed for the other commercially used sprout suppressants. The material below is intended as an introduction to the chemical and biological properties of this most unusual compound, and as an indication of the areas where problems have arisen through its use. More comprehensive surveys of the various aspects which have been investigated experimentally will be included where appropriate in subsequent chapters.

a) General

Tecnazene is the name approved by the International Organisation for Standardisation (ISO) and by the British Standard Institution (BSI) for 1,2,4,5-tetrachloro-3-nitrobenzene (IUPAC), also known as 2,3,5,6-tetrachloronitrobenzene or TCNB (see fig.3.3.6.). During the course of this thesis it will be referred to as tecnazene.

It is synthesised industrially by the nitration of 1,2,4,5-tetrachlorobenzene which can be produced by the chlorination of trichlorobenzenes resulting from the dehydrochlorination of hexachlorocyclohexane (HCH- now the BSI approved name for the insecticide BHC)(Galat, 1952). This process will be more fully discussed in Chapter 4. It yields colourless crystals, m.p.99^{o}C. Both the pure and technical product have a faint odour which from observations made during this study some people seem unable to detect.

Other properties of relevance to this study are its appreciable volatility at room temperature and its solubility. It is practically insoluble in water, soluble to about 4% in ethanol at 25^{o}C and readily soluble in most organic solvents (Martin,1972).

The discovery of the sprout suppressing properties of tecnazene was accidental. It resulted from studies by Brown and his co-workers at Imperial College, London into the control of various fungal pathogens of vegetables- principally Botrytis cinerea on lettuce - using a selection of chlorinated nitrobenzenes. Early experiments (Brown,1935; Smieton and Brown, 1940) were with

trichloro- and pentachloro- nitrobenzenes, but later experiments included tecnazene with considerable success (Brown and Montgomery, 1948).(Pentachloronitrobenzene - often referred to as PCNB is now called quintozene (BSI)).

Around the same period Brown, while conducting experiments on the control of the solanaceous strain of Rhizoctonia solani - a pathogen which causes considerable damage to potato sprouts - noted that both quintozene and tecnazene markedly reduced the amount of sprouting. Tecnazene as a 5% dust was more efficient than quintozene as a 20% dust in this respect and consequently Brown (1947) suggested the use of tecnazene as a sprout suppressant. Bayer Agriculture Ltd. promptly began marketing it as such under the trade name "Fusarex".

However, before discussing the sprout suppressing properties of tecnazene in greater depth, it is worth devoting a few lines to briefly reviewing its fungicidal properties.

b) Fungicidal properties

Tecnazene is not universally fungicidal. The early lettuce experiments mentioned above showed that although it gave substantial control of Botrytis cinerea and considerably reduced "damping-off" when caused by Rhizoctonia solani, it did not control "damping-off" when caused by Pythium spp., and had little effect on mildew, Bremia lactucae.(Brown and Montgomery, 1948; Last, 1952). The situation is similar with respect to potato pathogens - it controls some but not others.

Brown (1947) indicated that damage to sprouts on potatoes caused by the solanaceous strain of Rhizoctonia solani (which also causes the tuber conditions known as "black scurf") was reduced by a dust application of tecnazene. Dry-rot of the tuber was the next disease reported to be controlled. The interpretation of these experimental results however, is complicated by the fact that the condition can be caused by various Fusarium species or varieties (McKee, 1952). In Britain where the principal pathogen is believed to be Fusarium solani var. caeruleum a detailed investigation of the disease by the Agricultural Research Council and the Department of Agriculture for Scotland showed that good

control could be achieved with tecnazene (Foister and Wilson, 1951). Elsewhere others reported similar results (Anon, 1949; Mooi, 1949). Emilsson et al (1949; 1951) noted significantly less rot in treated samples and Brook and Chesters (1957a) reported good commercial control of dry rot caused by Fusarium solani var. caeruleum in five of the six cultivars tested, but stress that tecnazene should be present at the time of infection.

Ellison and Cunningham (1953) and Cunningham (1953) using artificial inoculation techniques with Fusarium sambucinum f.6 found only a very slight reduction of infection with tecnazene treatment. From the latter paper it is worth noting that when tecnazene is used as a liquid or as a dust at rates exceeding those recommended by the manufacturers, irregular wound cork formation results shortly after treatment but a continuous layer was subsequently formed so that damage due to dry rot was not greatly affected.

Detailed in vitro culture experiments confirm that the vapour of tecnazene will inhibit various potato pathogens. Reavill (1954) found that in an atmosphere saturated with tecnazene (1.32mg m^{-3} at $10^{o}C$, 4.67mg m^{-3} at $20^{o}C$ and 8.34mg m^{-3} at $25^{o}C$), the germination, colony growth and sporulation of various fungi including Rhizoctonia solani and Fusarium solani var. caeruleum was suppressed and that with two strains of the latter, one was more sensitive than the other. She also points out that the action of tecnazene is fungistatic rather than truly fungicidal. Brook and Chesters (1957b) confirm these findings and also report slight inhibition of Phoma exigua var. foveata in vitro although no reduction in gangrene has been noted in tuber work. They also note that tecnazene-resistant mutants of Fusarium first reported by McKee (1951) can be controlled by other tetrachloro-nitrobenzene isomers, namely 2,3,4,6- and 2,3,4,5- tetrachloro-nitrobenzenes. Both compounds are also more potent than tecnazene against normal strains of Fusarium (Brook, 1952; Brook and Chesters, 1957b). In general it appears that highly chlorinated nitro-benzenes possess high fungistatic activity (Eckert, 1962).

Tecnazene today is accepted commercially as an effective

potato fungicide principally used in this respect for the control of dry-rot. In the experiments described in the following chapters care has therefore been taken so that while investigating growth effects the beneficial effects of its fungicidal properties are minimised by removing from all treatments every tuber showing signs of damage and disease.

c) Sprout suppressing properties

As mentioned above the sprout suppressing properties of tecnazene were first described by Brown (1947) after experiments employing a 5% dust on tubers which were overwintered in clamps covered with straw and soil. This report stimulated a spate of research all over the world during the next decade.

Luckwill (1948) used tecnazene impregnated confetti paper at a rate of 20-100mg kg^{-1} which he found effective in inhibiting the sprouting of cv Arran Banner but not cv Kerr's Pink. Using cv Bintjie, Mooi(1949) reported successful suppression by using a 3% tecnazene dust at a rate of 135mg active ingredient (a.i.) per kilogram, provided that the clamp was covered with soil to prevent loss of vapour. Emilsson et al (1949; 1951; 1955) again using a 3% dust showed that under Swedish conditions 60-120 mg (a.i.) kg^{-1} was generally effective in controlling sprouting, and weight loss during storage was considerably reduced. This reduction in weight loss results in firmer tubers at the end of the storage period (Anon,1949).

The effectiveness of tecnazene in Australia and New Zealand has been somewhat variable. Downie (1949; 1950; 1952) reported that tecnazene used as a 3% dust at a rate of 135mg(a.i.) kg^{-1} applied immediately after harvest, prevented sprouting, and that the tubers were firm and attractive after 6 months storage, but these benefits were very much cultivar dependent. In fact he only recommends the treatment for the Australian cultivar Snowflake. Edwards (1952) demonstrated that propham was much more effective than tecnazene under Australian conditions. Similarly Driver (1957) had only limited success with tecnazene in New Zealand.

In America tecnazene has been found to be an excellent inhibitor when used in large bins at a rate of 100mg kg^{-1}, but

ineffective in small experimental batches. It was also found to be ineffective when treated tubers were exposed to free air circulation. Considerable sprouting occurred around the edges of treated bins (Ellison, 1952; Ellison and Cunningham, 1953). Despite these observations Sawyer (1967) found some ventilation with tecnazene treatment desirable to control storage temperature.

The experiments of Brown and Reavill (1954) help explain the mixed success of tecnazene in warmer climates. They showed that the reduction in sprouting resulting from tecnazene treatment is much greater at lower temperatures. They also report that tecnazene will volatilise rapidly from glass surfaces in high vebtilation conditions. The effectiveness of the chemical is very much dependant upon the rate at which it is lost from the store.

The sprout suppressing properties of other chlorinated nitrobenzenes have also been investigated. Brown and Mellor (1952) patented all chlorinated mononitrobenzenes containing at least three chlorine atoms, as sprout suppressants. The 2,3,4,5- and 2,3,4,6-tetrachloronitrobenzene isomers of tecnazene have been investigated,the latter being the more effective although not so potent as tecnazene (Brook, 1952; Brook and Chester, 1957a).

Tecnazene has found commercial acceptance in several European countries, Australia and the U.S.A. (Sawyer, 1967). It is an effective suppressant under the right conditions - a reasonably low temperature ($12^{o}C$ average storage temperature) coupled with very low to zero ventilation rates. It is however more costly and about 5 times less potent than chlorpropham, most recommended application rates being in the range 50-150mg kg^{-1} (cf.chlorpropham - 10-20mg kg^{-1})(Meigh et al, 1973). Such a treatment should be effective for up to 150 days. (Anon, 1975a)

Tecnazene is now available in a variety of formulations. Dust formulations (eg. Fusarex (Plant Protection Ltd.)(3% on kaolin) must be evenly distributed over the tubers as they are loaded into the store, either manually or using a special applicator attached to an elevator or conveyor. This formulation has one disadvantage in that it occasionally remains visible on the potatoes after removal from storage and can spoil their appearance. In these circumstances it is recommended that the tubers are brushed or

washed before sale. Granular formulations (eg. Bygran S - (Wheatley Chemical Co. Ltd.) - 7.5% on shale granules) can avoid this problem as they are easily removed during grading. There is also a fogable liquid formulation. (P.S. 100 - Wheatley Chemical Co. Ltd.) After application all of these formulations demand restricted ventilation for long-lasting control.

Results indicating the effectiveness of this chemical as a suppressant have been obtained during the course of this study and these will be described in Chapter 5.

d) Wound healing

During the preparation of this thesis only one reference describing the effect of tecnazene on wound healing has been discovered. Cunningham (1953) produced evidence indicating that tecnazene will delay and inhibit wound cork formation, but a continuous layer was eventually formed. However the application rates used in his experiments were in excess of the manufacturer's recommended rates. It is now generally accepted that correct application of tecnazene immediately after harvest or handling will produce no such deleterious effects, and indeed is recommended for the full expression of the chemicals fungicidal properties. Tecnazene is unique in this respect amongst the post-harvest applied sprout suppressants currently available.

e) Internal sprouting

Again very little work has been carried out on this aspect but Ewing et al (1968) have indicated that in experiments which showed that chlorpropham greatly increased this physiological disorder, tecnazene had no such effect. Brown and Reavill (1954) observed an abnormality in treated batches (especially c.v. Home Guard) which would appear to be a form of internal sprouting.

f) Processing characteristics

Little information is available on the effect of tecnazene on the important processing characteristics. What is available will be discussed in Chapter 6. Because of the ever increasing importance of the processing industry and the dearth of knowledge in this field a detailed study of this aspect has been

made, especially with respect to sugar levels. The results of this work can also be found in Chapter 6.

g) Residues

Again little information on this topic was available - indeed the methods for obtaining such information were not considered adequate for current requirements. Because of the acute public awareness of the chemical contamination of food - especially a basic food such as potatoes - it was essential that more information was obtained on this subject and on the effect of processing on tecnazene residues.

Another pressing reason for such a study is the widespread use of tecnazene on seed stocks (see below) and the anxiety of growers about the subsequent performance of treated seed. It was therefore felt desirable to obtain information on the influence of environmental factors on tecnazene residues.

What little historical information there is and the results of these investigations are discussed in the following chapter.

h) Mode of action

Despite having been in commercial use for nearly 30 years there is still no detailed biochemical information upon the way in which tecnazene inhibits potato sprouting. One factor however, which has been observed is that the compound inhibits sprout growth rather than killing it (cf nonanol). Work on this aspect is being incorporated into a future study on the metabolism of tecnazene by plants, which does not constitute part of this thesis.

i) Seed

In his original paper Brown (1947) used tecnazene on seed potatoes to prevent sprouting and wastage during storage. He found that if care was taken in planting these tubers they grew fairly normally. This property is unique amongst the currently used sprout suppressants.

Since then tecnazene has found widespread commercial acceptance as a fungicide and sprout suppressant for seed, especially

in the major seed producing countries such as Scotland. However the application of tecnazene can give rise to problems such as delayed emergence and reduced yield. These problems had not been investigated by experimental work which was strictly relevant to the Scottish situation. Investigations in this area constitute a major section of this thesis.

A survey of previous experimental work and details of the current investigation are included in Chapter 5.

j) Toxicology

It is felt that it would be of more relevance to discuss this topic after the investigation into tecnazene residues has been reported. It is therefore included in Chapter 4.

k) Summary

Tecnazene is a most unusual compound in that it possesses a) fungicidal properties b) sprout suppressing properties and c) can be used on seed potatoes. Consequently it has met with considerable success, and has made a major contribution to the Scottish potato industry.

Nevertheless, the full implications of its use as a sprout suppressant on seed and ware potatoes have never been fully evaluated and it is the aim of this study to contribute something to our knowledge of this compound and sprout suppressants generally, and to investigate some of the problems which have been reported in the literature or communicated during conversations with growers, merchants and processors.

CHAPTER 4

TECNAZENE RESIDUES AND THEIR IMPLICATIONS

4.1. INTRODUCTION

The most direct consequence of the use of any agricultural chemical is the almost inevitable residue which it leaves. In the case of tecnazene up to date information on this subject is lacking. Most of the data on this chemical were determined many years ago when public awareness was less acute and laboratory instrumentation less sophisticated.

Because of the important implications of such information, a detailed investigation using new techniques has been undertaken, not only to find absolute values for residues in commercial samples, but to study in detail the factors such as environment and processing which might affect these levels. The implications of the findings of this investigation are then discussed with respect to human health and toxicology in this chapter and with respect to the subsequent performance of treated seed in the following chapter.

The various experiments described below have been designed to provide information which is relevant to both fields and thus utilise the high requirements of such work for space, time and money more efficiently. Chapters 4 and 5 cannot therefore be read in total isolation of each other. Where previous work does exist and is relevant it will be discussed under the appropriate subheading.

4.2. DEVELOPMENT OF AN ANALYTICAL TECHNIQUE FOR TECNAZENE RESIDUES

4.2.1. INTRODUCTION

The work described in this section deals with the development of an analytical technique for low-level residues of tecnazene in fresh or frozen potato tissue. The analysis of other materials such as fried products which require modification to the basic technique will be described elsewhere. It should also be noted that no account was taken of any metabolites or degradation products of tecnazene - the technique was devised to isolate and quantitatively determine intact tecnazene.

4.2.2. POSSIBILITIES

Several colormetric, polarographic and gas chromatographic methods of analysis for tecnazene have been reported in the literature.

i) COLORIMETRIC

Auerbäch (1950) developed a colorimetric method which he applied only to pure tecnazene but which he states might be adapted to the assay of residual tecnazene. The method involves the formation of a red coloured derivative upon the interaction of tecnazene with anhydrous acetone in the presence of tetraethyl-ammonium hydroxide. His explanation of the chemistry involved is however somewhat dubious in the light of the experiments of Cänbach and Zajaczkowska (1950), but probably involves the formation of a coloured addition product (absorption max. 548nm) from reaction of the nitroquinoid ion (which is formed from tecnazene in alkaline solution and stabilised by the powerful electronegative chlorine substituents) with the active methylene group of acetone. Although neither a standard curvenor sensitivity figures are given it is implied that the method is suitable for analysis of 10µg amounts. The method is however extremely sensitive to interference from water, and the coloured product is very unstable.

Cänbach and Zajaczkowska (1950) also exploited nitroquinoid ion formation but recognised its instability and so further reacted the complex formed by reacting tecnazene dissolved in acetone with alcoholic potassium hydroxide with acid, producing nitrite ions which can then be determined by classical colorimetric procedures. The method preferred was the production of an azo-dye (using ∝ naphthylamine (carcinogenic !) as coupling reagent) giving a maximum absorption at 500nm. The lowest point on the published standard curve is 50µg. Others however have experienced difficulty in repeating this work using the prescribed conditions (see Higgons and Toms , 1957).

Higgons and Toms (1957) tried several colorimetric methods of analysis for tecnazene and eventually developed a method based on the methanolysis of tecnazene to 2,3,5,6-tetrachloroanisole with consequent release of nitrite, which they also determined using classical techniques (Snell and Snell,1949). Although the nitrite ion is not released quantitatively, its liberation by refluxing for 2 hours with 0.1 M sodium methoxide is reproducible, rendering the technique suitable for the analysis of 5-100µg of tecnazene. It has however only been applied to the analysis of tecnazene contaminated filter papers and not to potato residues.

Other methods investigated include hydrolysis in hot caustic ethylene glycol to unidentified chlorophenols which give an intense yellow colour in alkaline solution. This technique yielded reproducible results in the range 0-20mg.

They also investigated the possibility of reducing tecnazene to the corresponding chloroaniline, an approach which had been previously abandoned by Bray et al (1953). Higgons and Toms noted that if the reduction is to proceed readily, zinc and sulphuric acid in alcohol must be used rather than hydrochloric acid, due to the insolubility of the hydrochloride of the 2,3,5,6-tetrachloroaniline produced. (Conversely the sulphate of 2,3,4,5 tetrachloroaniline is insoluble but its hydrochloride is soluble so for this isomer hydrochloric acid should be used). The resultant amine will readily diazotise and couple to form compounds capable of being used as the basis for colorimetric procedures. Best results were obtained by coupling with m-toluidine ethyl hydrogen sulphate, 1-amino-8-naphthol-3,

6-disulphonic acid, 2-amino-5-naphthol-7 sulphonic acid and 1-naphthyl-amine-4,6,8-trisulphonic acid, but none of these reactions offer any advantage over the previously described methanolysis procedure.

None of these reported colorimetric procedures give details of their application to the analysis of homogenates of potato tissue and resultant recovery rates, nor is the sensitivity considered adequate by today's standards.

ii) POLAROGRAPHIC

Webster and Dawson (1952) described a polarographic estimation of residual amounts of tecnazene on tuber surfaces. They report recovery rates of 84-93% and state that the method is suitable for estimation of 10-500µg of tecnazene. Bray et al (1953) exploited a similar technique for the estimation of tecnazene in rat faeces but found it in this context suitable for the estimation of greater quantities

Like the colorimetric techniques polarography is insufficiently sensitive for the requirements of this work. It also demands specialised equipment and is apparently subject to interferences.

iii) GAS CHROMATOGRAPHIC

Klein and Gajan (1961) have reported a GC technique for the analysis of quintozene and tecnazene on lettuce, cabbage and string beans, using microcoulometric detection which they claim will detect 0.1-5mg kg^{-1}. This technique was tested using flame ionisation detection and was found to be most unsatisfactory and so was rejected. Although the technique eventually developed bears some resemblance in outline the results of the two techniques are not comparable in any way.

Because the literature yielded no totally satisfactory technique it was decided that a new technique had to be developed which would be highly specific and very sensitive towards tecnazene. It was also decided that this could be best achieved by exploiting the advantages of modern laboratory instrumentation, yet utilising only that which is common to most analytical laboratories.

Initially it was considered that it might be advantageous to use UV spectroscopy as tecnazene absorbs strongly at 296nm in hexane

sampling equipment was vigorously washed between each sample, including a wash with A.R. acetone. The 6-25 tuber pre-sample was further sampled by :-

i) CORING

Using a cork-borer 8mm cores were removed from 6-8 tubers and 100g of these cores weighed out. This was the most rapid of the 3 techniques and was widely used in the initial experiments. It should be noted that this technique can produce centre-biased samples i.e. a disproportionate amount of material from the centre of the tuber is included in the sample. As the bulk of sprout suppressant residues are near the surface of the tuber this can produce rather conservative estimates of residues. This problem was partially overcome by taking two cores from each tuber, the axis of the second being approximately 45^{o} from the axis of the first, such that the two axes intersected at the centre of the tuber. This method produces an acceptable sample.

ii) QUARTERING

The classical quartering method was also used, whereby the pre-sample was diced into 10mm cubes which were mixed, then the pile divided into 4 equal parts and the 2 diagonally opposite quarters discarded. The remaining two were bulked and the process repeated until only approximately 100g remained which was then weighed. This procedure has been used in some of the experiments but was found to be extremely time consuming, and has no great advantage over the diagonal coring method in terms of consistency of results.

iii) MASCERATION

The best sampling method used involved masceration of the complete pre-sample with a very powerful electric mincer capable of accepting small whole potatoes and maserating approximately 1kg in two minutes(made by Bauknecht, West Germany). This produced a thick mascerate which was then thoroughly mixed by stirring and 100g of this material weighed out. This technique has several advantages. Not only did it consistantly produce the best duplicate figures but it also was less demanding on the blenders used in the extraction process as the sample was already broken up. It is however rather messy to operate

and scrupulous cleaning between samples to prevent cross-contamination can be time-consuming. Despite this it was used in all the later samples and will be the preferred technique in future work wherever it is felt that enzyme action in the mascerate is of little or no relevance.

4.2.4. EXTRACTION AND PRELIMINARY CLEAN-UP

i) SOLVENT CHOICE

The ideal solvent for pesticide extraction is one which will extract the pesticide efficiently with a minimum of plant components and is easily removed by distillation under vacuum.

The alcohols were rejected because tecnazene has only limited solubility in aqueous alcohol. The chlorinated solvents (dichloromethane, chloroform and carbon tetrachloride) were also rejected - they extract too much plant material, cause emulsion problems (Van Vliet and Hertog, 1966) and can cause corrosion to flame ionization detectors (FID) and cause saturation of electron capture detectors (ECD). Ethyl acetate also forms stable emulsions. Ether can be hazardous to use in electric blenders. Benzene is too toxic, and difficult to remove. With due regard to the solubility data given in the previous chapter hexane was eventually chosen as the most suitable solvent for the extraction.

Hexane however presents one problem in that it does not adequately wet fresh potato tissue and therefore to achieve efficient extraction ethanol was added to assist the penetration of the hexane.

ii) SOLVENT QUALITY

From preliminary attempts to extract tecnazene from potatoes using a gas chromatographic detection method it soon became apparent that the quality of solvents, particularly hexane, was a key factor if satisfactory chromatograms were to be achieved. Because the extract is concentrated by a factor of several hundred during the determination even relatively small amounts of impurities - 0.001% and even less - become significant. All the readily available hexanes were therefore evaluated by placing 300cm^3 of the solvent in a scrupulously clean (chromic acid washed) 500cm^3 R.B. flask and evaporating off the solvent on a clean rotary evaporator at 30^o-40^{o}C, until only a few cm^3 remained. A 5µl aliquot of this material was injected into the gas

chromatograph under the conditions used for tecnazene determination (see later). Ideally the solvent concentrate should produce a narrow solvent front and an absolutely flat baseline.

The following hexanes were tested in this manner:-

a) Hexane fraction - General purpose reagent - BDH
b) Hexane fraction - General purpose reagent - Hopkins and Williams
c) Hexane fraction - Low in aromatic hydrocarbons " "
d) Hexane fraction - Spectrosol (spectroscopy grade) " "
e) Petroleum Spirit- Analytical Reagent " "
f) Hexanes - Nanograde (expensive) Mallinckrodt Chemical Works.

The resultant chromatogram for one of these solvents (solvent a) is reproduced in figure 4.2.1. This was the worst hexane tested but b,c,d and e were also considered unsatisfactory - even the spectroscopy grade contained considerable amounts of impurities. All of these materials can be purified by shaking with concentrated sulphuric acid, followed by a deionised water wash, refluxing over A.R. potassium hydroxide pellets and then distillation in an all glass system using a fractionating column.(Perrin et al, 1966). The resultant hexane then showed the desired characteristics.

Only hexane f (nanograde) was considered suitable without any pre-treatment. It should be noted, however, that this solvent is very expensive, nevertheless it was used in the majority of the analysis described below.

Recently another much cheaper hexane has also been found to be suitable - Hexane - Glass distilled grade - Raithburn Chemicals (Walkerburn) Ltd.

The ethanol used in the extraction was also of the highest quality (Absolute alcohol - Burroughs Ltd.) as were all other chemicals.

iii) EXTRACTION

The extractions were carried out in an electric blender (MSE - Ato-mix) by first blending with alcohol, then adding the hexane and blending for a further period. If the formation of a stable emulsion is to be avoided the initial homogenisation must be carried out solely in the presence of ethanol.

From a study of the distribution of tecnazene between aqueous

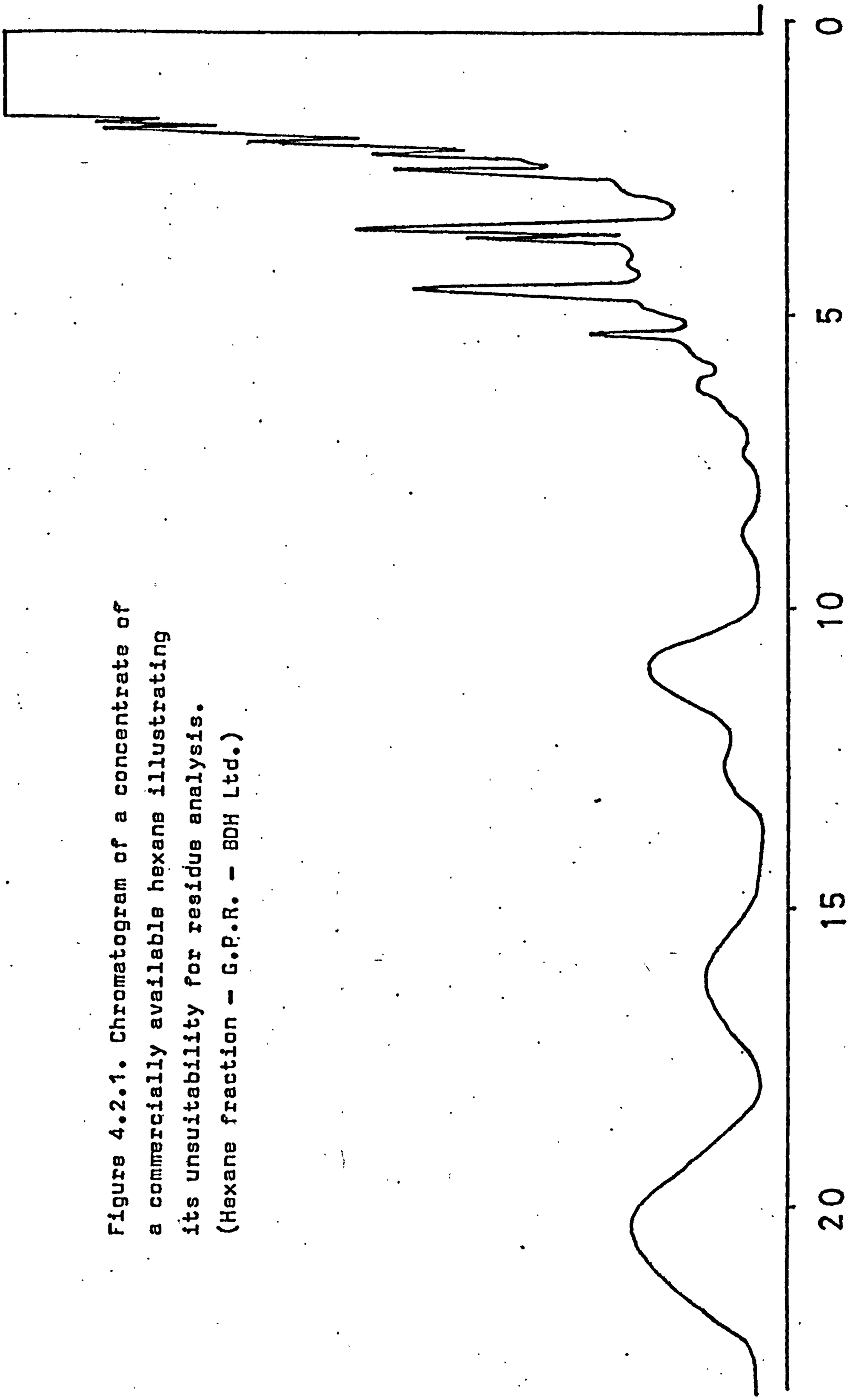


Figure 4.2.1. Chromatogram of a concentrate of a commercially available hexane illustrating its unsuitability for residue analysis. (Hexane fraction - G.P.R. - BDH Ltd.)

ethanol and hexane it was noted that provided the alcohol contains at least 40% water then the distribution is almost 100% in favour of hexane. The quantities of potato tissue (70% water) and ethanol were therefore chosen to satisfy this requirement. Thus when the homogenate is filtered and eventually forms two phases all the tecnazene should be in the hexane phase. The addition of saturated sodium chloride solution speeds up this separation and makes doubly sure that all the tecnazene is in the hexane layer.

After isolation of the hexane layer it was found desirable to further purify it with various washes. Of those tried 10% sodium carbonate produced the most dramatic improvement on the resultant chromatograms, removing several interfering peaks completely and so was included in the final method. These peaks were possibly derived from fatty acids or phenolic compounds.

v) EVAPORATION OF SOLVENT

After preliminary clean-up and drying thoroughly with anhydrous sodium sulphate it was necessary to reduce the large bulk of hexane to a volume suitable for application to a liquid chromatographic column for further purification. Tecnazene is fairly volatile and can easily be lost at this stage if the solvent is removed on a rotary evaporator at too high a temperature (Webster and Dawson,1952). The water-bath was therefore kept below $35^{o}C$ and the extract evaporated to small volume rather than taken to dryness. Under these conditions there was no tecnazene loss.

4.2.5. COLUMN CLEAN-UP

High levels of tecnazene residues ($>5 mgkg^{-1}$) can actually be determined in the crude extract produced as described above. However repeated injection of the crude extract onto a GC column drastically shortens its life due to the accumulation of charred plant residues. Low levels cannot be directly determined due to interference from natural components. For all levels it is therefore preferable to further purify the sample before final determination.

i) PRELIMINARY INVESTIGATIONS

Various attempts at solvent partitioning proved

unsuccessful in that they either involved substantial tecnazene loss, or inadequate removal of offending impurities. The quantity of impurity extracted also ruled out TLC as a possibility because of overloading problems. Column chromatography was therefore the chosen method.

There are two approaches to the use of columns in pesticide clean-up procedures. Either a column can be chosen which will preferentially adsorb the impurities and allow speedy elution of the pesticide or a column can be selected which retains some of the impurities strongly, some only weakly, and retains the pesticide long enough to allow complete separation from the weakly held impurities yet weakly enough to permit its elution in a reasonable time. The latter approach was to produce the purest samples and was therefore adopted although the former is much more rapid.

Various chromatographic materials were tested using TLC and column methods to gauge their adsorptive powers for tecnazene. These included silicic acid, florosil, cellulose and various grades of alumina, with a wide variety of eluants. From these experiments acidic alumina emerged as the most suitable material for column clean-up. It retained the pigments, allowed rapid elution of many other impurities and retained tecnazene long enough to allow its separation from the weakly held materials. More precise experiments were therefore carried out to quantify its properties.

ii) <u>DETERMINATION OF THE RETENTION VOLUME OF TECNAZENE ON ACIDIC ALUMINA</u>

The material selected from the above study was acidic alumina (Woelm) activity Grade 1. Columns of this material were prepared by pouring a slurry of alumina in "nanograde" hexane into glass chromatography columns of dimensions 300mm by 9mm diameter and fitted with a glass sinter, until the column was packed to a depth of 150mm. The taps on the columns which had previously been chromic acid washed were greased lightly with high boiling point silicone grease as Vaseline will produce various hydrocarbon peaks on the resultant chromatograms. The column was then connected to a $1.8cm^3$ siphon on an automatic fraction collector (Boulting) with narrow-bore silicone rubber tubing. Polythene tubing produced various GC peaks and teflon tubing was not flexible enough to allow efficient operation of the balancing mechanism of the fraction collector.

The level of hexane in the column was allowed to fall to the column surface during which time the flow rate was adjusted to 1.0cm^3 min^{-1}. An untreated potato extract prepared as described in the previous section was spiked with 1mg of tecnazene (1cm^3 of 1mg cm^{-3} in hexane) and applied to the column surface using a Pasteur pipette, the level being allowed to fall to the surface between each addition. Several washes of 1cm^3 of hexane were used to ensure quantitative transfer to the column and complete adsorption of the sample on the column. The column was then topped up with hexane and the flow rate checked. The fraction collector was started at the moment of application of the sample. Collection of samples was continued until 150cm^3 of hexane had passed through the column.

The various fractions were analysed for tecnazene as they appeared using gas chromatography and an elution profile constructed. Such a profile is illustrated in figure 4.2.2. From this work it was concluded that during routine analysis the first 50cm^3 eluted which contained substantial quantities of impurities and no tecnazene would be discarded and the next 100cm^3 would be collected, carefully concentrated (see previous section), made up to known volume, and an aliquot injected into the gas chromatograph for final determination.

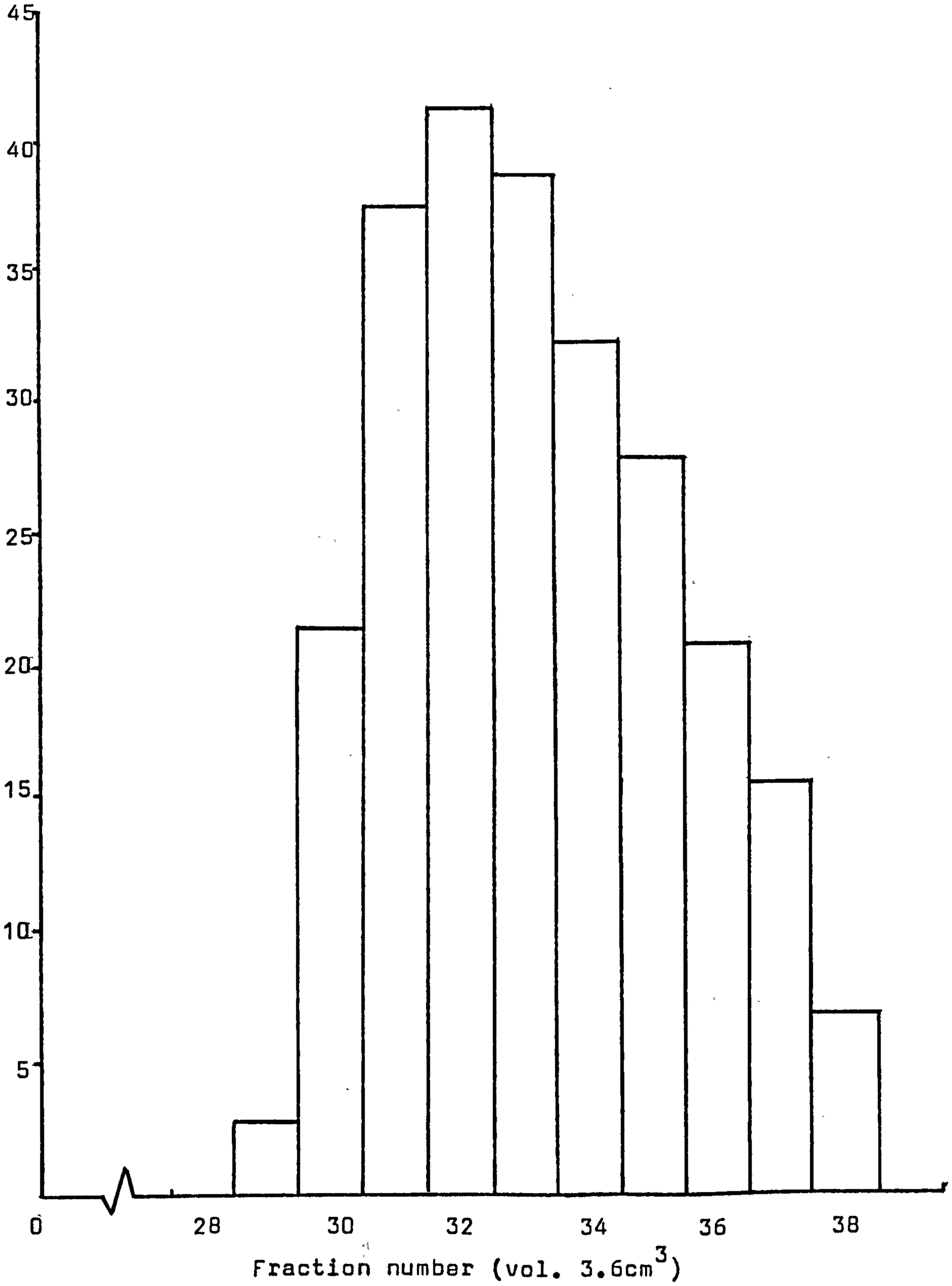
Using this technique all interfering substances can be removed and reproducible results obtained provided the various factors which can affect the separation are fully understood and a number of small but important points of technique are rigorously adhered to.

a) <u>variability of the alumina</u>

The alumina used was Woelm acid - nominally Grade 1 i.e. straight from the bottle. The method demands that the activity is such that tecnazene is retained until 50cm^3 of hexane have been passed through the column and then should be eluted quantitatively during the next 100cm^3. Any increase or reduction in the activity will therefore have adverse effects upon results. The magnitude of the variations in activity with respect to tecnazene adsorption was found to be relatively small in relation to the well documented variations eg. between grade 1 and grade 11.

Occasionally a batch was encountered which appeared to be very slightly more active than normal and hence retained tecnazene too

Figure 4.2.2. Elution profile obtained with 1mg of tecnazene on an alumina (Woelm acid) column of dimensions 150mm by 9mm diameter and a flow rate of 1cm^3 min^{-1}. (1.8cm^3 fractions bulked to give 3.6cm^3 fractions)



strongly. When this phenomenum was encountered 1% ethyl acetate in hexane was found to quickly remove the strongly adsorbed tecnazene, however such an abrupt change in eluant composition also removes other materials from the column, which contaminated the final sample. It was found more satisfactory to very slightly deactivate such batches by adding 5cm^3 of water to a 1kg bottle and shaking for several hours, or to leave it exposed to the atmosphere for a short period until the desired activity had been obtained.

On a few occasions the opposite problem was encountered i.e. the alumina was not active enough, resulting in the premature elution of tecnazene amongst the impurities. This problem was normally encountered after a bottle had been left uncorked or had been incorrectly corked, although on one occasion a sealed bottle produced such material. This fault was rectified by spreading the alumina on a clock-glass and heating at 110^{o}C for 2 hours to reactivate it.

As a matter of routine therefore every batch of alumina was pre-tested before use and its tecnazene retention properties adjusted if necessary. As an added precaution occasional samples were double-checked by :

i) concentrating the first 50cm^3 which were normally discarded, and subjecting it to GC to check that it contained no tecnazene and

ii) after the normal 150cm^3 of hexane a further 25cm^{3}of 1% ethyl acetate in hexane was passed through the column, concentrated and checked by GC.

Injections of both check samples were normally made at the end of the day or when no further samples were to be run, as up to an hour was required before the baseline returned to a level acceptable for accurate analysis, because of the large amount of contaminants which were contained in these fractions.

b) <u>anhydrous conditions</u>

Because the alumina is so sensitive to the effects of moisture it was found essential to maintain anhydrous conditions throughout the clean-up. All glass-ware must be dry and the sample

must be thoroughly dried with anhydrous sodium sulphate before application. The most common method of sample contamination with water was by opening the air valve on the rotary evaporator too quickly and allowing fine droplets of water on the cold condenser to be blown back into the sample.

As an added safeguard it was decided that as a matter of routine approximately 20mm of anhydrous sodium sulphate should be added to the top of the alumina column, before the sample was applied.

c) the effect of the plant extract

Plant extracts have a significant effect upon the chromatographic behaviour of tecnazene on alumina. Retention volumes calculated from runs using tecnazene alone bear little relation to the retention volume using tecnazene plus a potato extract. Such co-chromatographic effects are well documented (Heftmann, 1967). All preliminary runs and alumina testing were therefore carried out in the presence of the plant extract. It was found convenient to prepare such material in bulk using a scaled up version of the normal extraction process.

d) flow rate

The flow rate of $1.0cm^3\ min^{-1}$ was chosen after initial experiments as a compromise between rapidity and the ideal chromatographic conditions which produced near perfect equilibrium conditions resulting in optimum resolution and peak symmetry. As can be seen from figure 4.2.2. the peak is not quite symmetrical but to produce any significant improvement doubled the analysis time. It is however imperative that the flow rate never exceeds $1.0cm^3min^{-1}$ or tecnazene is eluted amongst the impurities.

Maintainance of the flow rate was achieved initially by simply adjusting the tap on the column, but in more recent work it has been found that more consistent flow rates could be achieved using a constant-head reservoir system connected to the column with teflon tubing.

It was noted that samples with unusually high lipid contents can cause reductions in flow rate. This was remedied by

stirring the top few millimetres of the anhydrous sodium sulphate with a long narrow nickel spatula.

e) contamination sources

Sample contamination during the clean-up stage is even more important to avoid than contamination during extraction. The clean-up procedure may remove some contaminants from earlier stages but any contaminant added at this stage must eventually reach the GC.

Solvent contamination was again found to be the most important single source, but this has been fully discussed in the extraction section.

Vaseline is the next most important contaminant, and in a laboratory where all types of analysis are being carried out with a common pool of equipment is the most difficult to remove. Sources include ground glass joints on all glass-ware - especially those on the rotary evaporator, and taps on chromatography columns. All glass-ware was scrubbed with very hot detergent solution, steeped in chromic acid and rinsed with deionised water.

To remove any chance of contamination from tecnazene vapour all glass-ware was rinsed in A.R. acetone and gently blown dry just before use.

Plastics of various sorts must be avoided at all times. Teflon and silicone rubber tubing were the only flexible materials of those which were tested which produce no GC peaks. Polyethylene produced very significant contamination.

f) recovery

If all the precautions described are rigoursly adhered to then the recovery from the column clean-up procedure was found to be 100%, calculated over several determinations.

4.2.6. FINAL DETERMINATION BY GAS CHROMATOGRAPHY

1) CHOICE OF COLUMN AND PACKING

Ideally in pesticide residue analysis the GC column should adequately resolve the pesticide from the solvent and any impurities with the minimum possible delay between each injection due to high boiling point impurities with long retention times. Therefore the selection of the various materials which constitute the packed column inevitably involves a series of compromises. There is no single solution to such a complex problem. However the reasoning behind the eventual choice is detailed below.

a) the column

Glass columns are generally more suitable for low-level work as they can be rendered reasonably inert by reaction of any active hydroxyls with hexamethyldichlorosilazane (HMDS) or other trimethylsilyl (TMS) group donating compound. Metal columns can cause adsorption problems. Short columns are preferred which permit rapid elution of high boiling contaminants - 1 and (occasionally) 2 metre columns were used.

b) the support

The best support materials for GC are those derived from diatomaceous earths which have been acid washed and rendered inert with a suitable reagent. Fine materials give better resolution eg. 100/120 mesh will give almost twice the resolution of 80/100. Gas Chrom Q 100/120 mesh was chosen.

c) the stationary phase

Because the GC is operating near maximum sensitivity it is essential that there should be little or no column bleed. The highly stable silicone oils are excellent in this respect and were therefore used throughout this work. For the same reason the amount of stationary phase should be kept below 10% - above this level excessive bleed due to "phase stripping" will occur.

As tecnazene is a fairly polar compound it is retained for too long on polar phases. Non-polar and semi-polar phases are

more suitable. The non-polar material used in this work was OV101- a methyl silicone oil which remains liquid at lower temperatures than the more usual OV1 or SE30. The semi-polar material was OV17, a methyl (50%) phenyl (50%) silicone oil. These materials were loaded at 10% and 5% respectively

The chosen column was therefore a 1m x 6mm OD glass column containing 10% OV101 or 5% OV17 on 100/120 mesh Gas Chrom Q. Figure 4.2.3. illustrates the separation of some organochlorine pesticides on an OV101 column.

2) PREPARATION OF COLUMNS (EXPERIMENTAL)

a) pre-treatment of glass columns

One end of a visibly clean column was connected via a liquid trap to a filter pump and the other to a filter funnel using silicone rubber tubing. It was then treated with the following materials by slowly adding them to the filter funnel whilst applying a gentle vacuum at the other end.

i) 100cm^3 A.R. hydrochloric acid.

ii) 100cm^3 de-ionised water.

iii) 100cm^3 A.R. acetone.

iv) 50cm^3 A.R. toluene.

v) The column was filled with 5% HMDS in A.R. toluene which was allowed to remain in the column for 10 minutes to silylate the active sites.

vi) 50cm^3 A.R. toluene.

vii) 100cm^3 A.R. acetone.

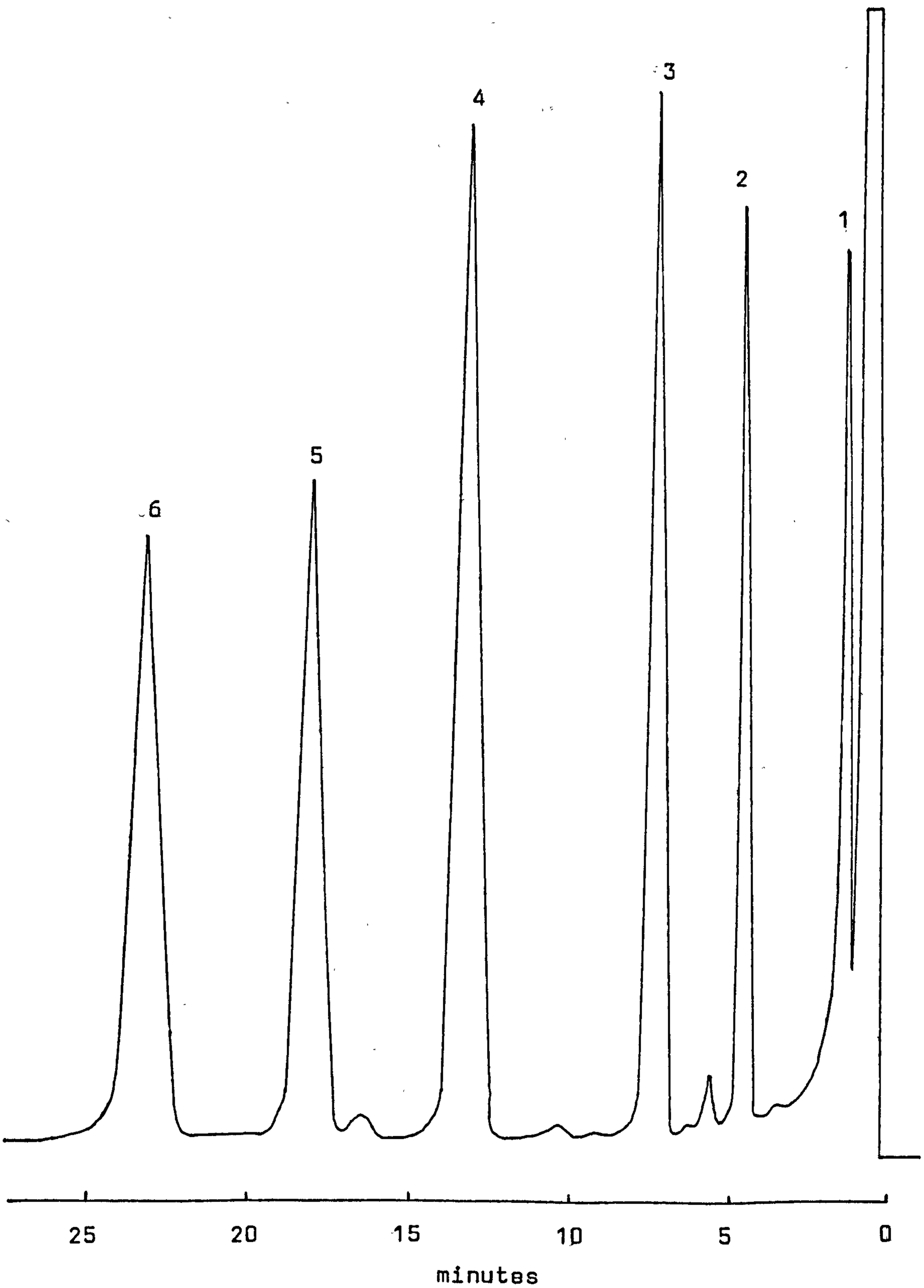
viii) The column was then dried in an oven at 110^{o}C for 20 minutes.

ix) The outlet end was then plugged firmly with a 5mm plug of silanised glass wool. (Prepared by steeping acid washed, dry glass wool in 5% HMDS in toluene for 1 hour and washing with toluene, then acetone, and drying at 110^{o}C)

b) preparation of packing material

i) The appropriate amount of silicone oil (OV101 or OV17) was weighed into a clean 100cm^3 Quickfit conical flask. This

Figure 4.2.3. Chromatogram of some organochlorine pesticides. Conditions: as for tecnazene (see text) except for column temperature which was 225°C, column 10% OV101 on Gas Chrom Q . Peak identification: 1, tecnazene; 2, HCH; 3, aldrin; 4,dieldrin; 5, DDE; 6, DDT.



procedure is made considerably easier if the stationary phase is first warmed slightly so that it can be handled with a Pasteur pipette.

ii) The phase is then dissolved in 75cm^3 of the appropriate solvent (toluene for OV101 and chloroform or toluene for OV17) warming if necessary.

iii) The support material (Gas Chrom Q) was weighed into a clean 1dm^3 Quickfit R.B. flask and made into a slurry with the solvent that was used for dissolving the phase.

iv) The phase solution was then added to the support slurry using 3 x 50cm^3 portions of solvent to ensure quantitative transfer, and the contents of the flask mixed by placing the flask on the rotary evaporator with the condenser switched off and with only enough vacuum to hold the flask securely in place, and allowing it to revolve slowly under these conditions for 30 minutes. Any vigorous shaking of support material can cause it to break down to finer material which leads to flow-rate problems.

v) The vacuum was then increased and the condenser switched on, and the water bath slowly warmed so that the solvent was slowly removed <u>without any frothing</u>.

vi) The flask was then placed in a vacuum oven at 80^{o}C overnight to remove all traces of solvent. The packing was then ready for use or was stored in air-tight containers. It was normally prepared in 25g or 50g batches.

c) <u>packing the column</u>

The filter pump was re-connected to the outlet end of the column and the filter funnel to the inlet end. The packing material was then added to the funnel with continuous vibration of the column using an electric vibrator, until the level of packing was approximately 50mm from the inlet end which was then plugged with 5mm of silanised glass wool. The column ends were then capped to prevent the entry of moisture when the column was not installed in the GC. Columns were replaced after 2-3 months continuous use.

d) conditioning the column

This step is especially important for low-level pesticide analysis as the GC is operated at high sensitivity. The following procedure was used.

i) The inlet end of the column was connected to the carrier gas supply which was then adjusted to 45cm^{3}min.$^{-1}$ The outlet end was left unconnected to avoid contamination of the detector with stationary phase etc.

ii) After sufficient time had elapsed for all the air to be expelled from the column the carrier gas supply was stopped and the oven heated to 250^{o}C for 2 hours. During this time the phase becomes very fluid and can flow around the particles of support covering any areas left exposed during the packing preparation.

iii) The oven was then cooled and the carrier gas flow re-started and the oven programmed to rise from 50^{o}C to 250^{o}C, by 2^{o}Cmin.$^{-1}$ which temperature was maintained for 48 hours. For ultra-low level analysis an even longer period was occasionally necessary.

iv) The oven was then set at 200^{o}C and several 5ul aliquots of BSA (N,O bis trimethylsilyl acetamide) injected. This was found to considerably improve both peak, shape and sensitivity during the early life of the column.

v) The oven was then cooled and the outlet end of the column connected to the detector.

vi) The temperature and the carrier gas flow rate were then adjusted as required and several large injections of tecnazene (µg amounts) made to saturate any active sites remaining.

vii) The column was then ready for use.

3) GAS CHROMATOGRAPHIC CONDITIONS (EXPERIMENTAL)

a) the gas chromatographs

At least 5 different GCs were used during the course of this work but most of the work was completed on the two machines described below.

i) A PYE 104 fitted with dual flame ionisation detectors,heated

injection ports and a workshop constructed detector heater. This machine was modified so that with the addition of an extra amplifier and recorder each column could be operated independently.

ii) A PACKARD (BECKER) 419 fitted with two independent flame ionisation detectors, proportionally controlled injection and detector heaters, rotameter monitoring of all gas flow rates, and full digital control of all temperatures.

The gas lines to both instruments were fitted with molecular sieve filters and the carrier gas lines also had a charcoal filter to remove any organic impurities.

b) GC conditions

The hydrogen flow rates to the FIDs were 40cm^{3}min.$^{-1}$ Oxygen was used as oxidant at 120cm^{3}min^{-1} instead of air as this not only involves less cylinder changing but increases the sensitivity of the FID. Extra care however must be taken during the ignition procedure. The detector was maintained at 225^{o}C in the PYE 104 and 285^{o}C in the BECKER 419. The injection heaters in both were maintained at 225^{o}C.

The selected flow rate of the nitrogen carrier gas for tecnazene analysis was 45cm^{3}min^{-1} and the column temperature 174^{o}C for 1m columns and 195^{o}C for 2m columns (when used). Using these conditions a typical chromatogram of a potato extract containing tecnazene is shown in figure 4.2.4.

The retention time of tecnazene on a 1m column of 10% OV 101 was 3.86 minutes and on a 1m 5% OV17 column was 5.01minutes.

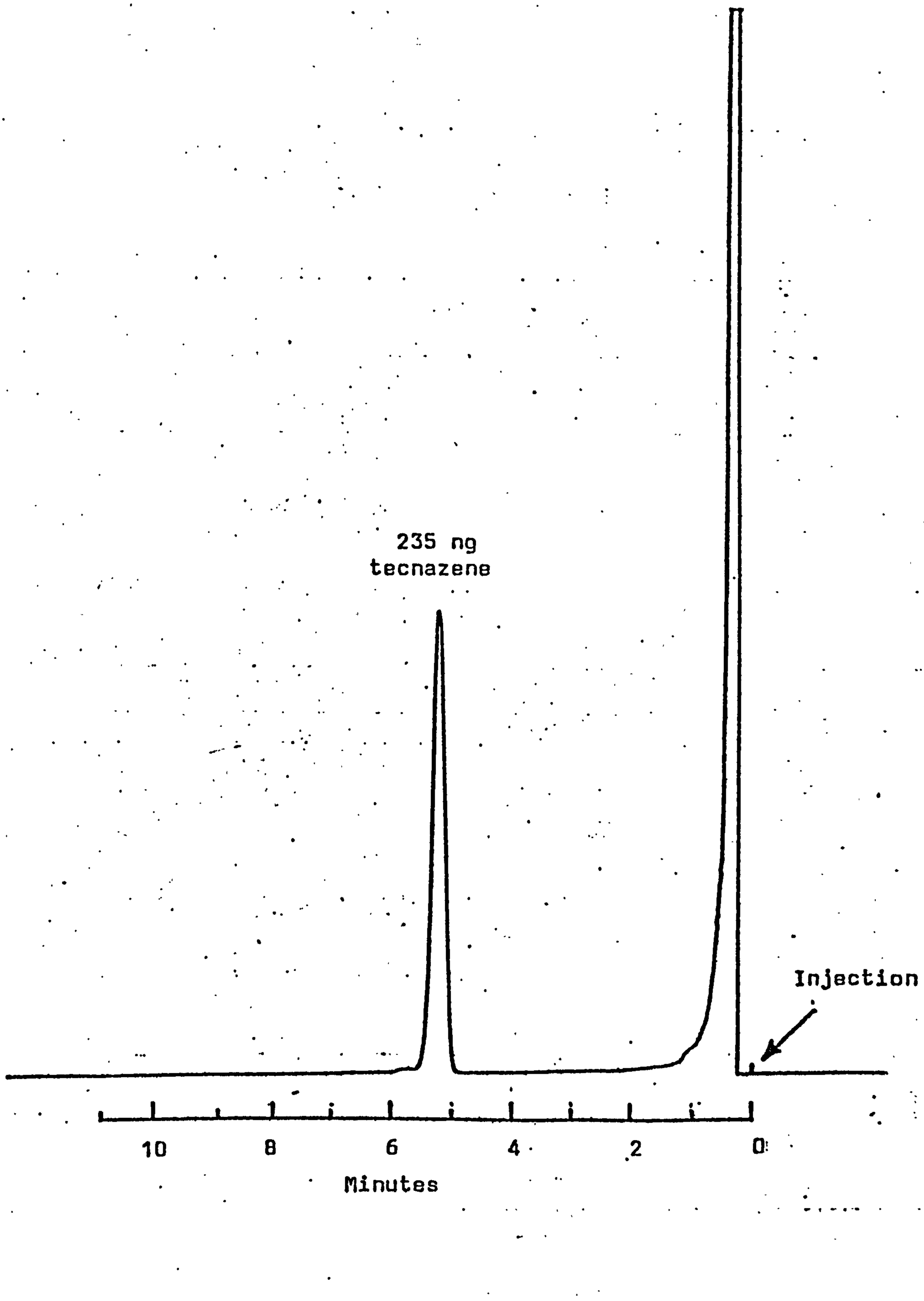
4) SAMPLE INJECTION

Two methods of sample injection were used - manual and automatic. However because of the problems encountered with automatic injection nearly all the results were obtained by manual injection.

a) Manual injection

Of the variety of syringes used during the course of this work, the most consistent results were obtained using Hamilton

Figure 4.2.4. Gas chromatogram of a hexane extract of potatoes which had been treated with tecnazene at a commercial dosage rate and held in store for 11 months. Sample size 0.7ul; column temperature 174°C; column 5%OV 17 on Gas Chrom Q; length 1m; Chromatogram represents 3.17mg of tecnazene/kg fresh weight of potato.



series 7000 syringes which were fitted with a "Reprojector" (Shandon Instruments - now unavailable).

Using these syringes samples of 0.5 - 5µl were injected. The most reproducible results were obtained injecting 0.7µl with a 7001 syringe (i.e. 70% of the syringe capacity). Duplicate injections were made of all samples, and where these varied by more than 3% further injections were made.

b) <u>automatic injection</u>

Because of the large number of samples which had to be analysed - especially during the development work when individual fractions from the fraction collector were being analysed, it was decided to investigate the possibilities of automatic injection.

Automatic syringe injection would have been ideal, but such an instrument can cost almost the same as a complete GC, and was not available.

An automatic solid injector was however available in a form suitable for attachment to the BECKER 419, and is illustrated in figure 4.2.5.

The sample is coated on to small stainless steel spirals and placed in the container which has a capacity for 24 such samples arranged in vertical holes near the circumference of a steel disc. Pressing the lever at the edge of the injection unit operates a ratchet mechanism which moves the disc through one twenty fourth of a revolution, bringing a sample into line with the GC column where it falls to the heated injection port and is volatilised. In automatic operation this mechanism is operated by a pneumatic plunger, connected to a pressurised gas supply controlled by a solenoid valve. The overall timing of the injections is automatically controlled by the discharge of a capacitor whose charging rate is controlled electronically and is variable (External timing unit - Jones Chromatography). This electronic unit will also trigger programmed operation of the GC.

A chromatogram of <u>n</u>-alkanes obtained using this system is illustrated in figure 4.2.6., the most notable feature being the absence of a solvent front.

However several problems arose during the application of this injection system to residue analysis.

Figure 4.2.5. Packard automatic solid injection unit. Illustrated with a metal spiral dropping on to G.C. column.

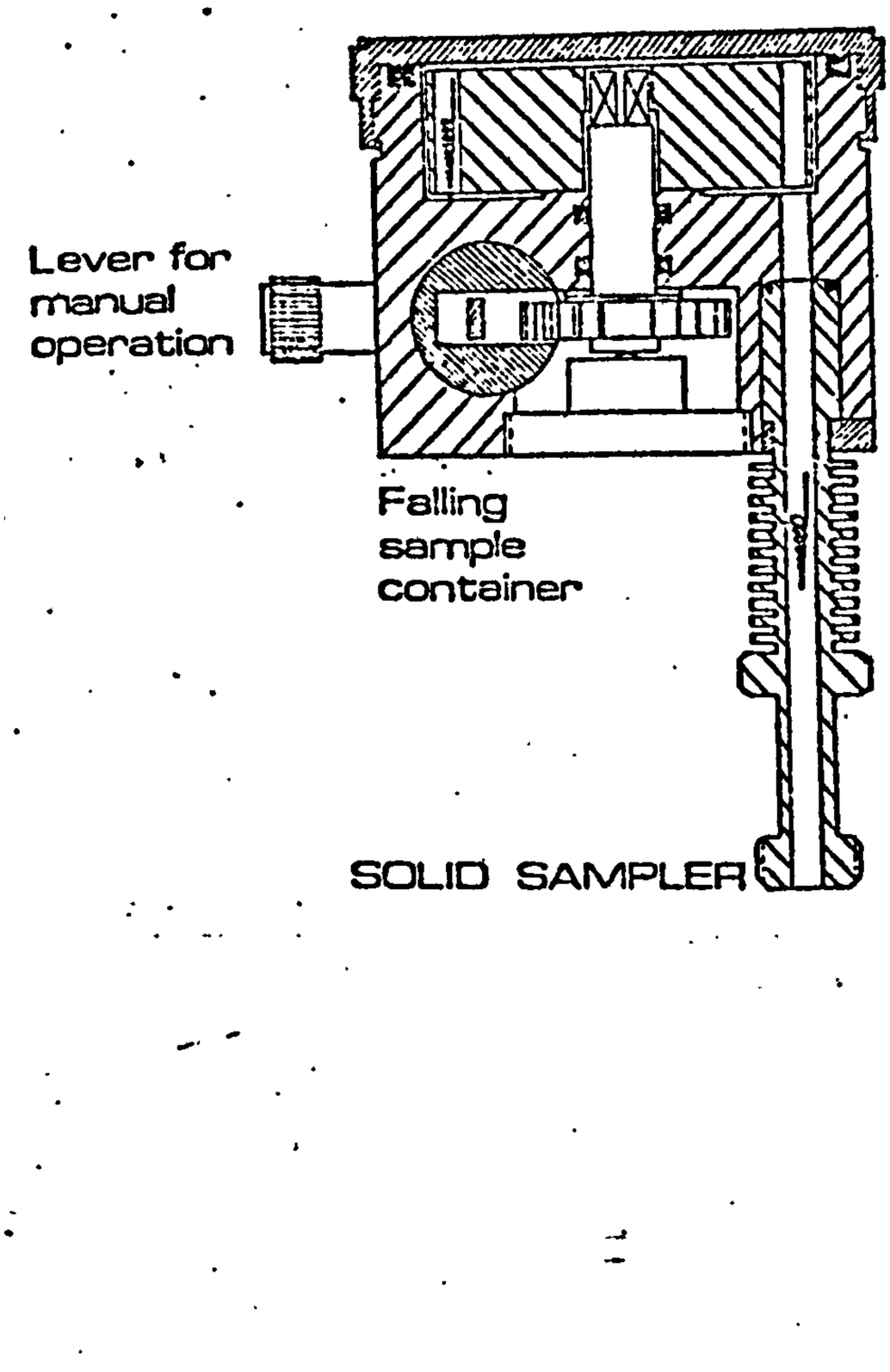
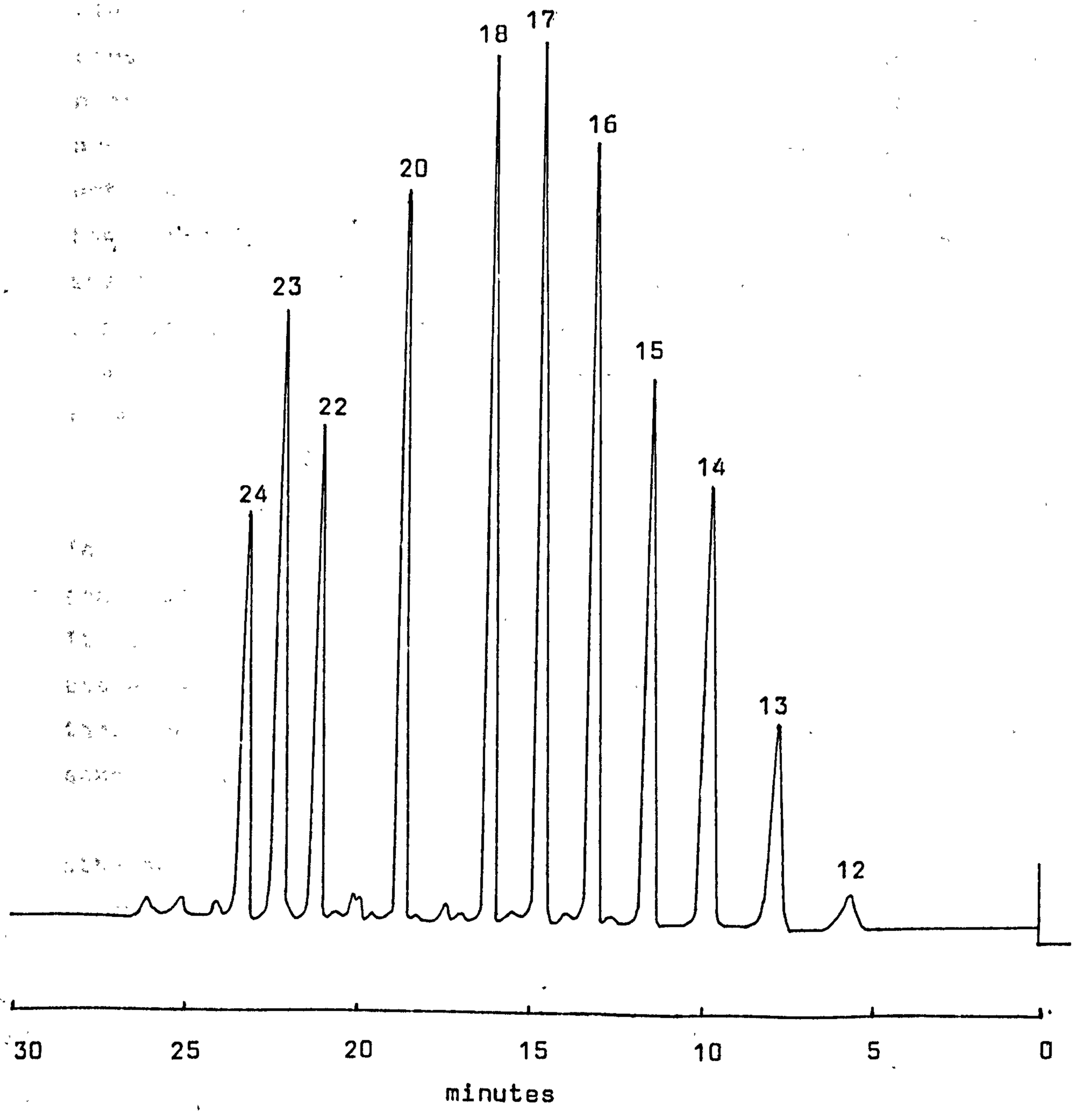


Figure 4.2.6. Chromatogram of n-alkanes injected on a metal spiral using an automatic solid injector. Alkanes: 12,13,14,15,16,17,18, 20,22,23,24 carbon atoms. Conditions: Initial hold at 100°C for 4 min., rising by 8°C min^{-1} to 280°C, column 2m 5% OV17 on Gas Chrom Q.



i) sample size

Sample size presented a double problem. Firstly the absolute size of the sample was unknown as sample spirals were prepared by dipping the metal spiral into the test solution and allowing the solvent to evaporate. This was overcome by the incorporation of HCH as internal standard and using relative weight response calculations.

The second problem was more difficult to solve. Because of the technique of sample preparation, the sample load and hence peak height was very variable. Some peaks in any run would be off scale and others too small to measure with the degree of precision required. An automatic attenuator and electronic integrater would have solved this problem but was not available.

Applying liquids to the spirals with a Hamilton syringe has to be accomplished very slowly so this solution was unacceptable.

It was found however that small glass tubes with one end closed were suitable for injection by the system. These were constructed by holding the end of a melting point tube with forceps, heating a section 5-15mm from the end in the flame of a micro-bunsen and drawing out. The tail of the very short piece was then heated until it ran into a small sphere at the end of the micro-tube. Samples of known volume could then be injected into these tubes. It was found that with suitable racks, 24 such samples could be prepared in less than 10 minutes. The solvent was then allowed to evaporate in an air or nitrogen stream at room temperature and the tubes placed in the injector.

ii) sample loss

It soon became apparent that samples prepared in either fashion (spirals or tubes) lost some of their more volatile components before they reached the GC column - tecnazene included. It was found that this loss occurred in two stages. Firstly there was a small loss during the solvent evaporation period and secondly there was a marked loss while the samples awaited injection in the sample disc.

To avoid this problem an aluminium cooling block was attached to the sample holder and an ethylene glycol - water mixture at $-15^{o}C$ was pumped through the block using a high output

centrifugal pump. A polyurethane foam block was cut to snugly fit the whole injection unit for insulation, and coolant lines were insulated using "Armaflex" insulation. These modifications resulted in a sample storage temperature of $0^{o}C$. Cooling below this level produced condensation problems when the sample chamber was opened.

Samples were then prepared by injecting into tubes and immediately installing them in the cooled chamber with their solvent. This however produced an excessive standing current in the detector due to very slow evaporation of solvent into the GC column. This problem was solved by introducing a very slow reverse bleed of carrier gas ($2cm^{3}min^{-1}$) into the injection unit and hence via a sensitive gas control valve (Brooks) to atmosphere.

Using this system it was possible with reasonable reproducibility to inject tecnazene samples automatically at 15 minute intervals over a period of several hours. Figure 4.2.7. shows the results of a reproducibility trial for tecnazene.

This system was therefore used during development work with some success but because of the instrument's eventual complexity and high maintenance requirement (pumps, chillers, valves, etc.) and because more reproducible results could be obtained using manual syringe injection it was rejected for routine potato sample analysis. It is worth noting however that the injector will produce good reproducibility in its standard state with high boiling point materials such as DDT.

5) <u>INTERPRETATION OF CHROMATOGRAM</u>

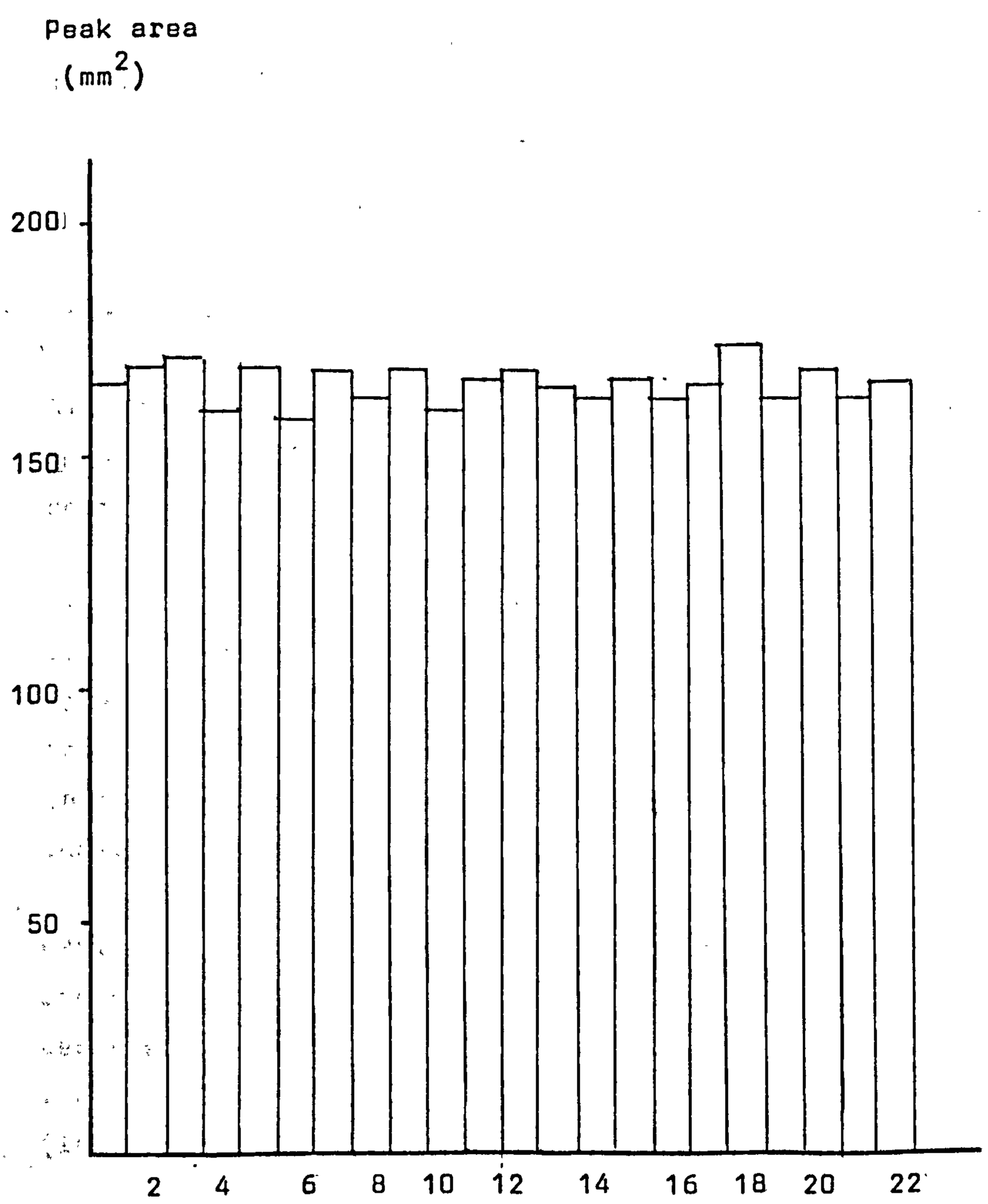
Various methods of quantifying peak area were tried including

1) using a planimeter
2) cutting out peaks and weighing
3) electronic integration
4) triangulation

The first two methods were found to be unsatisfactory unless very high chart speeds were used.

Electronic integration using a Kent Chromolog I was found useful only at high residue levels ($1.0mg\ kg^{-1}$) as for this particular integrator very flat base lines are necessary. Newer models of course could cope with this problem.

Figure 4.2.7. Reproducibility of automatic solid injector.
Repeated injections of 1µg of tecnazene (see text).



The method chosen was triangulation i.e. measuring peak height and width at half height and calculating the area of the peak. Faster chart speeds however than that illustrated in figure 4.2.4. were normally preferred for increased accuracy.

Some very recent statistical work (not included in this thesis) has indicated that for most pesticide work errors would have been even lower if peak height alone had been used in conjunction with computed response curves (linearity may no longer be assumed).

Using triangulation, peak areas for a series of standard injections were calculated and a standard curve (linear) constructed for 50 to 1000ng injections of tecnazene. Peak areas were similarly calculated for samples and the amount of tecnazene injected read from the standard curve. A typical standard curve is illustrated in figure 4.2.8.

6) ELECTRON CAPTURE DETECTION

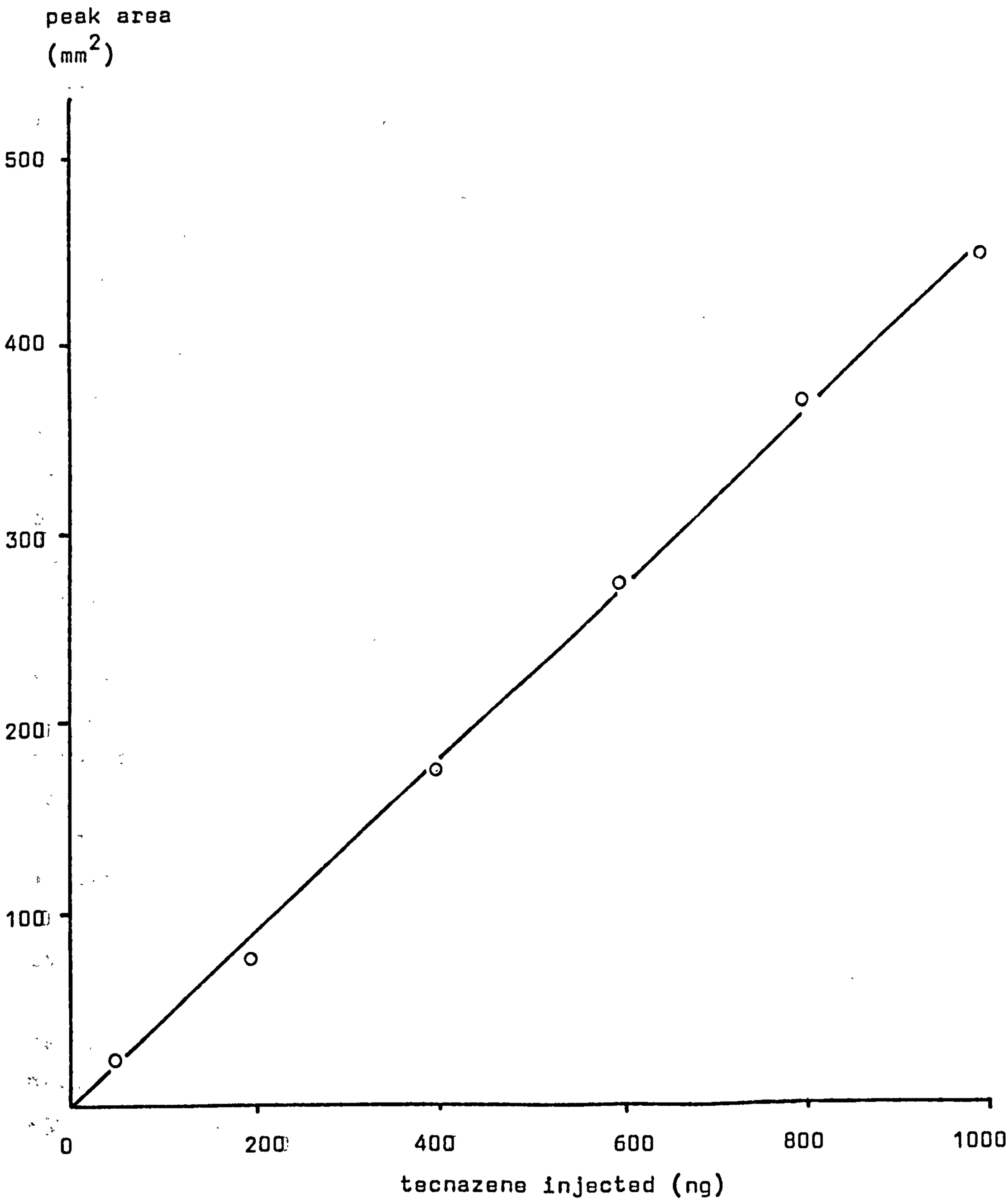
For some of the later work a GC fitted with an electron capture detector (ECD) was available and was used when necessary.

The gas chromatograph was a PYE GCD with a ^{63}Ni detector. Nitrogen supplied through copper tubing was used as carrier gas as copper tubing is impermeable to atmospheric oxygen which is very detrimental to detector performance.

Chromatographic conditions were as for FID instruments. Extra care however must be taken to ensure sample purity and to minimise column bleed as once contaminated an electron capture detector is difficult and expensive to fully decontaminate. Partial decontamination has been achieved by fitting an empty column and injecting 25µl aliquots of a 1:1 mixture of benzene and pyridine under normal running conditions.

The ECD is however an extremely useful instrument in estimating low-level tecnazene residues as it is selective towards organo-chlorine compounds, and it is especially sensitive towards tecnazene. In fact during the course of the work described in this thesis no other compound tested has produced such high responses (including HCH)

Figure 4.2.8. A tecnazene standard curve (flame ionisation detector).



When optimum conditions prevail as little as 0.1Pg can be quantitatively detected, increasing the sensitivity of the technique as a whole to 0.000001mg kg^{-1} (1ng kg^{-1}). Routinely the electron capture unit was used for injections in the range 0-1000Pg. Injection of more than 1000Pg causes saturation, so most samples have to be diluted for analysis.

A chromatogram of a typical tecnazene containing potato extract is shown in figure 4.2.9.

The response of an electron capture detector can only be considered linear over a very narrow range. It was found more accurate not to assume linearity at all but to fit the best curve and calculate results from this. In the early work this procedure was carried out graphically using a "Flexicurve".

However because a more precise method was desirable and as a new curve has to be constructed every day, a computer programme has been written for this purpose.

As an initial step a Fortran programme was written to conduct a search amongst various mathematical transformations for one which produced a good linear fit.(very similar to the one used for dose-response curves of sprout suppressant action). Of those initially tried, taking logarithims of both injected tecnazene and peak area (i.e. both X and Y axes) produced correlation coefficients (r) of 0.95 and better. This transformation was therefore used in the routine programme.

This programme not only computes the best fitting log-log regression line for the standard data, but also using a computer linked graph plotter produces a standard curve on linear axes. It also accepts sample data and produces a point and interval estimate (at the 5% level) of sample concentration taking into account the error involved in the standard curve and the error involved in the analysis of a single sample.

Figure 4.2.10. illustrates a standard curve produced in this manner.

Very recent statistical analysis of electron capture data has shown that this method can be even further improved by using peak height data alone when the best fitting line ($r>0.98$) becomes $y=ax^{0.75}$ where y is peak height and x is injected tecnazene.

Figure 4.2.9. Chromatogram of tecnazene extract of potatoes using electron capture detection. Sample cleaned up on alumina and volume adjusted to 50cm^3. Injection volume 0.5µl. Column: 5%OV17 on Gas Chrom Q. Peak is equivalent to a concentration of 0.23mg kg^{-1} in potatoes.

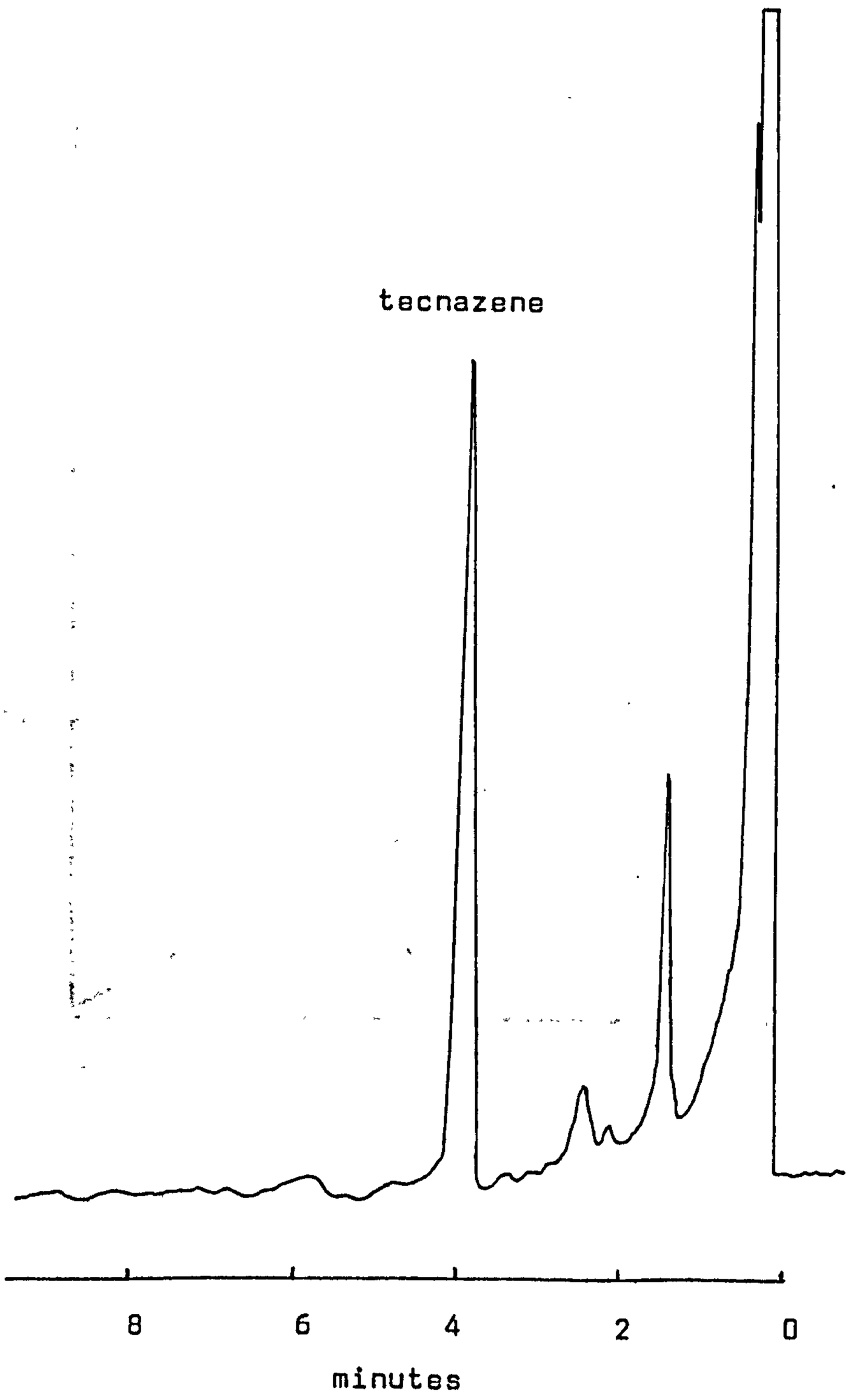
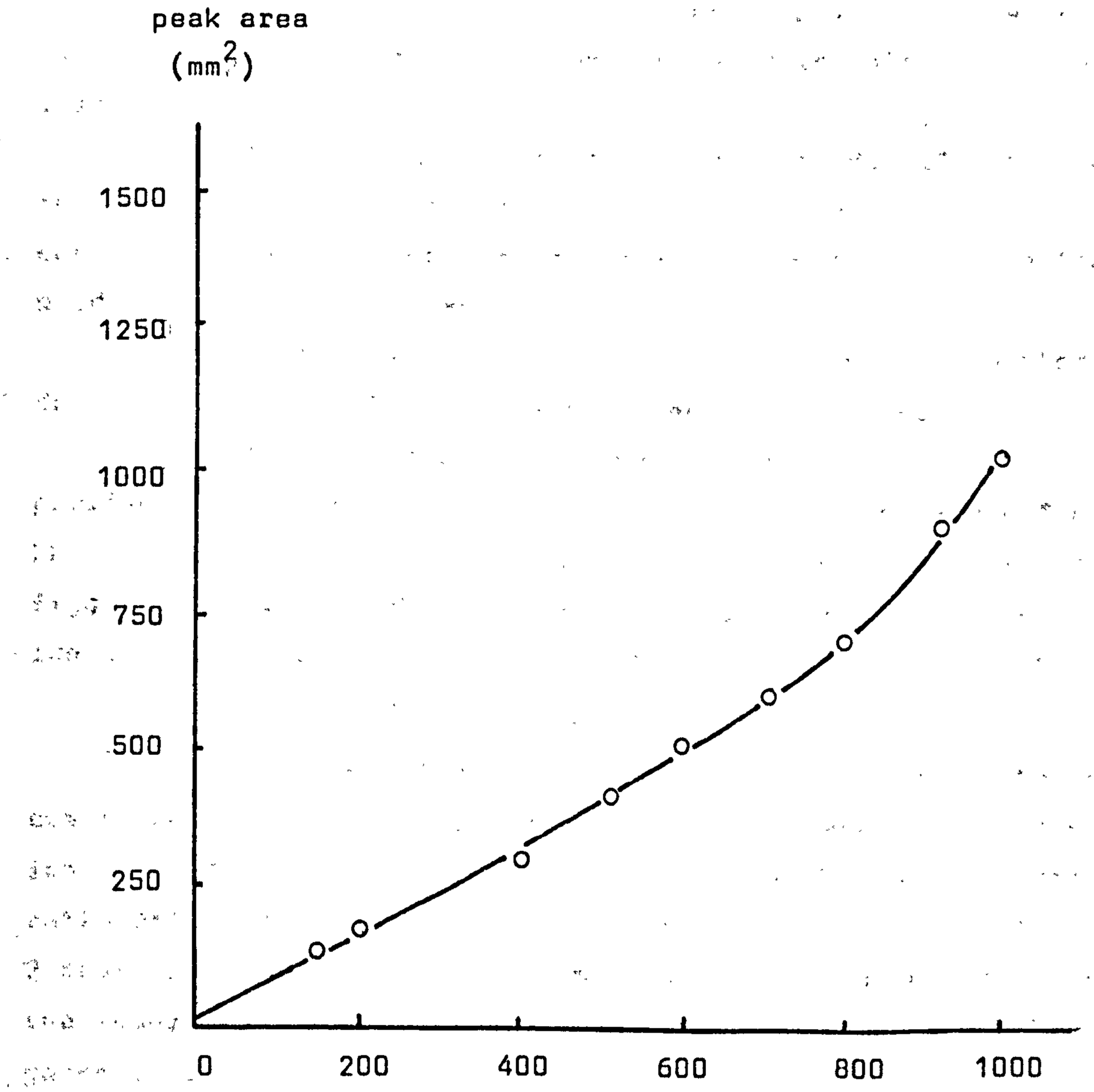


Figure 4.2.10. A tecnazene standard curve determined on a Pye GCD with electron capture detection.



Electron capture is then a very useful detection technique but has some disadvantages. These detectors are notoriously unstable, suffer from day-to-day variations, require a higher number of standard injections because of non-linearity and are easily contaminated. For these reasons it normally takes twice as long as FID analysis to process a given number of samples.

It was therefore only used on a limited number of occasions when its properties of high selectivity and high sensitivity were required.

7) GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GCMS)

Although a mass spectrometer can be used as a very sensitive detector for gas chromatography by focussing on a single characteristic peak it was not used in this manner during this work.

However on several occasions the identification of gas chromatographic peaks as tecnazene was confirmed by GCMS in its more conventional role of producing a complete spectrum of the fragments produced by electron bombardment.

Samples with chromatographic data were submitted to the departmental LKB GCMS service and spectra obtained.

The mass spectrum of pure tecnazene which has been published (Hutzinger et al) was similar to that obtained from the LKB GCMS unit which is illustrated in the form of a line diagram in figure 4.2.11. The characteristics of this spectrum make it easily identifiable and the most important of these are discussed below.

a) isotope distribution

The molecular ion region contains a cluster of peaks due to chlorine isotopes. The relative abundance of the common isotopes of chlorine are ^{35}Cl-75% and ^{37}Cl-25% i.e. they are in the ratio 3:1. This means that if a compound contains one Cl atom, 2 peaks can be expected in the molecular ion region - the one with the lower m/e value is referred to as M and the other as M+2. These peaks should be in the ratio 3:1.

Should a compound contain two Cl atoms then of course a molecule can contain two ^{35}Cl, one ^{35}Cl and one ^{37}Cl, or two ^{37}Cl atoms, producing peaks at M, M+2, and M+4 respectively. The relative

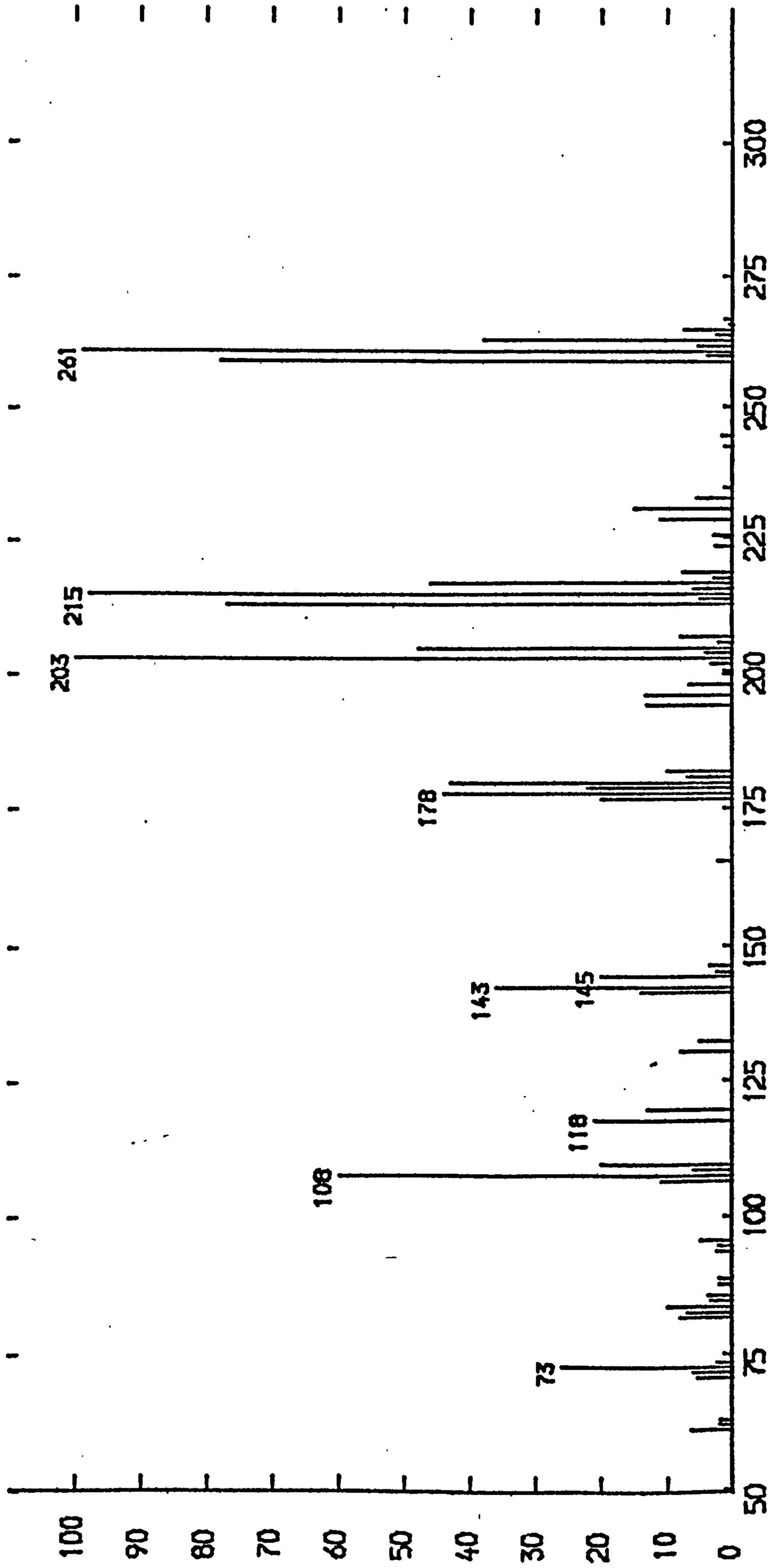


FIGURE 4.2.11

MASS SPECTRUM OF TECNAZENE

heights of these peaks should be 9:6:1. This ratio can be calculated by expanding the expression $(a+b)^x$ where a and b are the relative abundances of the two isotopes and x is the number of chlorine atoms.

Thus for tecnazene with 4 chlorine atoms the expression becomes $a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$, and by substituting the relative abundances the ratio of M:M+2:M+4:M+6:M+8 becomes 81:108:54:12:1 which is a very characteristic pattern. The observed ratio of the peaks at m/e 259, 261, 263, 265, and 267 is almost exactly this, allowing for the slight effect of ^{13}C on the lower peaks.

Isotopic distributions of fragments of tecnazene also have such distribution patterns according to the number of chlorine atoms they contain.

b) <u>fragmentation</u>

Detailed study of fragmentation patterns can be a fairly complex subject. However with tecnazene there is one very obvious fragment which is lost - namely NO_2, reducing the molecular ion's m/e ratio by 46 units and producing the characteristic 4 chlorine isotopic distribution of peaks at m/e 213 etc. More detailed studies show the loss of Cl from both these and the molecular ion peaks.

c) <u>doubly charged cations</u>

With compounds like tecnazene which because of the large electronegative groups and ease of charge delocalisation, doubly charged molecular ions are a possibility. If the m/e ratio of the molecular ion is odd as in the case of tecnazene then peaks are produced at fractional m/e ratios. Tecnazene shows very small peaks at m/e 129.5 and m/e 130.5. (They are so small that they have not been reproduced on the line diagram)

Using these characteristics (and of course the M.W.) GCMS was used to positively identify tecnazene even at very low levels. This was especially useful when analysing unknown samples.

4.2.7. THE FINAL METHOD

The following method evolved from the work described in the previous sections.

a) Extraction

1) Samples of potatoes were taken by coring, quartering or masceration after copious washing of tubers with water.

2) A 100g sample was homogenised with 100cm^3 ethanol for 1 minute in a high-speed blender.

3) 100cm^3 of hexane were added to the homogenate and the contents blended for a further 2 minutes.

4) The blender contents were filtered under reduced pressure through Whatman No.1 filter paper and the vessel rinsed with two separate 50cm^3 portions of hexane which in turn were passed through the filter pad.

5) The filtrate was transferred to a separating funnel using two 10cm^3 washings to attain quantitative transfer.

6) 100cm^3 of saturated sodium chloride were added and after shaking the aqueous layer was discarded.

7) The hexane layer was washed with 200cm^3 of 10% (w/v) sodium carbonate solution followed by a 200cm^3 deionised water wash.

8) The hexane layer was then collected and dried over anhydrous sodium sulphate.

9) After filtering off the sodium sulphate and washing the residue with 100cm^3 of hexane the combined filtrate was reduced in volume to approximately 2cm^3 using a rotary evaporator and keeping the water bath temperature under 40^{o}C.

b) Clean-up on alumina

1) Alumina columns were prepared by pouring a hexane slurry of alumina into a glass column of dimensions 300mm by 9mm and fitted with a glass sinter. The columns were packed to a depth of 150mm.

2) The extract was applied to the column surface quantitatively, using the minimum volume (2-3cm^3) of hexane washes and allowing the level to drop to the column surface before each addition. The flow rate was constantly maintained at 1.0cm^{3}min^{-1}.

3) The column was eluted with hexane and the first 50cm^3 of effluent discarded.

4) The subsequent 100cm^3 were collected and the solvent removed under reduced pressure, again maintaining a bath temperature of less than 40^{o}C, either to :-

i) dryness and the residue dissolved in 1cm^3 of hexane or preferably ethyl acetate, or

ii) very small volume and then made up to 1cm^3,2cm^3,5cm^3 etc. in a volumetric flask with ethyl acetate. The size of flask chosen was dependant on the expected residue level.

c) Gas chromatography

Samples of 0.5 to 5µl of extract were injected, using a Hamilton series 7000 syringe, into the GC fitted with FID detectors and silanised glass columns 1m x 6mmOD containing 10% OV17 on 100/120 mesh Gas Chrom Q. The oven temperature was 174^{o}C, injection heaters 225^{o}C and detector heaters at least 225^{o}C. Carrier gas flow-rate (nitrogen) was 45cm^{3}min^{-1}. Hydrogen 40cm^{3}min^{-1} and oxygen 120cm^{3}min^{-1}.

Under these conditions tecnazene had a retention time of 3.86 minutes on OV101 and 5.01 minutes on OV17 columns.

Positive identification was ensured by comparison of retention times on both columns with those of authentic samples. Concentrations were calculated using triangulated peak areas and comparison with a standard curve.

Confirmation of peak identity can be achieved using GCMS and low-levels quantified using electron capture detection.

d) Recovery factors

To determine overall recovery factors 1mg amounts of tecnazene were applied to five separate lots of diced potatoes prior to blending and isolation carried out as above using FID detection.

Recovery was found to be 91.6% s.d. $\pm$ 2.8 from potato samples and similar figures were recorded with other plant tissue viz. lettuce. All results subsequently obtained were corrected for this recovery factor.

e) Minimum detectable amounts

Spiked extracts containing only 1µg of tecnazene (i.e. equivalent to 10µg kg^{-1} or 0.01ppm in the potato) resulted in

similar recovery and could be accurately determined. However 0.1µg (i.e. equivalent to 1µg kg^{-1} or 0.001ppm) resulted in some loss of accuracy because of unstable base lines at very high sensitivities. For such quantities the electron capture detection was found to be more suitable (see earlier).

Some of the work described in this section has been published (Dalziel and Duncan, 1974).

4.3. RESIDUES IN LABORATORY TREATED AND COMMERCIAL SAMPLES

4.3.1. LABORATORY TREATED SAMPLES

Many laboratory treated samples were eventually analysed. This section includes only those from the 1972-73 storage season.

a) Experimental

50kg of freshly harvested potatoes cv Golden Wonder were treated with a commercial 3% dust formulation of tecnazene (Fusarex-Plant Protection Ltd.) at the recommended rate of 135mg kg^{-1} of active ingredient (4.5kg of formulation per tonne) by sprinkling the dust on successive layers of potatoes as they were placed in a wooden box of approximately 60kg capacity. The box was then covered with a loosely fitting lid and stored at 10°C in a constant temperature room. Samples of six tubers were removed at intervals and stored in a deep freeze at -18°C until required for analysis.

They were then washed, sampled by quartering and analysed by the method described above using FID detection.

b) Results

The results are shown in table 4.3.1.

Table 4.3.1. Tecnazene residues in laboratory treated samples

Months after tecnazene treatment	Tecnazene residue (mg kg^{-1} fresh weight)
2	4.54
5	1.97
11	3.17

c) Discussion

Too much weight should not be placed on the fluctuations in these results as so few samples were analysed. It was felt that the sample taken did not cope with the wide variations in tecnazene distribution found in dust treated tubers. Wilson and Dawson (1953) experienced similar problems. The results do however indicate that the levels of tecnazene found in treated potatoes are significant and worthy of more detailed examination.

4.3.2. COMMERCIAL SAMPLES

To check that laboratory experiments were producing comparable results to those found in large scale potato stores, commercial samples were obtained from a local farm and similarly analysed.

One of these samples (cv Maris Piper) had only been partially treated with tecnazene i.e. for reasons of economy only the top metre had been treated. This in fact a very common practice in Scotland. The results are shown in table 4.3.2.

Table 4.3.2. Tecnazene residues in commercial samples

Cultivar	Months after tecnazene treatment	Tecnazene residue (mg kg^{-1} fresh weight)
Maris Piper	4	2.64
Golden Wonder	1	7.66

The results from the laboratory experiments therefore lie within the range which may be found in commercial practice.

Both sets of results (laboratory and commercial) are apparently higher than those obtained by Wilson and Dawson (1953) upon analysis (by their polarographic method) of samples treated at the same rate (134mg kg^{-1}). However the two methods are not exactly comparable.

Their method involved no water washing of tubers and in fact in some of their experiments they make a deliberate attempt not to dislodge any surface accumulations. Using this technique one would have expected their figures to be very much higher.

However their analysis procedure, as was previously mentioned, estimates surface residues only (Webster and Dawson, 1952) and although they failed in their attempt to locate any residues below normal peeling depth the magnitude of the results from the present work suggested that it would be worth investigating the distribution of tecnazene within the tuber using the new analysis technique.

4.4. DISTRIBUTION OF TECNAZENE WITHIN THE TUBER

4.4.1. COMPARISON OF PEELED AND UNPEELED SAMPLES

Various samples were analysed before and after peeling, using the standard analysis technique with FID detection. The results of these analysis are shown in table 4.4.1.

Table 4.4.1. Residues in peeled and unpeeled tubers

Cultivar	Time since treatment (months)	Tecnazene residue		% Reduction by peeling
		Unpeeled	Peeled	
Pentland Crown *	3	2.91	0.21	92.8
King Edward*	5	1.10	0.16	85.4
Golden Wonder +	11	3.17	0.16	95.0

* Peeled using an electric abrasive peeler with a peeling loss of approximately 11%

\+ Hand peeled using a domestic peeler producing a peeling loss of approximately 15%

It is obvious from these results that a major part of the tecnazene present must lie very near the surface of the tuber. The amounts

found in the peeled tubers although small are still highly significant in comparison with other pesticide residues. Their relatively low magnitude probably accounts for the fact that Wilson and Dawson (1953) were unable to detect any residue below normal peeling depth.

Because most of the residue is near the surface of the tuber the possibility of using peelings only in future analysis was considered. The relatively high concentration of tecnazene in this material would certainly have been easier to analyse. However this idea was eventually rejected as it is very difficult to peel a potato in a standard fashion and hence allow comparison between results i.e. a figure of x mg kg^{-1} of peelings is almost meaningless because the composition of peelings is very much dependent on how the operation is carried out. On the other hand the analysis of whole tubers, although more difficult,,gives a value which is independent of any human bias, provided adequate sampling procedures are followed.

4.4.2. COMPARISON OF RESIDUES AT DIFFERENT DEPTHS WITHIN THE TUBER

As peeled tubers were shown to contain tecnazene residues the distribution of tecnazene at different depths from the tuber surface was further investigated.

Experimental

Tubers of 80mm diameter (cv Pentland Crown), which had been treated 4 months previously at the standard rate (135mg kg^{-1}) and stored at $10^{o}C$, were selected for analysis.

As the levels expected in some samples were low, ECD detection was used, and the more concentrated samples diluted where necessary.

Washed tubers were peeled as evenly as possible until the diameter was reduced by 20mm. The peelings from different tubers were bulked and sampled and the process repeated. The peelings were extracted in the normal manner.

Great care was taken to ensure that no tecnazene could be transferred on to the newly exposed surface. The peeler was repeatedly washed in A.R. acetone and new disposable gloves were worn for each 10mm layer which was removed.

Results

The results are shown in table 4.4.2.

Table 4.4.2. The distribution of tecnazene within potato tubers

Depth (mm)	Tecnazene residue (mg kg^{-1})	*Volume as % of whole tuber	+Residue contribution to an overall residue figure
0-10	8.2	57.8	96.30
10-20	0.54	29.7	3.23
20-30	0.15	10.9	0.33
30-40	0.23	1.6	0.07

* This figure was calculated by assuming the potato to be a sphere and expressing the differences between successive spheres as a % of the total volume.

\+ Calculated by multiplying the % volume by the tecnazene residue concentration, summing the products for all layers, and expressing each product as a % of the total.
Both figures would of course be slightly different for different sized tubers.

Discussion

Again these results indicate that the outer layer contains the majority of the residue and that there are small but significant amounts of tecnazene distributed throughout the tuber.

Similar analysis by various undergraduates in this laboratory under close supervision have confirmed this general pattern. In every case tecnazene has been isolated from the centre of the tuber.

It is interesting to compare this distribution with those obtained with other sprout suppressants.

Comparison with maleic hydrazide is of course invalid because its method of application demands translocation to the tuber.

MENA appears to penetrate to a depth of at least 10mm (Vadimov and Shtenberg, 1953) leaving a whole tuber residue similar to that of tecnazene (Denny, 1942)(See 3.3.1.3.).

With chlorpropham Van Vliet and Hertog (1966) detected no residue below a depth of about 8mm (See 3.3.2.3.)

The penetration of tecnazene into tuber tissue is therefore not unique although the depth to which it can be found is unusual. However with improved analysis techniques it is very likely that such penetration could now be detected for other volatile post-harvest applied suppressants.

The mechanism by which this penetration occurs is unknown, although from the distribution gradient and from brief translocation studies described later it would appear that diffusion is the most likely method (as with chlorpropham). However it is worth noting that MENA concentrates in meristematic tissue indicating that potato tissue has a role in the transport of some compounds and should not be discounted. No attempt was made in the current study to analyse the different anatomical regions of the tuber.

4.5. ANALYSIS OF FRIED PRODUCTS

The analysis technique described in section 4.2.7. is suitable for fresh or frozen tissue which contains relatively low amounts of fat. However deep-fried products of sliced potatoes (crisps) and the ever expanding range of deep-fried extruded potato products, contain large quantities of vegetable oil or fat. Crisps for example contain 35-40% vegetable oil so extraction of even a single 25g packet will produce 10g of oil, and such a quantity will grossly overload the clean-up column.

It was obvious therefore that the technique would have to be modified or a new technique developed to cope with this problem. Four different approaches were considered.

1) Saponification

The sample can be saponified by refluxing with alcoholic potassium hydroxide and the pesticide removed by organic extraction from the reaction mixture after dilution with water. This method however requires that the pesticide be very stable towards base.

Tecnazene is not (see section 4.2.) so this approach was rejected.

Retrospectively it is now felt that this method was deserving of closer scrutiny. If it is possible to determine DDT as its hydrolysis product DDE by this method, then it might be possible to determine tecnazene as its hydrolysis product(s), although a personal preference is held for methods which determine the intact molecule.

2) Solvent partitioning

The differing solubilities of impurities and pesticides are often exploited in pesticide clean-up procedures. The work described below although never coming to fruition in the form of a satisfactory analytical technique is nevertheless worth summarising as the information on the distribution of tecnazene between various solvents could prove useful.

The concept behind this work was to select a solvent which would extract tecnazene from another immiscible solvent which should retain the offending fat. In practice however this was found to be very difficult as the solubilities of both are similar.

Two very polar solvents which are immiscible (almost) with hexane were selected for this work, namely acetonitrile and dimethyl formamide (DMF). DMF is the more polar but is difficult to purify as it slowly oxidises (Perrin et al, 1966).

Originally it was intended that the method developed should be able to cope with both crisps and chips. As chips contain both water and oil a simple hexane extraction was rejected because of penetration problems.

Experimental and Results

The conventional extraction process was therefore used (see 4.2.7.), the only modification being that in the case of crisps (which contain very little moisture) 50% aq. ethanol was used instead of pure ethanol, to ensure the production of two phases and that the tecnazene was in the hexane. The stated quantities were used to extract 25g (1 packet) of crisps.

The hexane extract was then subjected to two DMF extractions, the first removing 84.5% and the second a further 13.1% of the tecnazene present. Thus 97.6% of the/

tecnazene could be removed from the hexane extract by two DMF extractions.

As DMF and acetonitrile are difficult to remove without loss of tecnazene because of their high boiling points it was decided to "push" the tecnazene back into fresh hexane by adding water. Various partitioning experiments showed that 40% water was required in the solvent to give near quantitative recovery in one extraction. However when this system was applied to crisp samples serious emulsion problems were experienced, which could only be partially cured with the addition of $2cm^3$ of acetic acid.

The hexane extract produced in this manner still contained too much fat for application to the normal clean-up column. Therefore although the overall recovery rate to this stage was good (approx. 85%) it was considered that the small improvement in sample purity did not justify the time and effort involved and so this approach was abandoned.

3) Modified column clean-up

Because the existing column clean-up was unsatisfactory due to overloading and because removal of the fat at an earlier stage was proving difficult it was decided that larger columns should be tried.

Experimental and Results

An alumina column was therefore prepared of dimensions 150mmx20mm i.e. approximately 5 times the surface area of the conventional column.

A spiked crisp extract was prepared as described in the previous section, applied to the column, eluted with hexane at $4cm^3min^{-1}$ and fractions collected and analysed for tecnazene by GC and for fat by weighing the tubes after the solvent had evaporated, cleaning them and reweighing.

Although fat and tecnazene were found to overlap there was sufficient evidence to suggest that some separation might be possible with even lower column loading. However time did not permit further investigation of this method although in future it might prove useful when coupled with ECD detection described below.

4) Selective detection by electron capture

The electron capture detector is highly sensitive and highly selective towards tecnazene as has been already explained. It produces a very low response to compounds with low electron affinity. This property was therefore exploited in the analysis of these most difficult samples with some success as is described below.

Firstly the decision was taken to abandon for the time being the search for a method for the analysis of all fried products and to limit the current investigation to crisps. This meant that the extraction method could be considerably simplified.

Experimental and Results

A 25g sample was therefore crushed into an extraction thimble and then placed in a Soxhlet extraction unit, and extracted with 75cm^3 of "nanograde" hexane for four hours. The condenser, Soxhlet extraction unit and 150cm^3 flat-bottomed flask were scrupulously cleaned before each analysis.

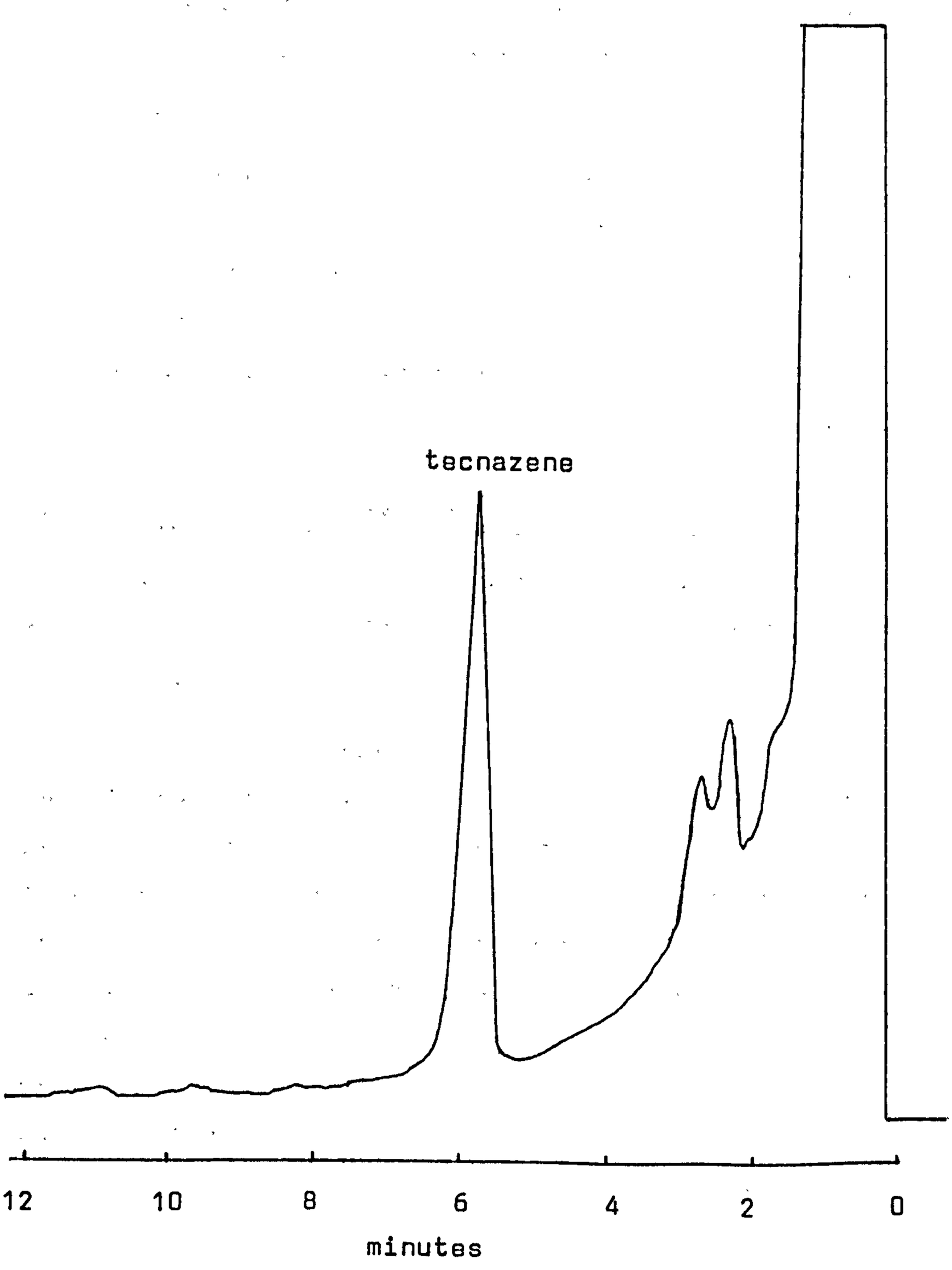
When cool the condenser was washed into the flask, with a few cm^3 and the extract was transferred quantitatively to a 100cm^3 volumetric flask and made up to the mark with hexane. 1µl aliquots of this material were injected into the GC fitted with the ECD (See section 4.2.6. for details) which was set at very high sensitivity, giving a working range of 0-10Pg injected. This ensured that only very small amounts of sample impurity were injected. Such a chromatogram of a crisp sample is shown in figure 4.5.1.

Recovery from spiked extracts was greater than 95% and repeated extraction of samples produced no significant amounts of tecnazene.

The procedure works well but unfortunately repeated use can lead to contamination of the detector. Therefore although it was used for the analysis of the samples below it is not recommended as a routine method.

Recent work indicates that a reduction in sample size to less than 5g followed by alumina clean-up on large columns and GC at high sensitivity will probably solve this problem, although the details of this technique have yet to be finalised.

4.5.1. Chromatogram of hexane extract of crisps made from tecnazene treated potatoes. Column: 5% OV17 on Gas Chrom Q, temperature 165°C: peak equivalent to 0.6mg kg^{-1} of crisps.



4.6. TECNAZENE RESIDUES IN COOKED SAMPLES

Because of the volatility of tecnazene it was considered likely that cooking might influence residue levels. Various experiments were therefore conducted to investigate such effects. As peeling is normally the first step in any preparation for human consumption, some peeling results are included so that losses at different stages in preparation are more easily compared (for discussion on peeling see 4.4.1.)

4.6.1. RESIDUES IN PEELED AND BOILED POTATOES

Residues in whole, peeled and boiled samples were determined by the method described in 4.2.7. using FID detection. Peeled tubers were boiled until cooked using normal domestic practice, drained and analysed. The results are shown in table 4.6.1.

Discussion

Boiling therefore, although marginally reducing the tecnazene residue did not eliminate the contamination.

Based on these figures, residents of the U.K. eating peeled and boiled samples would be expected to consume around 0.5g per annum of tecnazene, a quantity which cannot be treated as insignificant (see later).

4.6.2. TECNAZENE RESIDUES IN POTATO CRISPS

The assistance of Golden Wonder Ltd., Broxburn, Scotland, in providing facilities for this work is acknowledged.

Introduction

The changes in tecnazene residue levels during crisp manufacture under commercial conditions were investigated in some detail. Experiments were conducted to determine tecnazene losses at various stages of the process and also to investigate whether the frying oil could become contaminated and perhaps transfer residues to untreated material which was subsequently processed.

However before describing these experiments it might be useful for those not acquainted with the details of crisp manufacture to outline the process.

Table 4.6.1. Tecnazene residues after boiling

	Cultivar	Months since tecnazene treatment	Treatment prior to analysis	Tecnazene residue (mg kg^{-1} fresh weight)
1	Golden Wonder	11	washed	3.17
			washed and peeled	0.16
			washed, peeled and boiled	0.11
2	Golden Wonder	1	washed	7.66
			washed, peeled and boiled	0.57
3	Maris Piper	4	washed	2.64
			washed, peeled and boiled	1.04

Crisp manufacture

Incoming potatoes are discharged from lorries into a rotary washing unit where the bulk of the adhering soil is removed. They are then peeled in batches in an abrasive peeler. The period spent in the peeler is adjustable and is of course the minimum commensurate with producing an acceptable sample requiring the minimum of subsequent hand trimming, and usually removes around 10% of the weight of potatoes.(more after long storage periods). The peelings and trimmings may be further processed into animal food, and could be worthy of investigation with respect to sprout suppressant residues if samples could be obtained.

The peeled and trimmed tubers then pass into the slicer where they are sliced by rotating blades to a thickness of approximately 1.5mm, depending on variety and dry matter content.

The slices are then washed in a water spray to remove soluble sugars which may darken the product (see chapter 6). It also removes starch grains from ruptured cells.

After draining the slices drop into the deep fat fryer where they are cooked whilst moving through the tank. They emerge cooked and are cooled in an air stream.

Salt or flavour is then added by a powder applicator and mixed through the crisps in rotating drums where they cool further. They are then packaged and sealed.

The whole process takes approximately 10 minutes.

Experimental

To avoid any possible complications in the analysis, all the work described below was conducted on a line producing salted rather than flavoured crisps.

Experiment 1

Samples of 1 kg were taken at random at various points on the line during the processing of a 40 tonne batch of tecnazene (Fusarex) treated potatoes cv King Edward.

Sample	Description
1	The incoming potatoes after washing and before peeling.

2 After peeling and trimming but before slicing.

3 After slicing and washing of slices but before frying.

4 After frying but before cooling and salting.

5 After cooling, salting and packaging.

Experiment 11

A laboratory sample of several kg of cv Pentland Crown which had been treated as evenly as possible at the full commercial rate of 134mg kg^{-1} of tecnazene (Fusarex) was peeled in a factory laboratory peeler which produced similar peeling losses to the one on the line, cored and processed. Coring produces crisps with a disc removed from the centre and so they can be identified and sampled at any point on the line while still in full production.

This batch was sampled after peeling and after frying. The initial washed residue figure was already known.

Experiment 111

A laboratory sample of several kg of untreated Pentland Crown was processed and sampled as in Experiment 11 after 40 tonnes of treated potatoes had been processed. The original sample was also tested to make absolutely sure that no tecnazene contamination had occurred.

Experiment 1V

A $1dm^3$ sample of oil was taken from the fryer after the 40 tonnes had been processed.

Analytical methods

1) Samples containing no oil

These were extracted and analysed by the method described in section 4.2.7. using FID detection and ECD detection where necessary.

11) Samples containing oil

These were extracted and analysed as described in section 4.5.4. using ECD detection.

Results

The results are shown in table 4.6.2.

Table 4.6.2. Tecnazene residues during crisp manufacture

Experiment I - Commercial samples	
Sample	Tecnazene level (mg kg^{-1} fresh weight)
1 Incoming potatoes after washing	1.10*
2 After peeling	0.16
3 After slicing and washing	0.14
4 After frying	0.55+
5 Final product	0.25*+
Experiment II - Laboratory treated sample	
1 Washed	2.91*
2 Peeled	0.21
3 After frying	0.6+
Experiment III - Untreated laboratory sample after processing treated material	
1 Washed	0.00*
2 Peeled	0.00
3 After frying	0.34
Experiment IV - Oil sample after processing treated material	
1 1 dm^3 sample	0.07 mg dm^3 of oil

* Mean of duplicate results

\+ mg kg^{-1} of product

Discussion

1) Peeling as was previously stated makes a major impact on the total residue figure.
2) Washing of slices has very little effect.
3) Frying apparently increases residues due to the concentration effect of water loss. This effect was shown in both experiments 1 and II.
4) There appears to be a considerable loss during the cooling process.
5) Experiments III and IV indicate that crisps from untreated batches can be contaminated via the oil.
6) The residue figures in the final product must be considered to be significant in comparison with other pesticide residue levels in food.

4.7. ANALYSIS OF FORMULATIONS

On several occasions it was considered necessary to analyse various formulations of tecnazene to check that they contained their stated amounts of active ingredient.

Because of the high concentrations involved and the nature of the other components such analysis is relatively easy. However during this work one sample was analysed that contained another volatile ingredient.

This section then describes these estimations of tecnazene content and the identification of the mysterious component.

Experimental

The following formulations were investigated.

1) Fusarex (Plant Protection Ltd.) a nominally 3% formulation of tecnazene on kaolin dust.
2) Bygran S (sample gifted by the Wheatley Chemical Co.) a nominally 7.5% formulation of tecnazene on shale granules.
3) Bygran (sample gifted by the Wheatley Chemical Co.)(see below).

Analysis

Two 5g samples of each formulation were extracted with 3x50cm^3 portions of hexane (nanograde is not necessary) by shaking, allowing

to settle, and filtering the supernatant into a 250cm^3 volumetric flask. The residue was washed with a further 50cm^3 of hexane and the level in the volumetric flask made up to 250cm^3.

A 0.9µl aliquot of this extract was injected into the gas chromatograph using FID detection, and compared with standards.

Results

The results are shown in table 4.7.1.

Table 4.7.1. Tecnazene content of various commercial formulations

Formulation	% Tecnazene (mean of duplicates)
Fusarex	2.89
Bygran S	5.22
Bygran	2.13

Bygran was also found to contain another compound which had a slightly longer retention time than tecnazene.(see figure 4.7.1.). An attempt was then made to isolate and identify this material.

Experimental

The solvent was removed from the Bygran extract and the solubility of the residue tested. It was noted that it was insoluble in water, soluble in polar solvents but had only limited solubility in hexane. Tecnazene of course is fairly soluble in hexane.

The remainder of the residue was therefore fractionally crystallised from hexane, and after a single crystallisation was found to contain 90% unknown and 10% tecnazene. This process was repeated and the crystalline product dried in a vacuum oven to remove all traces of solvent. It was then submitted for analysis to the Departmental mass spectrometry service.

Results

The mass spectrum obtained is illustrated in the line diagram in

Figure 4.7.1. Chromatogram of hexane extract of Bygran (see text).
Column: 10% QV101, temperature 174°C, flame ionisation detectors.
Peak identification: 1, tecnazene; 2, chlorpropham.

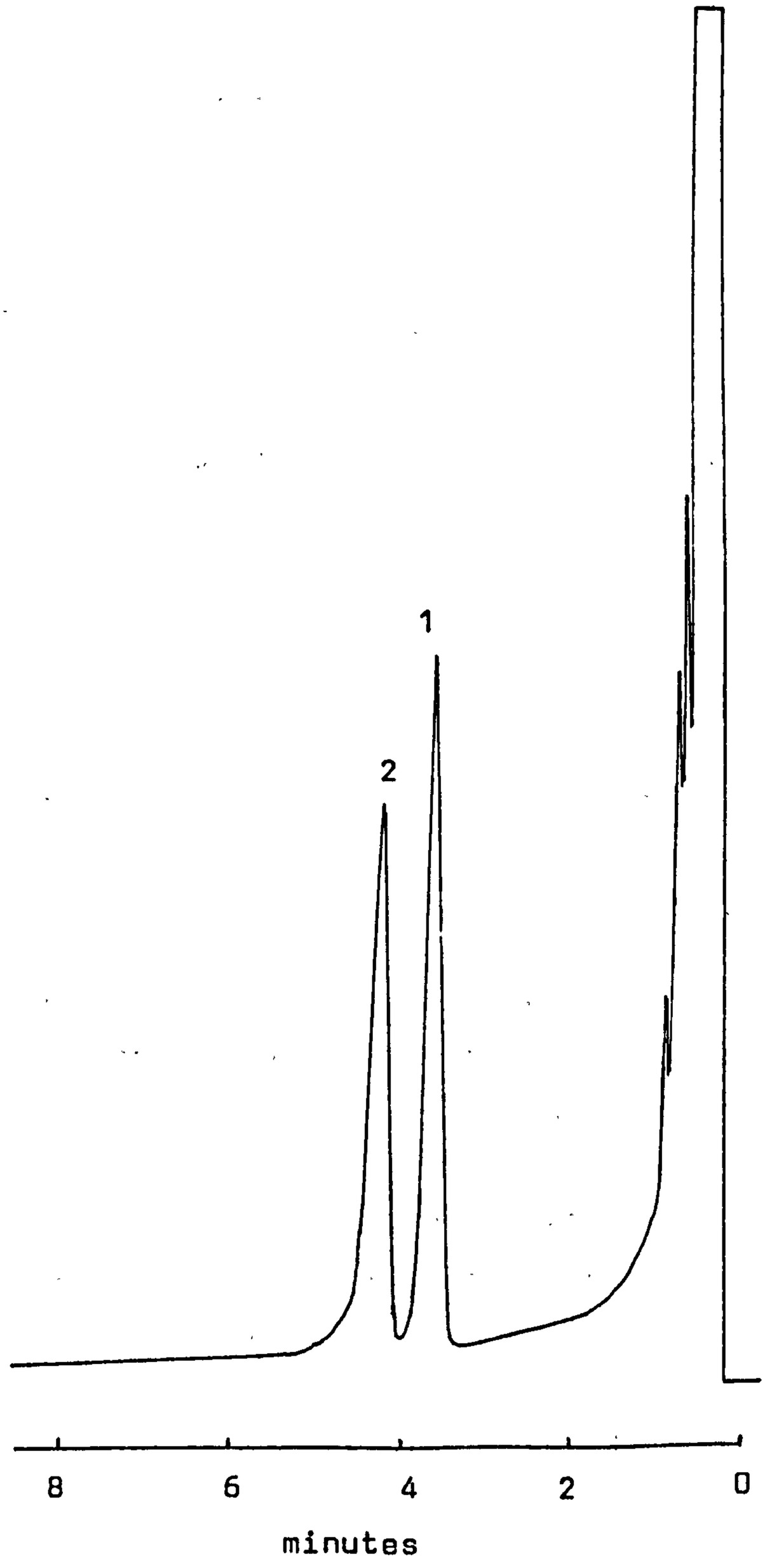


figure 4.7.2. from which the following deductions were made.

1) The mass of the molecular ion is at m/e 213 with a second peak at 215. These peaks are in the ratio of 3:1.

This immediately suggested that the compound contained a single chlorine atom and the odd molecular weight suggests that it may also contain nitrogen.

The possibility of the presence of another sprout suppressant compound was considered likely and various molecular weights were calculated.

Chlorpropham has a molecular weight of 213 (based on ^{35}Cl), contains one chlorine atom and one nitrogen and so was considered to be the most likely candidate.

2) This identification was confirmed by a study of the fragmentation pattern and from the presence of metastable ions. Using the relationship

$$\text{mass of metastable ion} = \frac{\text{mass of daughter}^2}{\text{mass of parent}}$$

confirmation of any proposed fragmentation process can be achieved if such a metastable ion is located in the spectrum.

Thus the loss of the isopropyl group as $CH_3CH{=}CH_2$, (a loss of 42 units resulting in a peak at m/e 171) is confirmed by the presence of a metastable peak at 137.1. Similarly the various fragmentation processes illustrated in figure 4.7.3. were confirmed.

Therefore it can be stated with 100% certainty that the compound is chlorpropham.

Discussion

Although this product (Bygran) and its composition was discussed with a representative of the Wheatley Chemical Co., the same representative later claimed that such a product containing tecnazene and chlorpropham did not exist, and the sample sent (although labelled Bygran) was an experimental batch and Bygran as commercially marketed was of different composition. Attempts made to obtain commercial samples manufactured at the time of supply of the "experimental sample" have been unsuccessful. The representative's claim cannot therefore be verified.

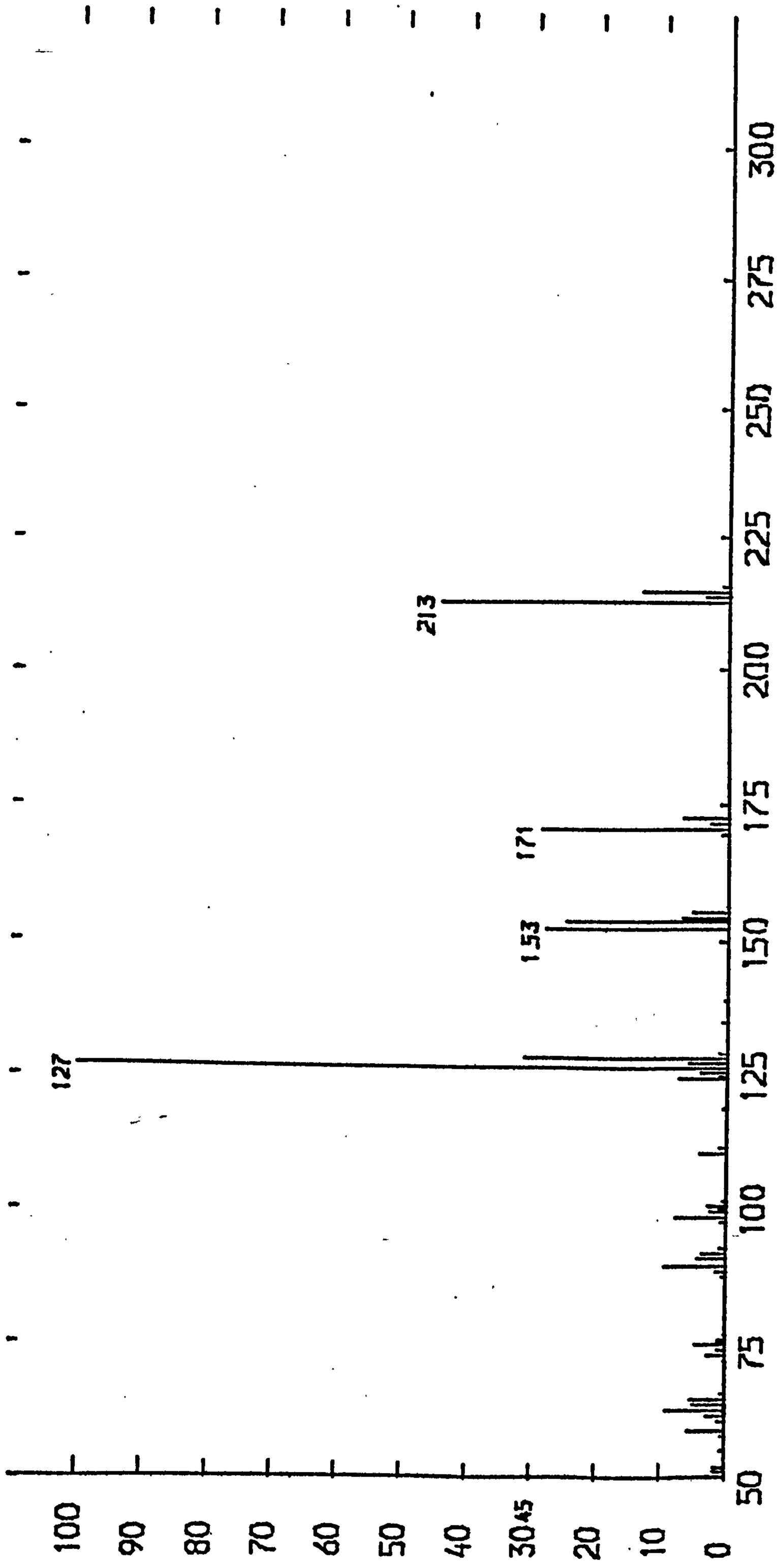
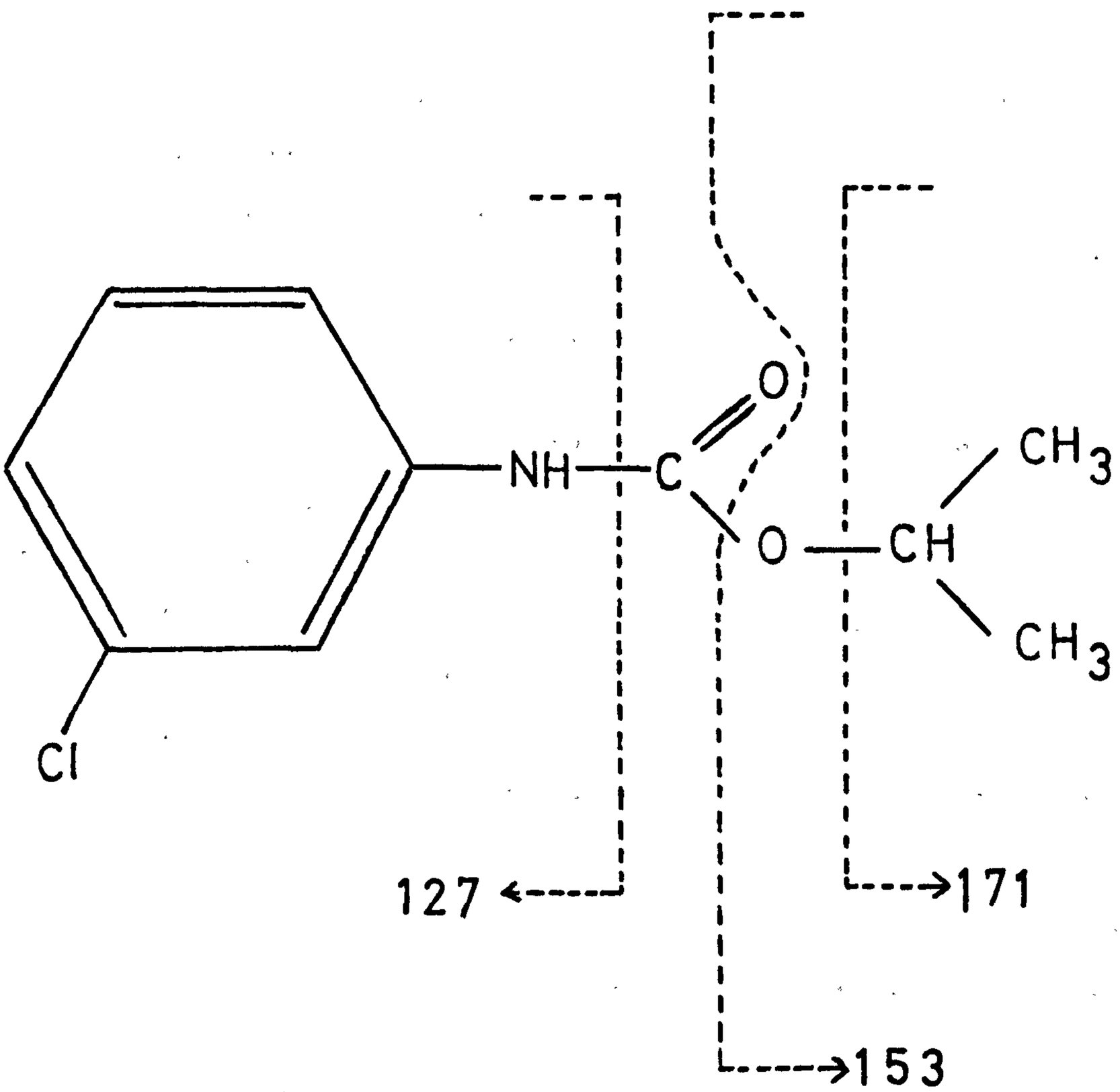

MASS SPECTRUM OF UNKNOWN COMPONENT OF BYGRAN
127
213
153
171
RELATIVE INTENSITY
100
90
80
70
60
50
40
30
45
20
10
0
MASS TO CHARGE RATIO
50
75
100
125
150
175
200
225
250
275
300

FIGURE 4.7.2

Figure 4.7.3. Fragmentation of chlorpropham.



4.8. THE INFLUENCE OF STORAGE CONDITIONS ON TECNAZENE RESIDUES

4.8.1. Introduction

As tecnazene is volatile it is believed that it is gradually lost from stored potatoes and such potatoes may eventually be used as seed (see earlier).

However for the following reasons it was decided to investigate this subject in greater depth.

1) Reports had been received from farmers concerning poor germination of tecnazene treated crops.

2) These reports had been supported by a field trial reported in chapter 5 which indicated that under certain conditions poor germination and yield reductions could be shown with tecnazene treated seed (see also Dalziel and Duncan,1975).

3) The toxicological implications of this process of tecnazene loss are important.

4) The volatility of tecnazene did not seem to be as great as some reports and the advertising literature imply. In fact when Roy (1947) exposed a sample of tecnazene to the atmosphere he found that at 15-20^{o}C only 0.34% of the sample was lost after 11 days. Even at 35^{o}C only 0.42% had volatilised after 28 hours.

5) The subject had never been investigated in any depth using analytical techniques.

It was therefore decided to conduct an experiment which would show how tecnazene residues are affected by various storage conditions and then to plant out the remainder of the tubers and investigate their subsequent performance, in terms of both emergence and yield.

The various treatments and storage conditions involved in this experiment and the residue estimations are discussed in this chapter. The effect of these treatments on sprouting, and the investigation of subsequent field performance are described in chapter 5. An additional investigation into the effect of the various storage treatments on compositional factors is described in chapter 6.

4.8.2. Experimental

a) Design

The design of experiments involving various changes of storage conditions with sampling up to the time of planting is very difficult because, of course, the planting date is weather dependent. This is especially so in Scotland, and in fact the proposed planting date for this experiment had to be postponed by 3 weeks.

Originally the plan was to treat 12 out of 14 batches of each of 4 cultivars, sample at 5, 2 and 0 weeks before planting, airing each batch after sampling. Two of the batches at each sampling date were to be transferred to 12°C storage and two left at 8°C, giving a fairly symmetrical experiment involving 3 airing times x 2 airing temperatures x 4 cultivars + 2 controls for each cultivar, thus making statistical analysis of the results relatively easy using a standard analysis of variance.

Because the postponement of the planting date was forseen just before the final sampling, modifications were made to cope with the fact that had the experimental design been allowed to stand, no sample would have been available which had remained unaired. Therefore on the initially proposed final sampling date only two batches of each cultivar were opened instead of four. As the symmetry of the experiment had therefore been lost anyway, and as only one further batch was now required for sampling at planting date it was decided that a forced ventilation treatment could be investigated using the extra batch.

In future experiments of this type extra batches were included to cope with this problem.

The eventual form of the experiment is illustrated in figure 4.8.1. It should be emphasised that this structure is fundamental to the work described below and as mentioned earlier to parts of chapter 5 and 6.

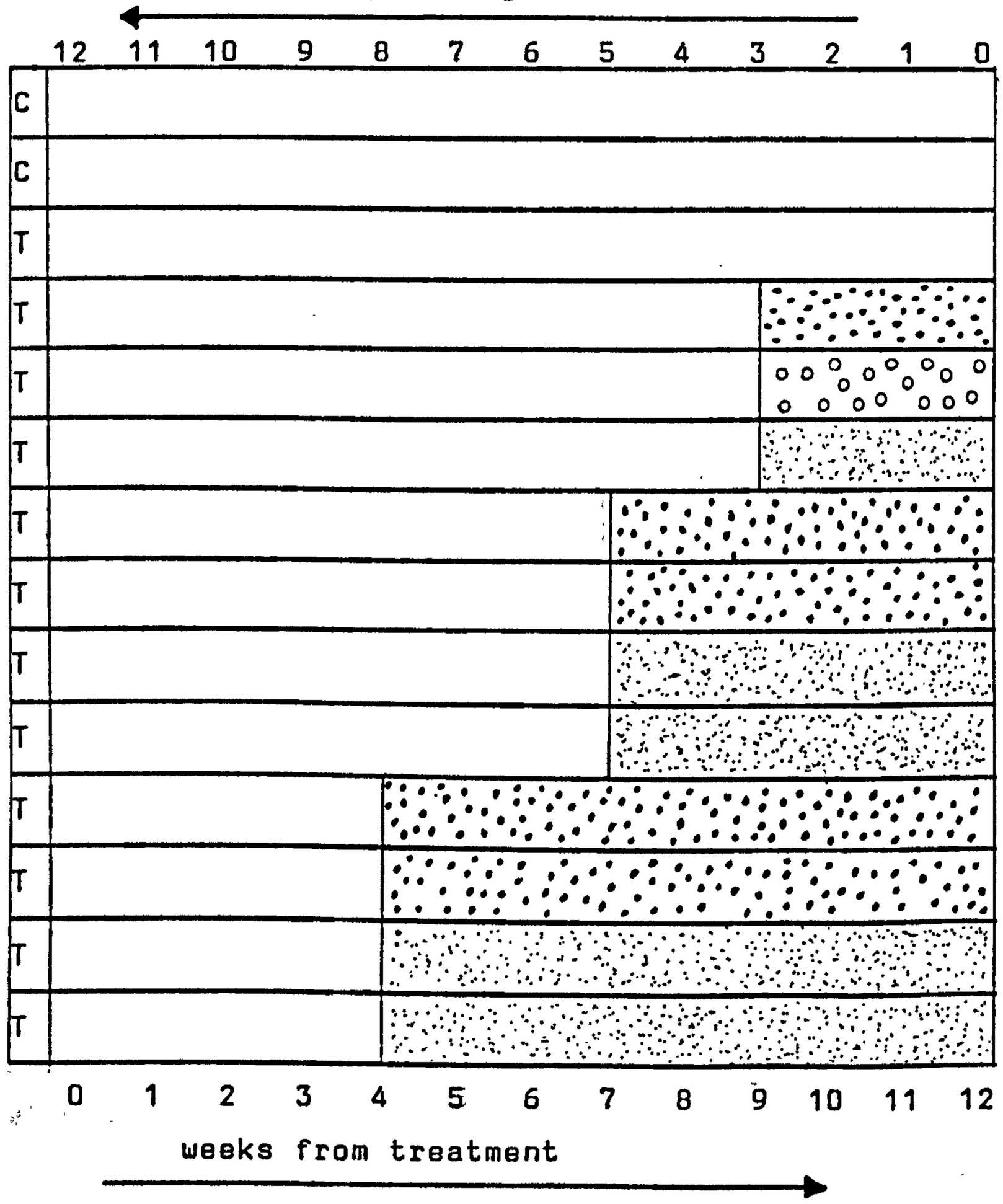
b) Materials and Methods

The experiment was conducted during the 1974-75 storage season.

Seed potatoes of the highest grades (FS where possible) were purchased and the experiment commenced as soon as the potatoes were

Figure 4.8.1. Experiment to investigate the effect of storage conditions on tecnazene residues

For each of 4 cultivars:-



C = Control

T = Tecnazene treated

Airing at 12°C

Airing at 8°C

Forced ventilation at 8°C

Closed storage at 8°C

available. Tubers were 32-57mm.

The four cultivars used were selected because of the range of sprouting characteristics they exhibit. They were Record and Redskin (vigorous sprouters) and Maris Piper and Pentland Crown (less vigorous)

Fourteen 10kg batches of each cultivar were weighed into identical good quality cardboard boxes with lids that overlapped the sides of the boxes completely (computer paper boxes). The 10kg almost filled the box completely.

Tecnazene was applied at 135mg kg^{-1} to 12 batches of each cultivar by sprinkling 45g "Fusarex" on each, replacing the lid and turning the box over several times to ensure even distribution.

They were stored at $8^{o}C$ in a constant temperature room, the control boxes being stored in a section of this room partitioned by a curtain of polythene and ventilated very slowly with enough air to maintain a slight positive pressure difference between that section and the rest of the room thus preventing any contamination with tecnazene from treated batches. This air was blown through a 50mm tube which passed through the main part of the room before entering the tent, thus allowing any temperature difference to be corrected.

The lids of the boxes were removed at various times indicated in figure 4.8.1. and the boxes either left at $8^{o}C$ or transferred to $12^{o}C$ conditions.

Forced ventilation was accomplished by blowing air over the surfaces of the opened boxes in a separate polythene enclosure with a powerful electric fan.

The air in the room as a whole was changed by pumping in fresh air and allowing air to escape at the opposite side of the room.

When each box was opened an eight tuber sample was taken, placed in a polythene bag and sealed, and stored at $-18^{o}C$ until it was analysed. A second sample from each batch was taken just before planting, when sprout counts and measurements were also made (see chapter 5).

The samples were washed, further sampled by coring and analysed by the method described in section 4.2.7. using FID detection.

4.8.3. Results and Discussion

The results of the analysis are shown in table 4.8.1. with the various airing conditions used.

The most obvious point about this data is its variability. The reasons for this have already been discussed in section 4.2.3.

Nevertheless on averaging the results of replicate treatments several trends become apparent. These means are shown in table 4.8.2. It should be noted that for some treatments there was no replicate so the result of a single analysis is employed.

Overall means have also been calculated from the raw data and these figures are shown in table 4.8.3.

The values in these tables (especially in 4.8.3.) indicate:

1) With closed boxes, analysis at various times after treatment shows a slight decay in tecnazene residues at $8^{o}C$.
N.B. 8,5,3 and 0 weeks from planting correspond to 4,7,9 and 12 weeks after treatment respectively (see below).

2) Airing periods apparently produce lower residue figures - especially at $12^{o}C$ (see below).

3) The significance of the forced airing treatment seems to be masked by the high variability.

4) There is a cultivar effect in the absolute value of the residues, Pentland Crown having consistently higher results. This factor indeed proved significant ($P < 0.05$) using an approximate analysis of variance technique, however because the experiment is only balanced with respect to cultivar a similar technique could not be used for the other variables.

Because of the importance of these observations on tecnazene residues during storage a statistical model was constructed to describe how they are affected by various storage conditions.

Table 4.8.1. The effect of storage conditions on tecnazene residues

1) Residue date from airing experiment (mg kg^{-1})

Airing conditions									
Time (weeks)	Temperature ^{o}C	Redskin S1[a]	Redskin S2[b]	Record S1	Record S2	P.Crown S1	P.Crown S2	M.Piper S1	M.Piper S2
0	8	1.4[c]	1.4[c]	1.1	1.1	1.6	1.6	0.96	0.96
3	12	1.4	0.69	1.5	1.3	1.4	1.3	1.3	0.81
3	8[d]	1.9	1.2	1.3	0.65	2.4	1.8	0.98	0.79
3	8	1.5	1.3	0.72	1.5	0.9	1.7	2.8	1.0
5	12	2.0	0.50	1.4	0.97	2.5	1.5	1.1	0.79
5	12	1.6	0.61	1.6	0.92	3.6	0.93	1.3	0.40
5	8	1.7	1.1	1.2	0.71	2.4	1.9	1.9	1.1
5	8	1.9	0.67	2.5	0.83	2.4	0.92	1.4	0.75
8	12	1.8	0.62	1.5	0.10	2.4	0.65	3.0	0.23
8	12	2.7	0.52	1.9	0.30	2.8	0.75	1.7	0.40
8	8	1.9	0.97	2.3	0.85	2.5	1.3	1.8	1.2
8	8	1.2	0.60	1.5	0.60	3.9	1.7	1.9	0.67

a Sample 1 taken at opening

b Sample 2 taken at planting

c Sample from unopened treatment taken at planting therefore S1=S2

d With added ventilation

Table 4.8.2. The effect of storage conditions on tecnazene residues

2) Means of replicates of residue data (mg kg^{-1})

Cultivar	Airing period	Residue at opening	Residue at planting after airing at 8°C	Residue at planting after airing at 12°C
Redskin	0	1.40	1.40	1.40
	3	1.60	1.30(1.20)*	0.69
	5	1.81	0.89	0.56
	8	1.90	0.79	0.57
Record	0	1.10	1.10	1.10
	3	1.17	1.50(0.65)	1.30
	5	1.68	0.77	0.95
	8	1.80	0.73	0.20
P.Crown	0	1.60	1.60	1.60
	3	1.56	1.70(1.80)	1.30
	5	2.73	1.41	1.22
	8	2.90	1.50	0.70
M.Piper	0	0.96	0.96	0.96
	3	1.69	1.00(0.79)	0.81
	5	1.43	0.93	0.60
	8	2.35	0.94	0.32

* With added ventilation

Table 4.8.3. The effect of storage conditions on tecnazene residues

3) Overall means of residue data (mg kg^{-1})

1. Cultivars over all airing times

Cultivar	Residue at opening	Residue at planting 8°C	Residue at planting 12°C	Residue at planting 8 and 12°C
Redskin	1.75	1.03*	0.72**	0.84***
Record	1.54	0.89	0.78	0.82
P.Crown	2.40	1.56	1.12	1.34
M.Piper	1.68	0.92	0.60	0.76
Overall	1.84	1.10	0.81	0.94

2. Airing times over all cultivars

Airing time	Residue at opening	Residue at planting 8°C	Residue at planting 12°C	Residue at planting 8 and 12°C
0 weeks	1.27	1.27	1.27	1.27
3 "	1.51	1.24	1.03	1.17
5 "	1.91	1.00	0.83	0.91
8 "	2.18	0.99	0.45	0.71
All times	1.72	1.13	0.90	1.02
All times (weighted)	1.84	1.10	0.81	0.94

* Including samples with added ventilation

** Also including unopened treatment

*** Including unopened once only and including samples with added ventilation

4.8.4. A statistical model for tecnazene residues under different storage conditions

The assistance of Mr T.C.Aitchison, Statistics Department, University of Glasgow is acknowledged in this work.

a) Introduction

The following sections are by necessity of a statistical nature- indeed some of the work involved mathematical processes which may be unfamiliar to agricultural chemists.

It has therefore been decided to present enough material to explain the process without assuming a detailed knowledge of the mechanics of some of the more complex operations which are implied (e.g. matrix algebra). Enough material is however presented such that any statistician or statistically orientated scientist could repeat the process, yet hopefully still rendering this section intelligible to all readers.

The aim of this work was to prepare a statistical model to describe the way in which tecnazene residues are affected by storage conditions and then to justify this model by comparison with the data obtained above. The model is concerned with residue decay or loss and not simply absolute values.

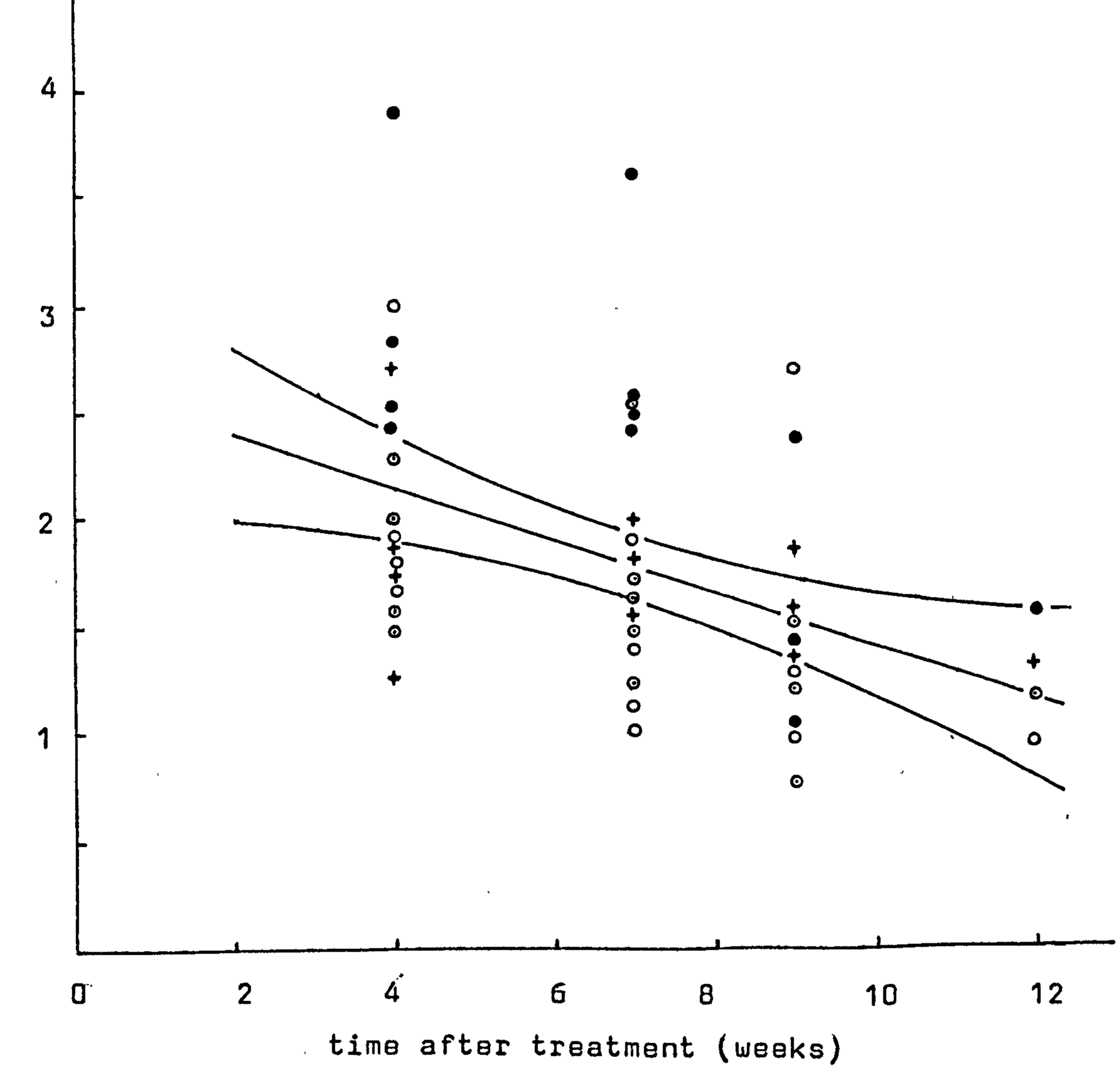
b) Graphical illustration of the data

Before describing the model it is felt that the results of some of the preliminary work might be useful in that it illustrates graphically the problem under consideration.

Firstly, considering only the residue data at point of opening, the values for all cultivars were plotted. This plot is illustrated in figure 4.8.2. with the 95% confidence curves for the best fitting straight line above and below the line. It indicates that there is a statistically significant decline in tecnazene residues with time when tubers are held in closed boxes at $8^{o}C$ (i.e. although r = only 0.44) the line has a negative gradient regardless of where it is drawn between the confidence curves.

Similar lines were then fitted to the data for each cultivar individually, as shown in figures 4.8.3., 4.8.4., 4.8.5. and 4.8.6.

Figure 4.8.2. Graphical illustration of experimental data showing the effect of time under closed storage conditions at 8°C on the decay of tecnazene residues.



4.8.3. Graphical illustration of data showing the effect of time on the decay of tecnazene residues under various storage conditions - cultivar Redskin.

o storage under closed conditions at 8°C.
● residue after airing at 8°C.
⊙ residue after airing at 12°C.
—— regression line for closed conditions.
..... line joining means before and after airing at 8°C.
_____ line joining means before and after airing at 12°C.

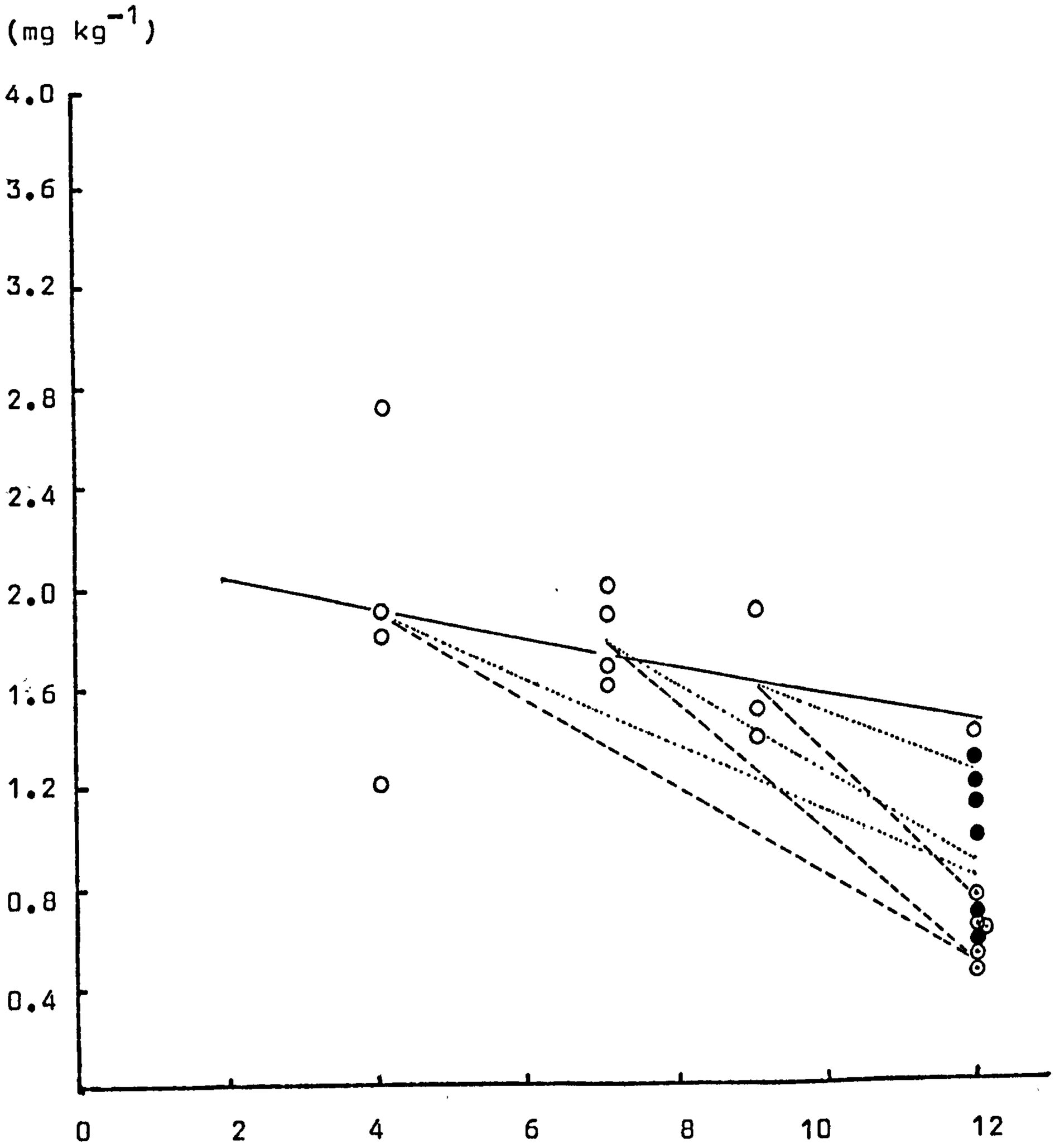


Figure 4.8.4. Graphical illustration of data showing the effect of time on the decay of tecnazene residues under various storage conditions - cultivar Record.

For key - see figure 4.8.3.

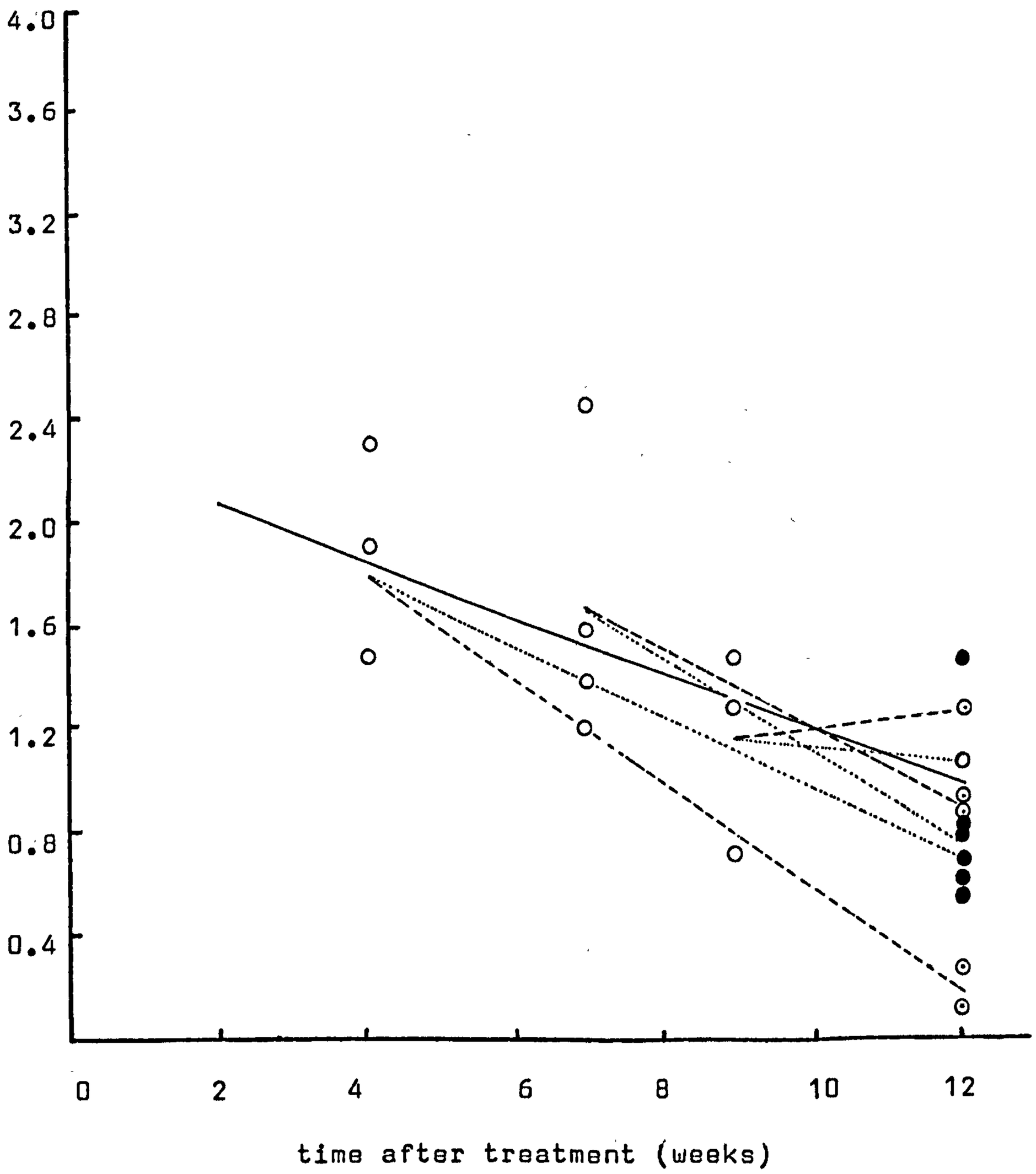


Figure 4.8.5. Graphical illustration of data showing the effect of time on the decay of tecnazene residues under various storage conditions - cultivar Pentland Crown.

For key - see figure 4.8.3.

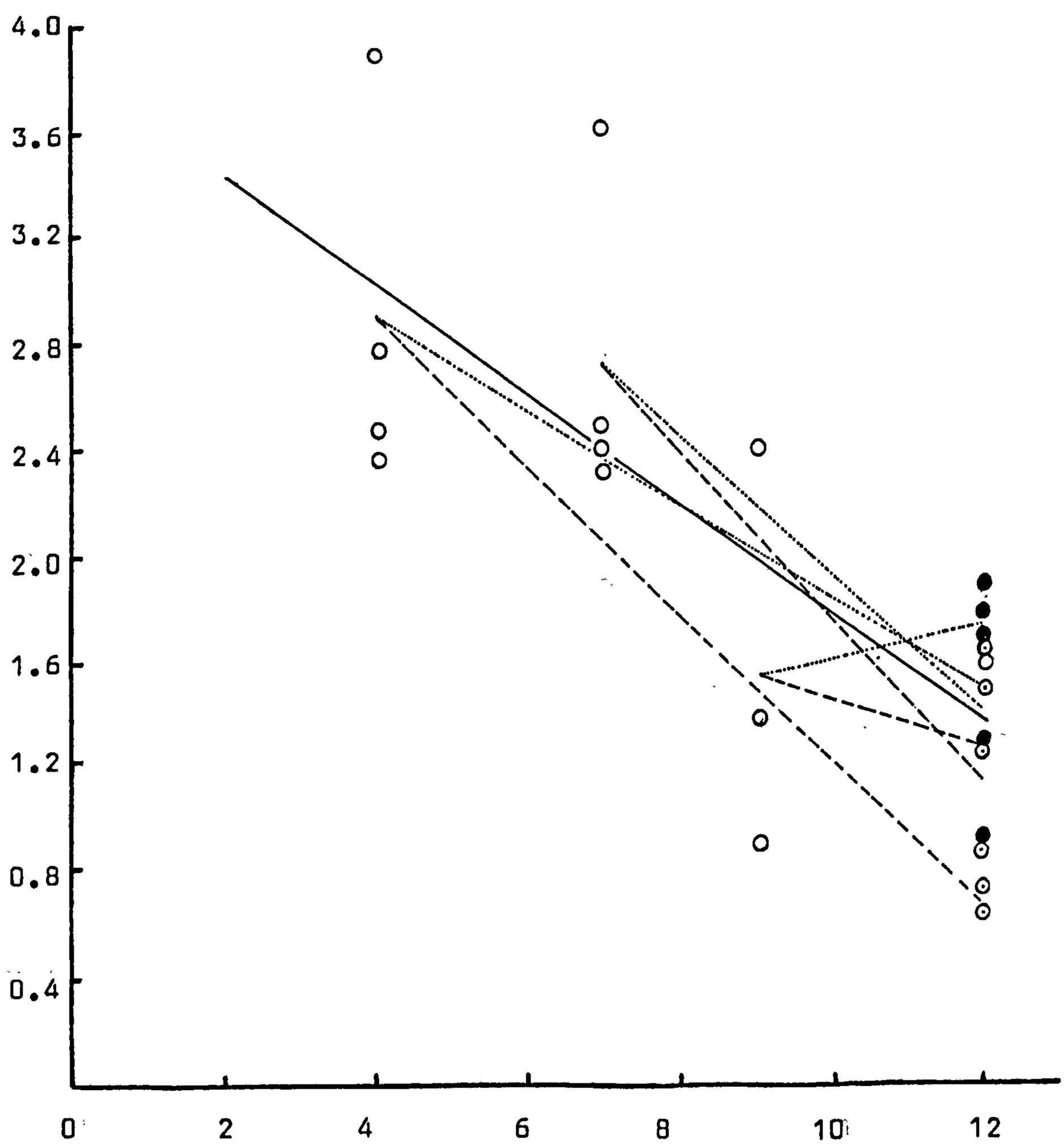
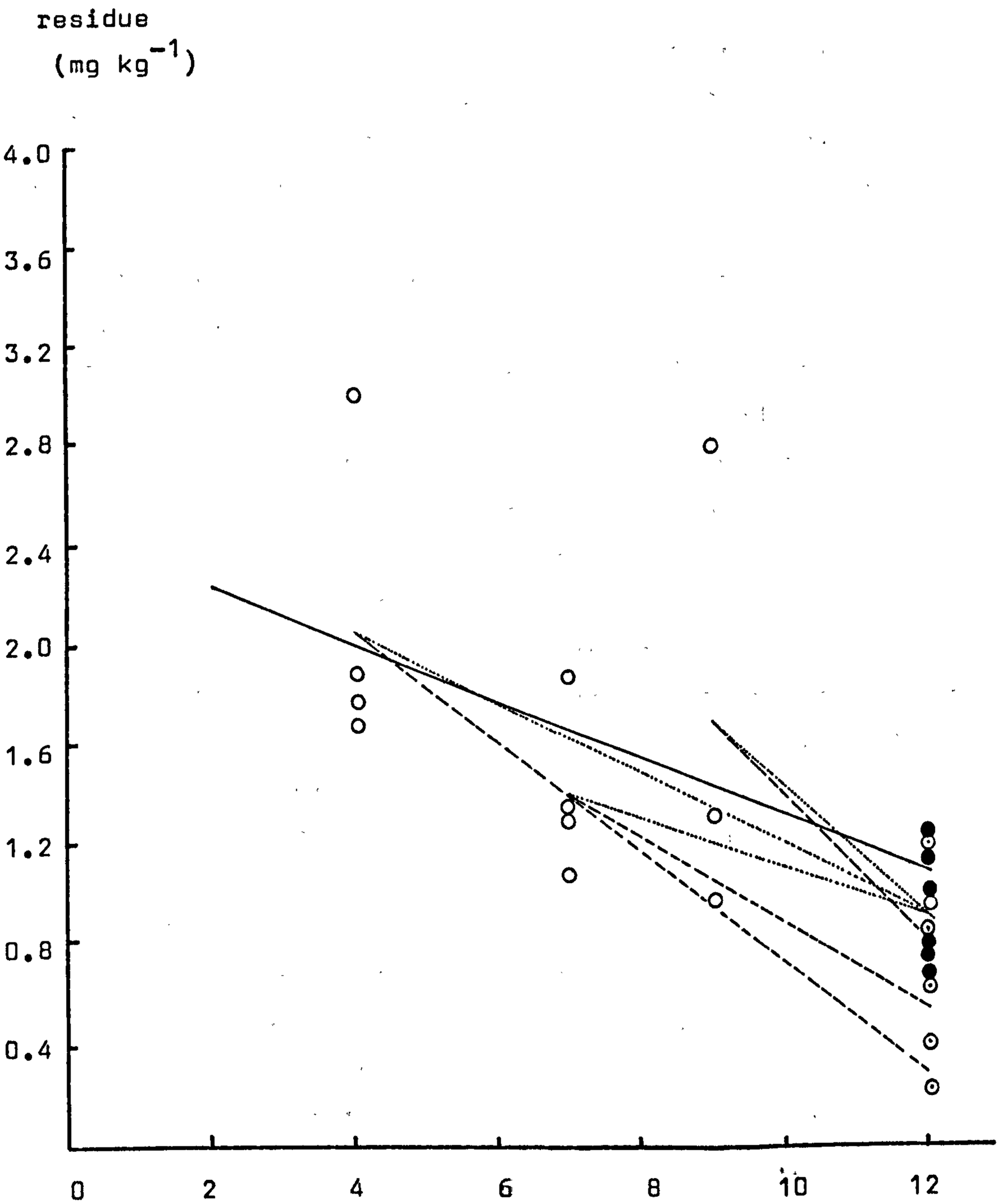


Figure 4.8.6. Graphical illustration of data showing the effect of time on the decay of tecnazene residues under various storage conditions - cultivar Maris Piper.

For key - see figure 4.8.3.



The data from the samples taken after airing, at time of planting were then superimposed on this plot, and the mean of each treatment at opening joined to the mean of each treatment at planting with straight lines. This illustrates the information from the experiment.

c) Transformations and assumptions

The simplest statistical model is a linear regression (as above) with constant variance. However, transformation of the data before fitting the line will often produce a better model than the raw data.

Therefore, considering only the residue data obtained from samples at opening, various mathematical transformations of both residue value and time after treatment were tested by calculating the correlation coefficient from the transformed data.

For 3 out of 4 cultivars the logarithm of residue versus time showed the greatest improvement over the original data, although for the cultivar Redskin a linear - linear fit was marginally better.

A log-linear assumption has the further advantage that it assumes that the rate of decrease in residue is proportional to the amount of tecnazene present - a not unreasonable assumption.

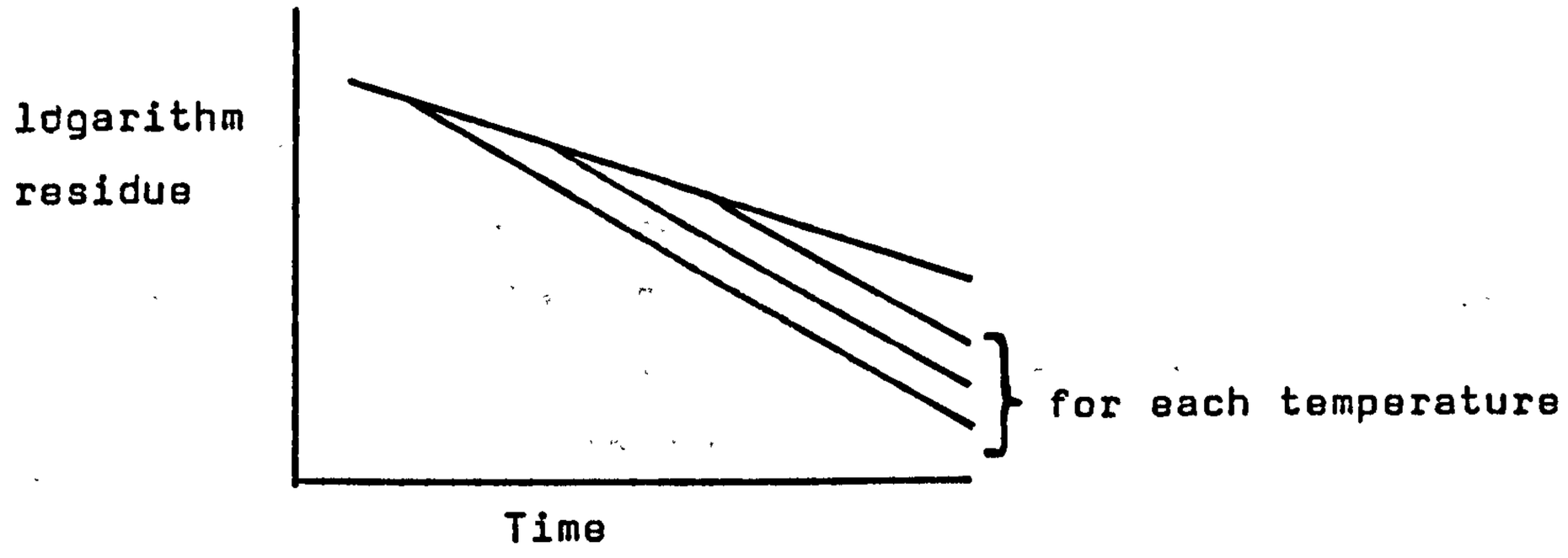
Another advantage is that using the logarithm of residue can cope with the fact that errors of the analysis technique produce a skew rather than a normal distribution of results. This is likely (and apparent) for two reasons. Firstly any contamination of the sample due to incomplete washing of the surface would produce a high result, and extra washing will not produce a low result. Secondly all the results must be positive, so that distribution is truncated. Logarithms of residue data should (and do) approximate to a normal distribution.

It was therefore considered very reasonable to adopt such a log-linear assumption for the model. Logarithms to the base e are used from this point on.

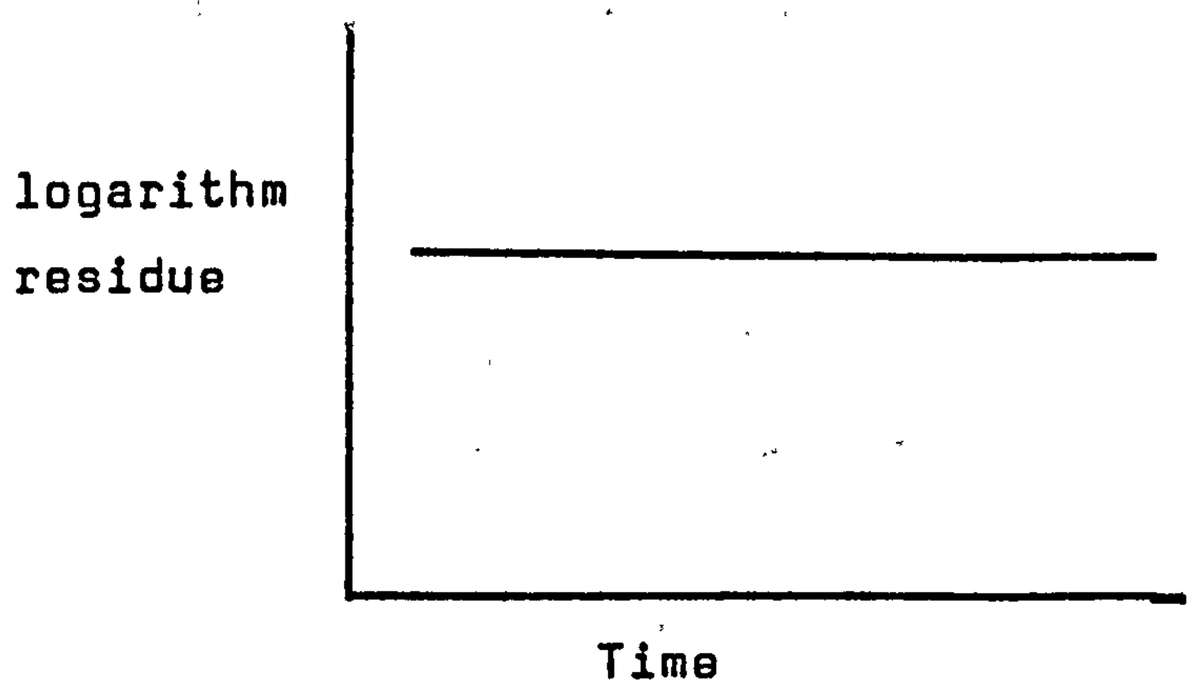
Then to include residues at planting in the model it was considered that the log-linear assumption was again appropriate (although because there are only 2 points on these curves this assumption cannot be validated as above).

It was also assumed (not unreasonably) that the form of the residue decay was constant i.e. the time of opening has no effect upon the shape of the decay curve but only affects the absolute starting value.

The model is therefore of the general shape:



It should be noted however that these assumptions could even encompass a graph such as that illustrated below:



i.e. that storage conditions have no effect whatsoever on tecnazene residues.

As the application rate for each of the cultivars was the same the model could have been forced to assume that the residues were the same for each cultivar in the early part of the experiment, however because of observations already made on the residue data

this was not assumed. i.e. different cultivars may have different log residue values at 0 weeks (time of treatment). However as there is no date before 4 weeks after treatment such extrapolation towards 0 weeks must be treated with caution, especially in the region very close to treatment where of course the actual residue determined by the method above should be zero.

d) <u>The model parameters</u>

The following notation is used:

Let x_{ijk} be the value of the $\log_e$ of the residue at opening of the k th replicate of the i th cultivar opened at the j th time (time t_i = weeks after treatment).

where

i = 1(Redskin), 2(Record), 3(P.Crown) and 4(M.Piper)

j = 1(12 weeks), 2 (9), 3(7) and 4(4)

k = 1 to n_j where n_j depends on time, because the number of replicates differs at different times.

Similarly

y_{ijkt} is the value of the $\log_e$ of the residue at planting of the i th cultivar, time t_j, k th replicate stored at t^oC

where i = 1-4 as above

j = 2-4 (9,7,4)

k = 1-n_j (as above)

t = 1 (8^oC) and 2 (12^oC)

The assumptions described above lead to the following

$$E(x_{ijk}) = \mu_i + \beta_i \cdot t_j$$

and $V(x_{ijk}) = \sigma_i^2$

where E = expected value and V = variance

and

$$E(y_{ijkt}) = \gamma_{ijt} + \sigma_{it} \cdot 12$$

and $V(y_{ijkt}) = \sigma_i^2$

As the expected residue must be the same at opening as at the beginning of airing, (see diagram above - where the lines intersect) it is necessary that:-

$$\gamma_{ijt} + \sigma_{it} \cdot t_j = \mu_i + \beta_i \cdot t_j$$

i.e. for every cultivar there are 4 parameters describing the linear model viz. μ_i, β_i, d_{i1}, d_{i2}.
Using least squares estimators of these parameters and least squares estimators of the variances the values in table 4.8.4. were obtained.

Table 4.8.4. Least squares estimators of model parameters for each cultivar

Cultivar	Parameter				
	μ_i	β_i	$d_{i(8)}$	$d_{i(12)}$	σ_i^2
Redskin	0.84	-0.05	-0.12	-0.18	0.05
Record	0.62	-0.03	-0.12	-0.22	0.19
P.Crown	1.32	-0.08	-0.09	-0.16	0.09
M.Piper	0.99	-0.08	-0.10	-0.22	0.10

Thus using these least squares estimators of the parameters the residue value at any airing time may be calculated from the equation:

Residue = Exponent of (μ_i + β_i x weeks closed + $d_{i(temp.)}$ x weeks open + $\frac{1}{2}\sigma_i^2$)

From this function the resultant decay curves have been plotted for each cultivar in figures 4.8.7., 4.8.8., 4.8.9. and 4.8.10. As the y axis is in mg kg^{-1} of tecnazene (and not log units) these best fitting log-linear plots appear as curves.

e) Residue decay during normal storage

Normal storage in the context of this work constitutes storage at 8°C in closed boxes. In fact storage in this manner seems to be fairly close to storage in a bulk store. Residue levels are similar and sprouting is comparable with that observed under

FIGURE 4.8.7 STATISTICAL MODEL DESCRIBING TECNAZENE RESIDUE DECAY UNDER DIFFERENT STORAGE CONDITIONS.

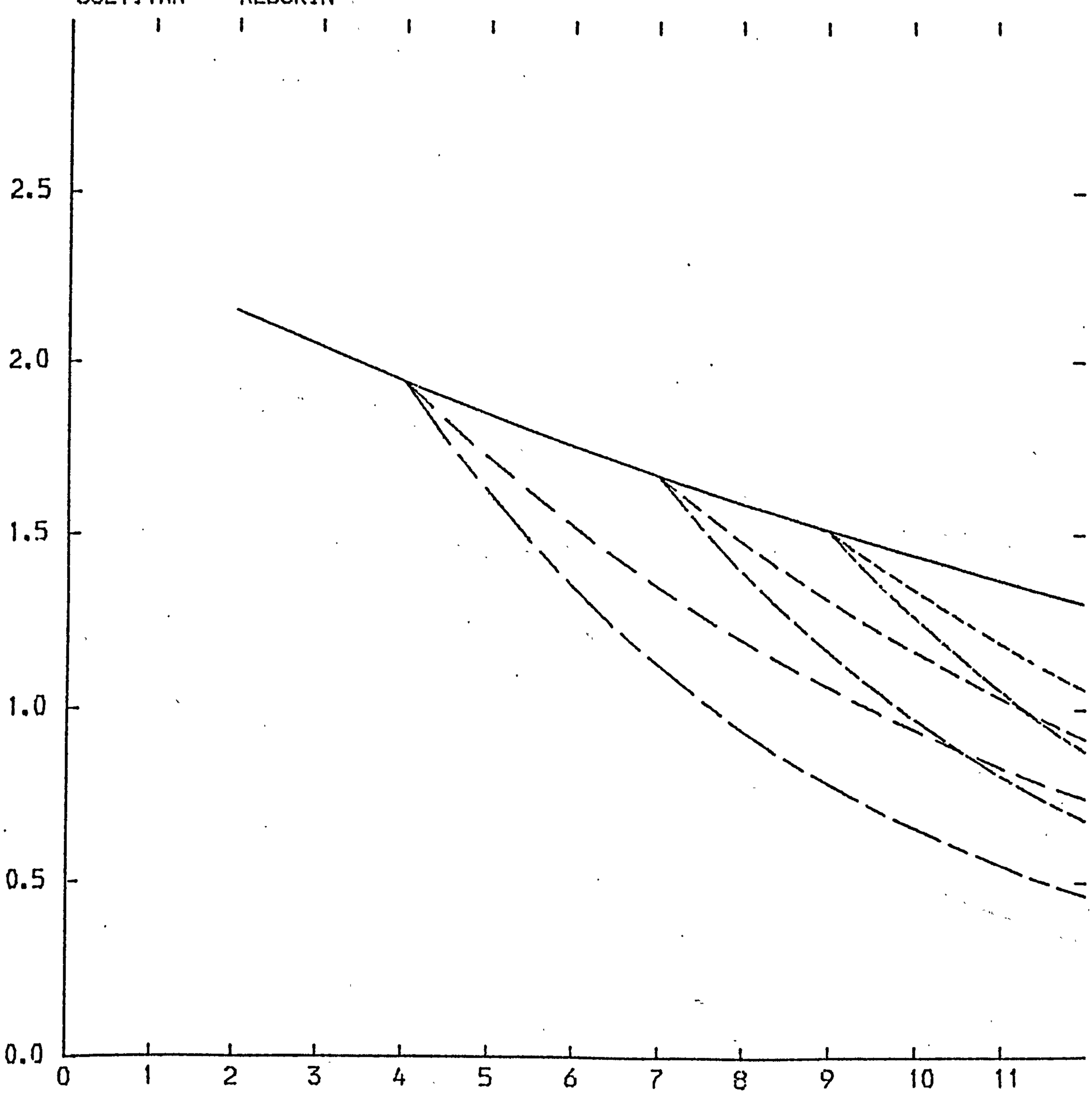


FIGURE 4.8.8 STATISTICAL MODEL DESCRIBING TECNAZENE RESIDUE DECAY UNDER DIFFERENT STORAGE CONDITIONS.

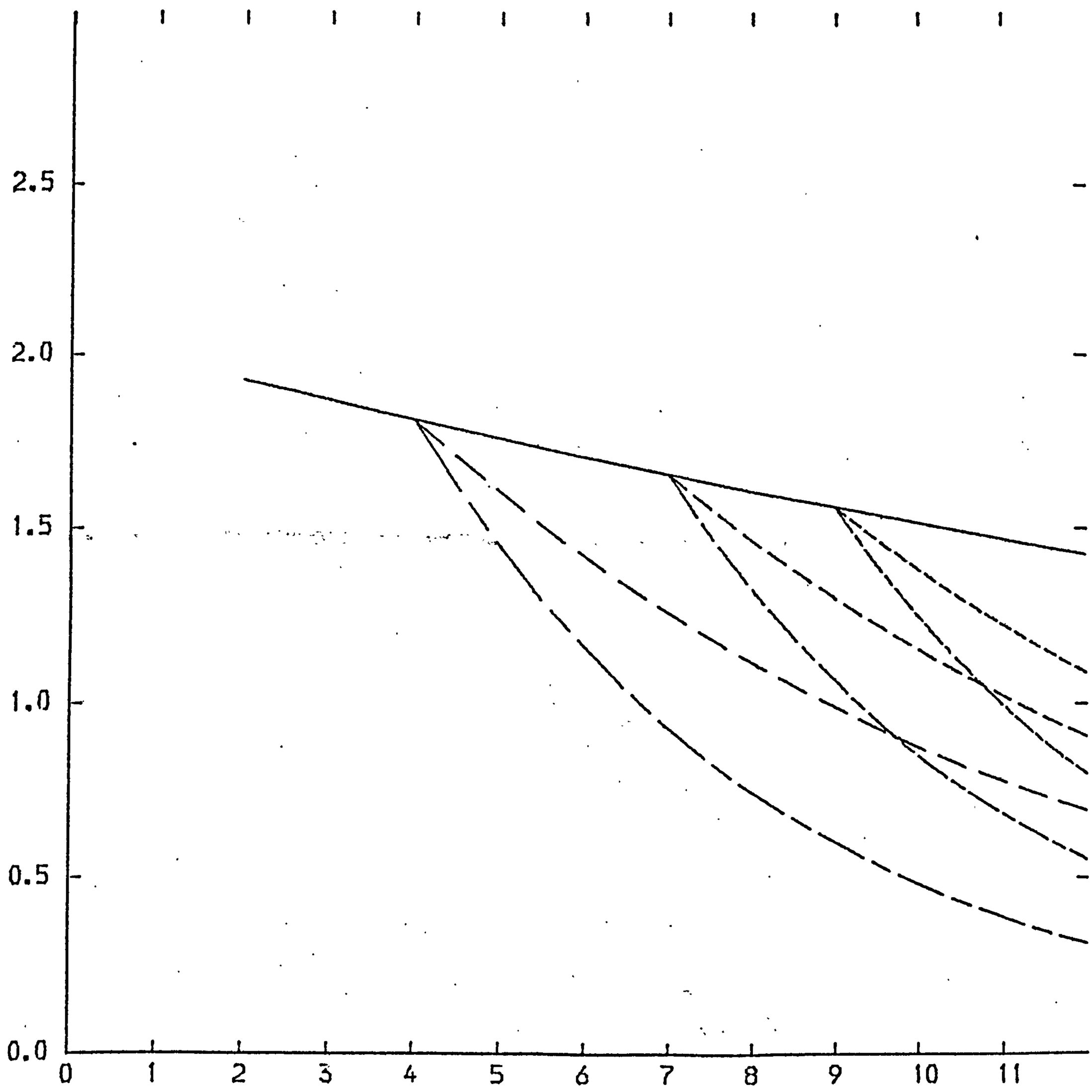


FIGURE 4.8.9 STATISTICAL MODEL DESCRIBING TECNAZENE RESIDUE DECAY UNDER DIFFERENT STORAGE CONDITIONS.

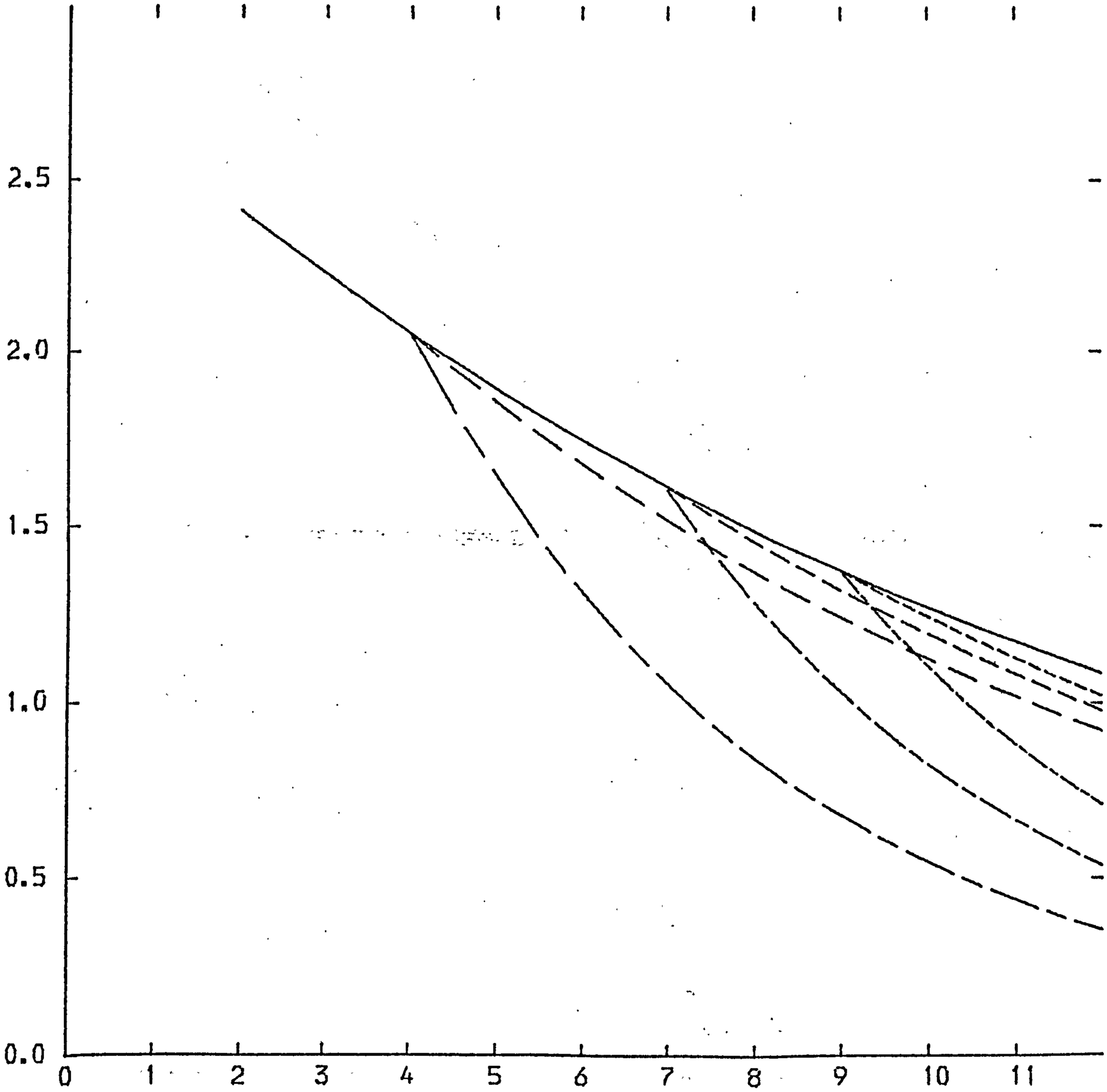
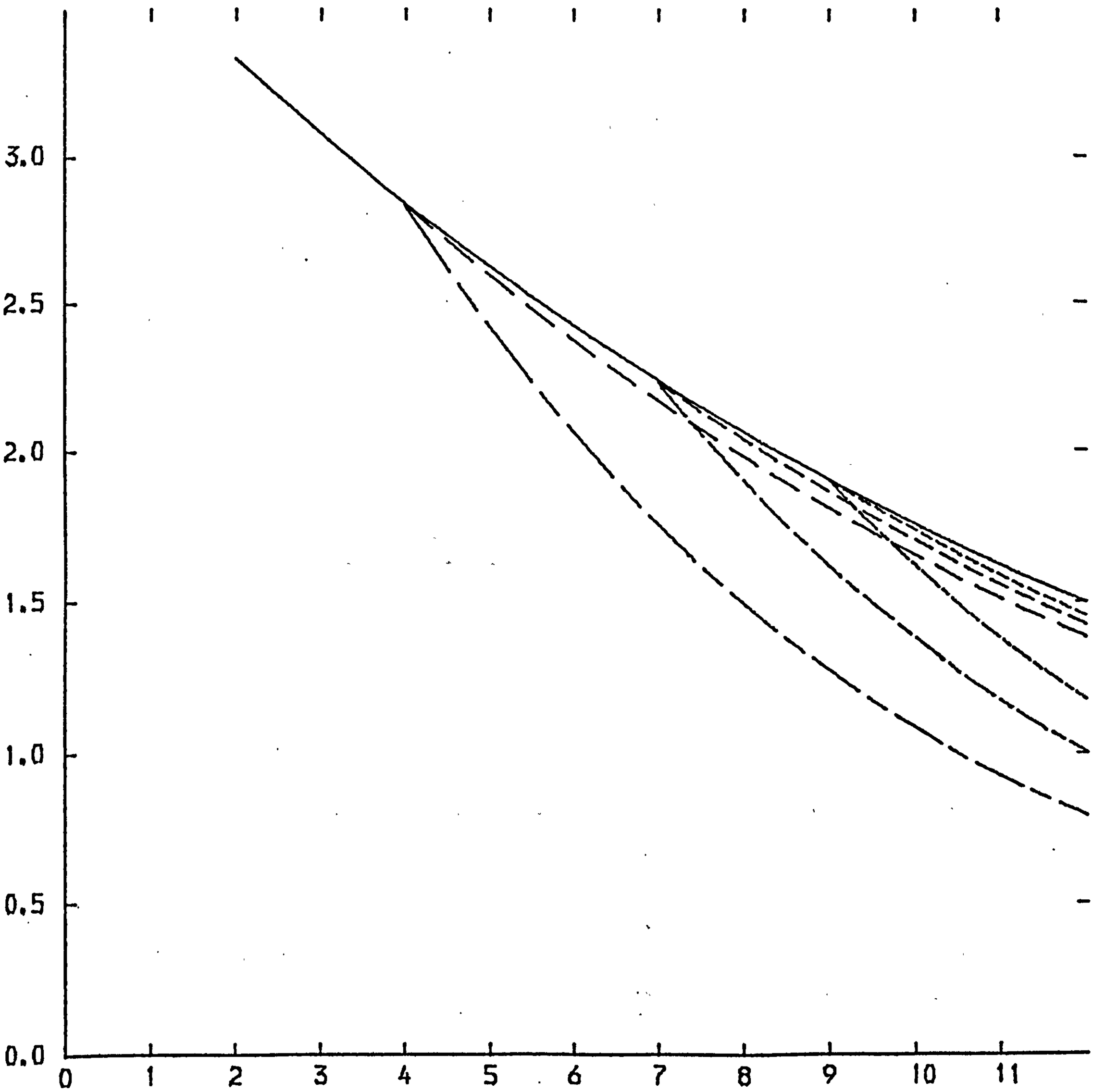


FIGURE 4.8.10 STATISTICAL MODEL DESCRIBING TECNAZENE RESIDUE DECAY UNDER DIFFERENT STORAGE CONDITIONS.



commercial conditions.

Now consider the null hypothesis $\beta_i = 0$

i.e. We are asking if the rate of residue decay under normal conditions (closed box at 8^oC) is significantly different from zero.

The estimated variance of $\hat{\beta}_i = *(0.00973).\hat{6}_i^2$

* is a function of the experimental design.

Therefore the T value $= \dfrac{\hat{\beta}}{\sqrt{0.00973.6_i^2}}$

Now as there are 23 observations for each cultivar and the model contains 4 parameters, there are 19 degrees of freedom.

T_{19} = 2.09 at the 5% level

The calculated T values for each cultivar are shown in table 4.8.5.

Table 4.8.5. The significance of residue decay during storage at 8^oC in closed boxes

Cultivar	T value	Significance
Redskin	-2.00	significant at 0.1>P<0.05
Record	-0.62	not significant
P.Crown	-2.70	significant at P<0.05
M.Piper	-2.54	significant at P<0.05

The decay of residues in closed boxes at 8^oC is therefore significantly different from zero in 3 out of 4 cases.

f) <u>Residue decay during storage with airing</u>

Let Ψ be the difference in the rate of decay of log residue values between normal storage and the rate of decay of log residue values under airing conditions.

Then for each cultivar

$$\psi_1 = \beta i - \alpha_{i(8)}$$
$$\psi_2 = \beta_i - \alpha_{i(12)}$$

Then a 95% confidence region of ψ_1 and ψ_2 is described by an ellipse of the form

$$\underset{\sim}{\psi}:(\underset{\sim}{\psi}-\hat{\underset{\sim}{\psi}})^T \; A \; . \; (\underset{\sim}{\psi}-\hat{\underset{\sim}{\psi}}) \leq d$$

(which translated means - all the values of ψ which are "close" to the least squares estimate taking into account correlations between the estimates, as there is some interdependence)

where $\hat{\psi}$ is the least squares estimator of ψ and

is the centre of the ellipse

A is a matrix determined by the design of the experiment, and so is the same for all cultivars, and determines the shape of the ellipse

d depends on the value of σ_i^2 and determines the size of the ellipse

(N.B. a statistical evaluation of the normality assumption was made and the model found to adequately fit the data).

The 95% confidence regions produced by this method for each cultivar are illustrated in figure 4.8.11.

The X axis is $\beta_i - \alpha_{i(8)}$ i.e. normal rate - rate at 8^oC with airing, and the Y axis is $\beta_i - \alpha_{i(12)}$ i.e. normal rate - rate at 12^oC with airing.

If the X axis cuts the ellipse then we cannot reject the hypothesis that normal storage and airing at 12^oC are the same at a level below 5%.

i.e. if the X axis cuts the ellipse then there is no statistically significant difference between normal storage and airing at 12^oC in terms of decay rate.

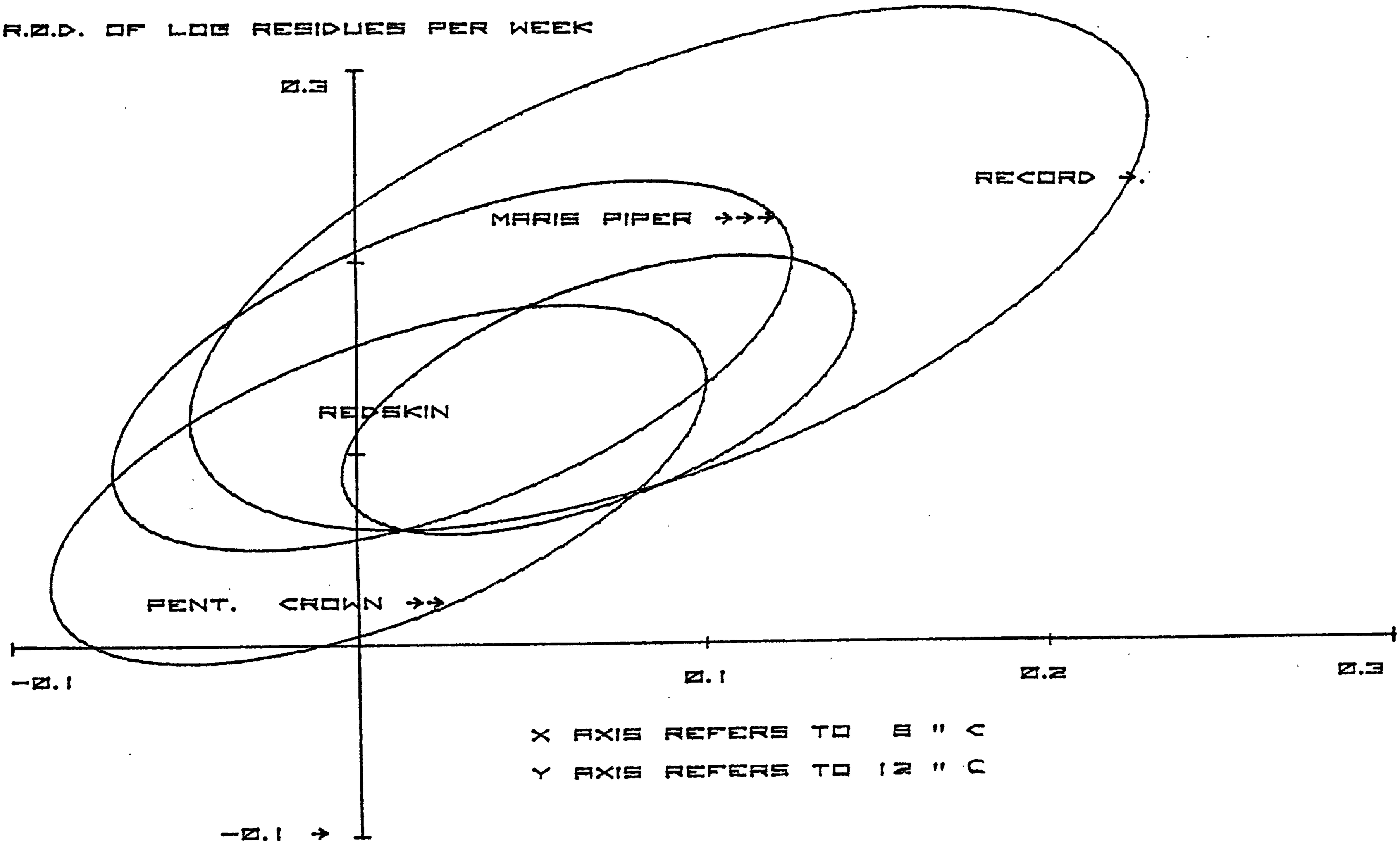
If the Y axis cuts the ellipse then we cannot reject the hypothesis that normal and airing at 8^oC are the same at a level below 5%.

i.e. if the Y axis cuts the ellipse then there is no statistically significant difference between normal storage and airing at 8^oC in terms of decay rate.

If the ellipse cuts the 45^o line through the origin then we cannot reject the hypothesis that airing at 8^oC and airing at 12^oC

FIGURE 4.8.11

CONFIDENCE REGIONS FOR DIFFERENCES IN R.Ø.D. OF LOG RESIDUES PER WEEK



X AXIS REFERS TO 8 " C

Y AXIS REFERS TO 12 " C

are the same at a level below 5%
i.e. $\psi_1 = \psi_2$ which implies $\beta_i - \alpha_{i(8)} = \beta_i - \alpha_{i(12)}$ which is equivalent to $\alpha_{i(8)} = \alpha_{i(12)}$
i.e. if the 45^o line cuts the ellipse then there is no statistically significant difference between airing at 8^oC and airing at 12^oC, in terms of decay rate.

From figure 4.8.11. it is therefore obvious that in three out of four cultivars airing at 12^oC produces a significantly greater rate of decay than normal storage at 8^oC in closed boxes, and in the case of P.Crown the difference is almost significant.

There are no such significant differences in the case of 8^oC airing and 8^oC closed storage. Although the bulk of each ellipse lies towards the left, it is cut by the Y axis so the difference in decay rates is not significant at $P < 0.05$.

For comparison similar ellipses using a linear model are shown in figure 4.8.12., where apparently only the difference in decay rate between normal and airing at 12^oC for the cultivar Redskin is significant at $P < 0.05$.

g) <u>Validation of the model</u>

The statistical model described above was validated by comparison with the most general model possible i.e. a model where different treatments have different expected values with no linear relationship and these expected values are estimated by the respective cell means.

This general model would have 10 parameters, estimated by the mean of each treatment cell i.e. the means of the replicates. There are 4 of these at time of opening, 3 after 8^oC airing and 3 after 12^oC airing.

The statistical model has only 4 parameters (see earlier).

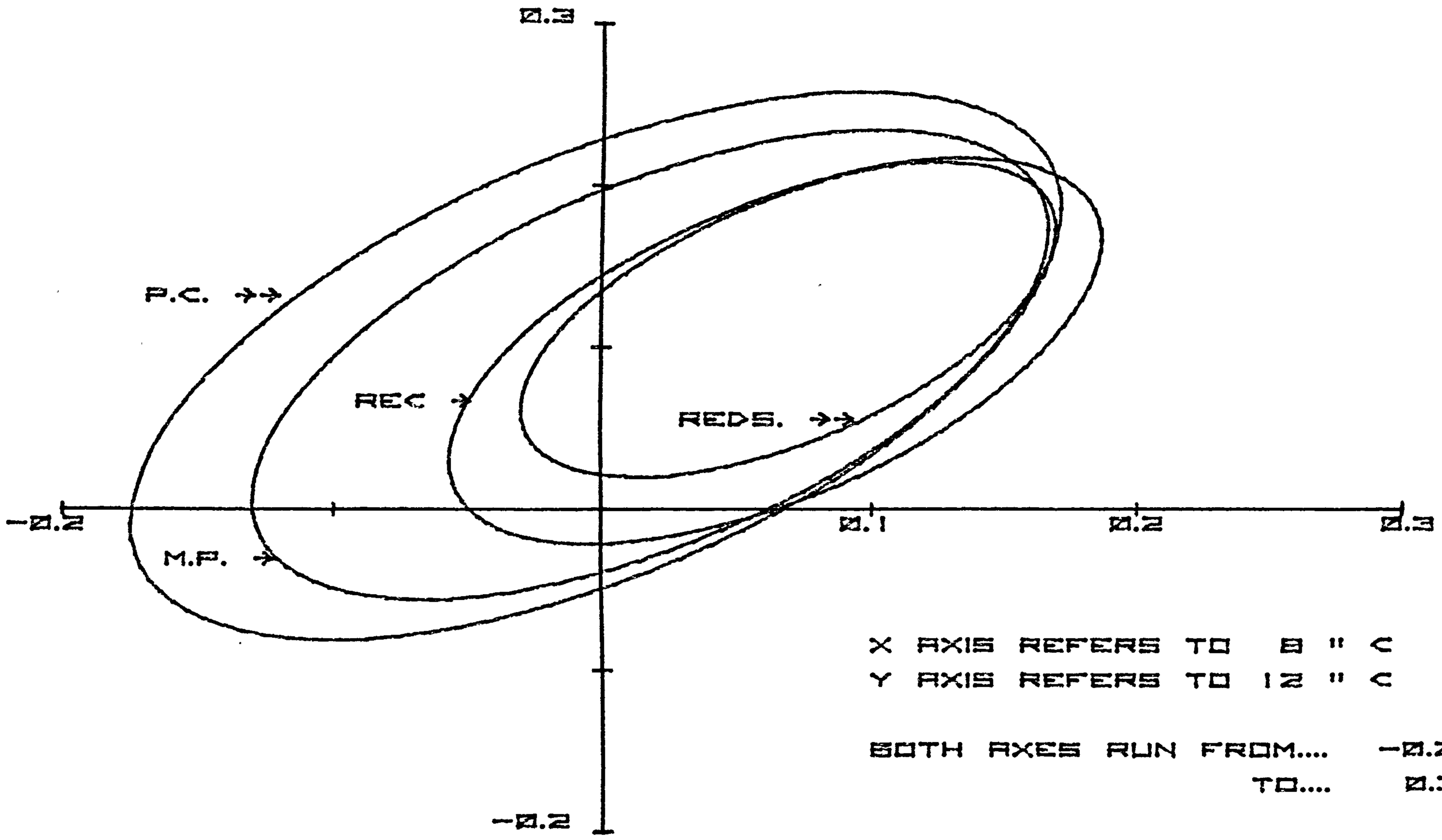
By comparison of the residual variations (i.e. that left unexplained by each model) the model can be validated.

If the statistical model produces unexplained variation which is only slightly greater but not significantly greater than that obtained from the general model then the model is justified.

This comparison is achieved for each cultivar by calculating an F statistic for each model from the residual sum of squares

FIGURE 4.8.12

CONFIDENCE REGIONS FOR DIFFERENCES IN RATE OF DECREASE OF RESIDUES PER WEEK →→→ DUE TO STORAGE TEMPERATURES



X AXIS REFERS TO 8 " C
Y AXIS REFERS TO 12 " C

BOTH AXES RUN FROM.... −0.2
TO.... 0.3

under each model and the degrees of freedom for each.

The residual sums of squares are given by:

1) for the general model

$$R_{gen} = \sum_{jk} (x_{ijk} - \bar{x}_{ij.})^2$$

where $x_{ij.}$ is the cell mean for that treatment

and

2) for the linear statistical model

$$R_{stat} = \sum_{jk} (x_{ijk} - E_{ij})^2$$

where E_{ij} is the predicted value for that treatment under the linear model

(The summation is over all observations in the experiment for that cultivar)

The F statistic is given by:

$$F = \frac{R_{stat} - R_{gen}}{R_{gen}} \cdot \frac{\text{difference in degrees of freedom between the models}}{\text{degrees of freedom for the general model}}$$

Degrees of freedom for general = 23 observations - 10 parameters

Degrees of freedom for statistical= 23 observations - 4 parameters

$$\text{i.e. } F = \frac{R_{stat} - R_{gen}}{R_{gen}} \cdot \frac{13}{6}$$

From tables

$F(6, 13) = 2.92$ at the 5% level

(N.B. the 5% test in this case is more severe than the 1% test as it is more likely that the hypothesis that the models are the same will be rejected)

As the values of R_{stat} are in log residue squared then of course the units of R_{gen} must be similar.

The calculated values are shown in table 4.8.6.

As can be seen from table 4.8.6. the F statistic for each cultivar is less than the value from the tables. This means in statistical terms that the null hypothesis that the linear model is true cannot be rejected, as the residual sums of squares for the models are not significantly different.

In non-statistical terms this implies that the statistical model fits the data.

Table 4.8.6. Validation statistics for the statistical model describing residue decay

Cultivar	R_{gen}*	R_{stat}**	F statistic
Redskin	0.690	1.032	1.07
Record	1.758	3.628	2.30
P.Crown	0.993	1.700	1.54
M.Piper	1.605	1.808	0.27

* Residual sum of squares from the most general model

** Residual sum of squares from the statistical model

As a further aid to the comparison of the experimental results with those predicted by the model, they have been plotted against each other in figure 4.8.13. It is obvious from this plot that the predicted values and the experimental observations are very closely related. The correlation coefficient has also been calculated and although it is not an accepted measure of "goodness of fit" for models, it is reported because of its general familiarity.

4.8.5. Summary

The main points to emerge from the above experiment were:

1) Absolute levels of tecnazene in the 92 samples analysed after treatment at 135mg kg^{-1} and storage under various conditions ranged from 0.1 to 3.9mg kg^{-1} in washed samples.

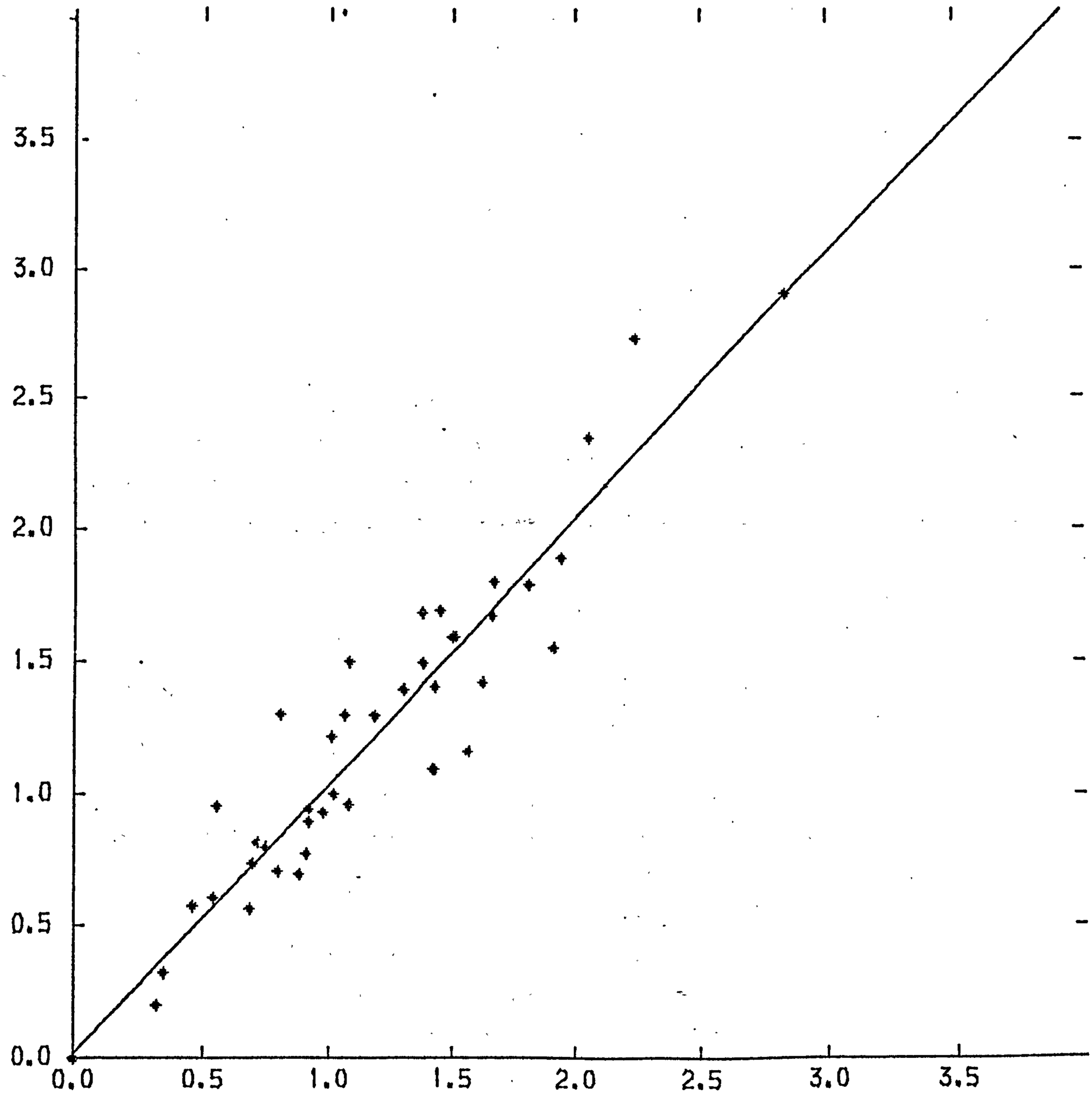
2) These levels are cultivar dependent. In the experiment above Pentland Crown had higher levels than the others tested.

3) Tecnazene residues decline with storage.

4) This residue decay appears to be logarithmic implying that the rate of decay is linearly dependent upon concentration of tecnazene present.

5) Storage conditions influence this decay process.

FIGURE 4.8.13 COMPARISON OF EXPERIMENTAL RESIDUE VALUES WITH THOSE OBTAINED FROM THE STATISTICAL MODEL FOR RESIDUE DECAY



EQUATION OF BEST LINE IS Y = 1.01 X + 0.02

CORRELATION COEFFICIENT = 0.95

SIGNIFICANCE =0.00001

6) Storage at 8°C in closed boxes produced a slight but significant reduction in residue levels in most cases.

7) Storage at 8°C with airing appears to be little different from storage at 8°C with no airing.

8) Storage at 12°C with airing however produced a significantly faster rate of residue decay than normal 8°C storage.

9) Storage at 12°C with airing produced a significantly faster rate of residue decay than 8°C with airing.

10) The statistical model which was constructed predicts that only storage for 5 to 8 weeks at 12°C will produce residue levels less than 1mg kg^{-1} in all cultivars (see chapter 5).

4.9. TECNAZENE IN POTATO STORES

As there was no information on whether tecnazene treatment of stored potatoes could lead to contamination of the store, which might have implications for the storage of subsequent potato crops, the following investigation was undertaken. Also investigated was the possibility that as tecnazene is volatile parts of the storage building not actually containing treated tubers might become contaminated from regions which did contain such material.

Experimental

1) Source of samples

Samples were taken from two adjacent 12 tonne storage bins at the Potato Marketing Board Experimental Station, Sutton Bridge, Lincs., which had well documented histories.

One of these bins (bin No. 10) had held tecnazene treated tubers during the previous storage season (1975-76). The other (bin No.11) had never held tecnazene treated tubers at any time. Both bins had been lying empty during the long hot summer of 1976.

Samples were taken during November by the staff of the Station by removing wall and floor drillings using a 25mm drill to a depth of 12.5mm, to produce individual samples weighing approximately 5g from various regions within the bin.

Dust samples were also collected from each bin, and all samples

were sealed in polythene bags and sent to Glasgow for analysis.

2) Analysis

a) determination of recovery rates

Samples of sawdust were spiked with 100µg tecnazene and placed in extraction thimbles. They were then extracted in a Soxhlet unit with 75cm^3 nanograde hexane for 4 hours.

After cooling and removal of the thimble the condenser was washed with 15cm^3 hexane and the extract transferred to a 100cm^3 volumetric flask which was then made up to the mark.

Aliquots were analysed by GC using electron capture detection because of interference with FID detection from an unknown compound with a retention time close to that of tecnazene.

Recovery was 96.2% (mean of duplicate samples). As a further check, a repeat extraction of several samples showed that most of the tecnazene (94%) had been removed by the four hour extraction.

b) analysis of samples

The samples were treated in exactly the same way as described above, except that in a few cases further dilution was necessary to allow analysis by ECD. The dust samples were analysed in the same manner.

Results and Discussion

The results of these analyses are shown in table 4.9.1.

There were tecnazene residues present in every sample tested, both from the treated bin (10) and the untreated bin (11).

Surprisingly the amount present in both bins was of the same order. The dust samples contained especially high concentrations of tecnazene.

However, on comparison with the formulation (30g kg^{-1}) and the recommended application rate of 135mg kg^{-1}, it would appear unlikely that these residues could produce a physiologically active concentration in subsequently stored tubers, so the storage of seed in bins contaminated at this level should produce no problems (see Chapter 5).

As far as ware potatoes are concerned it is almost certain that ultra-low level contamination could occur (detectable only using ECD

Table 4.9.1. Tecnazene residues in Sutton Bridge storage bins

Sample		Tecnazene (mg kg^{-1} of wood)			
		Bin 10		Bin 11	
Floor:					
	1	10.0	(8.4-11.7)*	11.7	(10.4-13.2)
	2	11.6	(9.8-13.7)	5.6	(5.0- 6.4)
	3	15.5	(13.2-18.2)	17.7	(15.0-20.9)
	4	16.8	(14.3-19.8)	13.6	(11.3-15.8)
	5	8.1	(7.2- 9.1)	8.5	(7.5- 9.6)
	6	8.5	(7.5- 9.6)	2.9	(2.5- 3.2)
	Mean:	11.7		10.0	
Walls:					
	1	6.5	(5.9- 7.1)	5.0	(4.2- 5.9)
Low	2	1.6	(1.4- 1.9)	3.6	(3.1- 4.3)
	3	6.1	(5.1- 7.1)	9.7	(8.2-11.5)
	Mean:	4.7		6.1	
	4	3.0	(2.8- 3.4)	21.8	(18.5-25.9)
High	5	3.7	(3.2- 4.3)	10.8	(9.1-12.8)
	6	4.4	(3.8- 5.1)	7.1	(6.0- 8.3)
	Mean:	3.7		13.2	
Dust:		34.5	(31.6-37.7)	22.1	(18.7-26.1)

* Values in brackets are lower and upper limits of the 95% confidence interval

techniques), however this would be of little toxicological significance (see 4.11.).

Carry-over contamination in potato stores is unlikely therefore to present a major problem with tecnazene. The levels on 0.5 and 1 tonne storage boxes would probably be even lower. However it should be borne in mind that this experiment measured residues after the bins had been empty for several months. Short term carry-over might still be worth investigating (see below).

The most significant information from this experiment is however the way in which the untreated adjacent bin has been contaminated. Tecnazene vapour must have passed over the dividing wall between the bins, which certainly must have produced substantial contamination of the potatoes stored in that bin during the 1975-76 storage season.

Observations from experiments conducted at Rothamsted would tend to confirm this latter point. In experiments which involved placing treated one tonne slatted boxes of potatoes in the same store with untreated boxes substantial tecnazene redistribution was noted, such that some untreated samples contained almost as much tecnazene as treated batches when assayed after 6 months storage (Fowden et al, 1975).

4.10. IMPLICATIONS OF TECNAZENE RESIDUES ON FLAVOUR

The flavour of potatoes is affected by many pesticides. Insecticides are the most common offenders.

Hexachlorocyclohexane, chlordane, parathion, heptachlor, dieldrin, aldrin and endrin have all been shown to adversely affect flavour under some conditions (Greenwood and Tice, 1949; MacLinn et al, 1950; Turner, 1950; Jameson and Peacock, 1953; Dawson et al, 1953; Kirkpatrick et al, 1955). It is interesting to note that all but one of these compounds are organochlorines.

With tecnazene early experiments produced no such off-flavours (Emilsson et al, 1949; 1951; Mooi, 1949). However more refined experiments by Wilson and Harris (1954) showed that tecnazene can significantly reduce flavour quality in tests involving the

presentation of boiled mashed potatoes alone to various taste panels. Propham which was included in these experiments had no effect.

The adjectives most commonly used by members of these taste panels to characterise the off-flavour were "metallic" and "bitter". It was also evident that the off-flavour was more pronounced in March than when sampled two months later in May.

Apparently the off-flavour cannot be detected when potatoes are eaten as part of a complete meal rather than alone.

Similarly Gooding et al (1956) noted that dehydrated potatoes made from tecnazene treated stocks possessed an "earthy" taint.

During the course of the work in this thesis a manufacturer of extruded fried products contacted this laboratory about a metallic taint in his product when using tecnazene treated potatoes.

Whether these off-flavours are in fact due to tecnazene itself, a metabolite of tecnazene (see below), an impurity in the tecnazene (see below) or to indirect effects of tecnazene treatment on natural flavour components is a matter of some doubt.

4.11. THE TOXICOLOGY OF TECNAZENE

N.B. The metabolism of tecnazene is discussed in section 4.12.

4.11.1. A survey of toxicological studies

1) Feeding experiments

Buttle and Dyer (1950) conducted an investigation into the effects of feeding tecnazene containing diets to rats and mice. Because of the importance of these investigations their results have been summarised below.

Feeding mice with diets containing 1.36, 13.6, and 136g kg^{-1} tecnazene (x 10, x 100 and x 1000 respectively, the rate at which potatoes are treated) they found that all levels produced reduced food intake. The lowest level-equivalent to 215mg kg^{-1} body weight/day produced normal growth, the intermediate level (1750mg kg^{-1}/day) completely inhibited growth, but no other effects were observed, and with the highest level (10,000mg kg^{-1}/day) 20% of the mice died

of fatty degeneration of the liver.

With rats fed in a similar fashion with diets containing enough tecnazene to produce daily doses of 57, 400, and 1111mg kg^{-1} body weight, it was found that tecnazene was more toxic. In fact all of the rats on the highest rate were dead at the end of the 5 week experiment. Those on the lowest level however showed no effects, whilst those on the intermediate level showed only 67% of the weight gain of the controls. At the two higher levels they found that a pigmented substance was being excreted in the urine.

Abrams et al (1950) have shown that the toxicity of tecnazene towards pigs fed on treated potatoes is of the same order as that in rats.

2) Inhalation tests

As tecnazene is very likely to be inhaled by workers during its application to potatoes, Buttle and Dyer (1950) subjected rats to tecnazene vapour. They noted no ill-effects.

3) Effects on eyes and skin

Buttle and Dyer (1950) also investigated possible irritation of the eyes and skin of rabbits. They found no irritation but noted that exposure of skin to tecnazene treatment for a few days can produce blackening and induration. The pigmentation could be produced even when the tecnazene was applied as a 2% dust in china clay i.e. a lower concentration than used in the commercial formulations.

More recently a case of occupational toxicodermia (dermatitis) which appeared after work with tecnazene in agricultural practice has been reported (Lopukhova, 1965). It was noted that this disorder was accompanied by increased subsequent sensitivity to tecnazene.

4) Carcinogenesis

Searle (1966) has studied the effect of tecnazene and its isomers on skin carcinogenesis in mice.

He found that when 0.2cm^{-3} of a 0.3% acetone solution was applied twice weekly to the clipped backs of mice, and croton oil employed as a promoting agent, then multiple papilloma formation

occurred. (Small skin tumours formed from hypertrophy of papilla) The 2,3,4,5 isomer appeared most active in this respect, but all isomers tested acted as mild tumour initiators.

Searle (1966) suggested that this tumour initiation is due to hydroxylamine derivatives formed as intermediates in the metabolic reduction of nitro groups (see section 4.12.1.).

Histological examination of the larger tumours showed one to be malignant in a tecnazene experiment although this cannot be treated as meaningful in a statistical sense (also see 3.3.2.1g. for problems in this type of study)

Apparently (from verbal communication with I.C.I.) further toxicological studies are being conducted but the results of these were not available at the time of writing.

4.11.2. Implications with respect to the current study

Burton (1974) states:

"If sprouting is suppressed there must inevitably be a physiologically active concentration of chemical present, at least in the region of the buds. This need not of course, imply that there is a physiologically active concentration in relation to the body weight of the consumer who has eaten a comparitively small quantity of treated potatoes".

Bearing this statement in mind, an attempt has been made to relate the results of the toxicological experiments described above to results of the experiments of the current study and to commercial practice.

From the evidence above tecnazene is probably best described as relatively non-toxic but not absolutely safe, but of course very few chemicals can be regarded as absolutely safe.

The American Food and Drug Administration have published a tolerance of 25mg kg^{-1} on or in potatoes - a figure which reflects their assessment of the toxicological data (Anon, 1966). At this level it is unlikely that any toxicity problems will arise.

The residue results produced in the current study are substantially below this level. From extrapolation of the results of Buttle and Dyer (1950) to humans, a 100kg man eating peeled potatoes containing 0.2mg kg^{-1} fresh weight, could eat 25 tonnes

per day with no toxic effects due to tecnazene (i.e. equivalent to 50mg kg^{-1} body weight). The safety margin is therefore substantial.

Eating unpeeled potatoes would reduce this safety margin by a factor of 10-20. Shaw et al (1973) showed that crisps made from unpeeled tubers are acceptable but the major disadvantage was likely to be sprout suppressant residues. Even so with tecnazene the safety margin would appear adequate.

However, it should be borne in mind that if the findings of Searle (1966) were further substantiated and tecnazene proven to be carcinogenic then of course the situation would have to be reviewed. Recent American thinking seems to indicate that there is no safe level once a compound is proven carcinogenic.

Another point which must also be considered is that most of the toxicological data have been obtained with highly purified tecnazene, and so no account has been taken of possible metabolites, impurities or the products of chemical degradation. These aspects are discussed below in sections 4.12., 4.13. and 4.14. respectively.

4.12. THE METABOLISM OF TECNAZENE

During the course of this study it has become increasingly obvious that the metabolism of herbicide derived anilines is extremely relevant to the metabolism of tecnazene. This section therefore should be considered in conjunction with 3.3.2.3h. where certain aspects of chlorinated aniline metabolism were discussed in considerable depth. It is perhaps worth repeating that as much of this information was derived from studies on the phenylamide and phenylcarbamate herbicides it was considered that it was more appropriate to review the subject under the heading of chlorpropham.

The present section has been divided, for convenience, into 3 sub-sections discussing the metabolism of tecnazene in animals, micro-organisms and in plants respectively. Although all 3 sections take the form of literature reviews, an investigation, outwith the scope of this thesis, is planned to supply further information on microbial and plant metabolism of tecnazene.

4.12.1. <u>The metabolism of tecnazene in animals</u>

The metabolism of tecnazene has been investigated in some depth.

Bray <u>et</u> <u>al</u> (1953) fed tecnazene (2,3,5,6) and its 2,3,4,5 isomer to rabbits and examined the resultant metabolites.

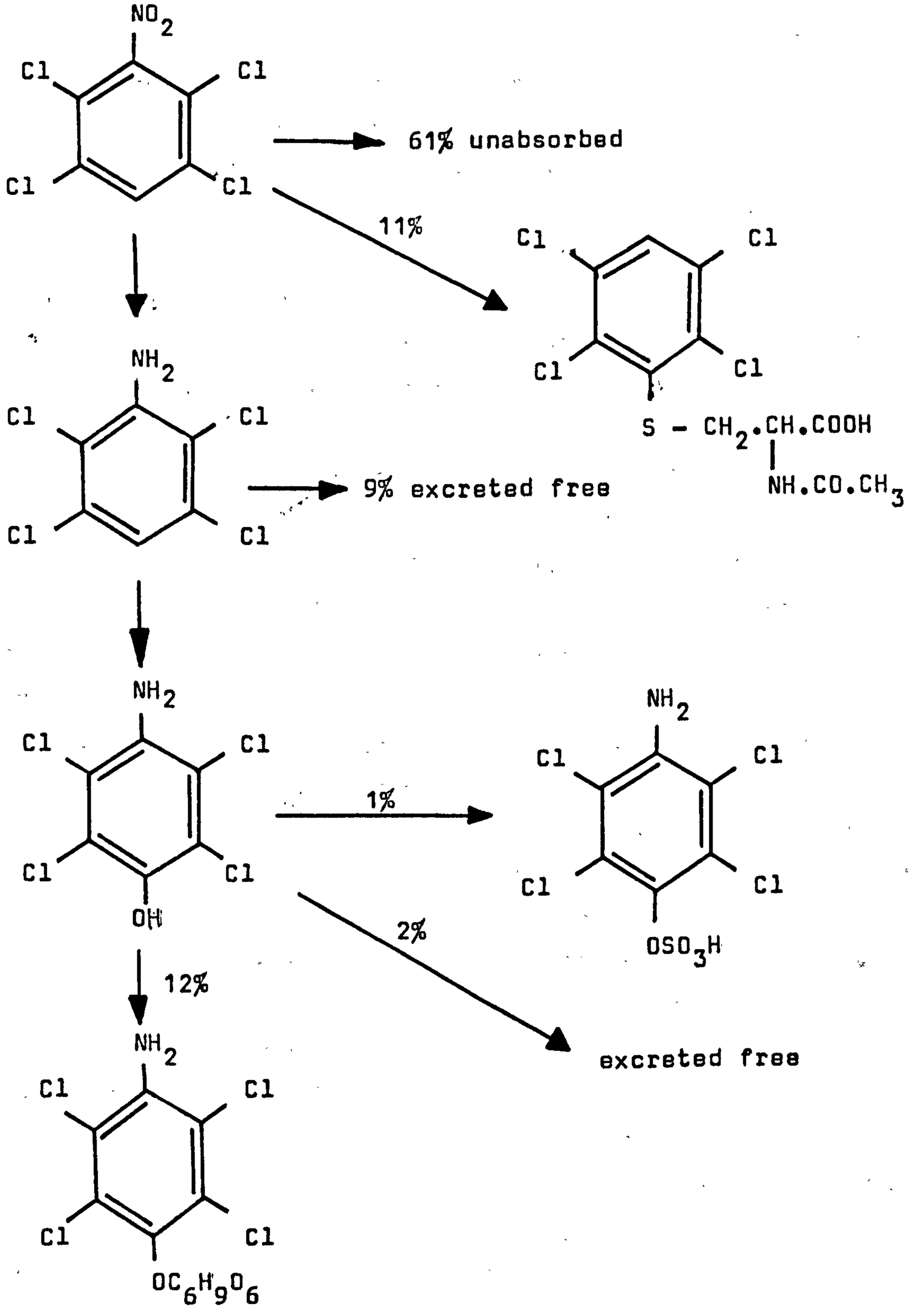
They found that tecnazene is not readily absorbed from the gut. However on feeding very small amounts the percentage absorbed increased substantially so that with a 10mg dose fed to a 2-3kg rabbit 70-80% was absorbed. It would seem likely therefore that when amounts such as those found in the present residue study are ingested by humans then the majority will be absorbed.

Of the 39% absorbed (see figure 4.12.1.) they found that 90% was excreted in the urine as various metabolites - 23% as 2,3,5,6-tetrachloroaniline, 5% as 4-amino-2,3,5,6-tetrachlorophenol, 31% as a glucuronide (not fully characterised), 3% as ethereal sulphate and 28% as a mercapturic acid, N-acetyl-S-(2,3,5,6-tetrachlorophenyl)-L-cysteine. No unchanged tecnazene was excreted in the urine. The pathways by which these compounds are formed are illustrated in figure 4.12.1. Tecnazene also apparently stimulates the production of <u>m</u>-hydroxybenzoic acid in the urine.

The isolation of the mercapturic acid was unusual in that the attachment of the cysteinyl group involved the elimination of the nitro function rather than the more common halogen replacement. Bray et al (1953) concludes that the mercapturic acid is formed either directly from tetrachloronitrobenzene or from some intermediate product of its reduction to tetrachloroaniline but not from tetrachloroaniline itself as no mercapturic acid is formed when it is administered. They found no increase in the excretion of nitrite in the urine which might be expected if nitrogen was liberated from some possible intermediates in the reduction process such as tetrachlorophenylhydroxylamine (but see below and Searle, 1966).

Also of interest is the fact that the metabolism of 2,3,4,5-tetrachloronitrobenzene is different from that of the symmetrical isomer in that no such mercapturic acid is formed. Tecnazene has

Figure 4.12.1. The metabolism of tecnazene in rabbits (after Bray et al, 1953)



three electron attracting groups in the vicinity of C_1, and the 2,3,4,5 isomer has only two. Later studies (Betts et al, 1955) have in fact shown that a mercapturic acid is formed from the 2,3,4,5 isomer but that it is formed by acetylcysteinyldechlorination rather than acetylcysteinyldenitration.

These later studies by the same group included a very interesting experiment in which the effect of treating various polychloronitrobenzenes with ethanolic sodium hydroxide is compared with their ability to form mercapturic acids in the rabbit. They found that the amount of nitrite liberated by this method was closely correlated with mercapturic acid formation. Tecnazene was 82% hydrolysed after 15 minutes reflux and 36% of an administered dose appeared as mercapturic acid in rabbit urine, whereas with the 2,3,4,5-isomer similar experiments produced no hydrolysis as determined by nitrite liberation and no mercapturic acid. An intense yellow colour is in fact produced with NaOH which is probably a phenol produced by displacement of a halogen group (see 4.2.2.).

Later work by the same group using some improved techniques (Betts et al, 1957) on the metabolism of closely related trichloronitrobenzene isomers is also of interest as such compounds might possibly be formed in soil (see below). In similar experiments to those described above, they found mercapturic acids with most isomers, formed by acetylcysteinyldechlorination with the 2,3,4-, 2,3,5-, 2,4,5-, and 3,4,5- isomers, and by acetylcysteinyldenitration with the 2,3,6- and 2,4,6- isomers. This indicates that nitro-lability is not the sole factor involved as the nitro-group of the 2,3,5- isomer is in fact more labile than that of the 2,4,6- isomer but as with the tetrachloronitrobenzenes both ortho positions must be chlorinated before acetylcysteinyldenitration is possible. The mechanism of mercapturic acid formation will be further discussed in a separate sub-section.

Betts et al (1957) also found that reduction was an important pathway in the metabolism of the trichloronitrobenzenes. All the isomers tested formed the corresponding substituted amines which are then excreted free or in acid labile conjugates or alternatively are further metabolised, to produce substantial quantities of

hexachloroazoxybenzenes in the cases of the 2,4,5- and 3,4,5- isomers. Should similar products be formed from tecnazene or any of its preconsumption metabolites this would have extremely serious toxicological implications, regardless of how minute a quantity was formed. Azoxy- and azo- benzenes were of course discussed in some detail in section 3.3.2.3h. It should be borne in mind however that azoxy- or azo-benzene production would be near impossible without first removing at least one of the chlorines from an ortho position in tecnazene. (Bordeleau and Bartha, 1972c), Dechlorination,of course,is a well documented metabolic pathway for pesticides, especially in soils (Kaufman, 1971). Poland (1977, private communication) has shown that some hexachloroazobenzenes can be as powerful inducers of hepatic aryl hydrocarbon hydrolyase activity as the most potent of the tetrachloro- compounds.

Mechanism of mercapturic acid formation

Booth et al (1961) discovered an enzyme which catalyses the formation of S-(2-chloro-4-nitrophenyl)-glutathione from 3,4-dichloronitrobenzene and glutathione (ɣ-L-glutamyl-L-cysteinyl-glycine). The best source of this enzyme is the soluble fraction of rat liver homogenate. They also demonstrated that this glutathione conjugate could be converted into S-(2-chloro-4-nitrophenyl)-L-cysteine by rat kidney homogenate, and this in turn could be converted to a mercapturic acid, N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine by rat liver slices.

They showed that the soluble rat liver fraction could also catalyse the formation of glutathione conjugates from 13 other mercapturic acid precursors including tecnazene. The elimination of nitrite during this conjugation with tecnazene and its 2,3,4,6-isomer was subsequently demonstrated (Al-kassab et al, 1962). Two moles of glutathione were used for every mole of nitrite liberated. Similarities in this behaviour with the carcinogens 4-nitroquinoline -N-oxide and 4-nitropyridine-N-oxide were indicated.

Later work demonstrated that the enzyme (glutathiokinase) in the soluble rat liver fraction, which catalyses the replacement of labile aromatic nitro-groups in compounds like tecnazene, is

identical to the enzyme which conjugates 3,4-dichloronitrobenzene and glutathione with the liberation of chloride (Al-Kassab et al, 1963).

With pigeon liver supernatant similar catalytic properties have been observed. Again nitrite is released during the conjugation of glutathione with tecnazene (Wit, 1969).

More recent work has shown that liver supernatant contains several of these glutathione transferases which will catalyse the conjugation with compounds bearing a nucleophilic substituent (Pabst et al, 1973). They are however relatively non-specific.

Summary

In the animal, tecnazene is readily metabolised, which probably accounts for its relatively low acute and chronic toxicity. The products of this metabolism are equally as readily excreted in the urine. Two factors are perhaps worthy of further investigation. Firstly some of the metabolic products from closely related compounds are toxic and animal studies on degradative products of tecnazene before consumption might therefore be worthy of consideration once the identity of such compounds has been established (see below). Secondly the eventual fate of the missing 10% in the experiments of Bray et al (1953) is also worthy of more consideration although inaccuracies in the complex estimation procedures undoubtedly account for some of this material.

4.12.2. The metabolism of tecnazene by micro-organisms

There are no reports in the literature on the metabolism of tecnazene by micro-organisms in potato stores. However there are several papers which discuss the metabolism of polychlorinated nitrobenzenes by bacteria and fungi in soil and in cultures. Most of this material deals principally with quintozene but as will become apparent below the metabolism of tecnazene is believed to follow similar routes.

Chacko et al (1966) examined the degradation of quintozene by a wide variety of actinomycetes and fungi in culture solutions and in every case found some degree of degradation. The actinomycete Streptomyces aureofacens was particularly potent, degrading 25-50% of of the quintozene in the culture solution within 6 days. A metabolite was subsequently isolated in crystalline form and identified as pentachloroaniline by various techniques including mass spectrometry.

Nakanishi and Okum (1969) using culture solutions of Fusarium oxysporum found quintozene was metabolised to pentachloroaniline and pentachloroanisole. They report that these compounds have low fungal toxicity and proposed that this detoxification might account for the tolerance of many fungi to quintozene. The tolerance of fungi to chlorinated nitrobenzene fungicides including tecnazene has also been investigated by Georgopoulos (1963).

Caseley (1968) investigated the loss of tecnazene and quintozene from sterile and non-sterile soils and found that in both cases most of the chemical applied was lost by volatilisation but approximately 20% of the loss of both tecnazene and quintozene could be attributed to microbial degradation. When the soil was enriched with sucrose the microbial degradation was considerably accelerated. Both chemicals were degraded more slowly than trichloronitrobenzenes and this is accounted for by the greater susceptibility of compounds with fewer chlorine atoms on the benzene ring, to microbial degradation. Caseley (1968) did not idenify the metabolites, but gas chromatography of soil extracts showed a variety of compounds were produced all of which contained chlorine. The general pattern of peaks from tecnazene was similar to those produced by quintozene.

There is, therefore, evidence to suggest that microbial degradation of tecnazene might lead to its conversion to tetrachloro-

aniline or tetrachloroanisole. This fact, however, requires confirmation. The extent to which these processes occur in the potato store is also worthy of examination.

4.12.3. The metabolism of tecnazene by higher plants

In light of the comprehensive studies of tecnazene metabolism in animals, surprisingly little has been published on its metabolism in higher plants. In fact, the only relevant work which could be located was that of Bokarev and Chertok (1969) who examined the reaction of various growth regulating compounds with cysteine. *In vitro* experiments showed that tecnazene, 2,4,5-trichloroanisole and methyl β naphthylether bound 40-50% of the available cysteine, whilst 2,4-dichloroanisole bound 83%. Application of solutions of 2,4-dichloroanisole to the eyes of potato tubers increased the content of sulphydryl groups in a subsequent water extract, when the chemical was applied at 100mg dm^{-3}. A concentration of 200-400mg dm^{-3} decreased the content of sulphydryl groups in the eyes, and they conclude that the interaction of these compounds with sulphydryl groups on proteins is worthy of consideration when explaining the mechanism of action of these compounds in plants.

In view of these findings and the earlier discussion on tecnazene metabolism in animals, the interaction of tecnazene with sulphydryl groups is certainly worthy of further investigation. Of particular interest would be investigations to demonstrate 1) whether tecnazene is bound to proteins in plants and 2) whether tecnazene interacts with glutathione or some other key sulphydryl metabolite in plants.

4.13. IMPURITIES IN TECNAZENE

Tecnazene is synthesised by nitration of 1,2,4,5-tetrachlorobenzene which is produced industrially by a variety of methods (Martin, 1972).

The starting material may be benzene, although the waste isomers from the production of the insecticide HCH can be dechlorinated to provide a mixture of trichlorobenzenes which may also be used (Galat, 1952).

The starting material must be chlorinated to produce 1,2,4,5-tetrachlorobenzene. Whether this is achieved by conventional techniques using ferric chloride as catalyst or by using antimony trichloride

and iodine as suggested by Fooladi (1971), a mixture of tetrachlorobenzenes results which is fairly difficult to separate. Inevitably, therefore, 1,2,4,5-tetrachlorobenzene must contain at least a very small amount of the 1,2,3,4 isomer, and nitration of this mixture must produce tecnazene and its 2,3,4,5 isomer. In the light of the evidence of Searle (1966) this observation must give cause for concern.

During the course of the present study no deliberate attempt was made to study the impurities in the commercial formulations of tecnazene. However, it has been consistently found that a very small peak is eluted from the gas chromatograph immediately after tecnazene. The data produced by Hammadmad (1967) suggests that this peak could be the 2,3,4,5 isomer.

Other impurities which are known to occur in tecnazene are 1,2,4,5 tetrachlorobenzene (the starting material) and the fungicide chloranil (Lawrence and Warren, 1966).

4.14. NON-BIOLOGICAL DEGRADATION OF TECNAZENE

Although no information has been located on the non-microbial degradation of tecnazene in potato stores Hammadmad (1967) has described the photochemical degradation of tecnazene and quintozene under laboratory conditions.

He found that irradiation of tecnazene in organic solutions with ultra-violet light at 250-260nm resulted in the formation of 1,2,4,5-tetrachlorobenzene, 2,3,5-trichloronitrobenzene and a small amount of 2,3,6-trichloronitrobenzene. Also of interest, in the light of the toxicological studies of Searle (1966) which were discussed in section 4.11., is the observation that 2,3,4,5-tetrachloronitrobenzene, but no tecnazene, was amongst the products from irradiation of quintozene.

The appearance of 2,3,5-trichloronitrobenzene from tecnazene is, however, the most important finding in view of the comments about the ultimate fate of this compound which were made in section 4.12. It should, however, be borne in mind that the level of uv light likely to be found in a potato store is extremely low, and this compound is, therefore, unlikely to be produced by this method.

The influence of alkali on tecnazene has already been mentioned in section 4.2. and this might be of relevance to the industrial technique of caustic peeling, where the surface of the tuber is

exposed to fairly concentrated caustic solutions for a few minutes at a temperature of approximately 90°C. During the current study it was found that when a few crystals of tecnazene were boiled with alcoholic KOH in a test-tube an intense yellow colour immediately developed. When extracted into hexane and injected into the gas chromatograph a variety of peaks are produced. The identification of these peaks and a study of the implications of these observations on caustic peeling is certainly worthy of further study.

CHAPTER 5

THE EFFECT OF TECNAZENE ON THE SUBSEQUENT PERFORMANCE OF TREATED SEED POTATOES

As the title suggests this chapter deals primarily with the effects of tecnazene but it also includes work on various other sprout suppressants and growth regulants. In some cases other sprout suppressants were included for comparison, in others the experiments were only indirectly related to the tecnazene work. However as these experiments were conducted concurrently with the tecnazene work it is considered that they are best included in this chapter rather than as an appendix.

5.1. INTRODUCTION

The treatment of seed potatoes with sprout suppressants is a subject of considerable controversy. Tecnazene as explained earlier is really the only one of the currently used compounds which can be used with any degree of confidence, but even here there appears to be some disagreement in the literature about the implications of its use.

Brown (1947) found that treated tubers would grow once planted out, yielding the same as untreated controls but less than chitted controls. He is however somewhat cautious about the exact application rate of the compound but it was probably in the range 50-80mg kg^{-1} active ingredient. Tubers were planted straight from clamps without airing. He concludes that tecnazene is probably too efficient at retarding sprouting to be used on seed.

Mooi (1949) found the germination of cv Bintjie was impaired by tecnazene treatment at a rate of 134mg kg^{-1}, and in the same year others noted improved yields when compared with untreated seed if the tubers were aired for at least one month before planting (Anon, 1949).

Downie (1949) despite finding inadequate sprout suppression in some cases observed that germination was depressed after treatment at 134mg kg^{-1} under Australian conditions.

In more detailed experiments under Swedish conditions Emilsson and Gustafsson (1951) conclude that tecnazene does not delay development, decrease the plant stand, nor decrease the yield if treated potatoes are chitted or aired sufficiently after treatment. They note however that application time is important. When tecnazene was used at levels of 75mg kg^{-1} and 225mg kg^{-1} there was a marked decrease in yield (64-66% of controls) when the potatoes were treated in January, but not when they were treated in November (92-95% of controls). They suggest that regardless of cultivar, dosage of tecnazene should not exceed 120mg kg^{-1} nor be applied later than the 1st. of January preceeding Spring planting, and that adequate airing requires at least 2 weeks. In experiments conducted during the same period the same group found a level of 120mg kg^{-1} very effective in inhibiting sprouting and under good conditions 60-80mg kg^{-1} was quite adequate (Emilsson et al, 1951).

Brown and Reavill (1954) conducted a detailed study under English conditions over a period of three seasons (1948-1951) two of which they state had "rather mild" winters. They used open-air clamps for these experiments dusting tubers of readily sprouting cultivars at a rate of 134mg kg^{-1} at various times throughout the storage season, and opening clamps and airing the tubers for various times before planting. They found that emergence was considerably delayed when no airing was employed, but with 7 weeks airing the emergence was equivalent to that of untreated, chitted seed. Delayed emergence, which was also found with tubers which had been hand desprouted at planting, was closely correlated with the yields produced, especially with early lifting. In later liftings the yield of tecnazene treated tubers with seven weeks airing was comparable to that produced by chitted seed. The average

number of shoots per plant was significantly increased by tecnazene treatment and this was reflected in a higher seed to ware ratio in the resultant crop.

Brook and Chesters (1957a) conducted a variety of experiments partly in Scotland but mainly in England, using tecnazene at 134 and 225mg kg^{-1}. They concluded that the marketable yield from tecnazene treated seed was sometimes below that from untreated seed, and that the crop usually contained a higher proportion of small tubers, despite long airing periods. They believe that when tecnazene is used as a fungicide on seed potatoes it is impracticable to recommend a minimum airing period which will be sufficient in all circumstances to overcome the check to sprout development.

Similarly Driver (1961) found that tecnazene delayed establishment of potatoes in New Zealand, although the effect was reduced by exposure to the air for 3-4 weeks. There was little effect on total yield but again the proportion of ware was decreased.

In America Murphy and Goven (1967) treated seed at a rate of 100 mg kg^{-1} during December, stored at 7.5^{o}C and aired for one week prior to cutting (a normal American practice) and planting in May and June. They found that emergence was not delayed nor yielding ability significantly different from that of chitted controls when the tecnazene was applied as a dust. "Emulsion grade" tecnazene applied as a fog however did have adverse effect, although "aerosol grade" produced no such effects.

In subsequent experiments again using seed pieces but carried out in 5 different locations, it was found that treatment of seed with tecnazene generally delayed emergence of all varieties tested, regardless of application time. In two locations this resulted in decreased yields of tubers but in the other three locations tecnazene in fact increased yields so that when all locations were considered there was no significant effect (Murphy _et al_, 1968). The same group reported similar results in later experiments (Murphy _et al_, 1969).

In Russia Bobryshev and Malakhov (1970) found that treatment of seed with tecnazene at rates of 67-133mg kg^{-1} increased the net photosynthetic rate and leaf area resulting in increased yields. Rakitin (1972) reported that similar rates retarded germination but

increased seedling biomass and in general improved the quality of seed potatoes.

Many authors have noted that the effects of tecnazene both on sprouting and subsequent seed performance are very much cultivar dependent (Downie, 1950; Brown and Reavill, 1954; Luckwill, 1957; Murphy and Goven, 1967; Murphy et al, 1968; 1969).

From this literature survey it appears that the effects of tecnazene on the performance of seed potatoes are various and variable. Most authors however agree that tecnazene can be used in this role with some confidence. Nevertheless it should be pointed out that in most of the experiments described above, variability was high and usually differences had to be of the order of 10% to be statistically significant - slight yield reduction of the order of 5% would therefore be missed. Yield reductions on this scale would not be noticed on individual farms but when taken on a national scale they become very significant indeed.

Despite these findings in the literature and the claims of publicity material, complaints have been received about the performance of tecnazene treated seed from Scottish growers, and similar complaints have been reported in the agricultural press from English growers. Both of these indicated that the effects of tecnazene may be more dramatic than previously indicated.

It was therefore decided to investigate this matter in some detail, however because the facilities for combined storage and field experiments were limited at the commencement of this investigation it was considered that the best approach was to conduct fairly small scale experiments at first and then on the basis of the results and on the experience gained in these investigations decide whether or not to carry the investigation further once facilities for larger scale experiments were available. Preliminary investigations were therefore conducted during the 1972-73 season to study the effect of tecnazene on subsequent emergence, yield and size distribution, and a more elaborate experiment was carried out, in conjunction with residue analysis work already described in chapter 4, during the 1974-75 season. (During the season 1973-74 the effects of maleic hydrazide were investigated as this did not require elaborate pre-planting facilities).

5.2. PRELIMINARY EXPERIMENTS (1972-73)

As explained above these preliminary experiments are concerned principally with the effects of tecnazene on subsequent seed performance. They also include other treatments likely to have an effect on sprouting e.g. nonanol was included in one experiment. Also of interest at this time were the effects of various growth regulants discussed in chapter 2 and their possible exploitation in the potato industry, so examples of these were included. Gibberellins IAA and cytokinins were all commercially available, however abscisic acid (ABA) was also considered worth investigating but was not commercially available in adequate quantities at that time - it was therefore synthesised.

5.2.1. Synthesis of abscisic acid

A review of the literature revealed that two methods were available.

Cornforth et al (1965a) reported a synthetic method involving the conversion of ethyl 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-cis, trans 2,4-pentadienoate to (RS)-abscisic acid in approximately 7% yield.

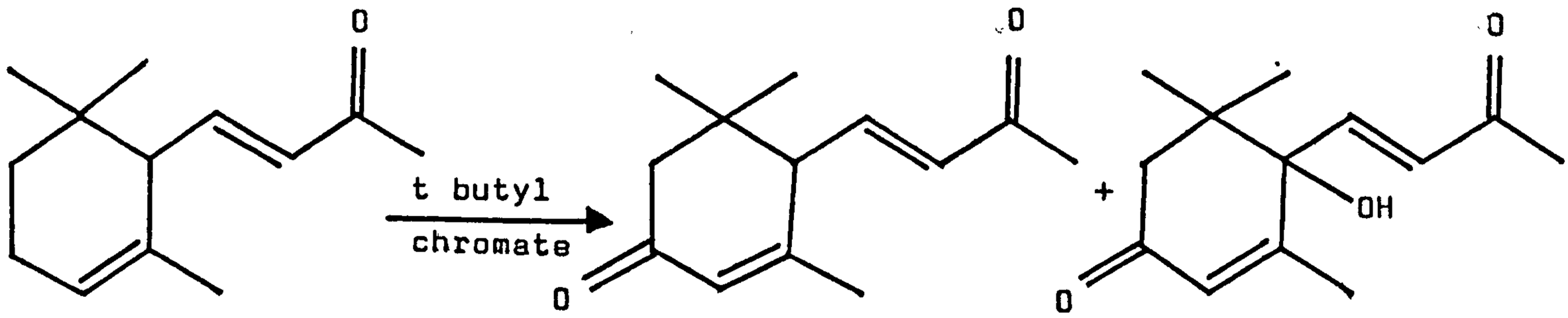
Roberts et al (1968) reported a method which started from readily available αionone and converted it in 22% yield to abscisic acid and its trans, trans isomer(i.e. approximately 11% (RS)-abscisic acid).

The latter method was chosen because of its slight yield advantage and because all the reagents required were available. The reaction scheme is shown in figure 5.2.1.

The α ionone (I) was oxidised using tertiary butyl chromate yielding a mixture of 4-keto-α-ionone (11) and 1-hydroxy-4-keto-α-ionone (III). The latter was isolated and the side chain elaborated using a Wittig reagent - carbethoxymethylenetriphenylphosphorane (IV) which was prepared by the reaction of triphenylphosphine with ethylchloroacetate (Considine, 1962). The resultant esters (V and VI) were then saponified and separated.

Cornforth et al (1967) have published a method for the resolution of the resultant mixture of R and S forms, but this was not considered

Figure 5.2.1. The synthesis of (RS) - abscisic acid

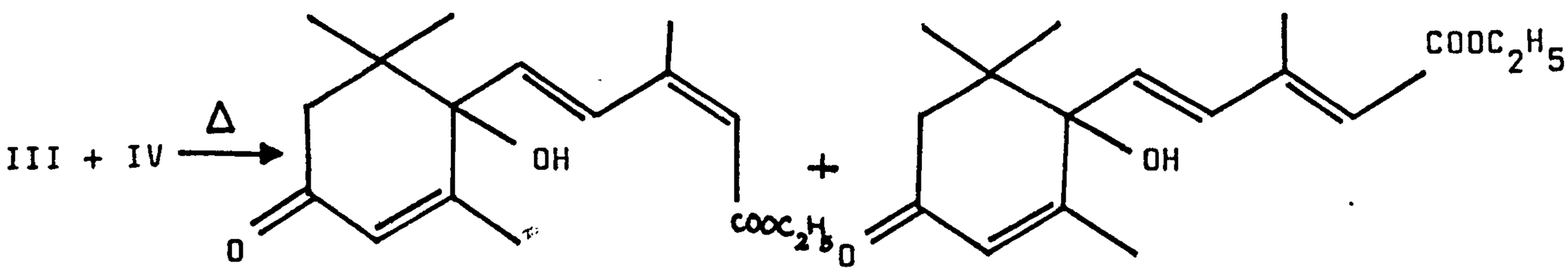


I II III

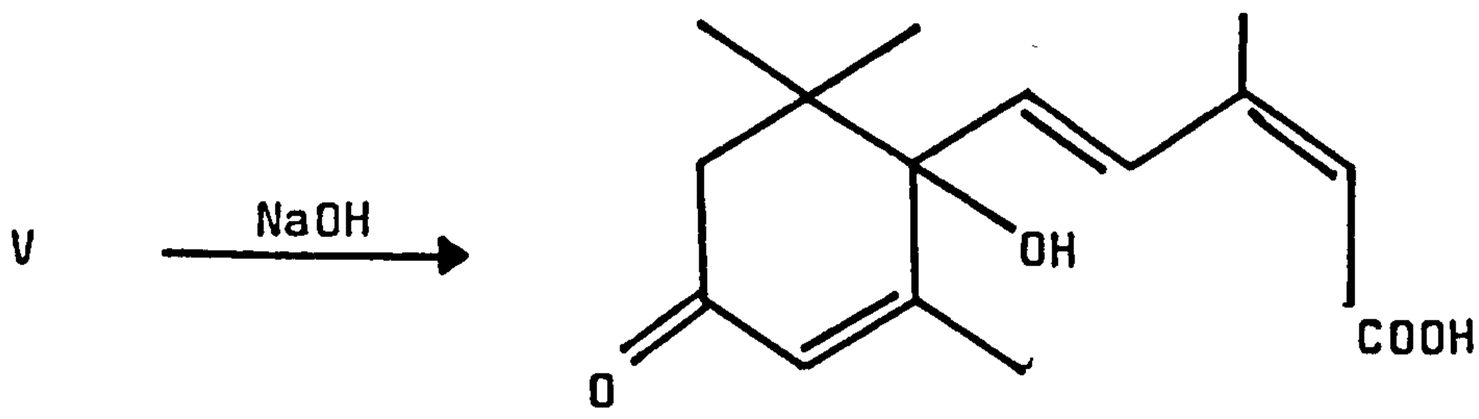
$(C_6H_5)_3P + ClCH_2COOC_2H_5 \longrightarrow (C_6H_5)_3\overset{\oplus}{P} - CH_2COOC_2H_5\overset{\ominus}{Cl}.2H_2O$

$\longrightarrow (C_6H_5)_3P = CHCOOC_2H_5$

IV



V VI



VII

necessary for the present investigation. The racemic mixture was however characterised using mainly instrumental methods.

Experimental

If the experimental procedures are to be repeated special attention should be paid to all aspects of safety.

1) t-butyl chromate reagent

This reagent was prepared exactly as described by Roberts et al. It is worth noting however that extreme care must be taken during the addition of chromium trioxide to the t-butanol, as flask fires can occur if any accumulation of solid material is allowed to form at the edge of the flask.

2) 1-hydroxy-4-keto-∝-ionone

This step was again carried out as described by Roberts et al but with some modifications.

a) The scale was doubled as a fairly large quantity of ABA was required.

b) It was found that the t-butyl chromate reagent must be added very slowly otherwise the reaction mixture will explode.

c) Because the scale was doubled the chloroform extraction of products in a separating funnel was found to be both inefficient and inconvenient. A continuous extraction apparatus was therefore preferred.

d) The silicic acid chromatography step on the filtrate was omitted as it was found that with some care nearly all of the product (III) as determined by gas chromatography could be recovered by crystallisation.

The composition of the crude crystalline product was shown by gas chromatography to be approximately 80% (III) and 20% (II). Repeated recrystallisation from toluene produced almost pure 1-hydroxy-4-keto-∝-ionone as determined by GC. Identification was checked by infra-red spectroscopy.

3) carbethoxymethylenetriphenylphosphorane

This material was prepared exactly as described by Considine (1962) using method B to convert the primary product,

carbethoxymethylenephosphonium chloride dihydrate to the required reagent (IV). The overall yield was approximately 90%.

4) abscisic acid

The Wittig reaction was carried out by method B as described by Roberts et al, and the ethyl esters of the cis, trans and trans, trans isomers saponified with alcoholic NaOH, acidified and extracted into ether.

The acids were then separated by fractional crystallisation from chloroform, the less soluble cis, trans ((RS)-abscisic acid-VII) coming out of solution first. The relative proportions of the two acids in the crystalline material were estimated by methylation using 14% (w/v) boron trifluoride in methanol and subsequent gas chromatography of the resultant esters at 265^{o}C on 5% OV17 (other conditions as per tecnazene analysis). The recrystallisation was repeated as necessary. The identity of the product was confirmed by mass spectrometry (see figure 5.2.2.), infra-red spectroscopy and nmr spectroscopy in deuterated chloroform. These spectra were compared with the data of Roberts et al and various handbooks. The overall yield after recrystallisation was 8%.

Later when ABA was commercially available a comparison by mass spectrometry showed that the ABA produced as described above was of higher purity than that supplied by the Sigma Chemical Co.

5) biological assay

The biological activity of the product was also checked using a wheat coleoptile test, the whole test being completed in the dark or under safelight conditions.

A sample of wheat was germinated for 4 days, 10mm lengths of coleoptile were cut and 20 such segments were floated in each of four 90mm Petri dishes containing water, 10^{-6}M ABA, 10^{-5}M ABA and 10^{-4}M ABA respectively. The coleoptiles were measured 48 hours later. The results are shown in table 5.2.1.

FIGURE 5.2.2

MASS SPECTRUM OF ABSCISIC ACID

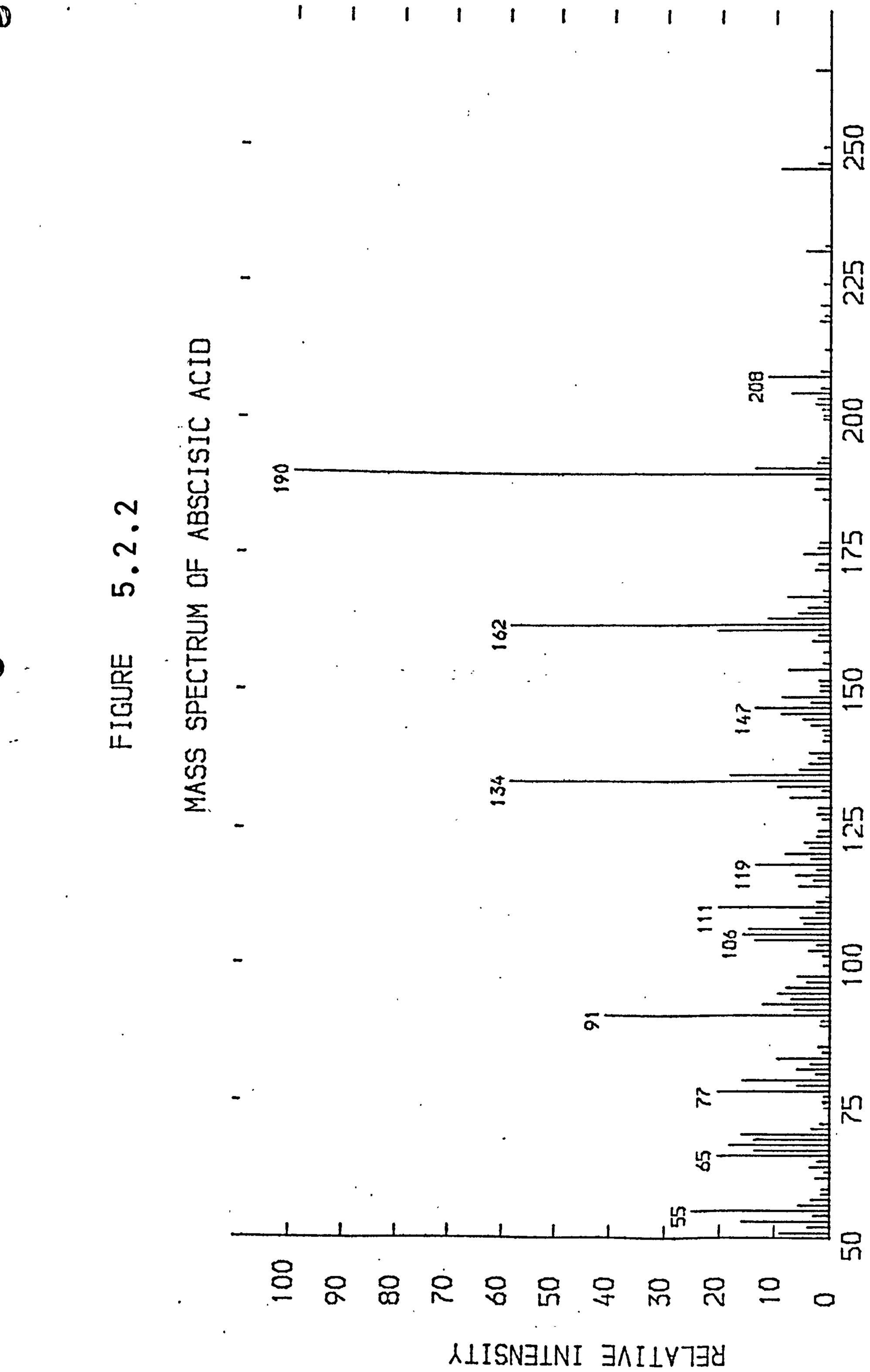


Table 5.2.1. The biological activity of synthesised ABA as determined by a wheat coleoptile test

Solution	Mean length (cm)	S.D.
H_2O	1.56	0.28
$10^{-6}M$	1.39	0.17
$10^{-5}M$	1.33	0.10
$10^{-4}M$	1.13	0.10

These results indicate that coleoptile elongation has been substantially inhibited by the ABA and that the biological activity is high.

5.2.2. The effect of various storage treatments upon the subsequent performance of the cultivar Golden Wonder

Introduction

As the experiment below was intended not only to supply information and experience as outlined above, but also to supply material for an investigation into compositional changes, the treatments chosen were as diverse as possible. Both temperature and chemical treatments were therefore included.

Experimental

a) Treatments

Seed of the cultivar Golden Wonder was supplied by a local merchant approximately five days after it was harvested. The treatments were carried out, on 50kg batches in wooden bins, as soon as possible after delivery on 16th October, 1972.

1) Control - untreated stored at $10^{o}C$
2) Untreated stored at $4^{o}C$
3) Untreated stored at $20^{o}C$

4) Tecnazene treatment - Tubers were treated with Fusarex at the recommended rate of 135mg kg^{-1} tecnazene as they were placed in the bin. A loosely fitting lid was then fitted and the box stored at $10^{o}C$. One month before planting the lid was removed to allow air to circulate freely. Samples were taken for residue analysis just before planting.

5) Nonanol treatment - Nonanol was gifted by I.C.I. The tubers were placed in a large plastic sack fitted with inlet and outlet tubes. This sack was contained in a wooden bin stored at $10^{o}C$ and the inlet tube was connected to an air pump (Dymax - Charles Austin Pumps Ltd.) via a Dresch bottle containing nonanol (Plant Protection Ltd.). The glass tube in the bottle was shortened so that the air passed over the surface of the nonanol rather than bubbling through it. It was found that the rate of application could be controlled by mounting the Dresch bottle over the pump which produced some heat, and then adjusting the distance between the two (5-40mm). Intermittent application as recommended by Burton (1966) was commenced as soon as the tubers began to sprout. When nonanol was not being applied the Dresch bottle was by-passed so that ventilation could be continued. Treatment was halted 4 weeks before planting.

6) Naphthalene acetic acid (NAA) was applied by dissolving 2g in $50cm^{3}$ acetone (40mg kg^{-1}) and spraying on to the tubers with a Shandon TLC spray-gun whilst filling the bin. The lid was not fitted until there was no trace of acetone remaining. This treatment was also stored at $10^{o}C$.

7) Abscisic acid (ABA). The ABA was applied in a similar manner to NAA. 0.5g were dissolved in aqueous acetone to give an application rate of 10mg kg^{-1}.

Photographs of each treatment were taken just before planting and general observations noted, but no quantitative estimation of sprouting was undertaken.

b) Planting

All treatments were planted out on the 8th and 9th May, 1973 in plots which received a uniform treatment of FYM and seed potato

fertilizer. Tubers were planted at 300mm spacing in 700mm drills. Each treatment was planted in triplicate in single drill plots of 33 tubers in a randomised design with guard rows at the edge of the of the experimental area.

A score of the number of plants emerged was taken at 2 day intervals.

c) Harvesting

The crop was lifted by hand on 14th-15th October 1973, graded and yields recorded.

Results

The results of this experiment are reported below in some detail to illustrate the way in which factors such as mean emergence time were calculated from the raw data. To save space in subsequent more complex experiments only these calculated factors and various graphical summaries are reported as inclusion of raw data would greatly expand this thesis.(The emergence data for one experiment amounted to 163 pages of computer output).

a) Sprouting

Of the chemical treatments only tecnazene and nonanol provided control of sprout growth (see plate 5.2.1a.and b.). NAA and ABA had no visible effect upon sprouting at the concentrations used. Nonanol caused some increase in rotting due to penetration of micro-organisms at eyes where the sprouts had been killed (see plate 5.2.2a).

Sprout control was satisfactory when tubers were stored at $4^{o}C$ and limited at $10^{o}C$. As expected sprouting at $20^{o}C$ was excessive and tubers had shrunk considerably by planting (see plate 5.2.2b.). Residue analysis showed tecnazene treated tubers to contain a mean of 2.0mg kg^{-1} at planting.

b) Emergence

From the scores of emergence, the number of plants emerged since the last count was calculated and this figure was called interval emergence. As a score was taken of each plant, the number of replicates was considered as the total number of plants in each

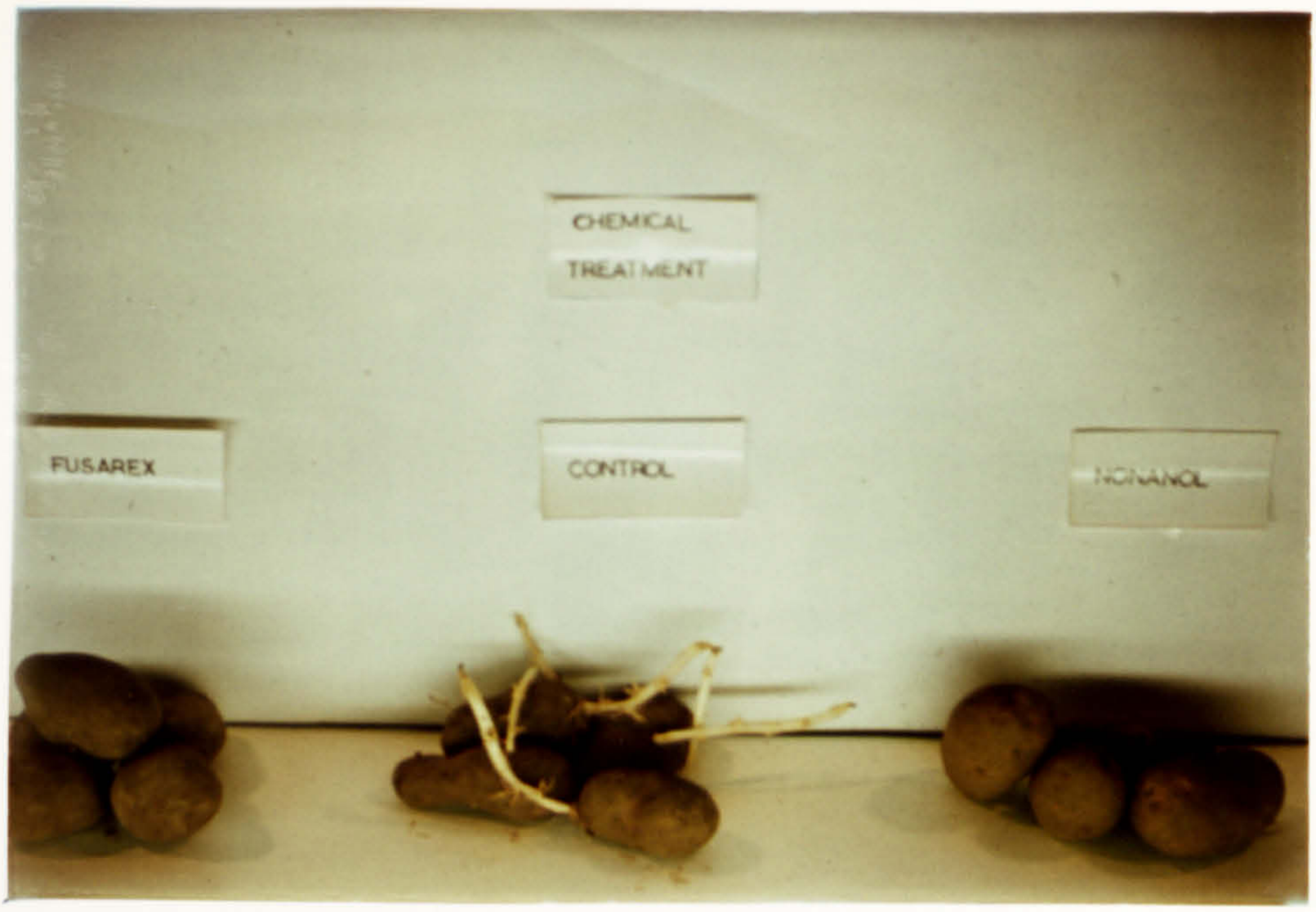


Plate 5.2.1a. The effect of tecnazene and nonanol on the sprouting of potatoes at 10°C (cv. Golden Wonder).

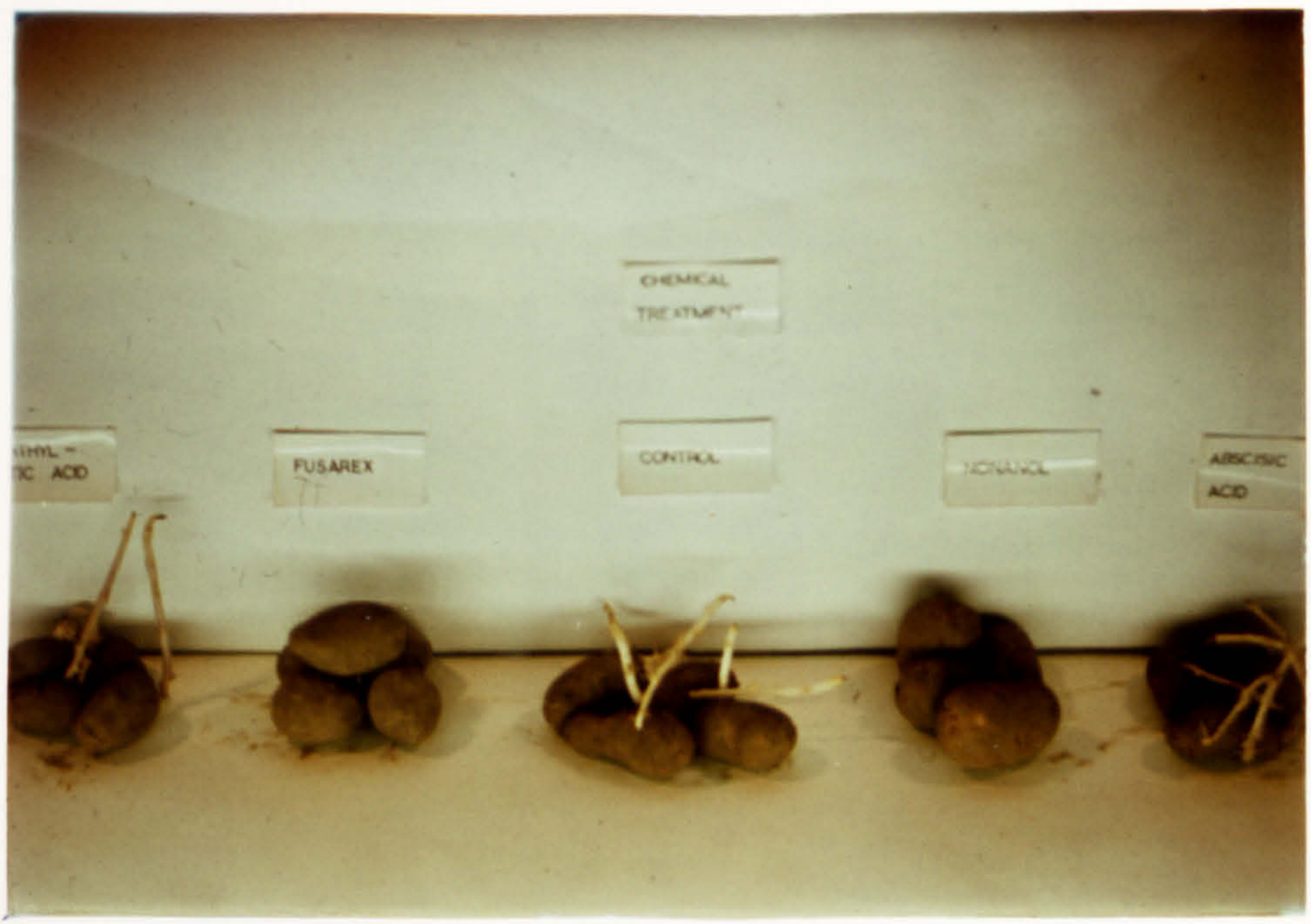


Plate 5.2.1b. The effect of tecnazene, nonanol, naphthaleneacetic acid and abscisic acid on the sprouting of potatoes at 10°C (cv. Golden Wonder).

Plate 5.2.2a. The effect of temperature on the sprouting of potatoes (cv. Golden Wonder).

Plate 5.2.2b. Rotting due to penetration of micro-organisms at eyes where sprouts have been killed by nonanol (cv. Golden Wonder).

treatment rather than the number of plots. Results from triplicate plots have therefore been pooled. Table 5.2.1. shows the pooled interval emergence for each treatment. From this frequency distribution the mean emergence time (MET) was calculated, for those plants which did emerge i.e. non-emerged plants are disregarded by this quantity. To cope with this omission the percentage emergence was also calculated.

Another quantity which appears in the literature is "time to 75% emergence". In this experiment this quantity was taken as the first day on which more than 75% of the plants had emerged. In later experiments when computer analysis was used this figure was more accurately estimated by drawing a straight line through the points in the region of 75% emergence and hence calculating the time to 75% emergence.

In the present experiment differences in MET between treatments and the 10^{o}C control were analysed using a simple t-test, after normality had been checked on probability graph paper.

These calculated quantities and the results of the statistical analysis are shown in table 5.2.2. As the tecnazene results are of special interest they have been illustrated in the histograms in figure 5.2.3.

c) <u>Yield and size distribution</u>

Data for individual plots is shown in table 5.2.3., and the means of each treatment are shown in table 5.2.4. with the results of statistical analysis of the raw data.

<u>Discussion</u>

The following conclusions were drawn from this experiment.

1) MET was found to be the most satisfactory quantity to describe emergence behaviour as individual plants can be treated as replicates when undertaking statistical comparisons, thus reducing the least significant difference to a few days.

2) MET does however have two disadvantages in that it neglects any effect on total emergence and can be affected adversely by one or two tubers which take a very long time to germinate. It should not therefore be considered on its own but in conjunction with %

Table 5.2.1. Emergence frequency table for the cultivar Golden Wonder after various storage treatments (1972-73)

Days after planting	Interval emergence*						
	10°C	Tecnazene	Nonanol	ABA	NAA	4°C	20°C
19-20	0	0	0	4	3	2	22
21-22	1	2	1	7	11	8	24
23-24	10	0	0	15	12	3	15
25-26	13	2	0	17	14	7	11
27-28	16	1	3	14	12	7	6
29-30	14	1	5	14	12	31	11
31-32	14	1	8	10	9	27	9
33-34	12	1	9	9	6	6	1
35-36	6	0	13	2	2	2	0
37-38	7	6	12	3	3	1	0
39-40	5	8	11	1	2	0	0
41-42	1	31	7	2	1	1	0
43-44	0	31	4	1	0	1	0
45-46	0	11	0	0	0	0	0
47-48	0	4	0	0	0	0	0
Total emergence**	99	99	73	99	87	96	99

* Number of plants emerged in the interval specified

** 99 tubers of each treatment were planted

Table 5.2.2. The emergence characteristics of the cultivar Golden Wonder after various storage treatments (1972-73)

Treatment	MET** (days)	%Total emergence	Time to 75% emergence (days)
10°C Control	30.3	100	34
Tecnazene	41.2*	100	44
Nonanol	35.8*	74	-
ABA	28.1	100	32
NAA	27.7	88	34
4°C	29.3	97	32
20°C	24.1*	100	28

* Significantly different from 10°C Control ($P < 0.001$)

** Least significant differences in MET (days)

$P < 0.001$	2.3
$P < 0.01$	1.8
$P < 0.05$	1.3

Figure 5.2.3. The emergence profile of tecnazene treated seed compared with untreated seed (cv. Golden Wonder).

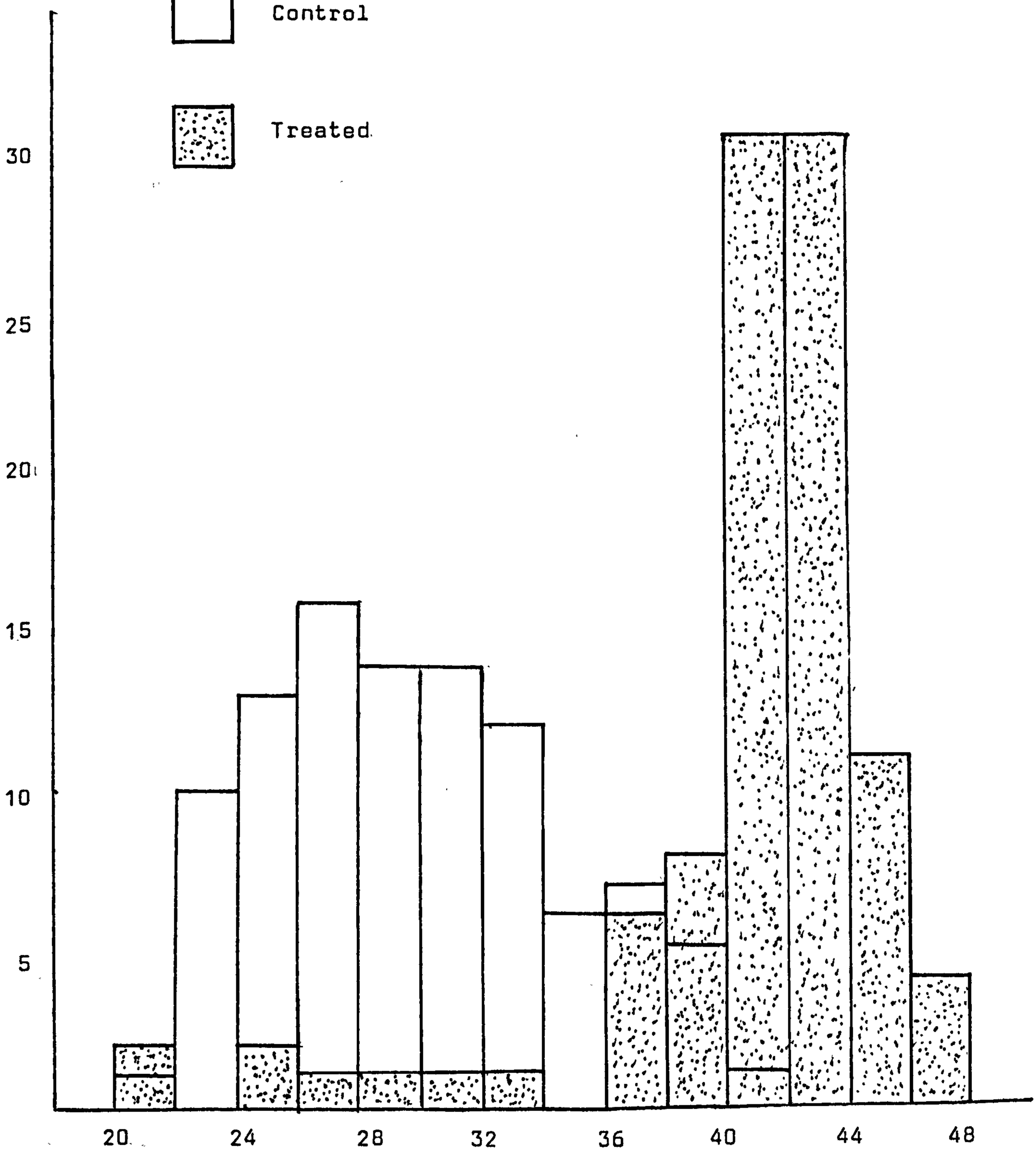


Table 5.2.3. The effect of various storage treatments on the yield and size distribution of the cultivar Golden Wonder (1972-73) 1. Individual plot data

Treatment	Replicate	Yield kg/plot	% > 42mm
Control 10°C	1	16.4	58.8
	2	17.0	66.2
	3	14.3	53.5
Tecnazene	1	12.5	45.6
	2	9.0	45.5
	3	11.7	39.3
Nonanol	1	8.1	34.7
	2	6.0	29.9
	3	9.5	40.3
ABA	1	15.5	64.8
	2	16.7	68.8
	3	16.3	69.9
NAA	1	16.7	77.2
	2	14.0	63.7
	3	15.3	68.3
4°C	1	18.1	67.9
	2	16.4	63.2
	3	15.5	74.8
20°C	1	17.8	64.5
	2	19.1	72.0
	3	16.2	65.7

Table 5.2.4. The effect of various storage treatments on the subsequent yield and size distribution of the cultivar Golden Wonder (1972-73) 2. Means of replicate plots

Treatment	Yield kg/plot	Yield as % of control	% > 42mm
Control	15.9	100.0	59.5
Tecnazene	11.1*	69.8	43.5*
Nonanol	7.9**	49.7	35.0**
ABA	16.2	101.9	67.2
NAA	15.4	96.9	69.7
$4^{o}C$	16.7	105.3	68.9
$20^{o}C$	17.7	111.3	67.4

* Significantly different from control ($P < 0.05$)

** Significantly different from control ($P < 0.01$)

Least significant differences

1) Yield - ($P < 0.05$) 3.2kg/plot i.e. approximately a 20% difference in yield.

2) Size - ($P < 0.05$) requires approximately 12.5% change in % > 42mm i.e. approximately 20% difference taking the control as 100%.

total emergence, and as an insurance against the latter disadvantage it is also worth considering a figure such as time to 75% emergence.

3) The number of plants used in each treatment (99) was adequate to allow fairly small differences in MET to be detected with some confidence.

4) The variability of yield and size distribution data was high, and as only 3 replicates were used only differences of 20% or more could be considered statistically significant. For the more detailed investigations which were conducted in subsequent seasons, steps were taken to reduce variability by increasing the plot size and the number of replicates.

5) The MET of Golden Wonder seed was significantly increased by tecnazene and nonanol treatment when compared with an untreated control. Tecnazene treatment however, when followed by a 4 week airing period prior to planting did not affect the eventual plant density - every tuber in fact germinated. Nonanol on the other hand adversely affected total emergence, as did the synthetic auxin NAA. The decrease in MET produced by storage at 20^{o}C is of little practical importance as the sprouts produced by this treatment would be removed by normal commercial handling. More worthy of note is the fact that the MET of tubers stored at 4^{o}C was no different from those stored at 10^{o}C despite the fact that the former showed very few sprouts when planted.

6) The delayed emergence in the tecnazene and nonanol treatment resulted in significantly lower yields and a decrease in the percentage of tubers suitable for ware use.

7) The 4 week airing period used resulted in a mean tecnazene residue level of 2.0mg kg^{-1}. This residue adversely affects the subsequent performance of the cultivar Golden Wonder.

5.2.3. The effect of tecnazene on twelve different cultivars

Introduction

The principal aim of this experiment was to check that the cultivar Golden Wonder was not unique in its response to tecnazene. The conditions chosen for this experiment are not representative of commercial practice but were chosen simply to investigate whether

the performance of other popular cultivars could be adversely affected by tecnazene. At the time of conducting this experiment the cultivars chosen probably accounted for at least 80% of the British crop (The most obvious omission, Maris Piper was not available).

It was also considered that this experiment might produce useful information on whether tecnazene could be used in a deliberate attempt to increase the proportion of seed in the resultant crop, a suggestion which has been aired frequently in the industry.

Experimental

Seed potatoes of 12 cultivars were obtained from the Department of Agriculture and Fisheries for Scotland, Scientific Services, East Craigs, Midlothian. All 12 cultivars had been grown in the same field.

42 tubers of each cultivar were treated at twice the recommended rate with Fusarex on 25th March 1973 and these and untreated controls were stored at 10°C without airing until planting.

Drills of 21 tubers of each cultivar were planted out in duplicate with controls which were randomised within the experimental plot. Retrospectively it is considered that a split plot design would have been superior.

Details of husbandry, observations and harvesting were identical to those already described in section 5.2.2.

Results

The results from the emergence counts are summarised in the form of MET values in table 5.2.5. As in the previous experiment MET values were calculated from pooled emergence scores. T-tests were carried out on the emergence data to test if treated material was significantly different from untreated, after normality had been established.

The yield and size distribution data are recorded in table 5.2.6. and 5.2.7. Each variable was subjected to analysis of variance and an analysis of variance ("ANOVA") table produced. The ANOVA table for yield of tubers > 42mm is shown in table 5.2.8. From the mean squares, F statistics were calculated and these are shown in table 5.2.9. for all seven variables in table 5.2.6.

Table 5.2.5. The effect of tecnazene on the emergence characteristics of twelve cultivars (1972-73)

Cultivar	MET[+]		Total emergence as % of control[++]
	Control (days)	Treated (days)	
Desiree	21.3	29.2*	92.3
Golden Wonder	35.9	41.7*	100.0
Kerr's Pink	22.5	25.2**	100.0
King Edward	23.5	36.1*	97.6
Majestic	23.0	31.5*	83.3
Maris Peer	21.2	27.8*	61.1
Pentland Crown	24.5	32.1*	83.3
Pentland Dell	24.7	30.7*	100.0
Pentland Hawk	21.5	27.5*	83.3
Pentland Ivory	21.4	29.6*	82.9
Record	21.7	30.2*	87.8
Redskin	22.9	31.4*	95.2

\+ Mean emergence time

++ Control emergence was greater than 98% except for Maris Peer (86%) and Pentland Dell (81%).

* Significantly different from control $P < 0.01$

** Significantly different from control $P < 0.05$

Table 5.2.6. The effect of tecnazene on yield and size distribution of twelve different cultivars (1972-73)+

Cultivar	Treatment	>42mm*	32-42mm*	<32mm*	Total*	%>42mm	%32-42mm	%<32mm
Desiree	C**	25.4	2.8	0.5	28.7	88.2	10.0	1.9
	T	19.4	3.3	0.7	23.5	82.6	14.5	3.0
Golden	C	5.3	4.1	1.1	10.5	50.4	38.9	10.7
Wonder	T	3.0	3.4	1.3	7.7	39.6	43.5	17.1
Kerr's	C	26.8	0.9	0.1	27.8	96.2	3.4	0.5
Pink	T	22.1	3.2	0.6	25.9	85.3	12.4	2.3
King	C	10.9	3.7	1.0	15.6	69.6	23.9	6.4
Edwards	T	6.4	3.4	1.1	10.9	56.1	33.8	10.0
Majestic	C	16.0	3.0	1.4	20.4	77.5	15.5	6.9
	T	10.9	3.3	1.2	15.5	70.5	21.4	8.0
Maris	C	10.4	4.4	0.8	15.6	66.5	28.3	5.3
Peer	T	6.9	4.2	1.0	12.1	56.9	35.3	8.1
Pentland	C	25.5	1.5	0.2	27.3	93.7	5.4	0.9
Crown	T	17.4	1.5	0.3	19.2	90.5	7.9	1.6
Pentland	C	18.2	5.7	1.2	25.1	72.5	22.3	4.6
Dell	T	10.8	2.9	1.3	14.9	71.7	20.4	8.6
Pentland	C	22.5	3.4	0.8	26.8	84.1	12.9	3.0
Hawk	T	19.8	3.1	0.5	24.0	82.8	13.0	2.3
Pentland	C	21.9	1.4	0.3	23.5	92.8	5.9	1.4
Ivory	T	13.6	1.5	0.4	15.5	88.2	9.4	2.4
Record	C	18.5	3.6	0.5	22.6	81.6	16.0	2.3
	T	14.2	2.2	0.3	16.7	84.4	13.6	2.1
Redskin	C	18.3	1.7	0.4	20.4	89.7	8.1	2.1
	T	15.7	2.2	0.5	18.4	85.3	12.1	2.6

\+ Mean of duplicate plots

* kg/plot

** C = control and T = tecnazene treatment

Table 5.2.7. The effect of tecnazene on the subsequent yield and size distribution averaged over twelve cultivars (1972-73)

Variable	Control	Tecnazene treated
Yield >42mm*	18.3	13.4
Yield 32-42mm*	3.0	2.9
Yield <32mm*	0.7	0.8
Total yield*	22.0	17.0
% >42mm	80.2	74.5
% 32-42mm	15.9	19.8
% <32mm	3.8	5.6

* Yields in kg/plot

Table 5.2.8. The effects of tecnazene on the yield of tubers > 42mm of twelve different cultivars investigated by analysis of variance (1972-73)

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F statistic*
Cultivar	11	1719.0	156.3	12.82
Treatment	1	293.2	293.2	24.06
Interaction	11	48.5	4.4	0.36
Replicates	24	292.5	12.2	--
Total	47	2353.2	--	--

* Significant values of F statistic from tables (Lindley and Miller, 1968)

$F(11,24)$ = 2.22 at the 5% level
= 3.10 at the 1% level

$F(1,24)$ = 4.26 at the 5% level
= 7.82 at the 1% level

If the calculated F statistic is greater than the values obtained from the tables for the appropriate number of degrees of freedom, then the effect is significant.

Table 5.2.9. Summary of analysis of variance on yield and size distribution data from twelve different cultivars (1972-73)

Variable	F statistics for each source of variation		
	Cultivar	Treatment	Interaction
>42mm	12.82*	24.06*	0.36
32-42mm	12.34*	1.00	4.60*
<32mm	15.17*	1.32	1.01
Total yield	8.79*	22.14*	0.52
% > 42mm	32.18*	14.57*	0.85
% 32-42mm	22.87*	8.24*	0.71
% < 32mm	36.60*	25.76*	2.69**

* Significant at the 1% level

** Significant at the 5% level

Discussion

a) Effects on emergence

Tecnazene treatment as described above produced a statistically significant delay in emergence with all 12 cultivars tested. The MET values were increased by 3-13 days (mean 7.4 days). As the MET of Golden Wonder was increased by 5.8 days as compared with 10.9 days in the previous experiment, it would appear that the late treatment used in this experiment produced less dramatic effects than when tecnazene was applied at a time more comparable with recommended practice.

Tecnazene treatment also reduced the total number of plants emerged in 9 out of the 12 cultivars. This effect was particularly large in the case of Maris Peer.

During the accumulation of this data, discolouration and malformation of the young growth of tecnazene treated batches were noted. These effects were particularly obvious with the cultivars Maris Peer, Pentland Dell and Majestic, where red pigmentation in newly formed leaves was much more distinct than in untreated batches. Such leaves were often grossly distorted in a manner not unlike the familiar effects produced by paraquat.

b) Yield and size distribution

As can be seen in tables 5.2.6. and 5.2.7. tecnazene treatment produced substantial reductions in the yield of large tubers and in total yields. These reductions are substantially larger than those found by Brown and Reavill (1954) with similar late applications of tecnazene. This point is of some practical significance as it is common agricultural practice to apply tecnazene to seed tubers late in the storage season when sprouting has begun.

The effect of tecnazene on the yield of seed sized tubers (32-42mm) was more variable although with 11 of the 12 cultivars the proportion of such tubers increased because of the reduction in large tubers.

When the raw data for each of the seven variables was subjected to an analysis of variance several facts emerged.(see table 5.2.9.).

1) As expected, cultivar produced a highly significant effect upon all seven variables.

2) Tecnazene treatment produced a significant effect upon the yield of large tubers, total yield and upon all the distribution variables. The effects upon the yield of seed and upon the yield of small tubers are non-significant as might be expected from an inspection of the overall means in table 5.2.7.

3) Of more direct consequence to the aims of this experiment are the interactive effects between tecnazene treatment and cultivar. The interaction between chemical and cultivar are non-significant with respect to yield of large tubers, yield of small tubers and total yield i.e. the effects of tecnazene upon these variables are independent of cultivar. With regard to yield of seed tubers there is however a significant interaction. Apparently tecnazene is producing different effects upon different cultivars. Similarly the interaction is significant with respect to the % of small tubers in the total yield.

Overall it would appear from this investigation that the major effects of tecnazene are independent of cultivar and that Golden Wonder is not an atypical cultivar.

c) <u>The relationship between emergence delay and yield reduction</u>

Obviously the larger the growing period of any crop the more likely it is to express its full genetic potential. With potatoes the well publicised advantages of chitting are simply an exploitation of this fact.

Conversely any delay in emergence is likely to produce a reduction in yield, but is the delay produced by the use of sprout suppressants the sole factor which produces yield reduction or is there some prolonged chemical influence upon the physiology and biochemistry of the plant after it has emerged? If the former were true then future experiments could be greatly simplified by observing only the effects upon emergence and hence predicting the effect upon yield.

In an attempt to answer this question the data from this experiment has been more closely examined.

The delay in emergence for each cultivar has been plotted against the reduction in yield and the resultant graph is shown in

figure 5.2.4.

Although there appears to be a general trend that when emergence delay is high yield reduction is high the relationship between the two is fairly loose (r=0.34).

In the case of Golden Wonder for example a delay of only 5.8 days was associated with a yield reduction of 27.0% whereas with Redskin a delay of 8.5 days was associated with a yield reduction of only 10.0%.

Apparently the effects of tecnazene on the subsequent growth of seed potatoes persist even after the plant emerges. Consequently future experiments must include yield measurements and not simply emergence counts.

5.2.4. The effect of various growth compounds applied at planting to the cultivar Golden Wonder

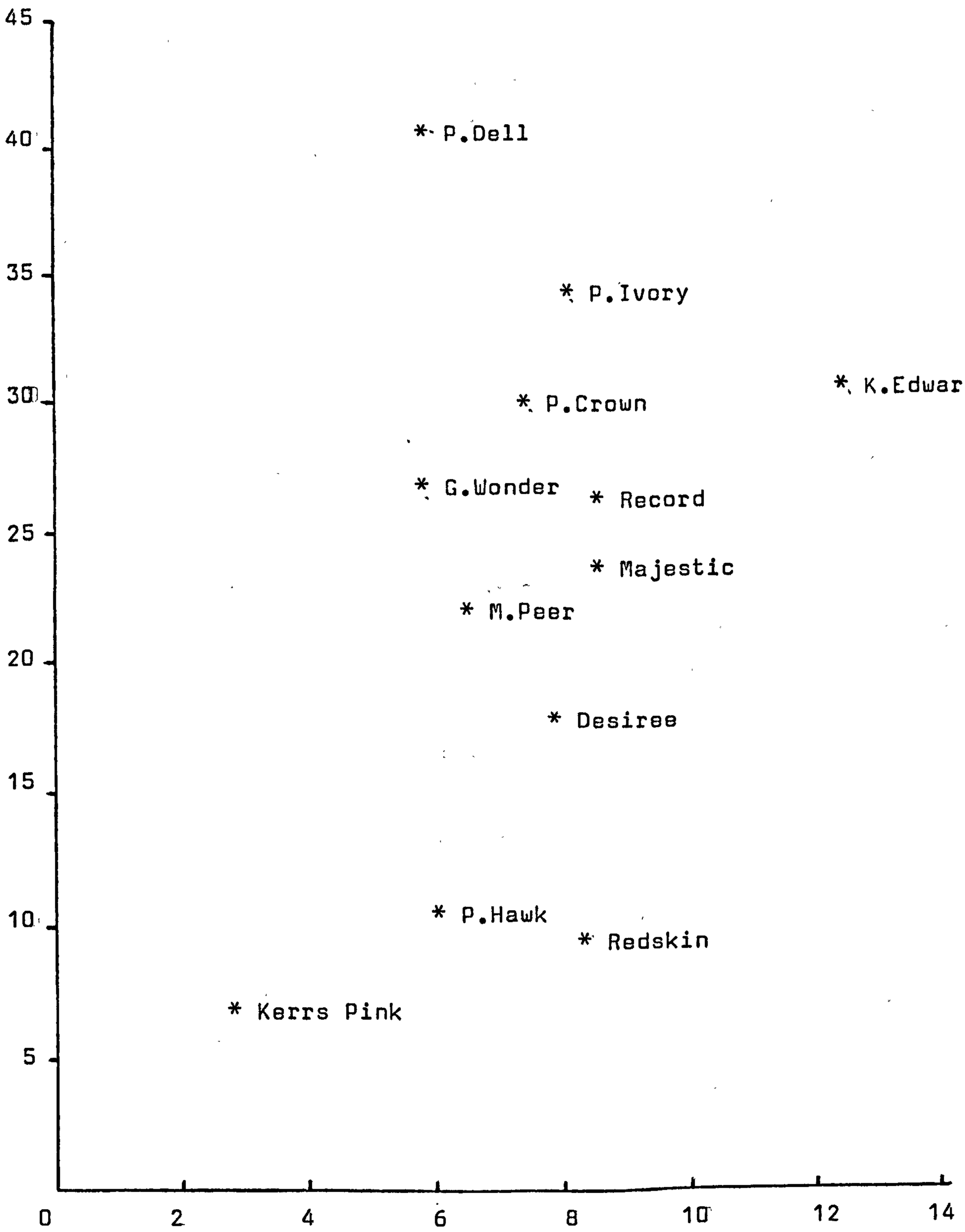
Introduction

If the growth of plants is affected by a chemical compound often these effects can be overcome or at least diminished by using another biologically active compound. With potatoes various authors have reported attempts to overcome the effects of sprout suppressants with various sprout stimulants such as ethylene chlorohydrin and thiourea (Burton, 1966).

As discussed in chapter 2 and reviewed by Rappaport and Wolf (1969) hormonal control of dormancy and sprouting is receiving considerable scientific attention, and the exploitation of the implicated growth compounds in agricultural situations is under active consideration (Wittwer, 1971). For example, Rappaport (1957) reported that 0.5-1.0µg cm^{-3} gibberellin can be used to break the dormancy of seed prices when used as a spray or as a dip. It might therefore be possible to use these natural growth regulants to overcome the effects of sprout suppressants.

An experiment was therefore initiated to study the effects of these chemicals under conditions which closely resemble commercial practice. As this was an exploratory exercise it was decided to restrict the experiment to untreated tubers initially and if

Figure 5.2.4. The relationship between the delay in emergence caused by tecnazene and the subsequent reduction in total yield.



significant effects were found to conduct further studies to investigate antagonistic effects with sprout suppressant treated tubers. As the inhibitor ABA was available it was also included.

Experimental

Certified seed of the cultivar Golden Wonder was purchased from a local merchant during March 1973 and stored at 10°C till treatment on 4th May 1973, by which time buds were apparent on all tubers.

Chemicals were applied as a 10 minute dip, of 10kg batches of unwashed tubers, in aqueous solutions of gibberellic acid (GA3), IAA, ABA and kinetin at concentrations of 1,10 and 100µg cm^{-3}. Control tubers were dipped in deionised water.

Drills of 33 tubers of each treatment were planted in duplicate in a randomised design.

Husbandry, observations and harvesting were exactly as described in section 5.2.2.

Results

The results of the emergence counts and the yield measurements are summarised in table 5.2.10.

Discussion

The various treatments had remarkably little effect. Only GA3 at a dip concentration of 100µg cm^{-3} produced significantly faster emergence than the control, and even in this case the difference was less than 3 days.

The yield data is variable and none of the differences is statistically significant.

It would appear unlikely therefore that any of these compounds holds much promise as a commercial solution to delayed emergence caused by sprout suppressants. Higher concentrations would prove prohibitively expensive.

The most likely explanation of this lack of activity is probably the inability of these mainly water soluble compounds to penetrate the skin of an over-wintered seed potato. This would also explain the apparent discrepancy between these findings and those of Rappaport (1957) who used seed pieces rather than intact tubers.

Table 5.2.10. The effect of various growth regulants on seed performance (1972-73)

Treatment	Dip concentration ($\mu g\ cm^3$)	MET* (days)	Yield*** (kg/plot)
Control	--	36.3	15.3
Gibberellin	1	35.1	17.0
	10	35.1	14.2
	100	33.5**	15.2
ABA	1	36.2	16.5
	10	37.2	15.8
	100	35.1	14.2
IAA	1	35.7	16.4
	10	35.5	13.8
	100	35.6	15.9
Kinetin	1	36.3	16.5
	10	37.7	14.9
	100	36.0	17.1

* Emergence for all treatments was greater than 94%

** Significantly different from control ($P < 0.01$)

*** Mean of duplicate plots

5.3. AN INVESTIGATION INTO THE EFFECT OF TECNAZENE ON SEED PERFORMANCE DURING THE 1974-75 SEASON.

5.3.1. Introduction

The aim of this experiment was to investigate in some detail the effect of tecnazene used under different storage regimes on the subsequent performance of treated seed and to compare this material to untreated controls.

As previously explained this work was a continuation of the storage experiment already described in Chapter 4. Detailed information about residues was therefore available and an attempt has been made to correlate subsequent performance not only with storage conditions but with the residue levels they produce.

The areas of seed performance investigated were:

a) degree of sprouting at planting

b) emergence characteristics

c) yield and size distribution

The assessment of various other factors such as leaf area, and plant height was also considered and although these might have been of some interest their estimation was not feasible with the available manpower.

Because of the vast amount of individual plot data accumulated during these experiments an attempt has been made to condense it as much as possible by using treatment means in the various tables of results. However as such figures give no idea of the original distribution from which the treatment means were calculated, graphical methods and various statistics have been used to overcome this problem where necessary.

To describe the relationships between the independent variables (airing time and tecnazene residues) and the more important dependent variables simple linear regression models have been constructed and some of these are illustrated graphically using axes annoted in the original units of X and Y rather than using transformations, because it is easier to relate this type of plot to what is occurring in the field. Transformations will only be used where the situation is obviously non-linear or where a

significant improvement in fit as estimated by the correlation coefficient is obtained (at least 0.05).

5.3.2. Experimental

The materials used, tecnazene treatment and storage have been fully described in section 4.8.

For the purposes of this experiment the replicates within the 14 treatments described in figure 4.8.1. were combined to give 9 treatments for each cultivar.

1. Untreated control stored at $8^{o}C$
2. 8 weeks airing at $8^{o}C$
3. 5 weeks airing at $8^{o}C$
4. 3 weeks airing at $8^{o}C$
5. 0 weeks airing
6. 8 weeks airing at $12^{o}C$
7. 5 weeks airing at $12^{o}C$
8. 3 weeks airing at $12^{o}C$
9. 3 weeks forced ventilation at $8^{o}C$

It should be noted that treatment 5 acts as the 0 weeks airing treatment for both $8^{o}C$ and $12^{o}C$ as the temperature only applies to the period after opening. All samples were stored at $8^{o}C$ until opening.

Assessment of sprouting

The degree of sprouting was assessed immediately prior to planting i.e. 12 weeks after the tecnazene had been applied. The longest sprout of each of twenty randomly selected tubers was measured and recorded. Sprouts of less than 1mm were recorded as 0mm.

Planting out

The experimental plot was situated at Hattrick Farm, Bridge of Weir, Renfrewshire (Map Reference NS 355673) and was approximately 0.5ha of sandy loam soil. It had been uniformly treated with FYM and received 1400kg ha^{-1} seed potato fertilizer (Scottish Agricultural Industries) immediately before planting on 19 April 1975. Tubers were planted by hand at 300mm spacing in drills 710mm apart. Each plot consisted of 3 drills 6m long and containing 18 tubers in each

drill. The experimental area was protected by two guard drills.

Experimental design

The experimental design consisted of four independently randomised blocks each containing all 36 treatments (4 cultivars x 9 storage treatments). Each block was 9 plots wide by 4 plots long and was approximately square. The 4 blocks were combined such that the whole experimental area was also square.

Husbandry

Weed control was achieved by applying 3kg ha^{-1} linuron and 2kg ha^{-1} paraquat using a 4 nozzle knapsack sprayer just before emergence (12 May). Blight prevention was achieved using the same knapsack sprayer and captafol sprays at 14 day intervals during August, but on one occasion the whole experimental area was accidentally sprayed by a tractor whilst spraying the rest of the field (see later).

Emergence counts

Scores of emergence were taken at 2 day intervals from 13 May to 29 June as described in section 5.2. Because of the time involved only the centre drill of each of the 144 plots was assessed.

Harvesting and Grading

The crop was defoliated using diquat on 3 September and harvested on 23-25 September. The tubers harvested from the centre drill of each plot were subsequently graded mechanically over 50mm and 32mm riddles. Yields of large (> 52mm), medium (32-50mm - seed sized) and small tubers (< 32mm) were recorded in kilograms.

5.3.3. Results and Discussion

1) Sprouting

The relationship between sprouting, tecnazene residues and storage conditions has been investigated in some depth because, of course, any recommendations made after analysis of emergence and yield data must take into account that the primary function of tecnazene is a sprout suppressant, and if the recommended storage conditions do not permit it to act as such then there is little point in its application.

From the twenty sprout measurements for each treatment the mean sprout length and percentage of tubers with sprouts were calculated and are reported in table 5.3.1. (Maris Piper and Pentland Crown) and 5.3.2. (Record and Redskin).

Sprouting and tecnazene residues

The range and distribution of the original measurements are illustrated in figure 5.3.1. with the corresponding tecnazene residues in the form of a "box and whisker" diagram. The forced airing treatment has not been included as the statistical model described in section 4.8. did not encompass this treatment because of insufficient data.

(The box and whisker diagram is an extremely useful way of representing data where numerous observations of Y have been made for a single value of X. The upper and lower bars on the "whiskers" describe the maximum and minimum values respectively. The central line indicates the value of the median data point and the box contains the middle 50% of the values (the interquartile range). The upper and lower limits of the box are therefore specified by the median value between the true median and the upper and lower extremities respectively. A FORTRAN programme was written which carries out a search for these points and plots the diagram on a computer linked graph plotter).

Figure 5.3.1. and tables 5.3.1. and 5.3.2. show that the sprout length for all four cultivars is very much dependent upon tecnazene residue.

To further investigate this relationship a linear regression search was conducted by computer, amongst various mathematical transformations of both sprout length and tecnazene residue, for the transformation which produced the best linear fit as estimated by the correlation coefficient. Data from both $8^{o}C$ and $12^{o}C$ airing conditions were used.

Of the 50 transformations tried on each cultivar it was found that raising X (tecnazene residue) to a fractional power gave the best fit in all cases.

$$\text{i.e.} \quad y = \alpha + \beta x^{t}$$

The correlation coefficients, power of X and the least squares

Table 5.3.1. The effect of storage conditions on the sprouting of tecnazene treated seed potatoes (1974-75) (Cultivars - Maris Piper and Pentland Crown)

Cultivar	Storage conditions			Tecnazene residue		Mean sprout length	% Sprouting
	time closed (weeks)	time open (weeks)	temp.[a] (°C)	actual[b] (mg kg^{-1})	predicted[c] (mg kg^{-1})	(mm)	
Maris Piper	- untreated control -			0.00	0.00	42.1	100
	4	8	8	0.94	0.92	3.2	50
	7	5	8	0.93	0.98	2.7	35
	9	3	8	1.00	1.03	1.1	20
	12	0	-	0.96	1.08	1.1	20
	4	8	12	0.32	0.35	17.2	70
	7	5	12	0.60	0.54	5.8	55
	9	3	12	0.81	0.71	2.4	40
	9	3f[d]	8	0.79	-	1.2	30
Pentland Crown	- untreated control -			0.00	0.00	34.1	100
	4	8	8	1.50	1.38	4.2	55
	7	5	8	1.41	1.43	0.8	15
	9	3	8	1.70	1.45	0.6	10
	12	0	-	1.60	1.50	0.6	10
	4	8	12	0.70	0.79	10.2	60
	7	5	12	1.22	1.01	5.0	55
	9	3	12	1.30	1.18	3.0	50
	9	3f[d]	8	1.80	-	3.6	30

a The airing temperature applies to the airing period only as all batches were stored at 8°C until airing commenced.

b Experimentally determined tecnazene residue at time of planting - mean of replicates where applicable.

c Tecnazene residue predicted by the statistical model described in previous chapter.

d Forced ventilation.

Table 5.3.2. The effect of storage conditions on the sprouting of tecnazene treated seed potatoes (1974-75) (Cultivars - Record and Redskin)

Cultivar	Storage conditions			Tecnazene residue		Mean sprout length	% Sprouting
	time closed (weeks)	time open (weeks)	temp.[a] (°C)	actual[b] (mg kg^{-1})	predicted[c] (mg kg^{-1})	(mm)	
Record	- untreated control -			0.00	0.00	43.2	100
	4	8	8	0.73	0.69	12.7	80
	7	5	8	0.77	0.91	5.4	55
	9	3	8	1.50	1.08	4.8	35
	12	0	-	1.10	1.42	3.0	30
	4	8	12	0.20	0.32	33.5	85
	7	5	12	0.95	0.55	14.2	65
	9	3	12	1.30	0.80	6.6	50
	9	3f[d]	8	0.65	-	9.2	45
Redskin	- untreated control -			0.00	0.00	28.3	100
	4	8	8	0.79	0.74	9.1	80
	7	5	8	0.89	0.92	7.1	70
	9	3	8	1.30	1.06	4.3	40
	12	0	-	1.40	1.30	2.9	30
	4	8	12	0.57	0.46	16.5	85
	7	5	12	0.56	0.68	6.4	55
	9	3	12	0.69	0.88	5.2	50
	9	3f[d]	8	1.20	-	3.0	40

a The airing temperature applies to the airing period only as all batches were stored at 8°C until airing commenced.

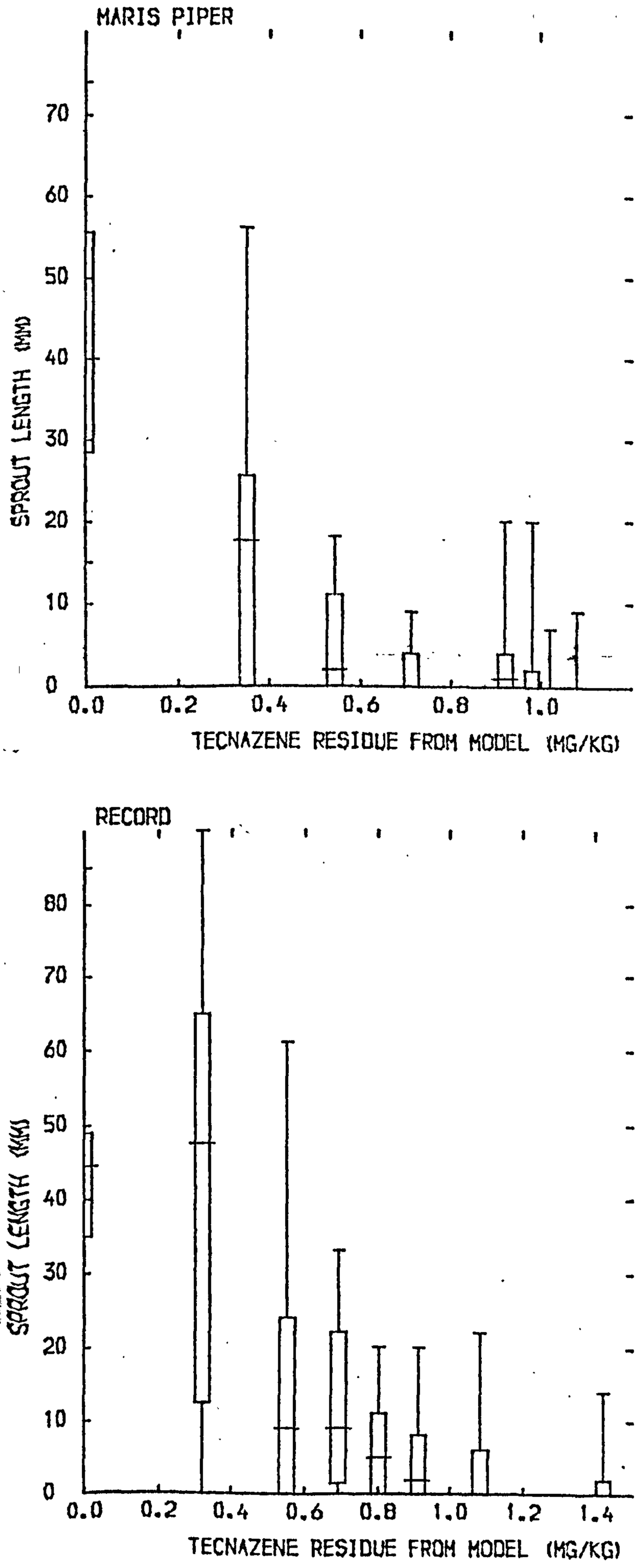
b Experimentally determined tecnazene residue at time of planting - mean of replicates where applicable.

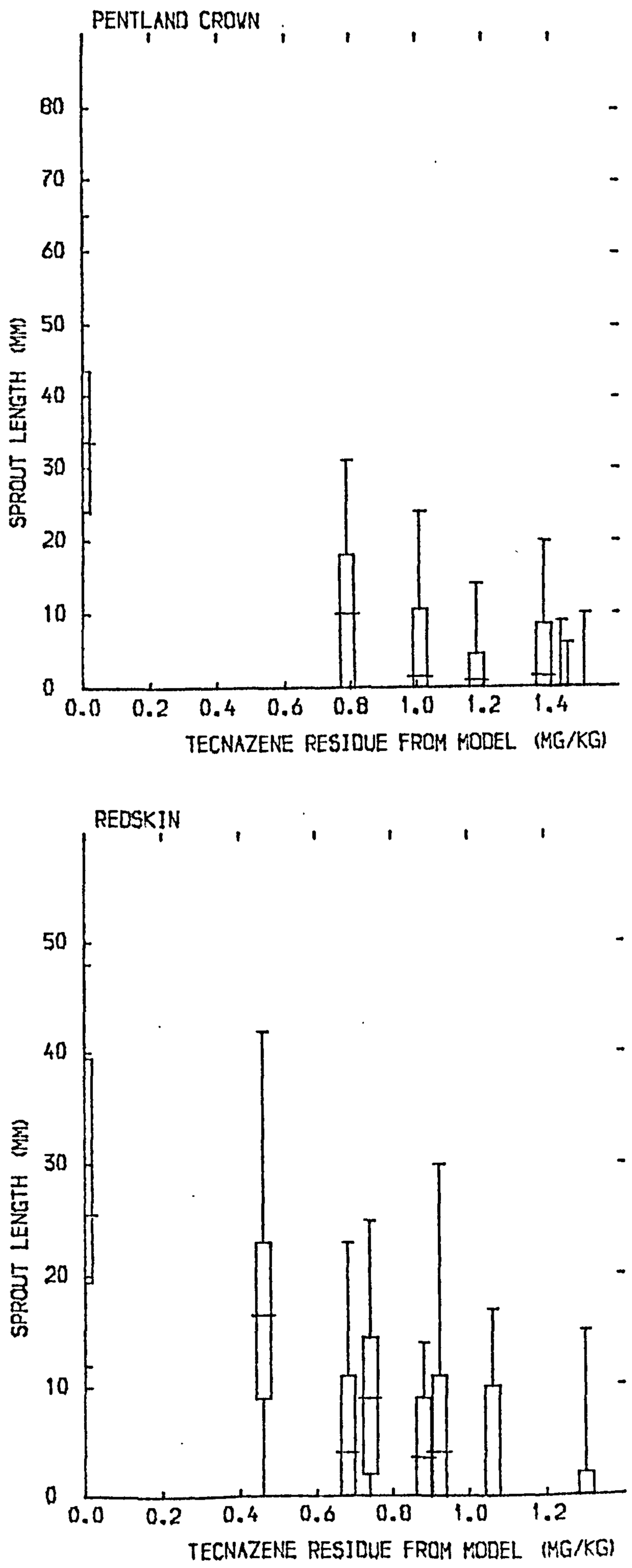
c Tecnazene residue predicted by the statistical model described in previous chapter.

d Forced ventilation.

TEXT BOUND INTO THE SPINE

FIGURE 5.3.1 THE EFFECT OF TECNAZENE RESIDUES ON THE SPROUTING OF SEED POTATOES (1974-75)





estimators of α and β are given in table 5.3.3. for the best fitting straight line for each cultivar.

Table 5.3.3. Parameters of the linear model $y = \alpha + \beta x^t$ describing the relationship between mean sprout length and tecnazene residues

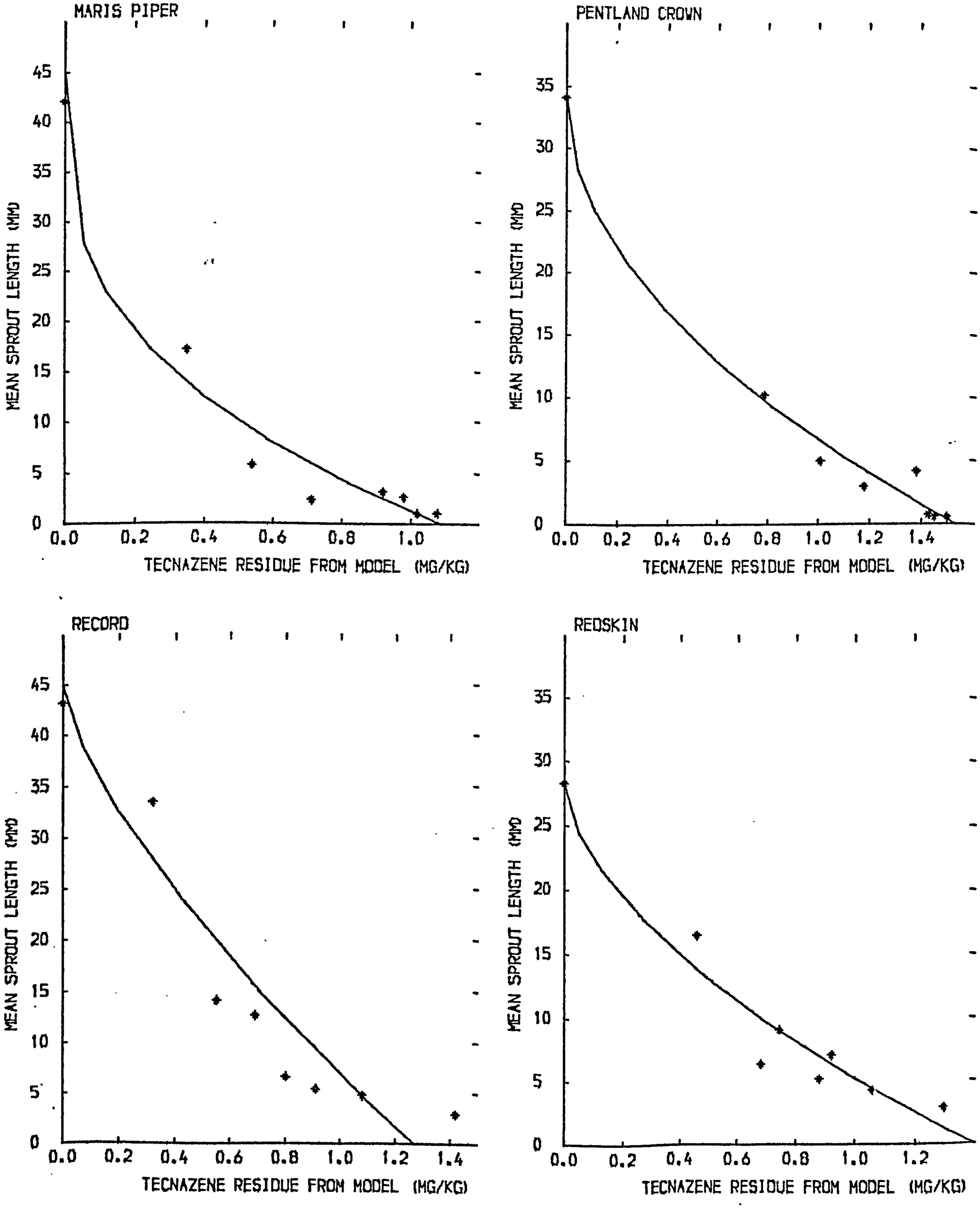
Cultivar	Power of X (t)	Intercept (α)	Gradient (β)	Corr. Coeff. (r)
Maris Piper	0.30	45.1	-43.8	-0.81*
Pentland Crown	0.50	34.2	-27.6	-0.80*
Record	0.70	44.9	-38.1	-0.66*
Redskin	0.60	28.3	-23.1	-0.69*

* Significantly different from zero at $P < 0.001$

It should be noted that the original measurements were used in the construction of the model not the mean values in table 5.3.1. and 5.3.2. If the latter are used correlation coefficients 0.95 are obtained. It is also worth noting that this model is not quite the best available as it assumes constant variance of Y. A marginally superior but more complex model could be constructed by taking logarithms of sprout length to equalise the variance. However the simple model described above is an adequate description for the present purpose.

Using the parameters in table 5.3.3. the model has been plotted for each cultivar in figure 5.3.2. Linear axes have been used for the reasons previously explained and so the functions are curved. The mean sprout lengths from tables 5.3.1. and 5.3.2. have been superimposed to indicate graphically the "goodness of fit". Detailed examination of the points which deviate substantially from the model indicates the possibility of physiological effects of storage

FIGURE 5.3.2 THE EFFECT OF TECNAZENE RESIDUES ON THE SPROUTING OF SEED POTATOES (1974-75)



conditions which are independent of tecnazene residue. For example the data point corresponding to 8 weeks airing time at $8^{o}C$ is consistently above the model line (see below). In general however the models appear to fit the observations fairly well.

From table 5.3.3. it is obvious that the power to which tecnazene residue must be raised to produce the best linear fit is higher for the cultivars Record and Redskin than for Maris Piper or Pentland Crown. In the region under consideration, low powers produce highly concave curves and higher powers produce less concave curves. This indicates the greater effectiveness of tecnazene at low concentrations on the latter cultivars.

As this effect is somewhat masked in figure 5.3.2. because of the different values for mean sprout lengths for each cultivar visual comparison is difficult. If however the sprout lengths for each cultivar are expressed as a percentage of that obtained with zero tecnazene residue (control) then direct comparison is much easier. Relative sprout lengths for various values of tecnazene residue were therefore calculated in this way and are shown in table 5.3.4.

Table 5.3.4. Relative sprout lengths calculated from the linear model for various levels of tecnazene residue

Tecnazene residue ($mg\ kg^{-1}$)	Sprout length as a % of control			
	M.Piper	P.Crown	Record	Redskin
0.00	100	100	100	100
0.25	36	60	68	65
0.50	21	43	48	46
0.75	11	30	31	31
1.00	3	19	15	19
1.25	0	10	6	7
1.50	0	1	0	0

These findings would seem to indicate that different application rates for different cultivars might be a subject worthy of investigation. In commercial practice, store managers through experience, apply tecnazene at different rates to different cultivars but the manufacturers give no information on this subject.

From the results above it would appear that the criterion which is important in such decisions is not so much the propensity of a given cultivar to sprout but the vigour of the sprouts. For example Maris Piper whilst having a high propensity to sprout tends to produce sprouts with rather lower vigour than Record or Redskin. This may be a reflection of the number and type of sprouts produced.

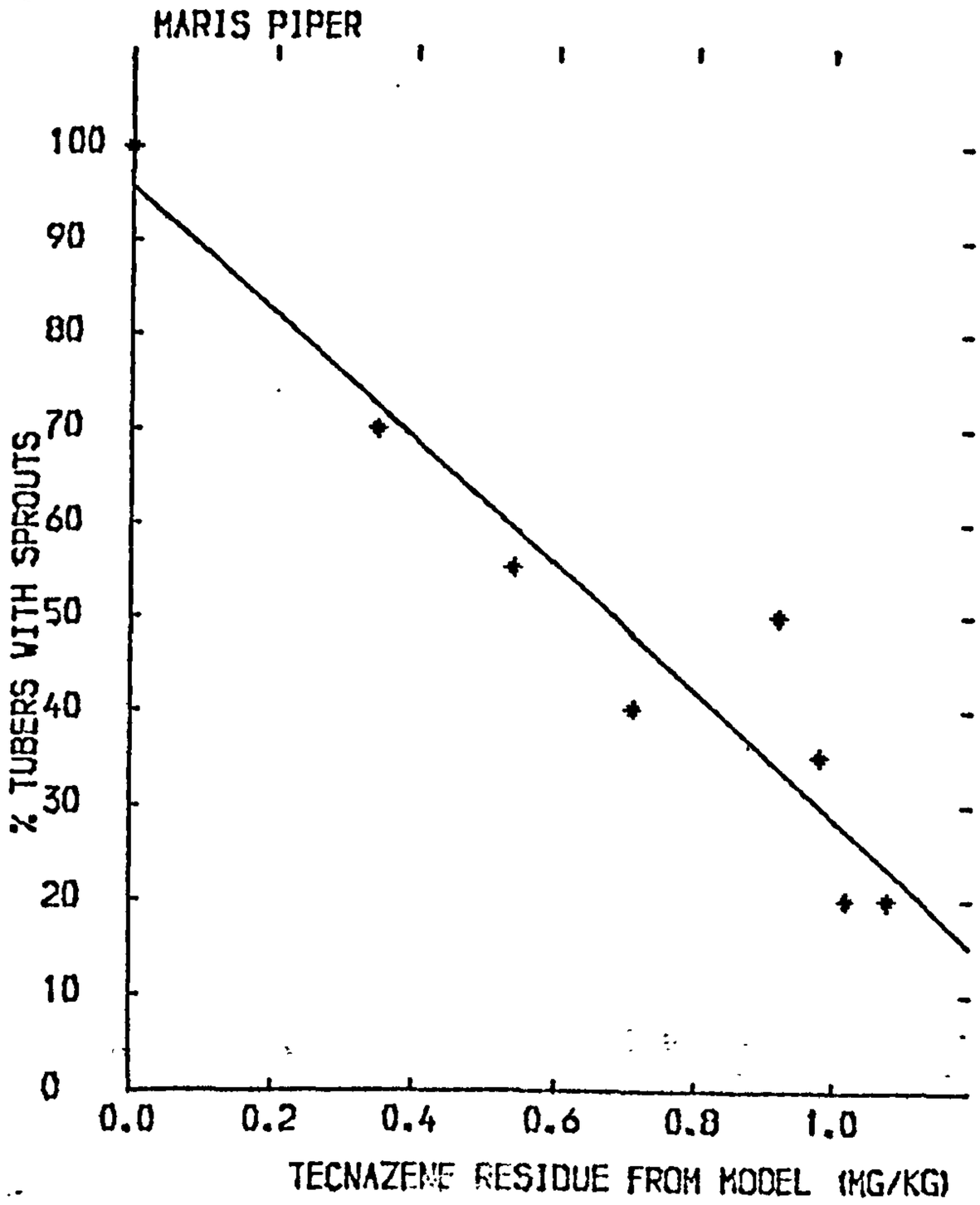
Further investigations into the relationship between the percentage of tubers with sprouts and tecnazene residues were also undertaken.

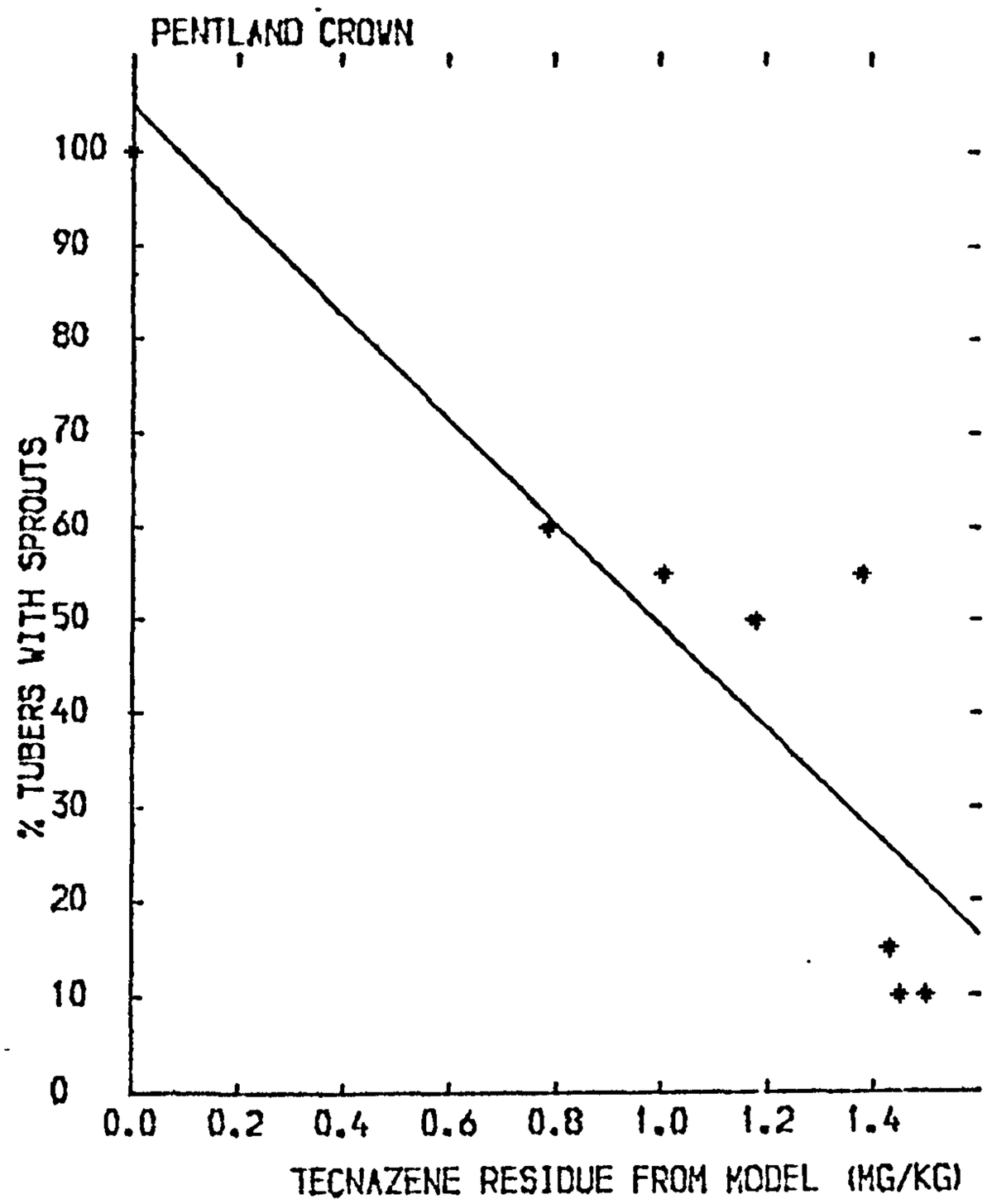
The percentage of tubers with sprouts (% sprouting) has been plotted against tecnazene residues and the resultant diagrams for each of the four cultivars are shown in figure 5.3.3. The best straight line has also been plotted although it is not suggested that this represents the true relationship. Careful examination reveals that the situation is more complex than that described for sprout length, and that the results do not lend themselves to simple model construction as various tecnazene independent, environmentally produced physiological effects are apparent. For example the point corresponding to 8 weeks airing at $8^{o}C$ is consistently above the regression line.

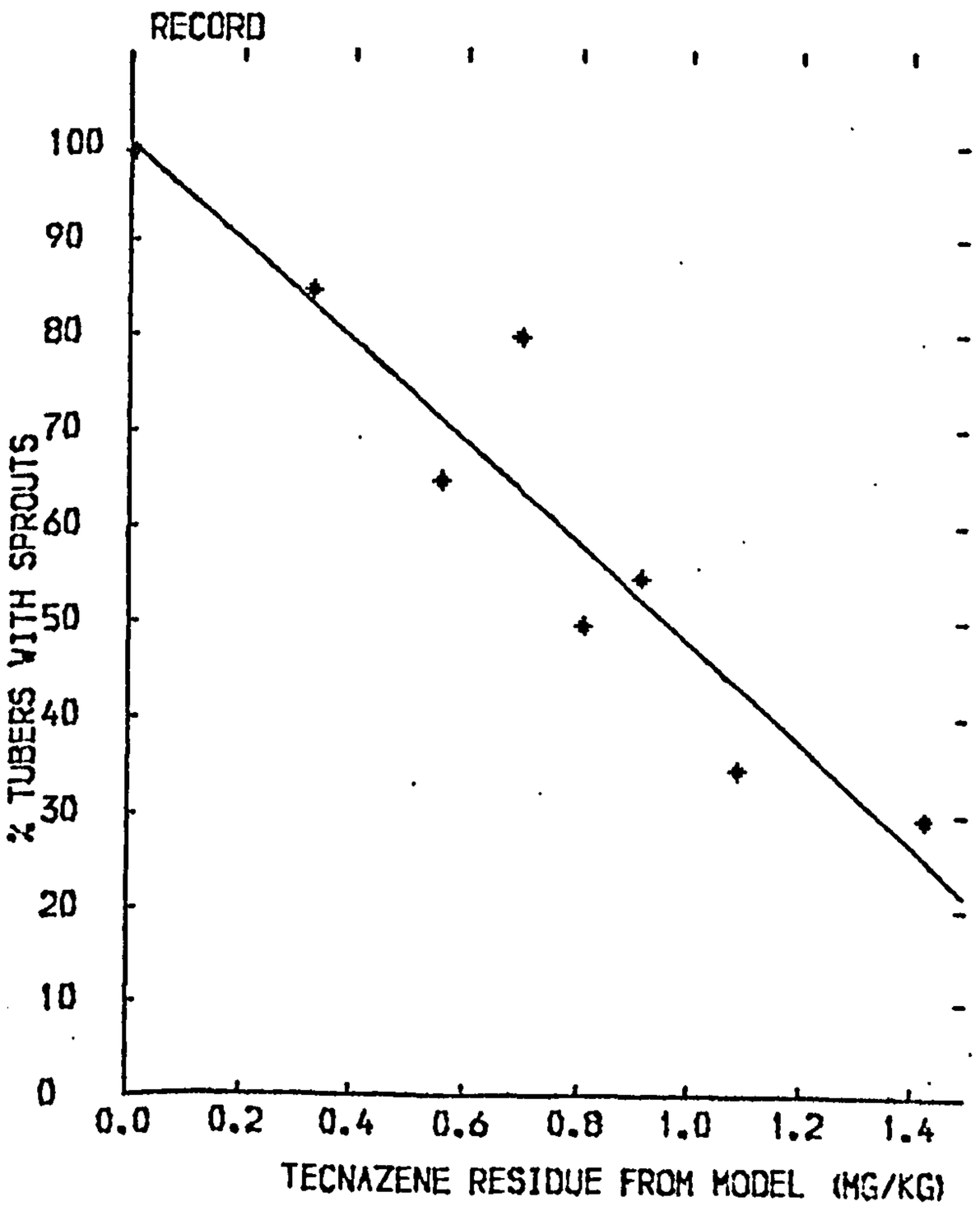
Despite these environmental effects however, it is obvious that tecnazene residues are not reflected in such a dramatic fashion in terms of % sprouting as with sprout length. This can be interpreted in two ways not wholly independent of each other.

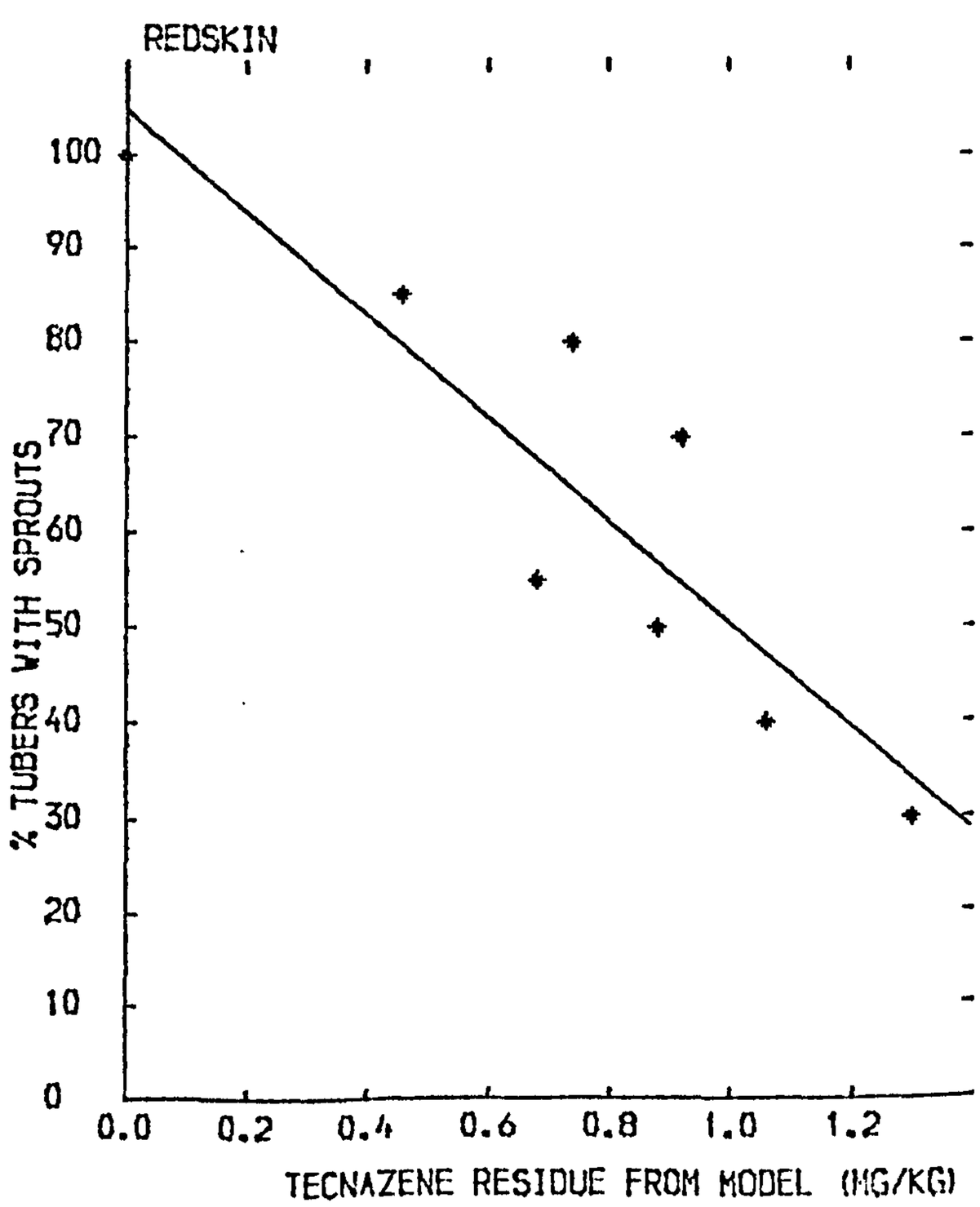
Firstly, the system under examination is dynamic. Residues fall due to suitable airing conditions then the tubers begin to sprout. The definition of % sprouting implies that this would be reflected in this term long before it would make any impact on mean sprout length. Consequently fairly high residue levels can produce high % sprouting figures. Alternatively if the degree of sprouting is controlled by the tecnazene on the tuber surface which slowly

FIGURE 5.3.3 THE EFFECT OF TECNAZENE RESIDUES ON THE SPROUTING OF SEED POTATOES (1974-75)









volatilises and inhibits sprout growth by entering the bud rather than the tuber itself, then if the surface concentration drops sprouting will immediately commence. Tecnazene residues under this hypothesis are an indirect consequence of the surface concentration and would slowly decrease. The difference in rates at which the two processes occur would again mean that high tecnazene residues could be associated with high % sprouting values and low mean sprout lengths.

The second explanation for the discrepancy between the effect of tecnazene on % sprouting and mean sprout length concerns its mode of action. If tecnazene inhibits cell elongation but not cell division then the above effects would be expected. Evidence to support this explanation may be found if the sprouts on tecnazene treated tubers are closely examined, in that such sprouts have a stubby swollen appearance concurrent with the fact that cell division is not inhibited. Interference with IAA function would also be concurrent with the well documented loss of apical dominance discussed previously. This latter explanation would not, of course, conflict with the comments about the route of tecnazene entry in the former.

Sprouting and storage conditions

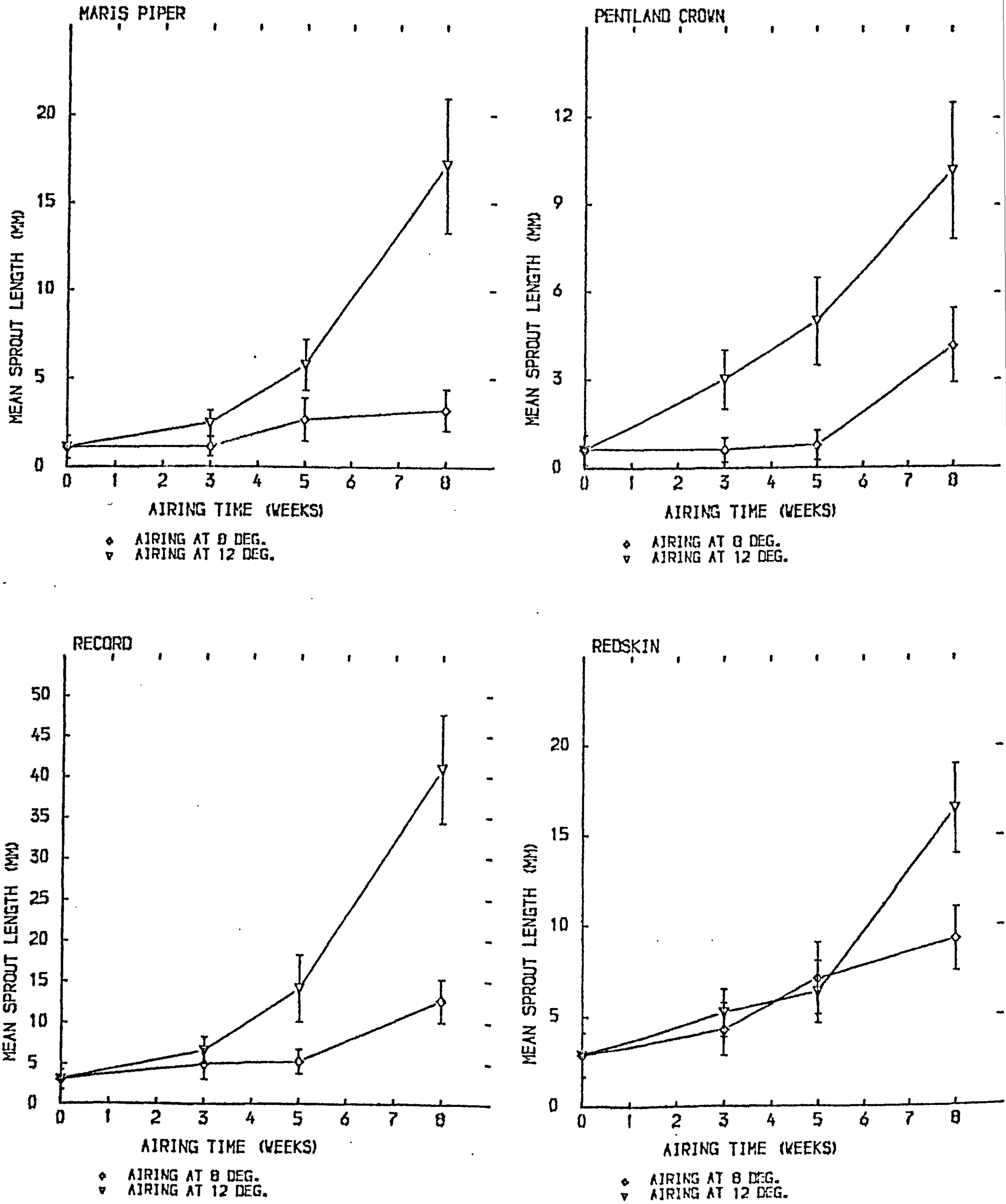
From the comments made in the previous section it would be expected that storage conditions and sprouting of tecnazene treated seed should be closely related, because tecnazene residues and the tecnazene independent environmental effects are dependent upon storage conditions.

The effect of storage conditions on mean sprout length is illustrated in figure 5.3.4. (The error bars represent the standard error of the mean).

A search amongst mathematical transformations of X and Y (using the raw data) to find the best regression line revealed that in 6 out of 8 data sets, ($8^{o}C$ and $12^{o}C$ for each cultivar), the best fitting model was of the form $y = \alpha + \beta x^2$ i.e. mean sprout length is proportional to the airing time squared. This is not the best model from the statistical view-point as it assumes constant variance. It is however adequate for the present purpose.

These findings imply that doubling the airing time will produce a four-fold increase in sprout length. This of course has serious

FIGURE 5.3.4 THE EFFECT OF STORAGE CONDITIONS ON THE SPROUTING OF SEED POTATOES (1974-75)



implications if long airing times are recommended for the reduction of tecnazene residues in that the sprouts produced are unacceptably long and would undoubtedly be removed during commercial handling (see later).

From figure 5.3.4. it can also be seen that airing at 12°C will produce much longer sprouts than airing at 8°C. This is especially obvious after 8 weeks airing and this must also be taken into consideration when making recommendations.

The effect of storage conditions on the % sprouting of tecnazene treated seed is illustrated in figure 5.3.5. and requires no explanation.

2) Emergence

From the 2-day emergence scores, interval emergence frequencies and hence the various emergence characteristics discussed in section 5.2. were calculated using a specially written computer programme.

Using pooled interval emergence frequencies the "emergence profiles" for the various treatments were constructed in the form of histograms using a computer linked graph plotter. These histograms are illustrated in figures 5.3.6., 5.3.7. (Maris Piper), 5.3.8., 5.3.9. (Pentland Crown), 5.3.10., 5.3.11. (Record), 5.3.12., 5.3.13. (Redskin) and 5.3.14. (forced airing treatment for all 4 cultivars).

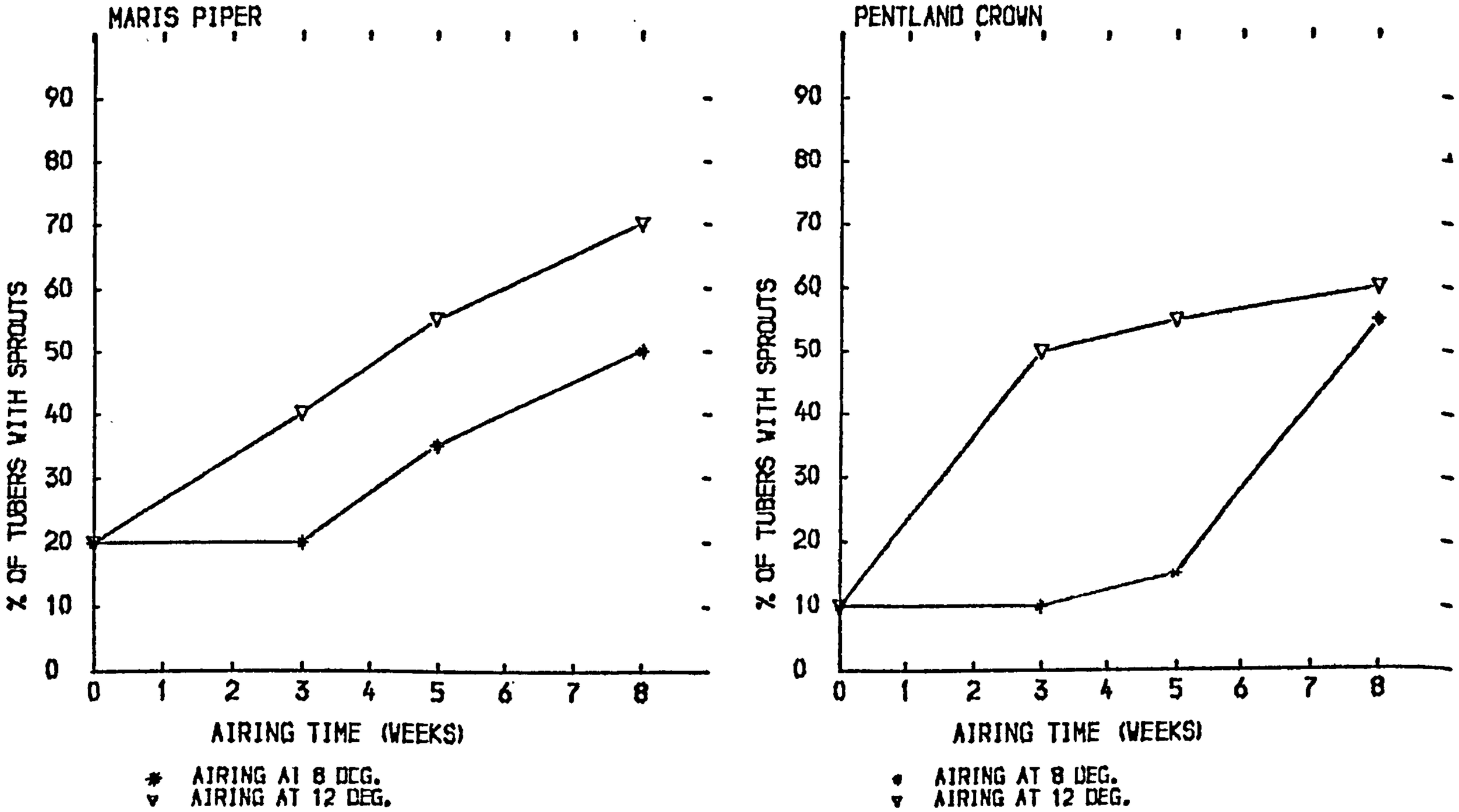
The calculated emergence characteristics (again using pooled data) are listed with the associated storage conditions in tables 5.3.5. (Maris Piper and Pentland Crown) and 5.3.6. (Record and Redskin).

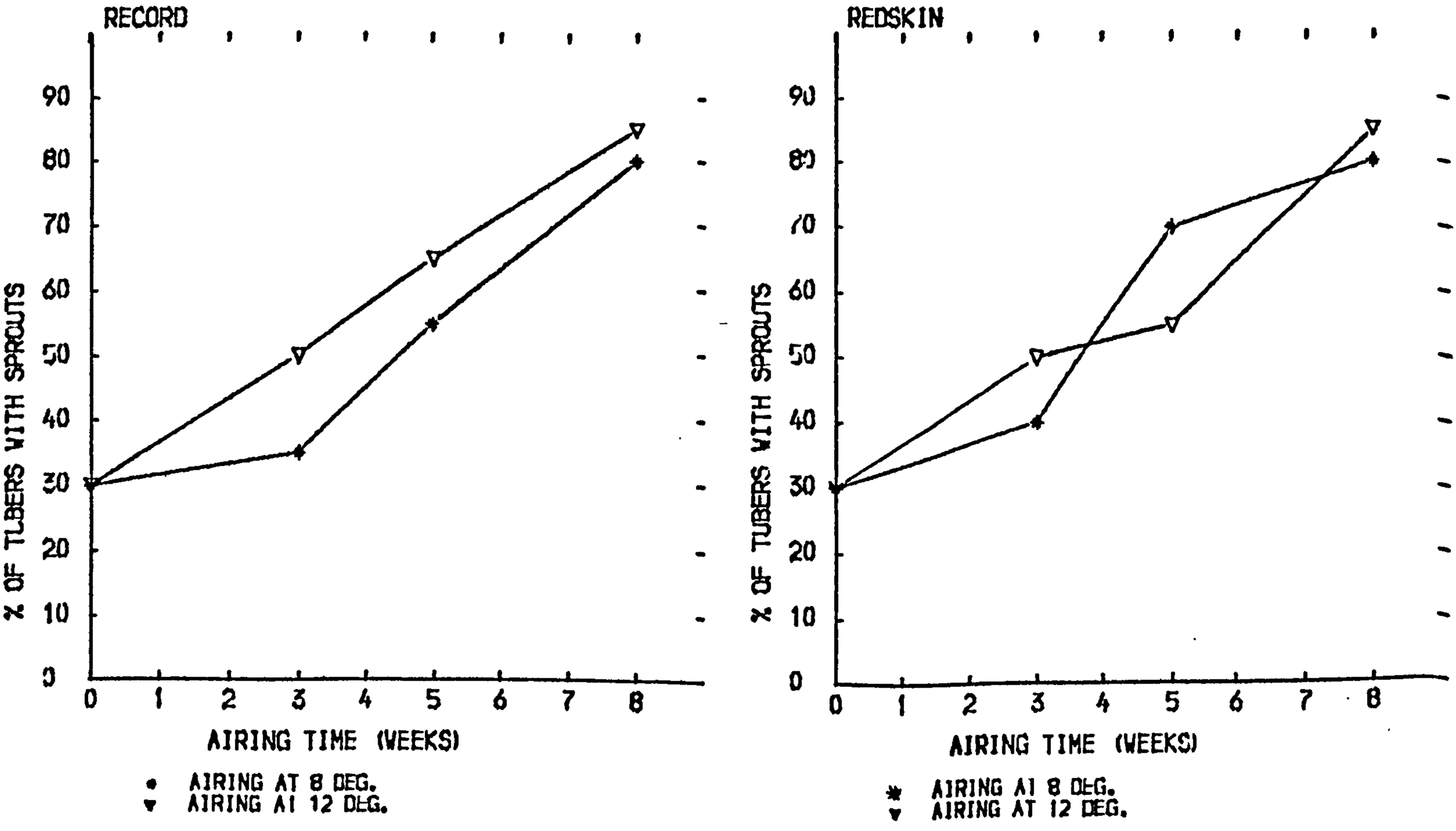
From the histograms and the tables it can be seen that in general tecnazene treated seed emerged more slowly than the untreated controls, and that the effects of tecnazene can be minimised by using appropriate storage conditions. An attempt has been made below to correlate these changes in performance with residues at planting and with storage conditions.

More detailed examination of the histograms reveals two other factors which should be mentioned.

In general tecnazene treated seed produced more squat emergence profiles than those untreated controls. This could affect chemical weed control under commercial conditions as,ideally, for optimum results plants should emerge rapidly immediately after spraying.

FIGURE 5.3.5 THE EFFECT OF STORAGE CONDITIONS ON THE SPROUTING OF SEED POTATOES (1974-75)





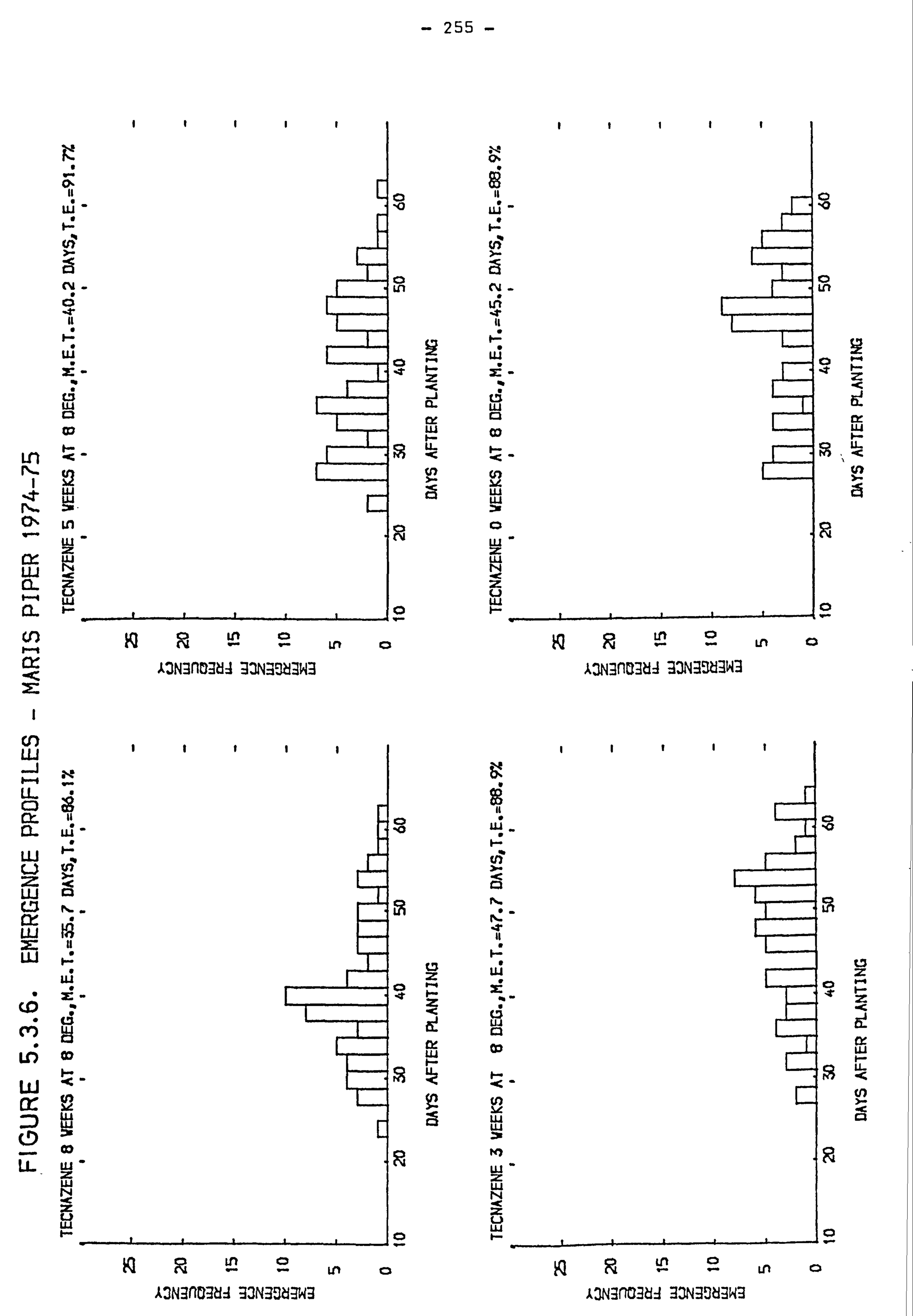


FIGURE 5.3.6. EMERGENCE PROFILES - MARIS PIPER 1974-75
TECNAZENE 0 WEEKS AT 8 DEG., M.E.T.=45.2 DAYS, T.E.=88.9%
TECNAZENE 5 WEEKS AT 8 DEG., M.E.T.=40.2 DAYS, T.E.=91.7%
TECNAZENE 3 WEEKS AT 8 DEG., M.E.T.=47.7 DAYS, T.E.=88.9%
TECNAZENE 8 WEEKS AT 8 DEG., M.E.T.=35.7 DAYS, T.E.=86.1%
EMERGENCE FREQUENCY
DAYS AFTER PLANTING
0
5
10
15
20
25
10
20
30
40
50
60

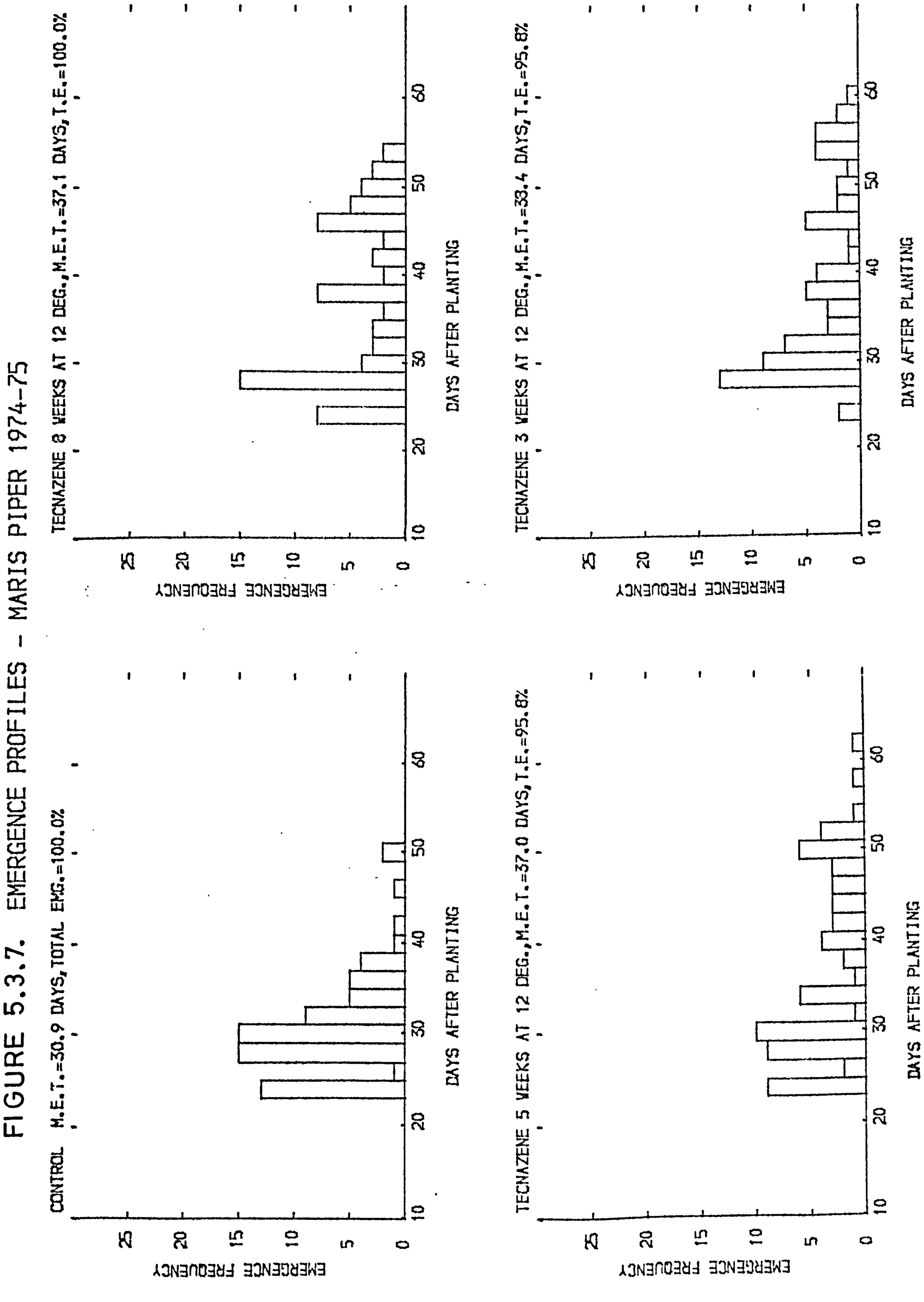
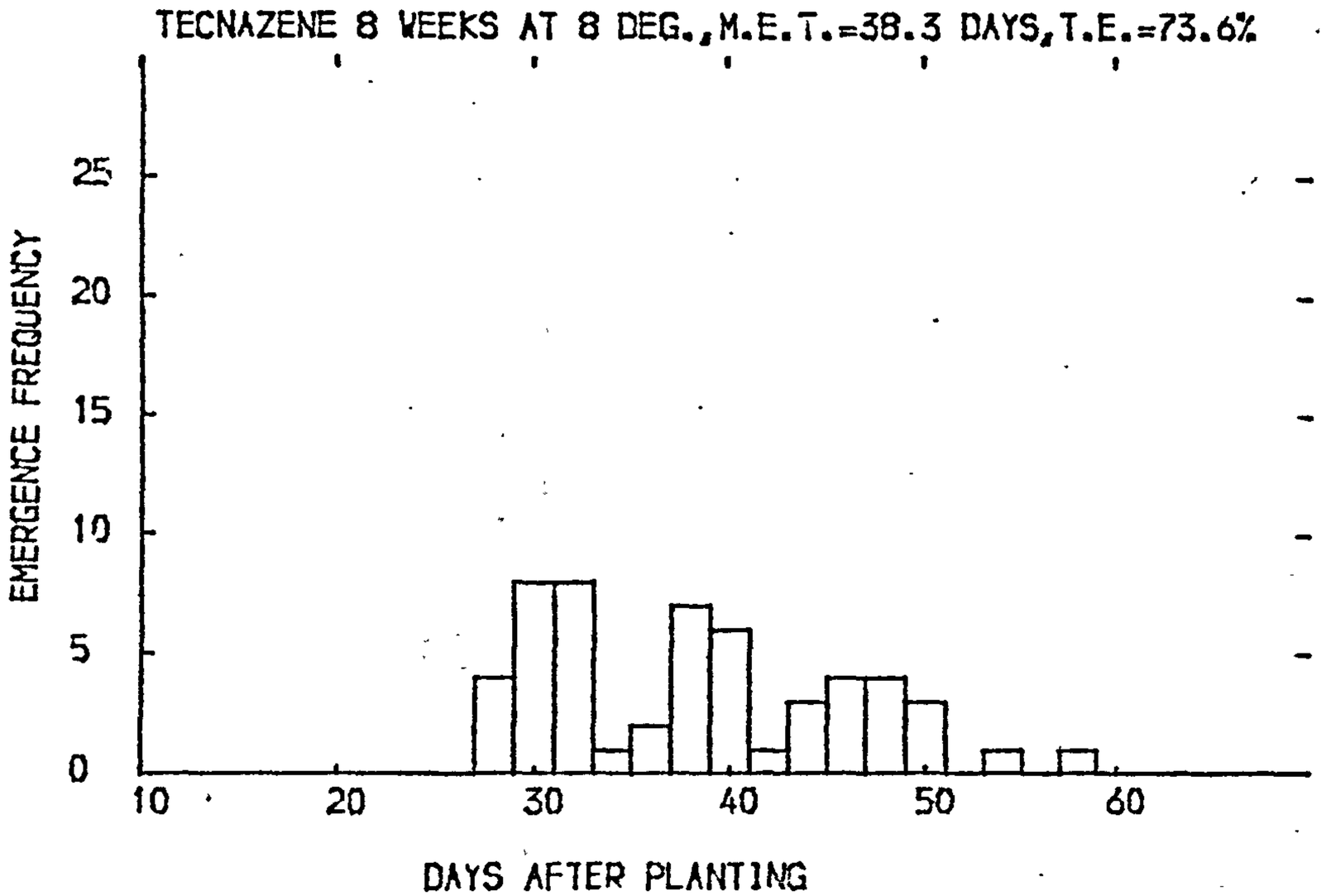
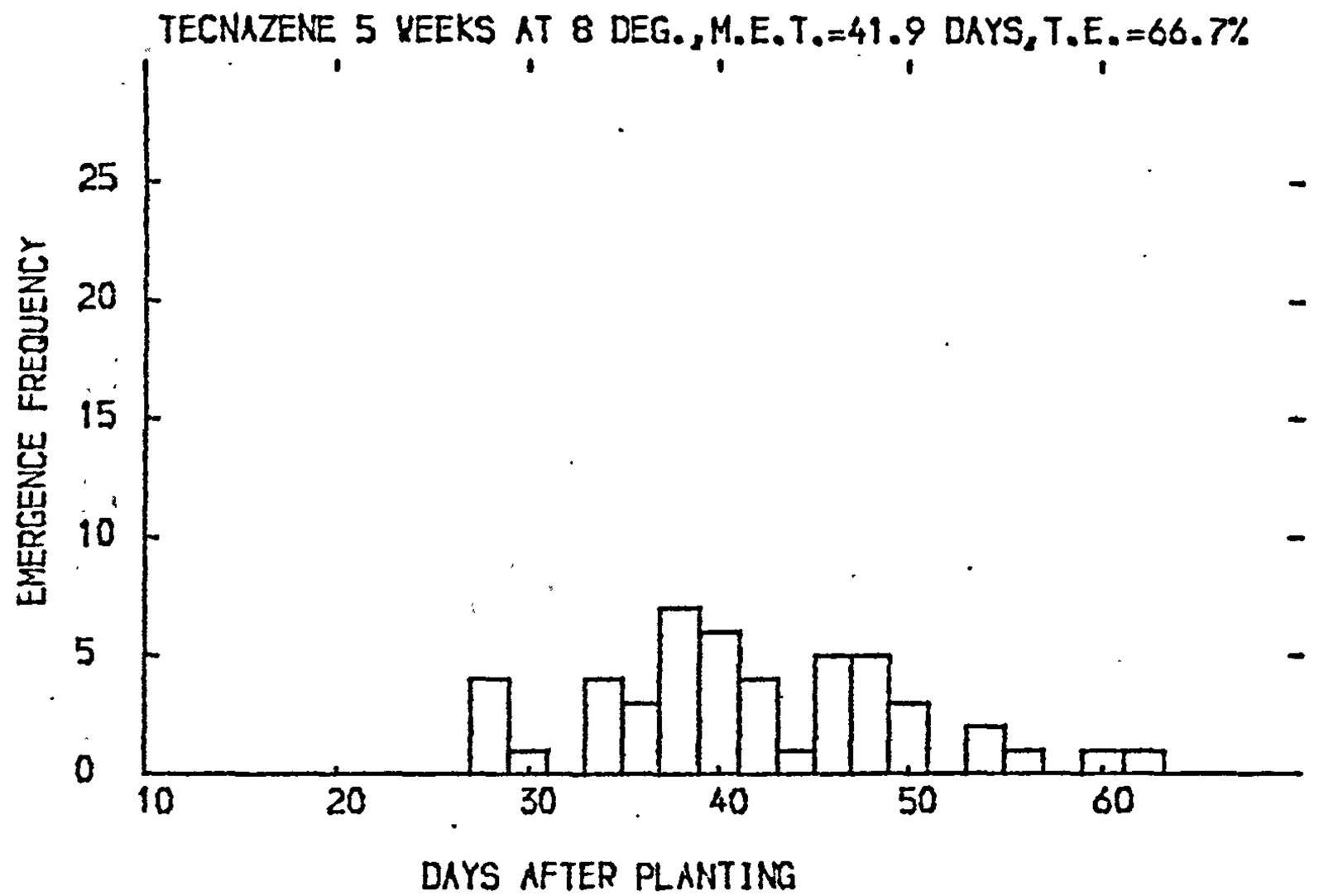
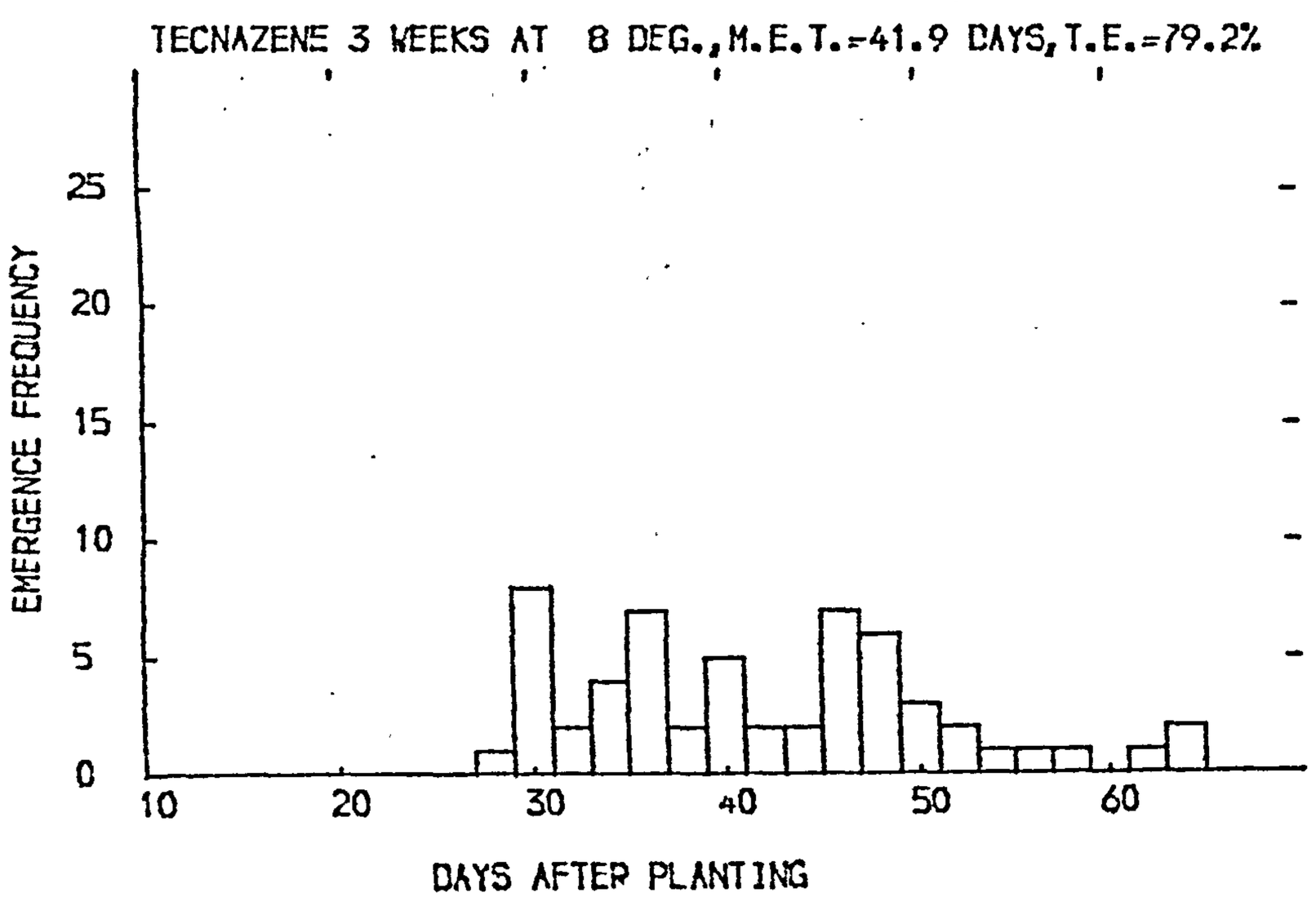

EMERGENCE PROFILES - MARIS PIPER 1974-75
CONTROL M.E.T.=30.9 DAYS, TOTAL EMG.=100.0%
TECNAZENE 2 WEEKS AT 12 DEG., M.E.T.=37.1 DAYS, T.E.=100.0%
TECNAZENE 3 WEEKS AT 12 DEG., M.E.T.=33.4 DAYS, T.E.=95.8%
TECNAZENE 5 WEEKS AT 12 DEG., M.E.T.=37.0 DAYS, T.E.=95.8%
EMERGENCE FREQUENCY
0
5
10
15
20
25
10
20
30
40
50
60
DAYS AFTER PLANTING

FIGURE 5.3.7.

FIGURE 5.3.8. EMERGENCE PROFILES - PENTLAND CROWN 1974-75







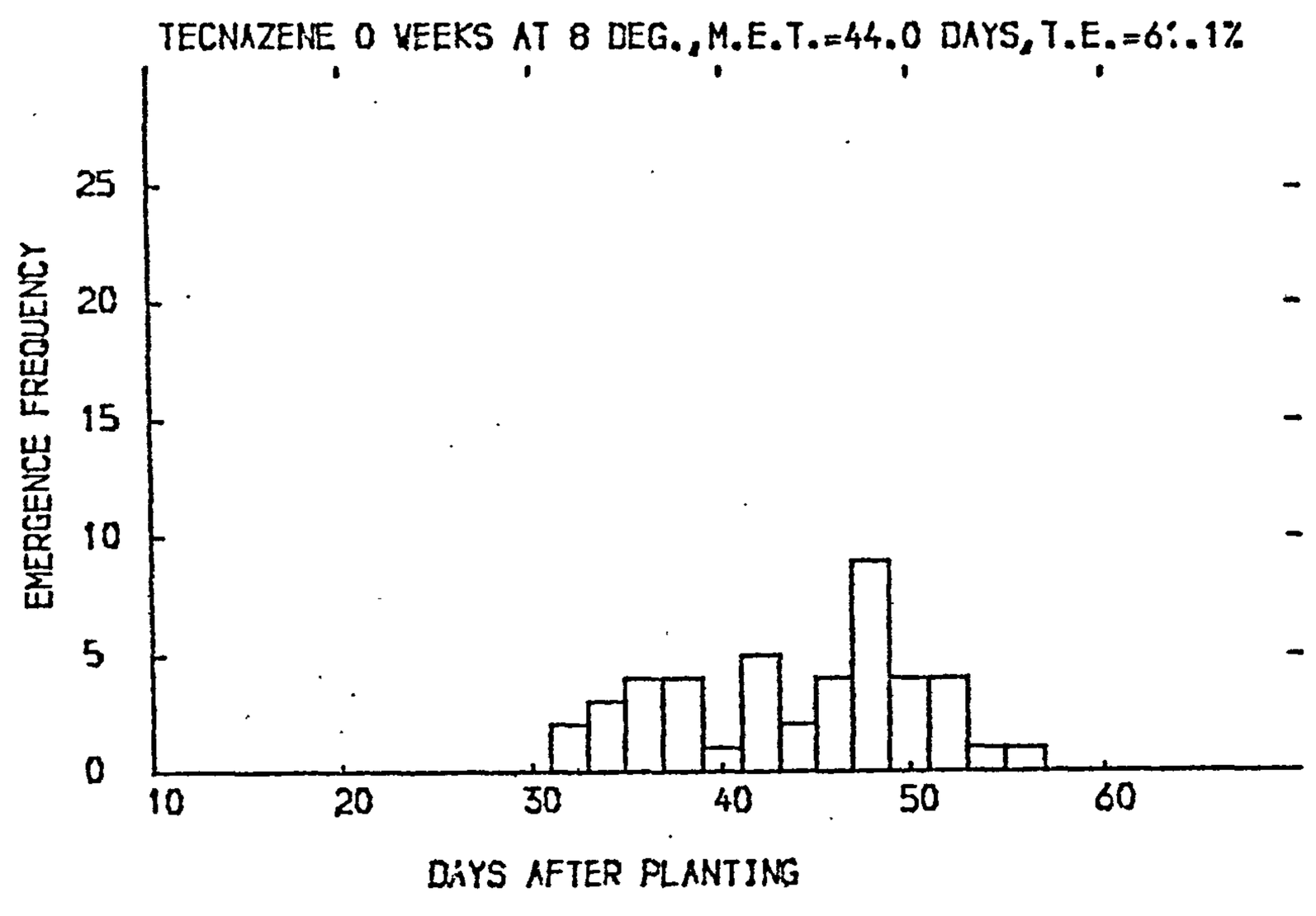
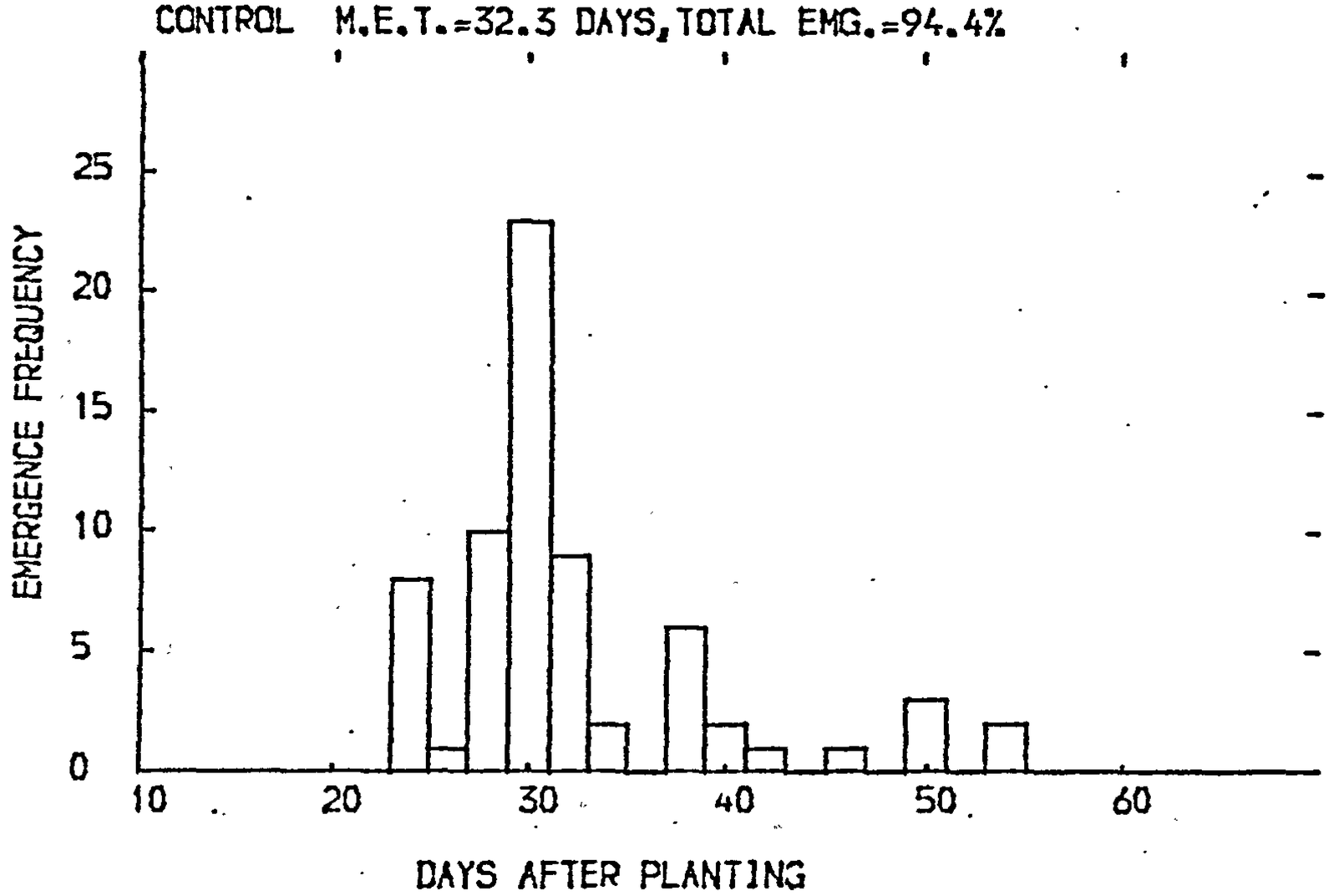
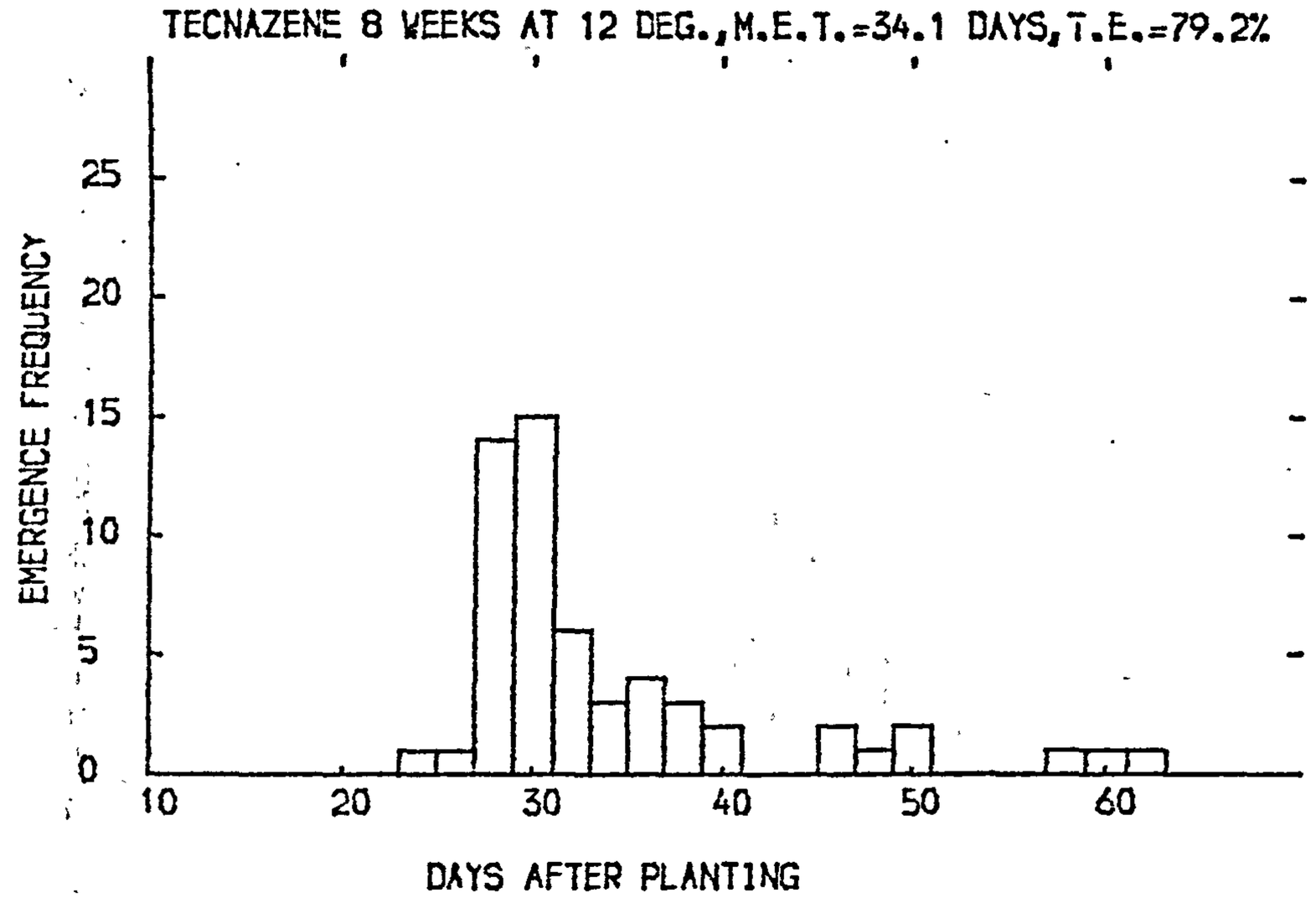
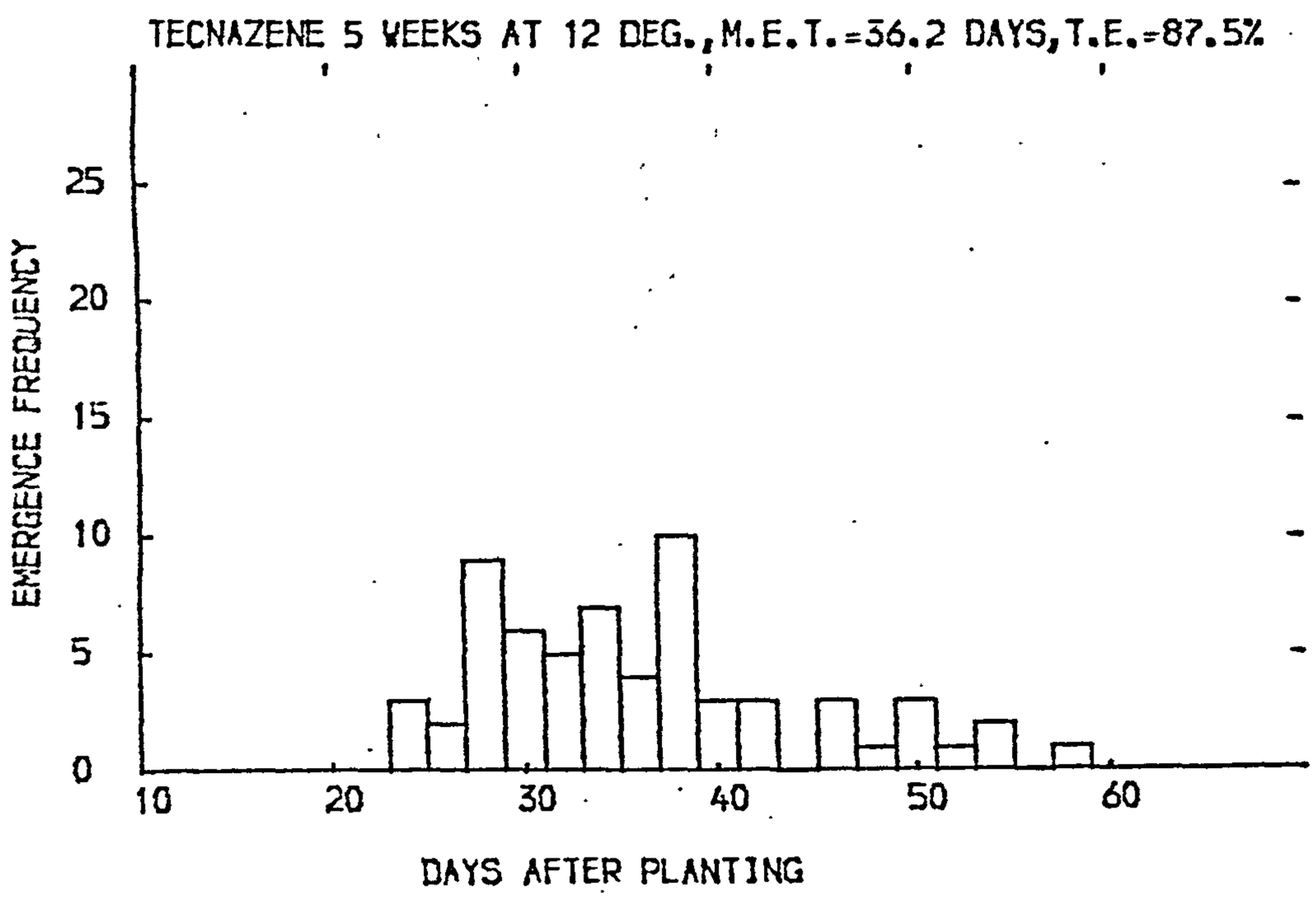


FIGURE 5.3.9. EMERGENCE PROFILES - PENTLAND CROWN 1974-75







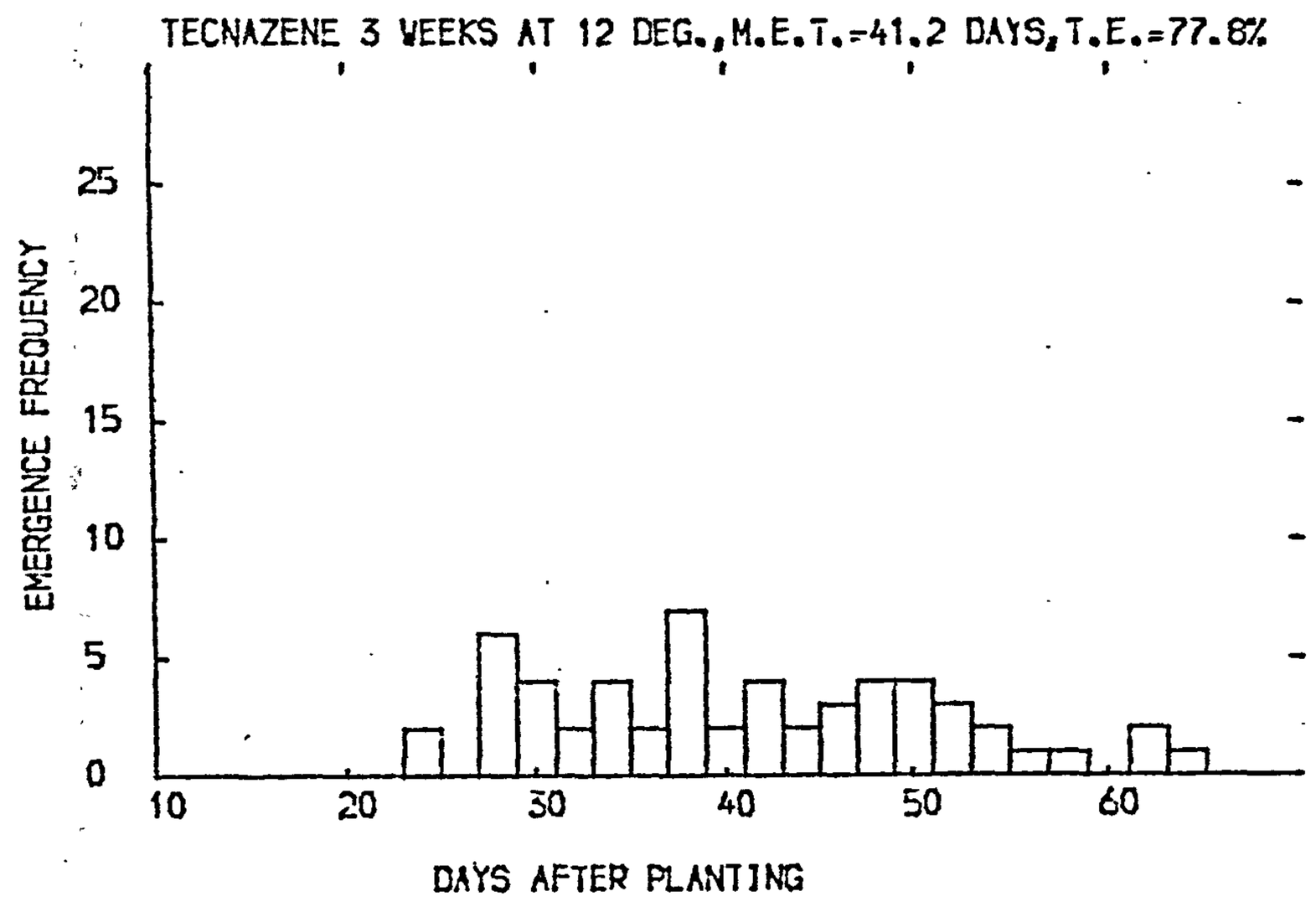
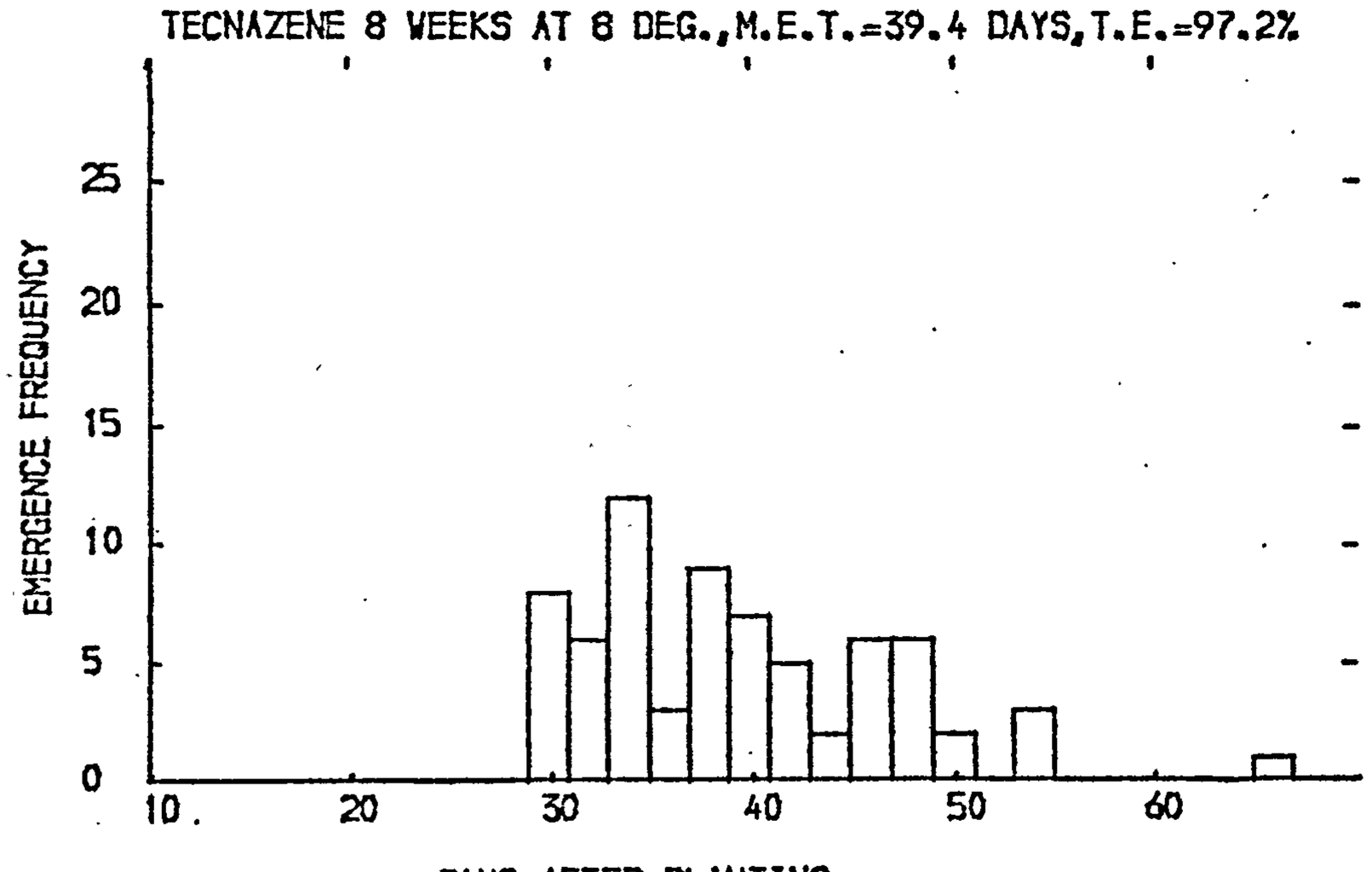
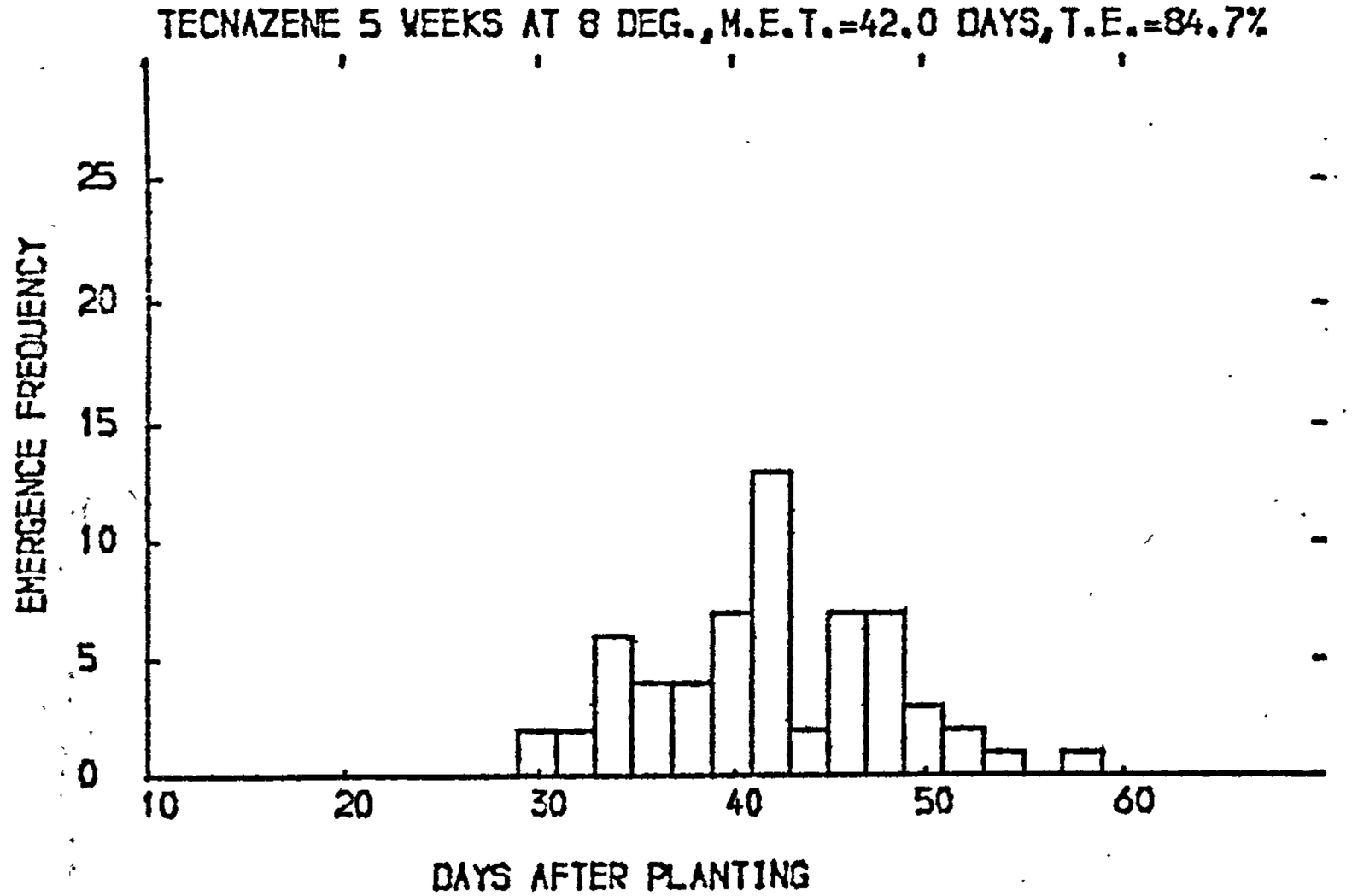
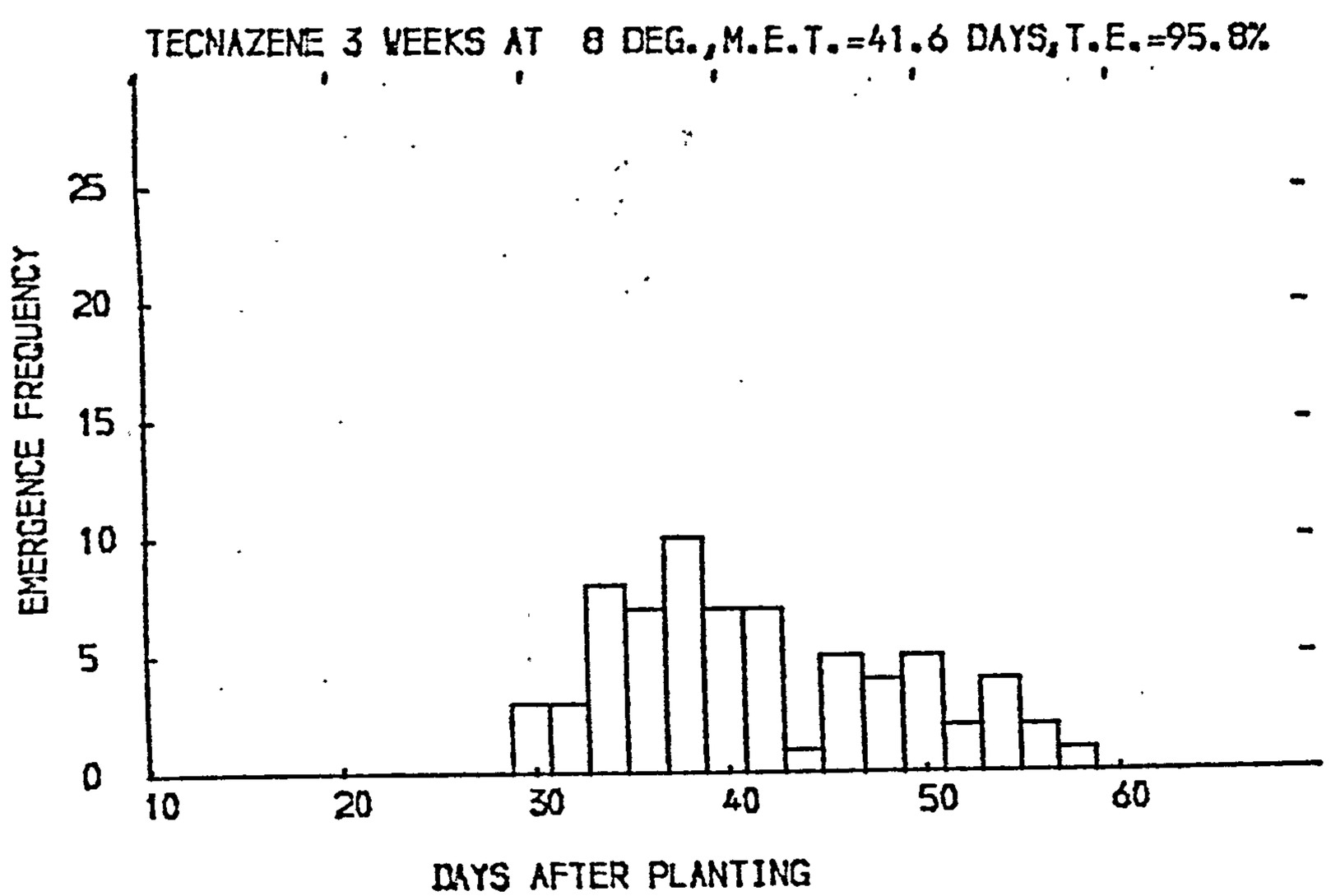


FIGURE 5.3.10. EMERGENCE PROFILES - RECORD 1974-75







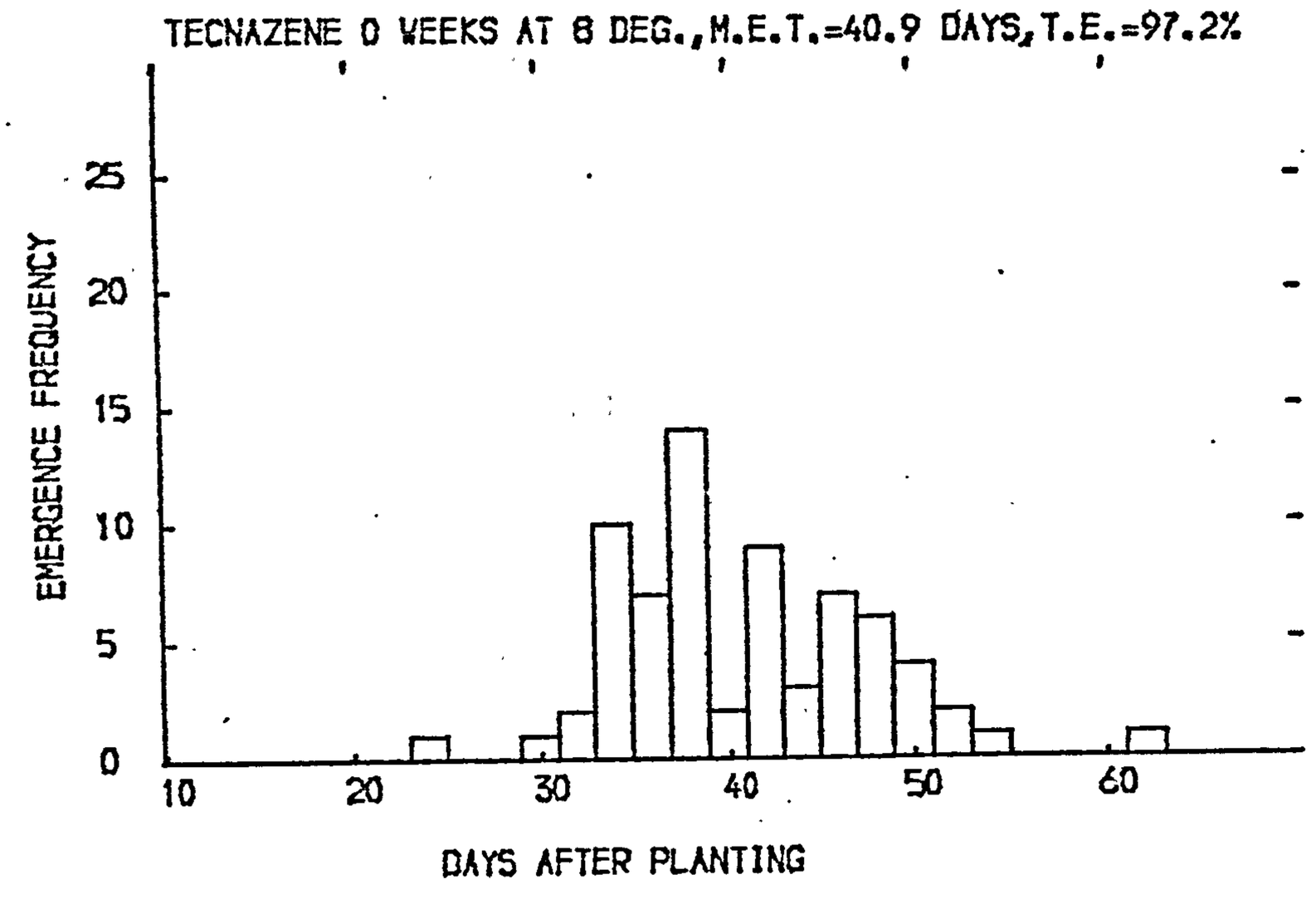
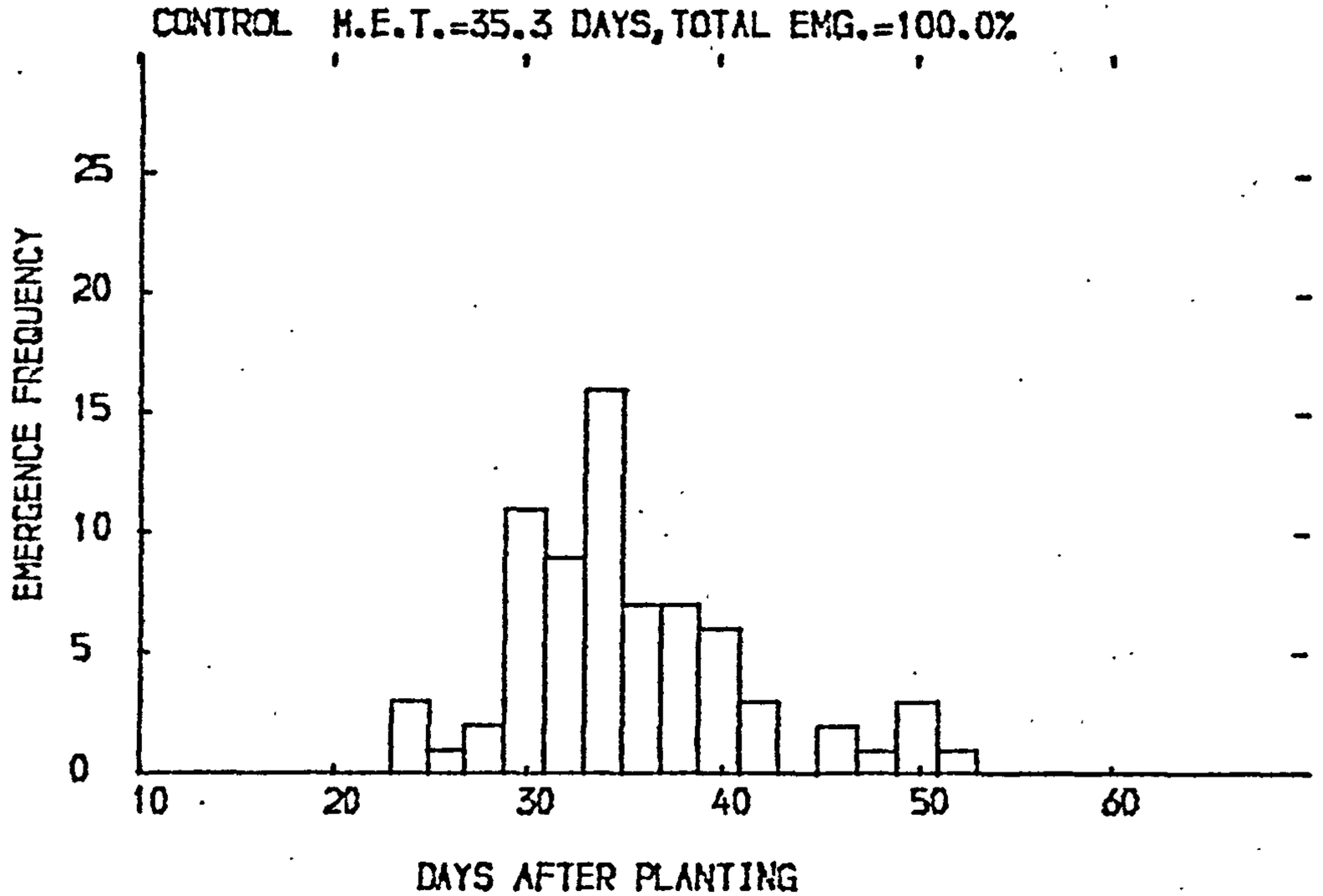
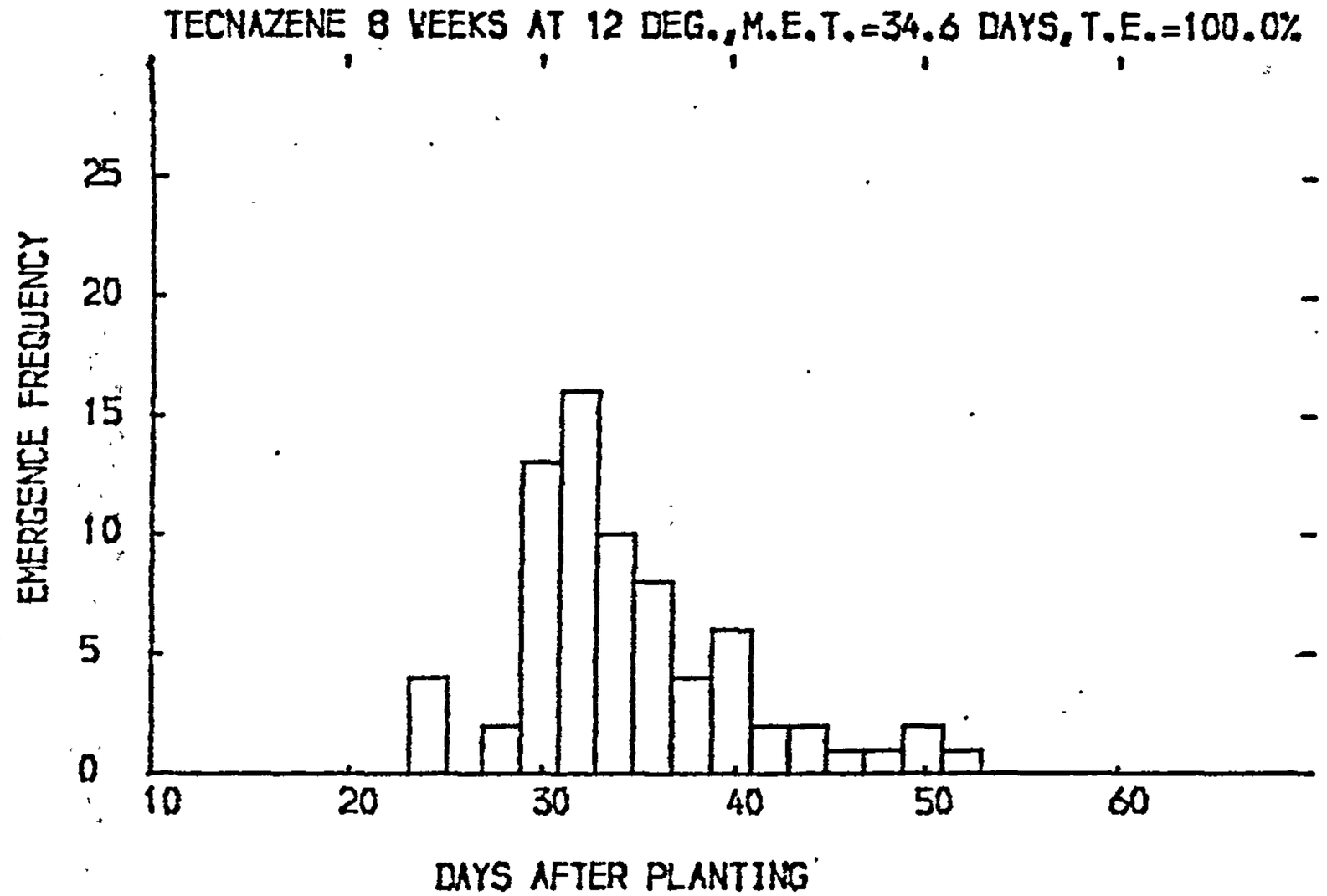
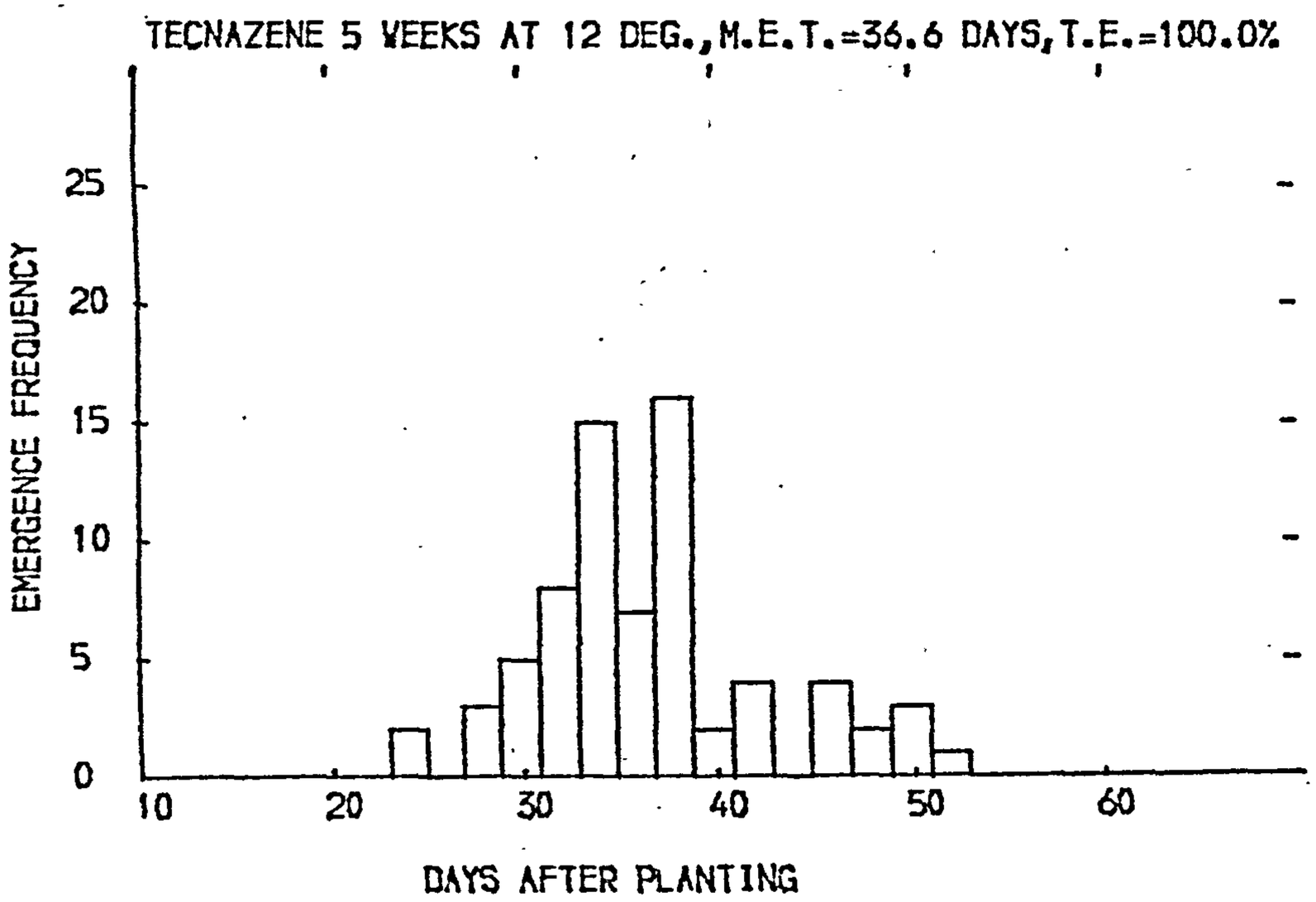


FIGURE 5.3.11. EMERGENCE PROFILES - RECORD 1974-75







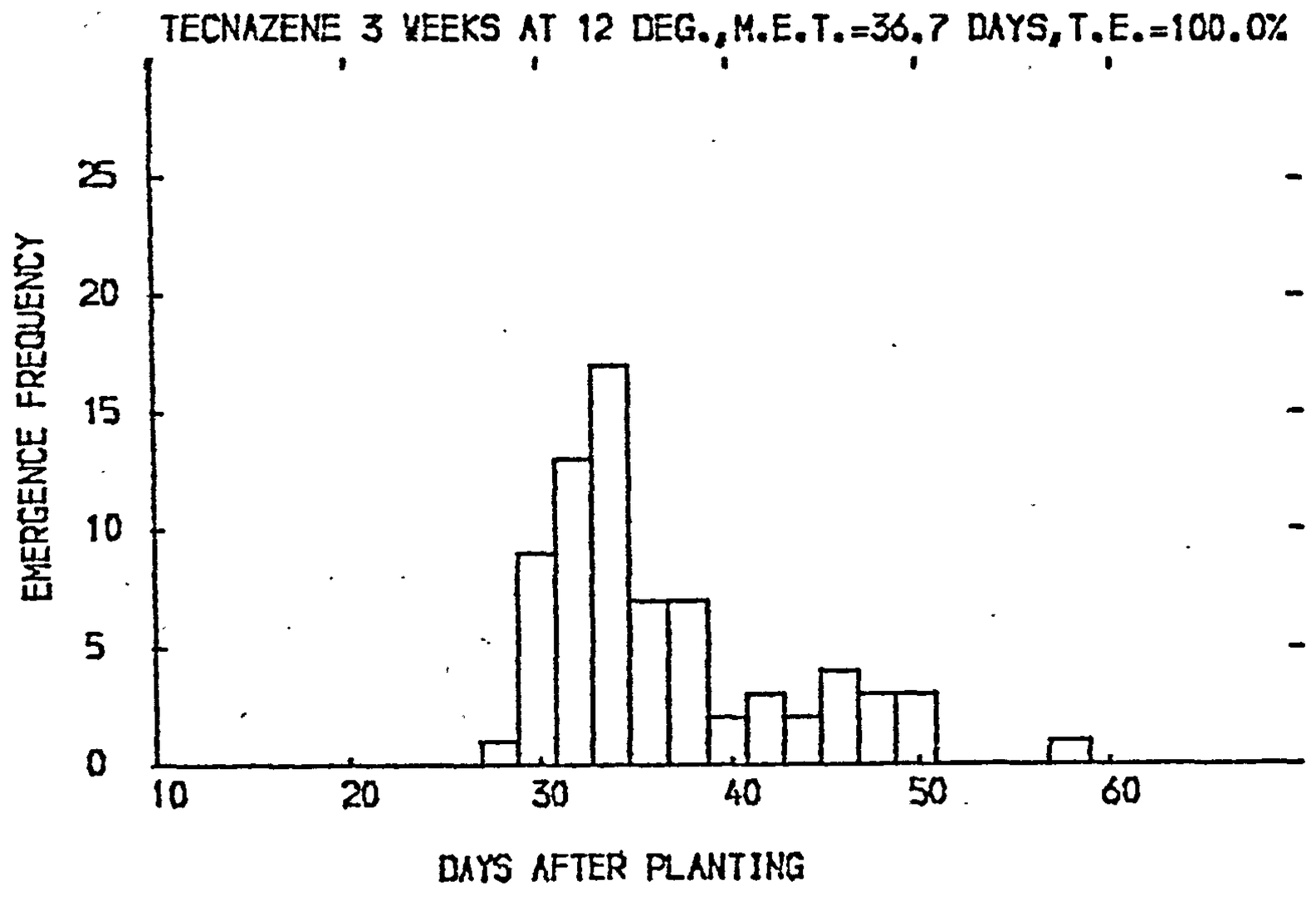
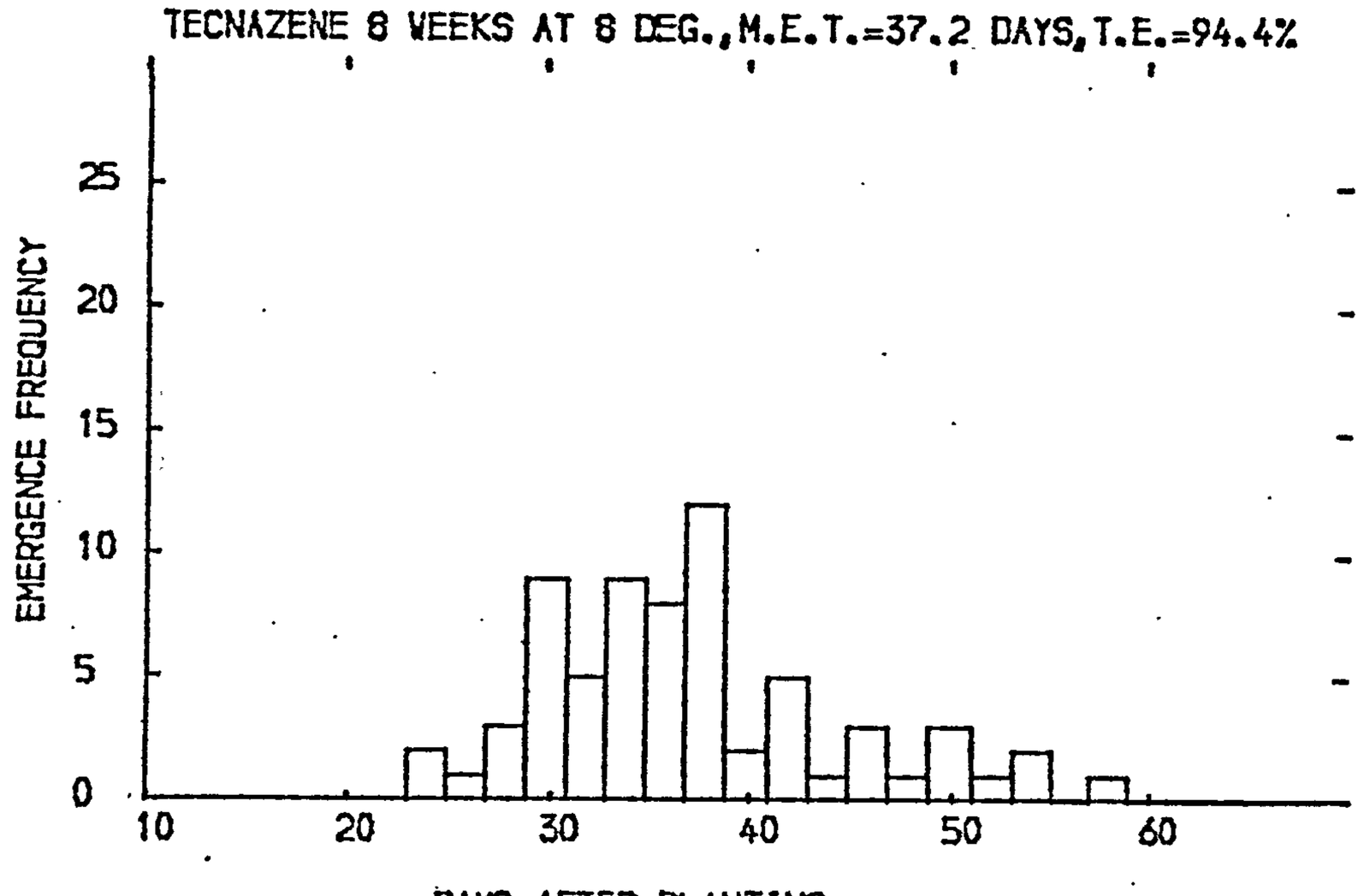
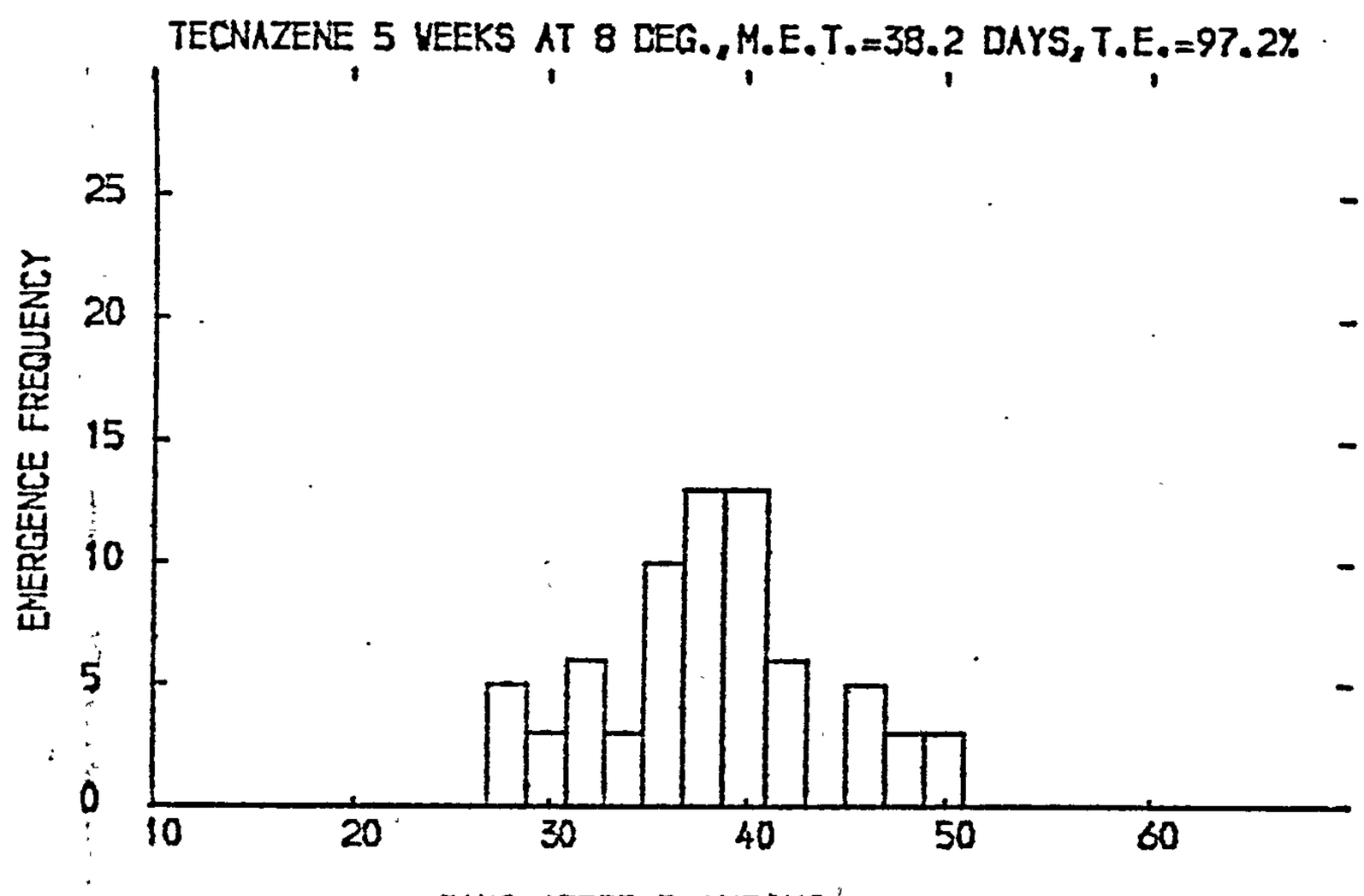
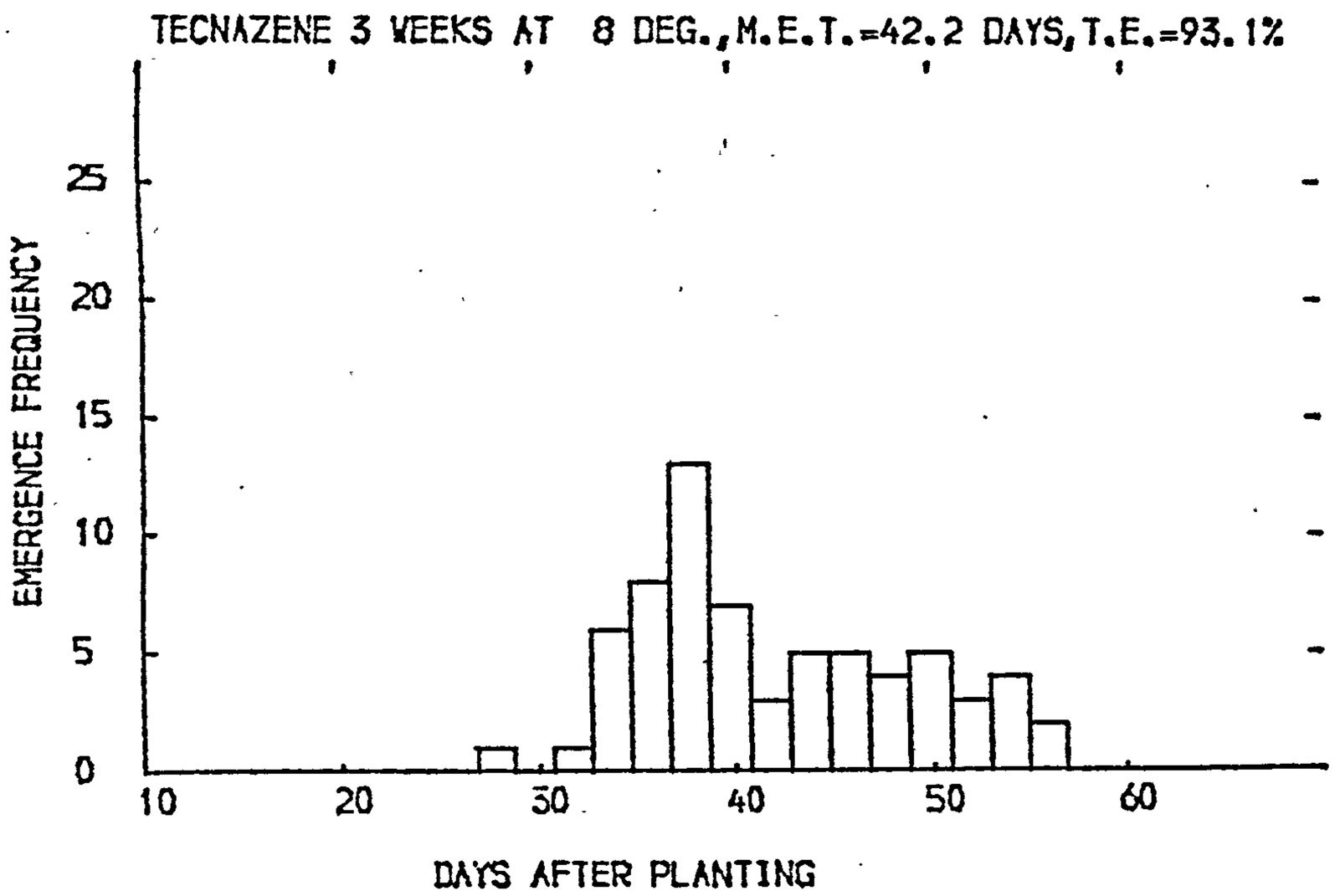


FIGURE 5.3.12. EMERGENCE PROFILES - REDSKIN 1974-75







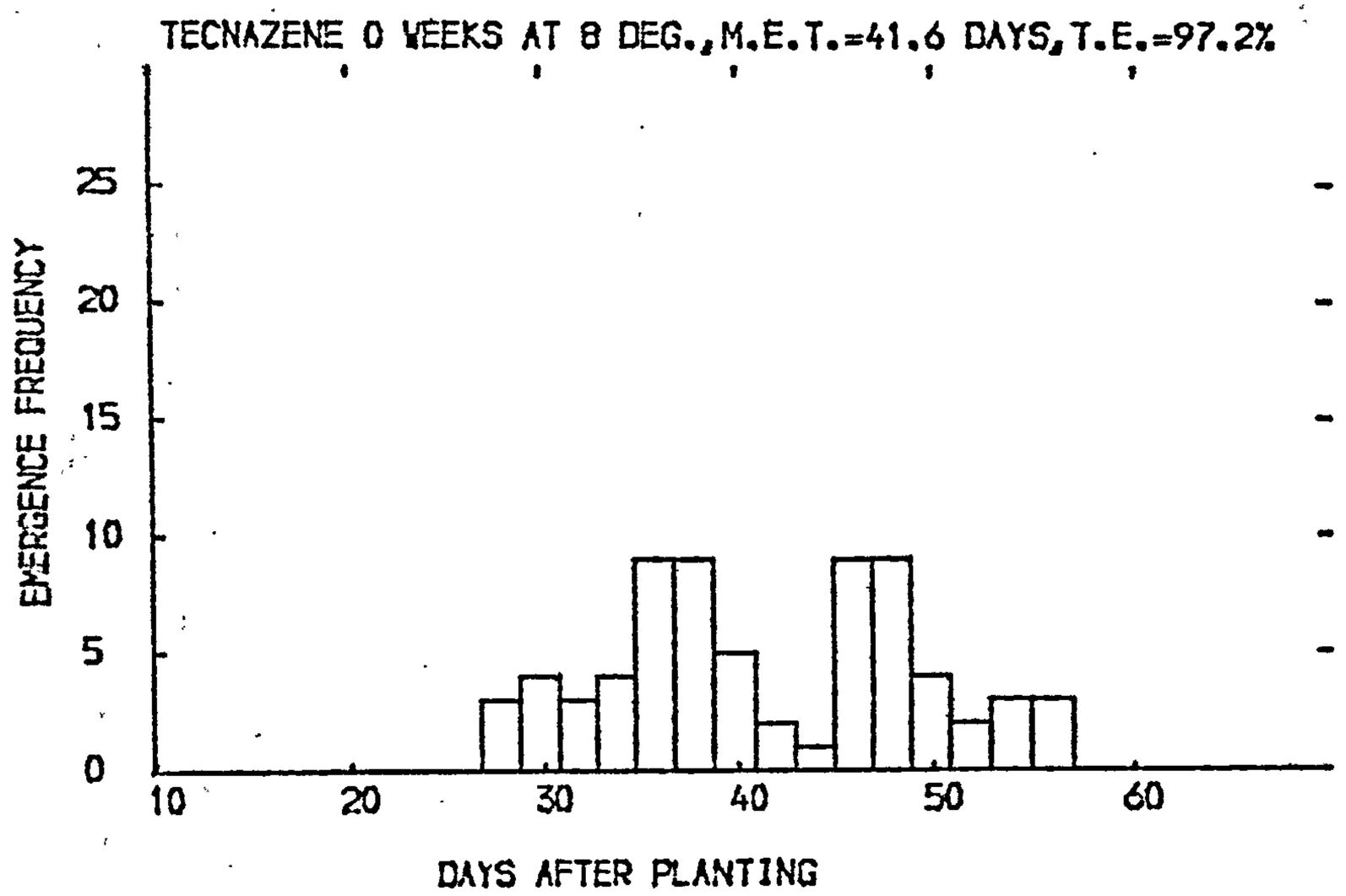


FIGURE 5.3.13. EMERGENCE PROFILES - REDSKIN 1974-75

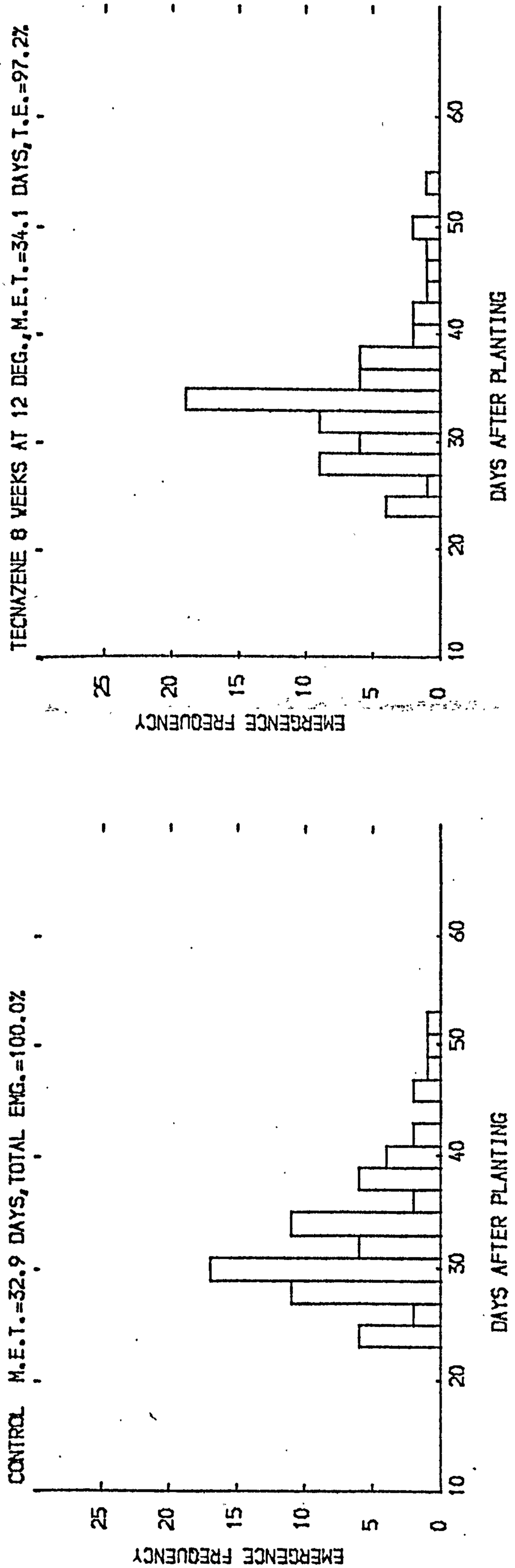


FIGURE 5.3.14. EMERGENCE PROFILES - 3 WEEKS FORCED VENTILATION 1974-75

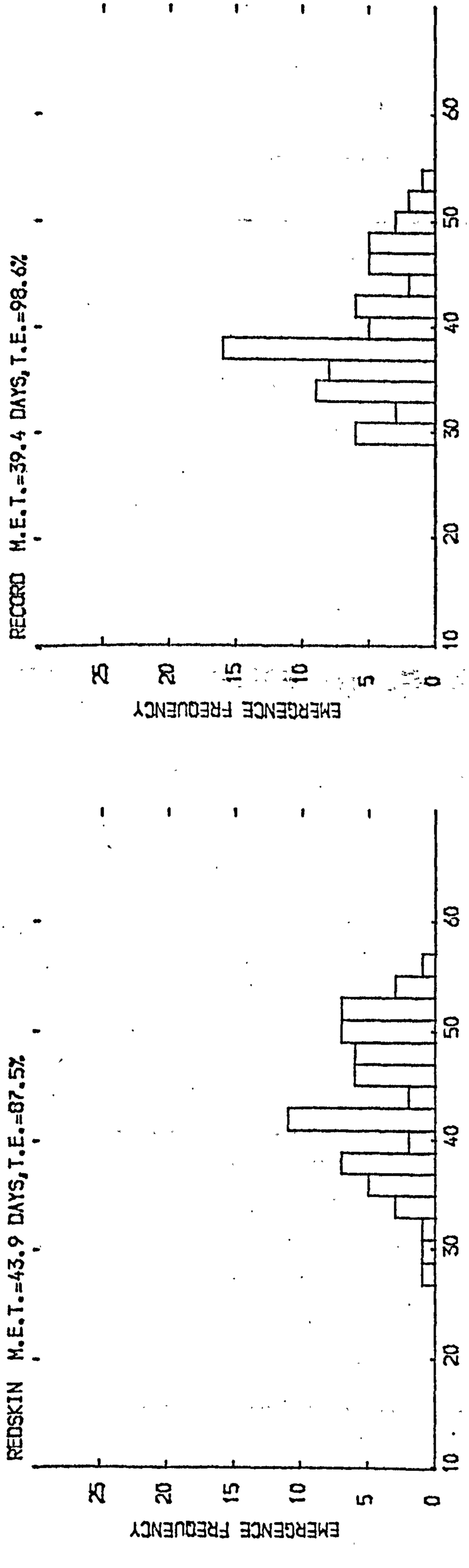


Table 5.3.5. The effect of storage conditions on the subsequent emergence behaviour of tecnazene treated seed potatoes using data pooled from replicate treatments (1974-75) (Cultivars - Maris Piper and Pentland Crown)

Cultivar	Storage conditions			Emergence characteristics			
	time closed (weeks)	time open (weeks)	temp. (°C)	MET[a] (days)	T50[b] (days)	T75[c] (days)	Total[d] emergence (%)
Maris Piper	- untreated control -			30.9	29.9	33.4	100.0
	4	8	8	40.8	40.6	53.0	86.1
	7	5	8	40.2	41.7	49.4	91.7
	9	3	8	47.7	50.6	56.2	88.9
	12	0	-	45.2	47.9	55.0	88.9
	4	8	12	37.1	37.3	46.0	100.0
	7	5	12	37.0	34.7	47.7	95.8
	9	3	12	38.4	36.3	48.0	95.8
	9	3f[e]	8	42.2	43.0	49.0	94.4
Pentland Crown	- untreated control -			32.3	30.5	37.3	94.4
	4	8	8	38.3	41.0	-	73.6
	7	5	8	41.9	47.4	-	66.7
	9	3	8	41.9	45.9	59.0	79.2
	12	0	-	44.0	50.0	-	61.1
	4	8	12	34.1	32.7	51.0	79.2
	7	5	12	36.2	37.0	46.3	87.5
	9	3	12	41.2	45.7	62.0	77.8
	9	3f[e]	8	41.5	47.0	-	70.8

a Mean emergence time

b Time to 50% emergence

c Time to 75% emergence

d Total emergence expressed as a % of the number of tubers planted

e Forced ventilation

Table 5.3.6. The effect of storage conditions on the subsequent emergence behaviour of tecnazene treated seed potatoes using data pooled from replicate treatments (1974-75) (Cultivars - Record and Redskin)

Cultivar	Storage conditions			Emergence characteristics			
	time closed (weeks)	time open (weeks)	temp. (°C)	MET[a] (days)	T50[b] (days)	T75[c] (days)	Total[d] emergence (%)
Record	- untreated control -			35.3	34.3	38.4	100.0
	4	8	8	39.4	38.6	45.7	97.2
	7	5	8	42.0	42.7	49.0	84.7
	9	3	8	41.6	40.4	48.4	95.8
	12	0	-	40.9	40.0	46.5	97.2
	4	8	12	34.6	33.2	37.5	100.0
	7	5	12	36.6	35.9	38.8	100.0
	9	3	12	36.7	34.5	39.0	100.0
	9	3f[e]	8	39.4	38.3	44.0	98.6
Redskin	- untreated control -			32.9	31.0	36.0	100.0
	4	8	8	37.2	36.8	42.2	94.4
	7	5	8	38.2	38.4	41.3	97.2
	9	3	8	42.2	41.0	49.4	93.1
	12	0	-	41.6	40.6	48.1	97.2
	4	8	12	34.1	33.7	37.0	97.2
	7	5	12	38.9	38.1	45.7	97.2
	9	3	12	39.9	39.8	47.4	91.7
	9	3f[e]	8	43.9	46.0	51.6	87.5

a Mean emergence time

b Time to 50% emergence

c Time to 75% emergence

d Total emergence expressed as a % of the number of tubers planted

e Forced ventilation

Also apparent in the histograms is the effect of the prevailing climatic conditions. In general warm wet conditions produced more rapid emergence and dry conditions delayed emergence, thus the theoretical normal distribution is not obtained under field conditions.

The effect of the forced airing treatment is somewhat variable and has not been included in the analysis below. There is however, sufficient evidence to suggest that this treatment is worthy of further investigation.

Emergence and tecnazene residues

To investigate the relationship between tecnazene residues and emergence characteristics a regression search was conducted amongst various transformations as previously described.

It was decided for emergence characteristics any transformation adopted, besides showing the required improvement in correlation coefficient discussed earlier, must also show a consistent improvement over at least three of the cultivars. Such transformations were then adopted for all four cultivars, allowing the slope and intercept terms to cope with cultivar differences.

The transformations adopted and the parameters of the resultant linear models were obtained for mean emergence time (MET), time to 50% emergence (T50) and total emergence (TE), and are shown in table 5.3.7. with their associated correlation coefficients.

From table 5.3.7. it is obvious that there is a close relationship between MET, T50 and tecnazene residues. To further illustrate this point the functions have been plotted on linear axes in figures 5.3.15. (MET) and 5.3.16. (T50) with the mean of the observations at each residue level.

For TE no transformation produced a consistent improvement so a simple linear model has been adopted and is illustrated in figure 5.3.17. In the cases of Maris Piper and Pentland Crown high tecnazene levels are associated with sizable and significant reductions in TE. For the cultivars Record and Redskin the effects are much smaller if indeed they exist.

Table 5.3.7. Parameters of linear models of the form $y = \alpha + \beta x$, describing the relationship between tecnazene residues (x)* and emergence characteristics or transformations thereof (1974-75)

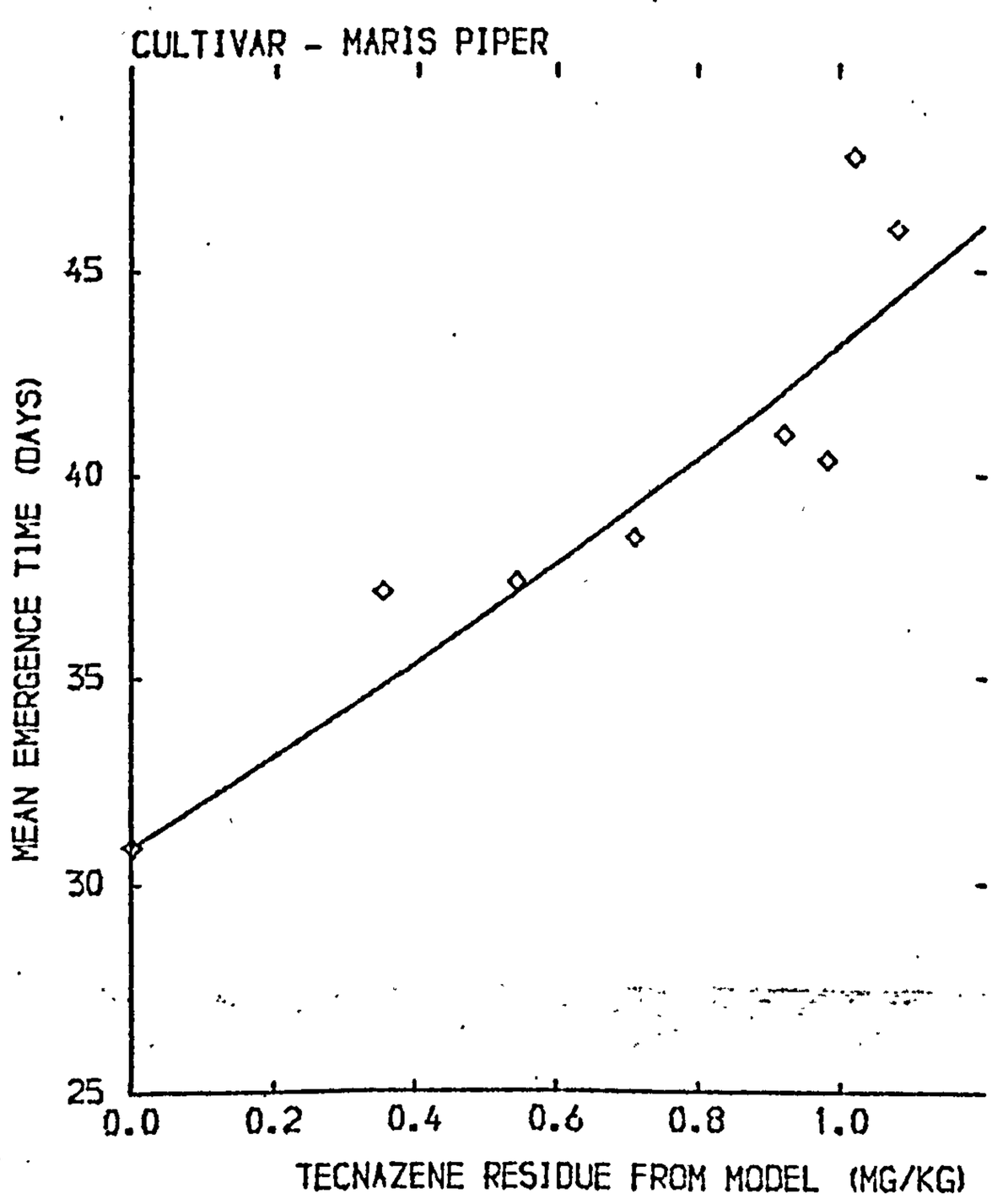
Emergence characteristic	Transformation	Cultivar	Intercept α	Slope β	Correlation coefficient
Mean emergence time (days)	$\log_{10}$***	M.Piper	1.49	0.145	0.81**
		P.Crown	1.49	0.088	0.69**
		Record	1.54	0.059	0.61**
		Redskin	1.51	0.091	0.69**
Time to 50% emergence (days)	$\log_{10}$***	M.Piper	1.47	0.175	0.73**
		P.Crown	1.47	0.125	0.71**
		Record	1.53	0.069	0.57**
		Redskin	1.50	0.105	0.69**
Total emergence (%)	none	M.Piper	102.1	-12.4	-0.51**
		P.Crown	96.2	-17.1	-0.49**
		Record	99.7	-3.9	-0.25
		Redskin	98.8	-3.7	-0.26

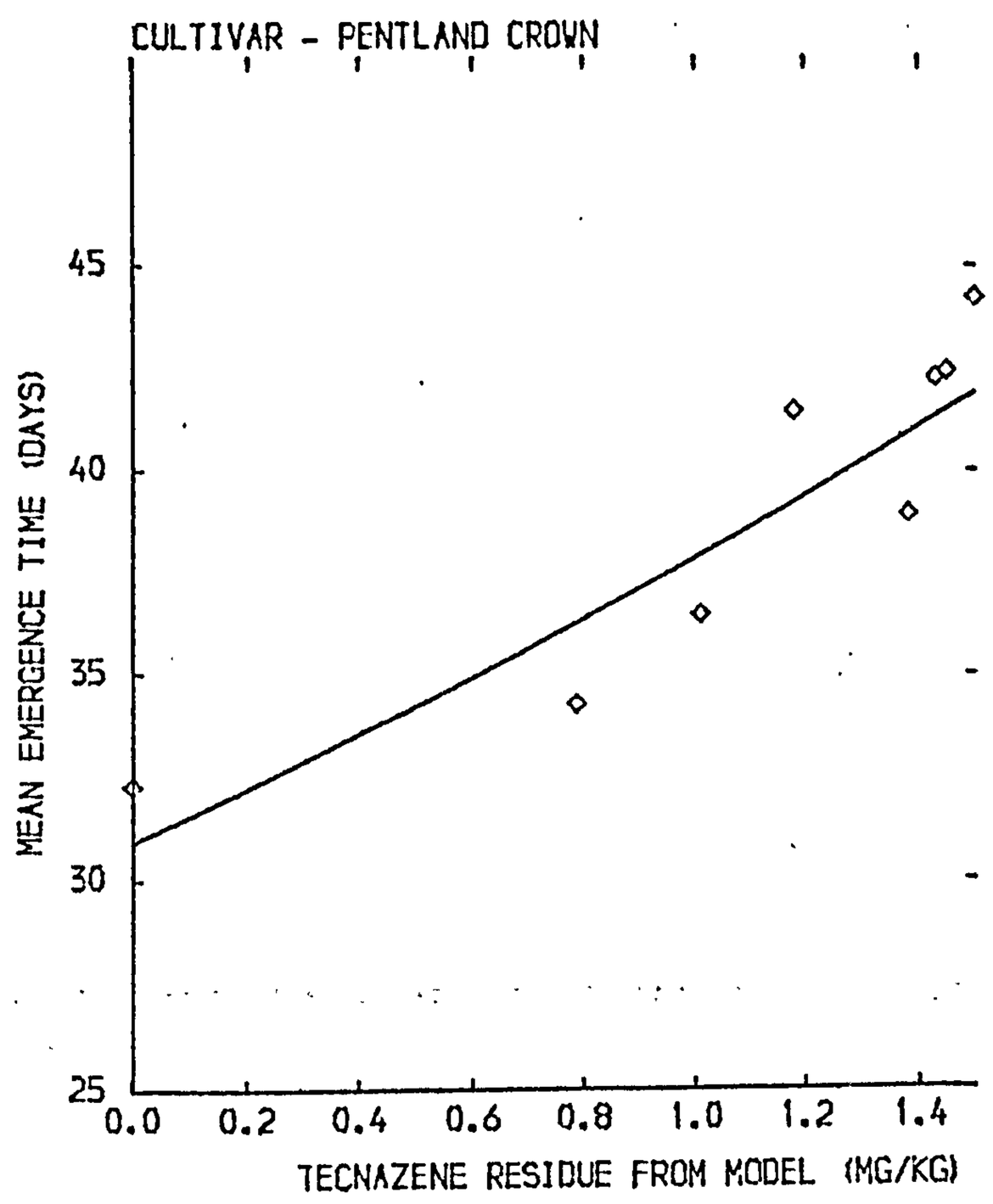
* Tecnazene residue in mg kg^{-1}

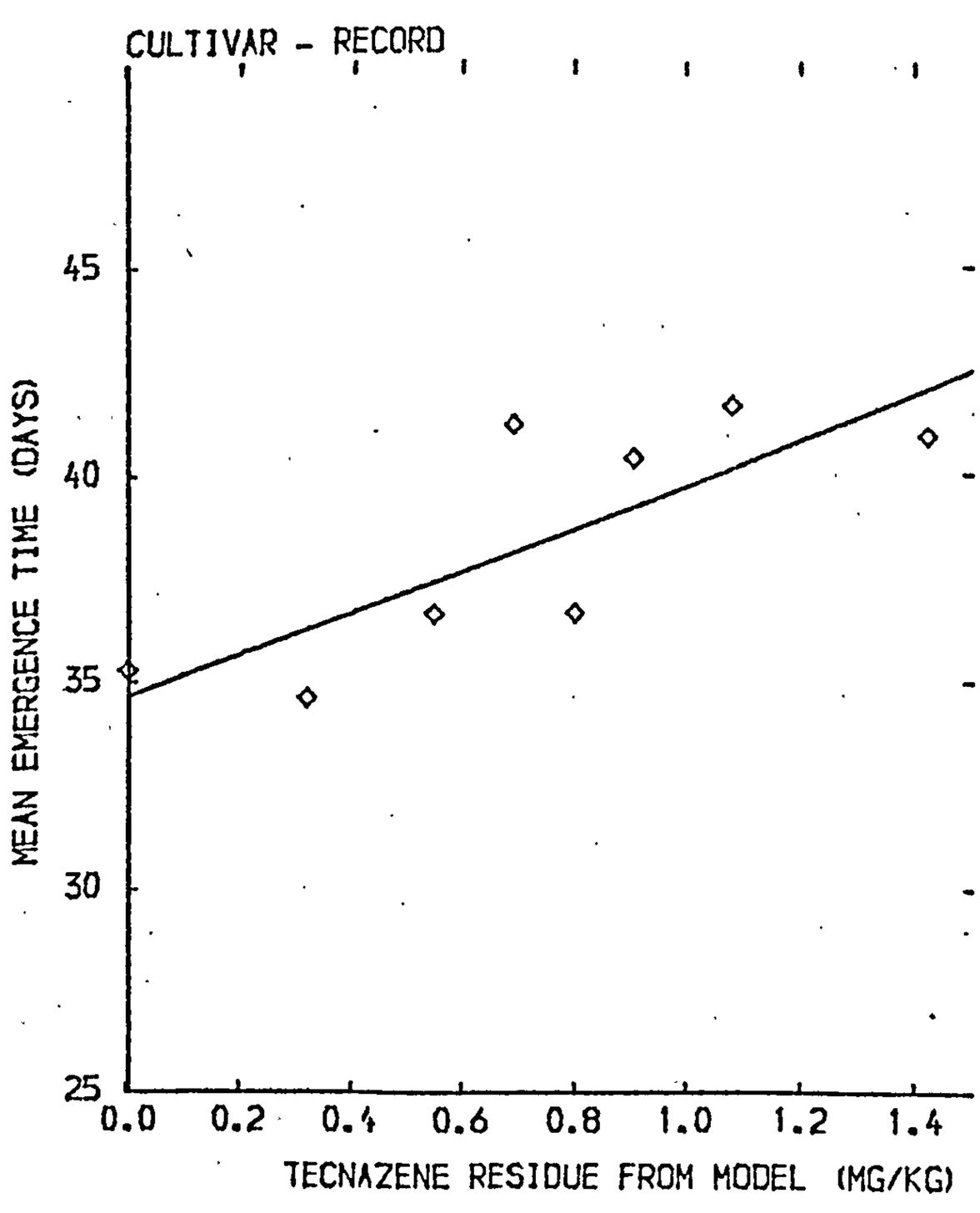
** The probability of obtaining a more extreme value of the correlation coefficient if the true value is zero is less than 0.01

*** Value of emergence characteristic at any point given by $y = 10^{(\alpha + \beta x)}$

FIGURE 5.3.15 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND MEAN EMERGENCE TIME







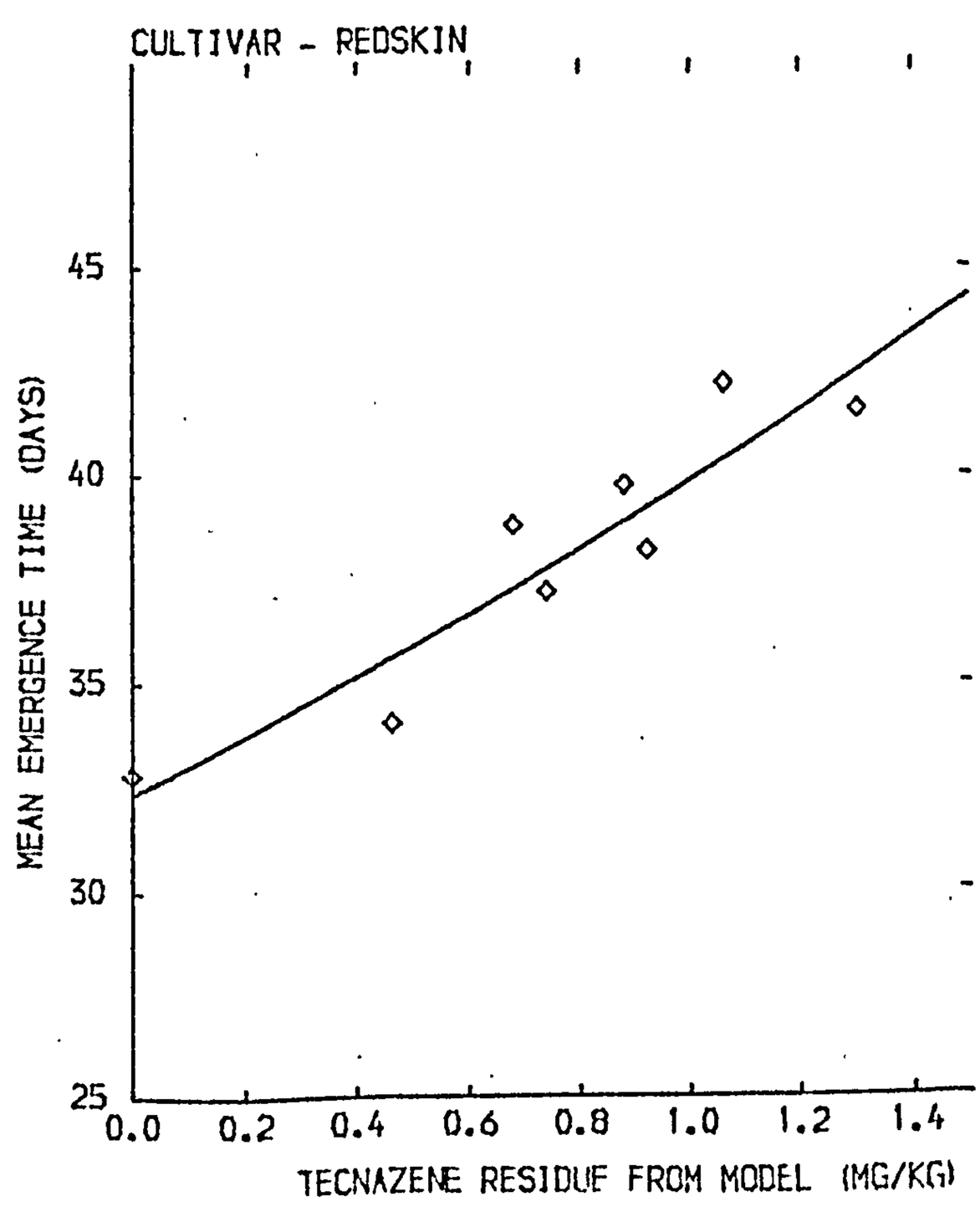


FIGURE 5.3.16 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND TIME TO 50% EMERGENCE

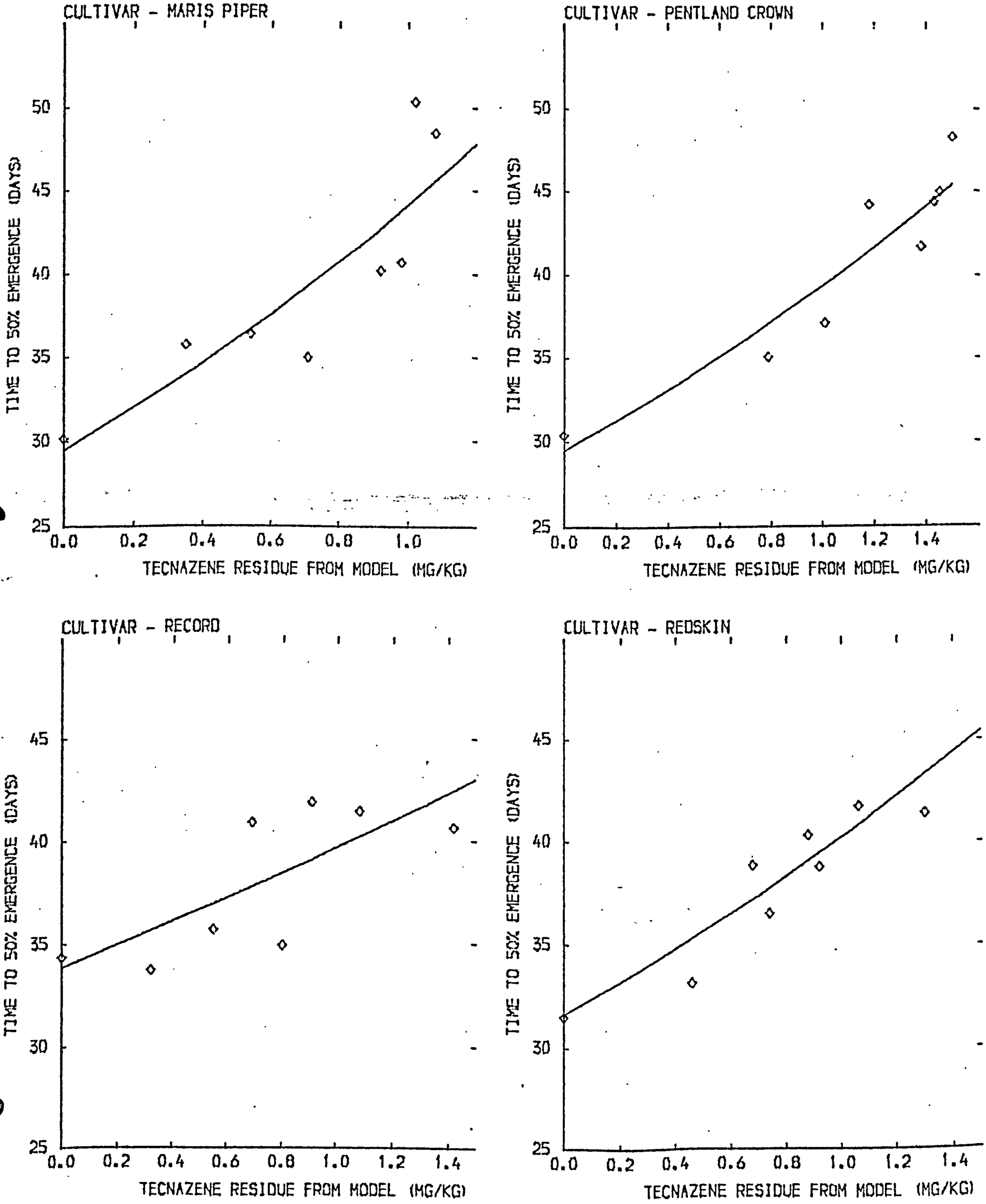
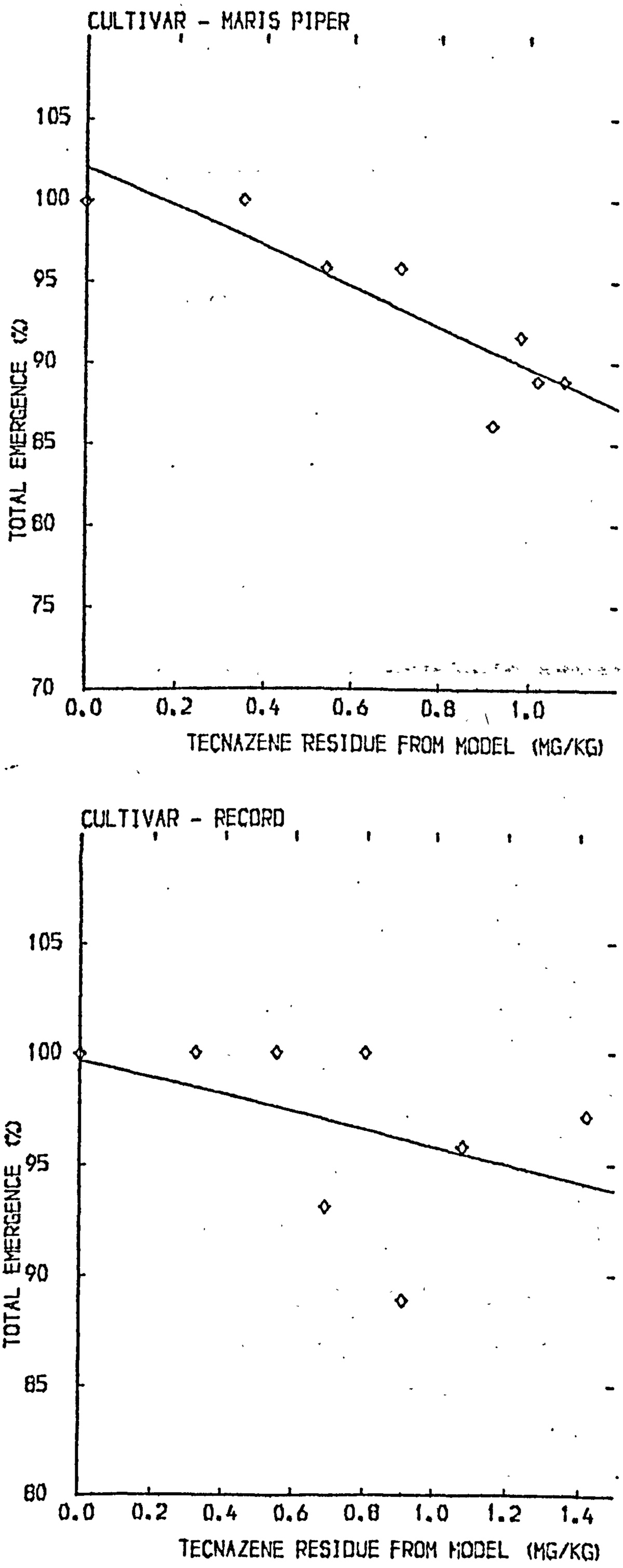
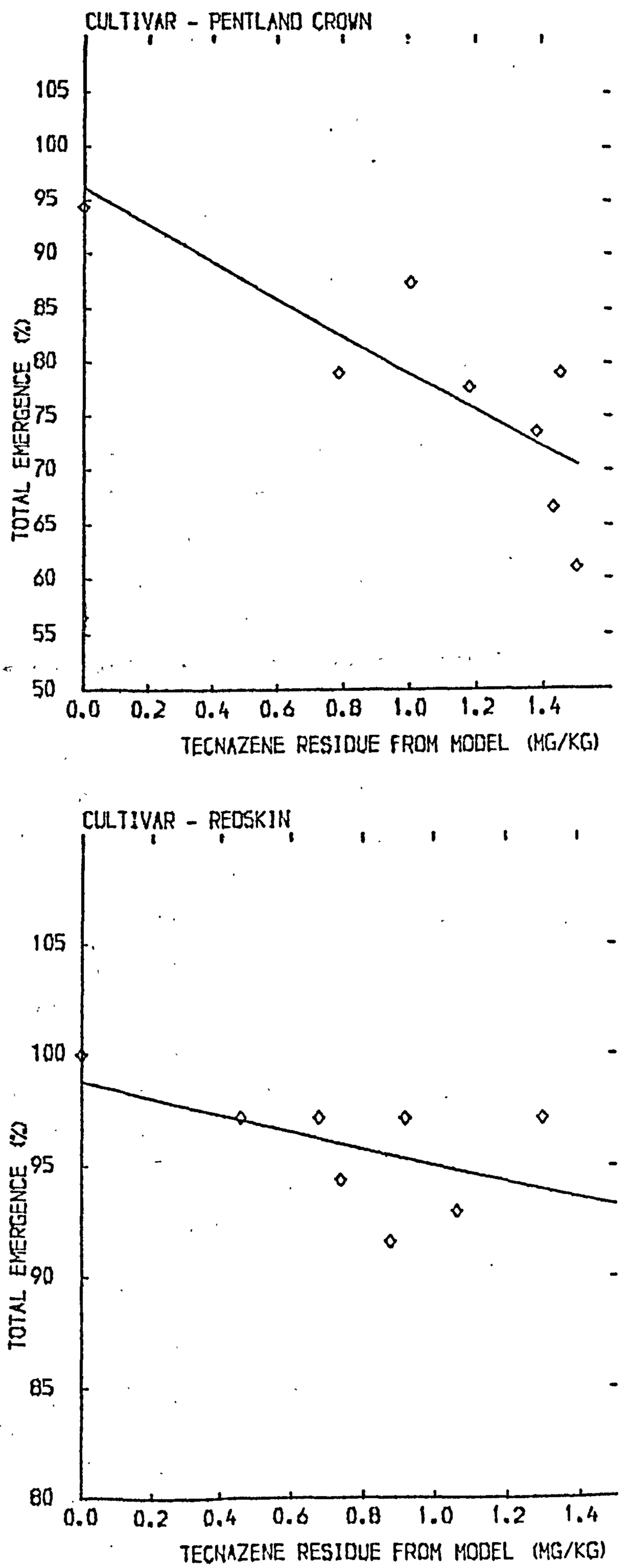


FIGURE 5.3.17 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND TOTAL EMERGENCE





Emergence and storage conditions

The relationship between MET, T50, TE and storage conditions are illustrated in figures 5.3.18., 5.3.19. and 5.3.20. respectively. Standard error bars indicate the variability between different plots.

To further quantify these relationships a regression search was carried out in the normal manner. However as the various factors have been estimated by only 4 observations at only 4 airing times for each temperature, and because of the high variability it has been decided to use simple linear models for all three emergence characteristics.

As the regression lines were obtained independently at the two storage temperatures, two values for the intercept were produced, but under the conditions of the experimental design these values must be equal and so the two values have been averaged and it is this average value which is reported. This procedure is not strictly correct in the statistical sense as a more complex model could have been constructed to cope with this complaint, but in practice it was found that the independent estimations were very close and rarely differed by more than 2% and thus estimations of the slope are still reasonably accurate and certainly adequate for the present purpose. The parameters thus determined are reported in table 5.3.8.

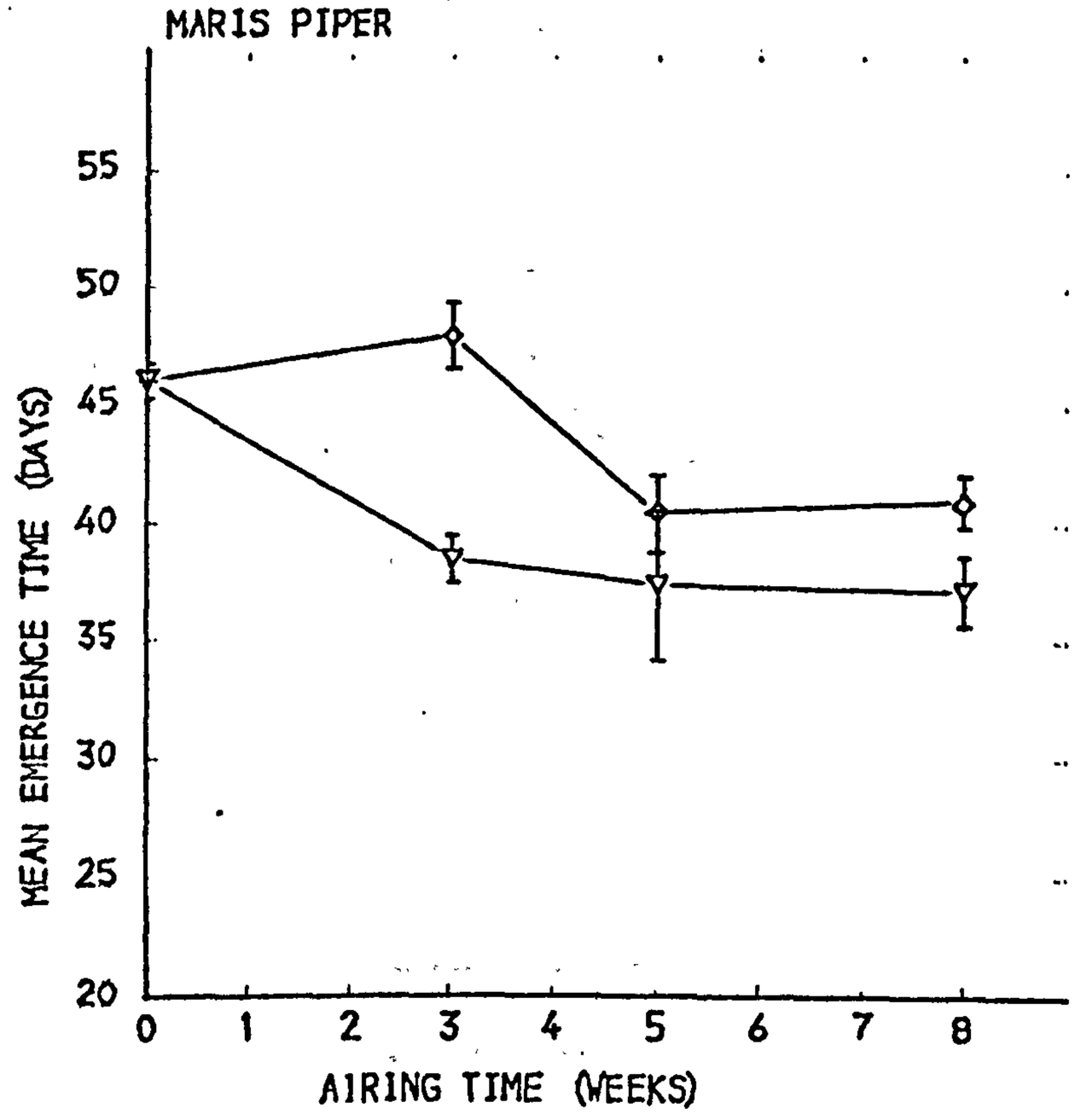
As might be expected from the discussion on tecnazene residues above, the correlation coefficients between airing time MET and T50 are fairly high at 12^{o}C but generally somewhat lower at 8^{o}C. The slope parameter indicates the effect on MET or T50 produced by increasing the airing period by 1 week. For all four cultivars airing at 12^{o}C produces faster emergence than airing at 8^{o}C. For example for the cultivar Pentland Crown, 8 weeks at 8^{o}C will reduce MET by approximately 5 days whereas airing for a similar period at 12^{o}C will reduce MET by approximately 10 days.

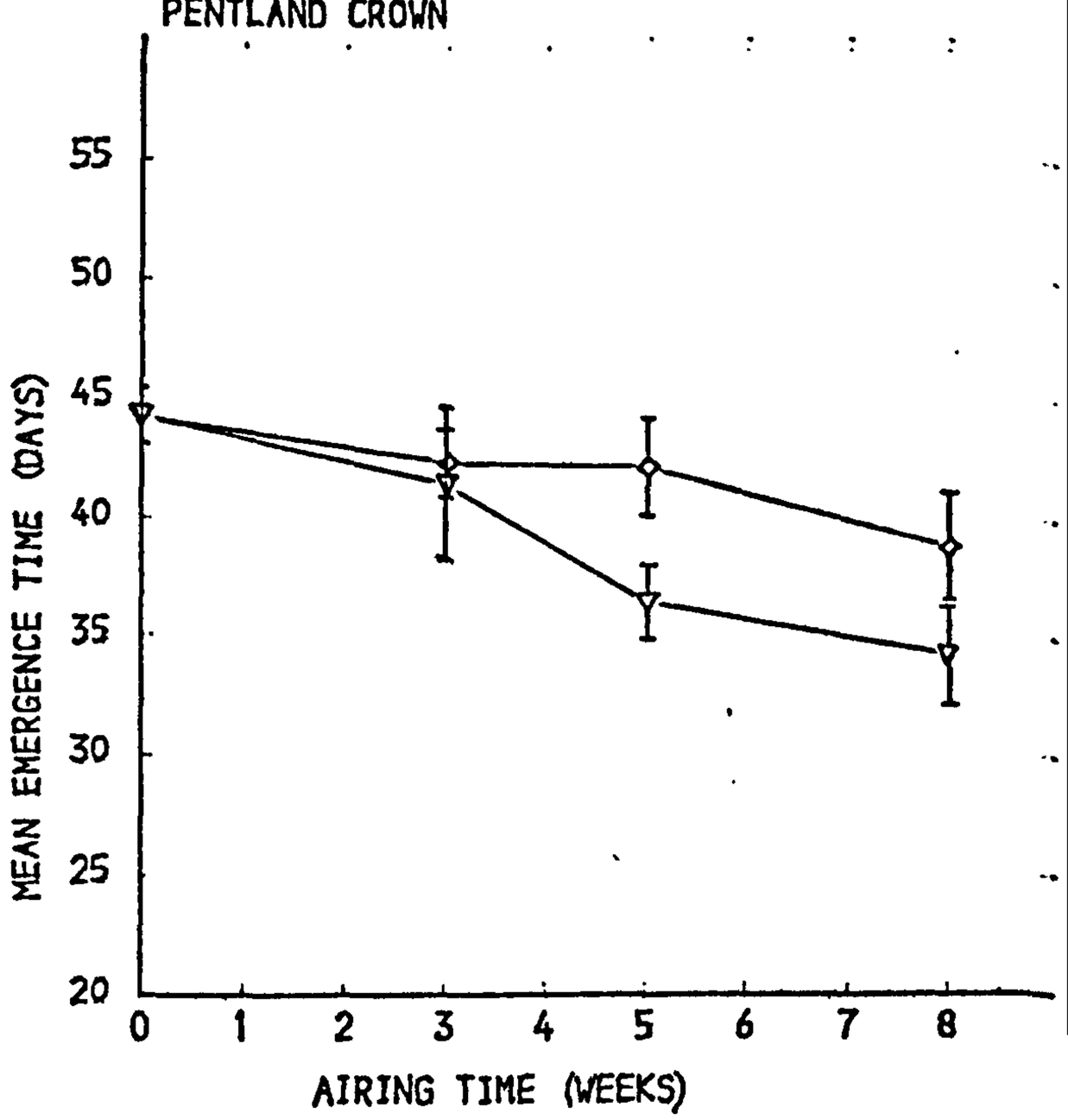
TE is improved in all cases by airing at 12^{o}C but airing at 8^{o}C produced no consistent or significant effect.

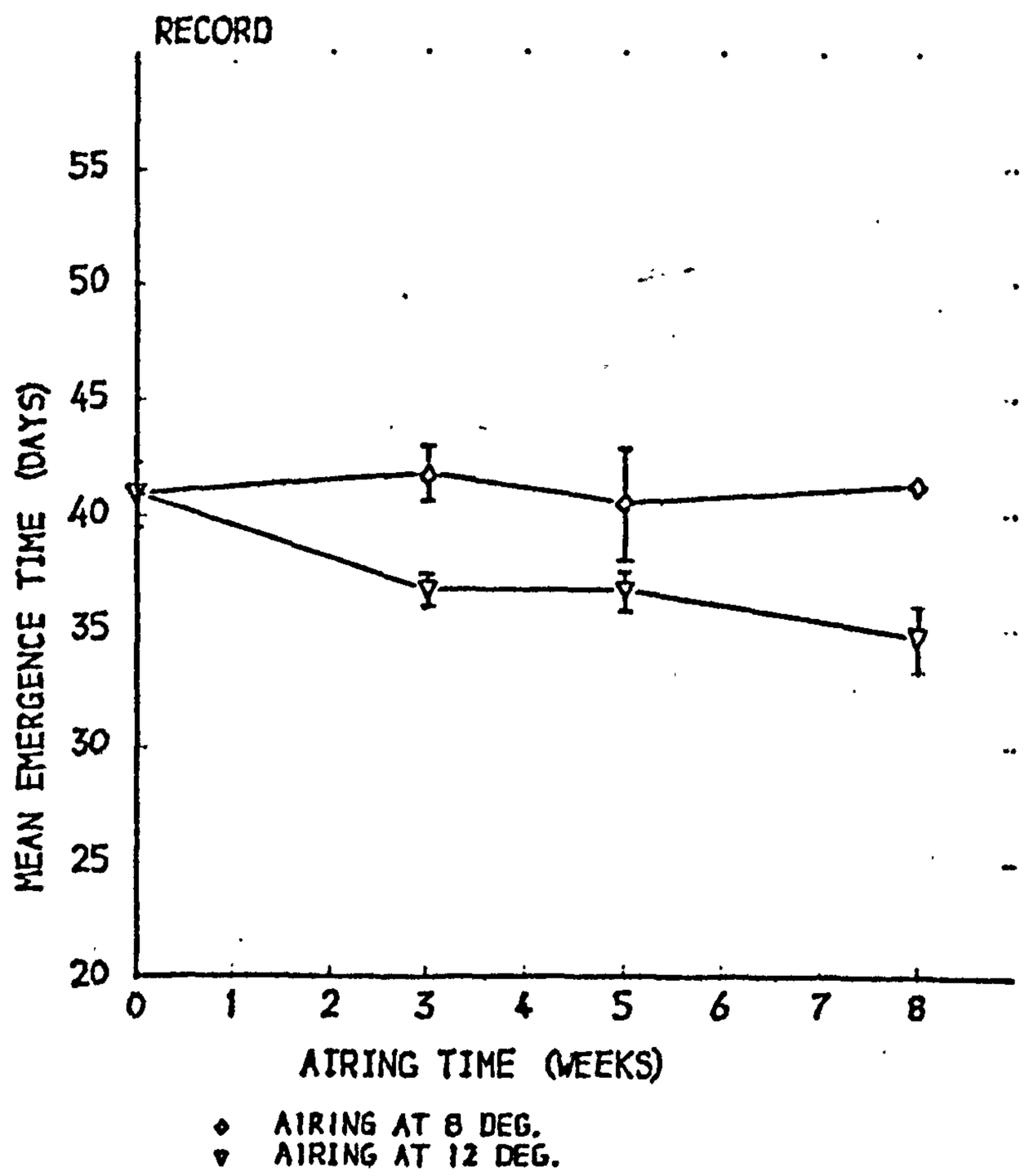
3) Yield and size distribution

From the recorded data the means of the seven yield and size distribution variables were calculated for each treatment and are reported in tables 5.3.9. (Maris Piper and Pentland Crown) and

FIGURE 5.3.18 THE EFFECT OF STORAGE CONDITIONS ON MEAN EMERGENCE TIME 1974-75







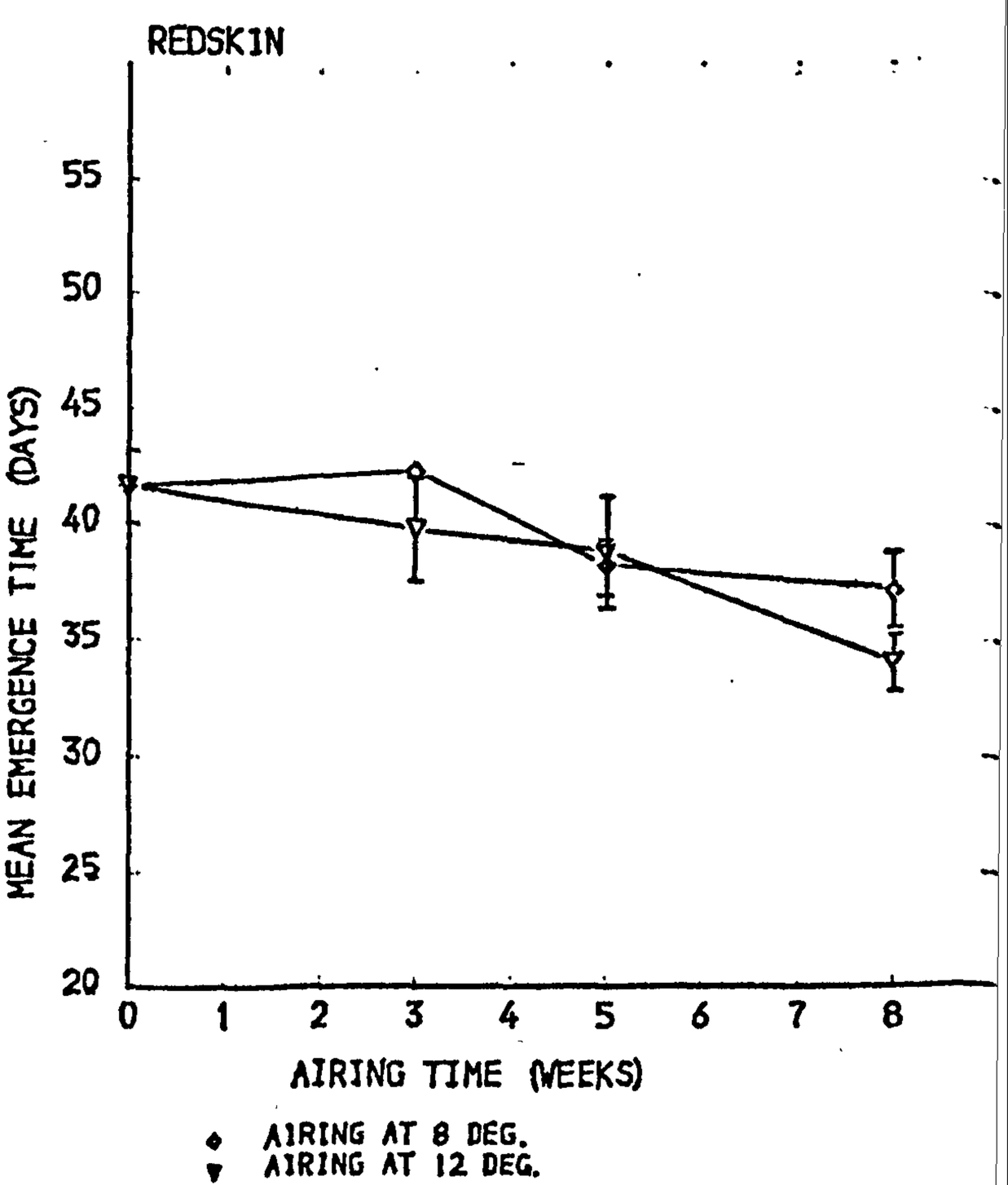


FIGURE 5.3.19 THE EFFECT OF STORAGE CONDITIONS ON TIME TO 50% EMERGENCE

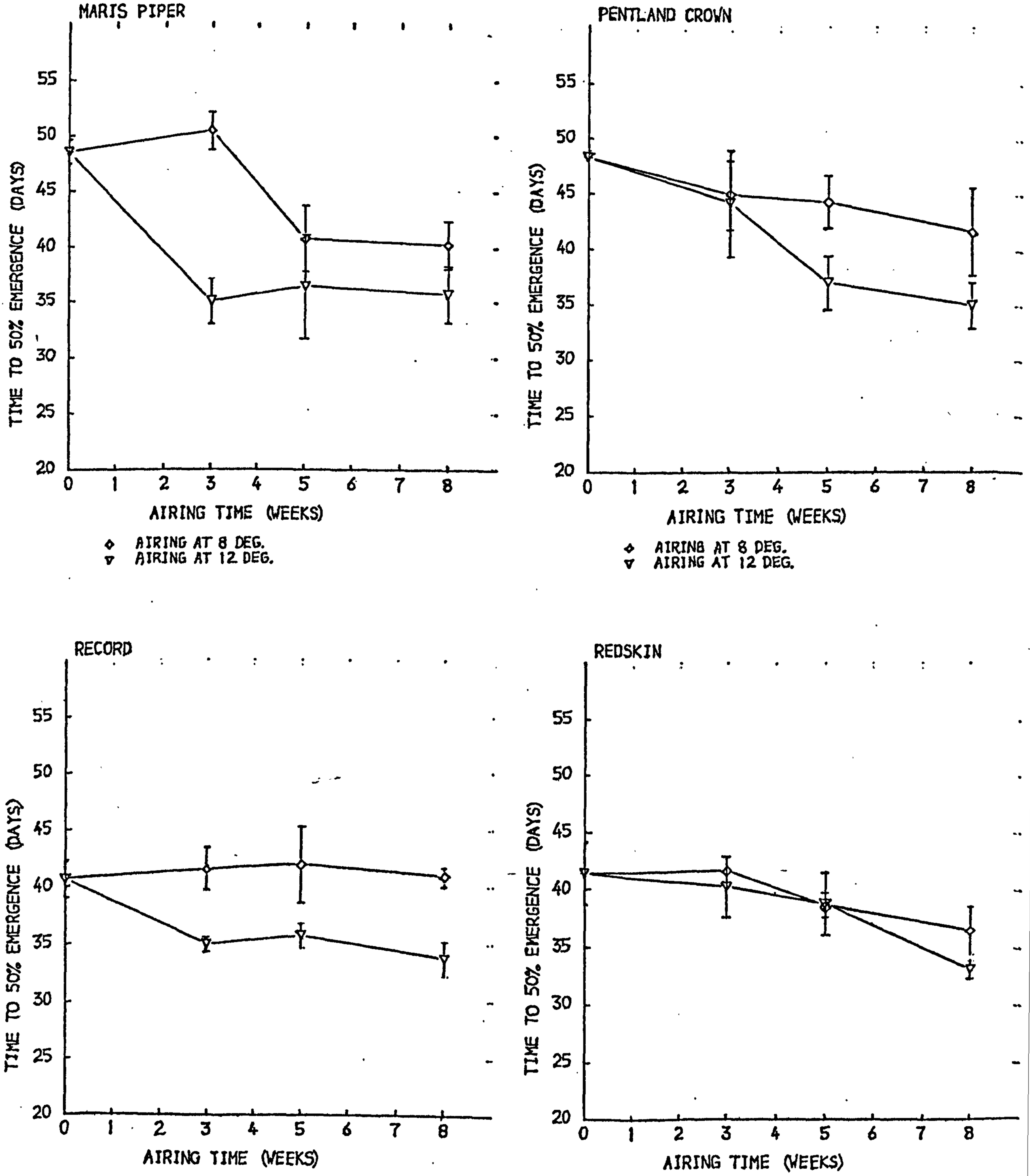
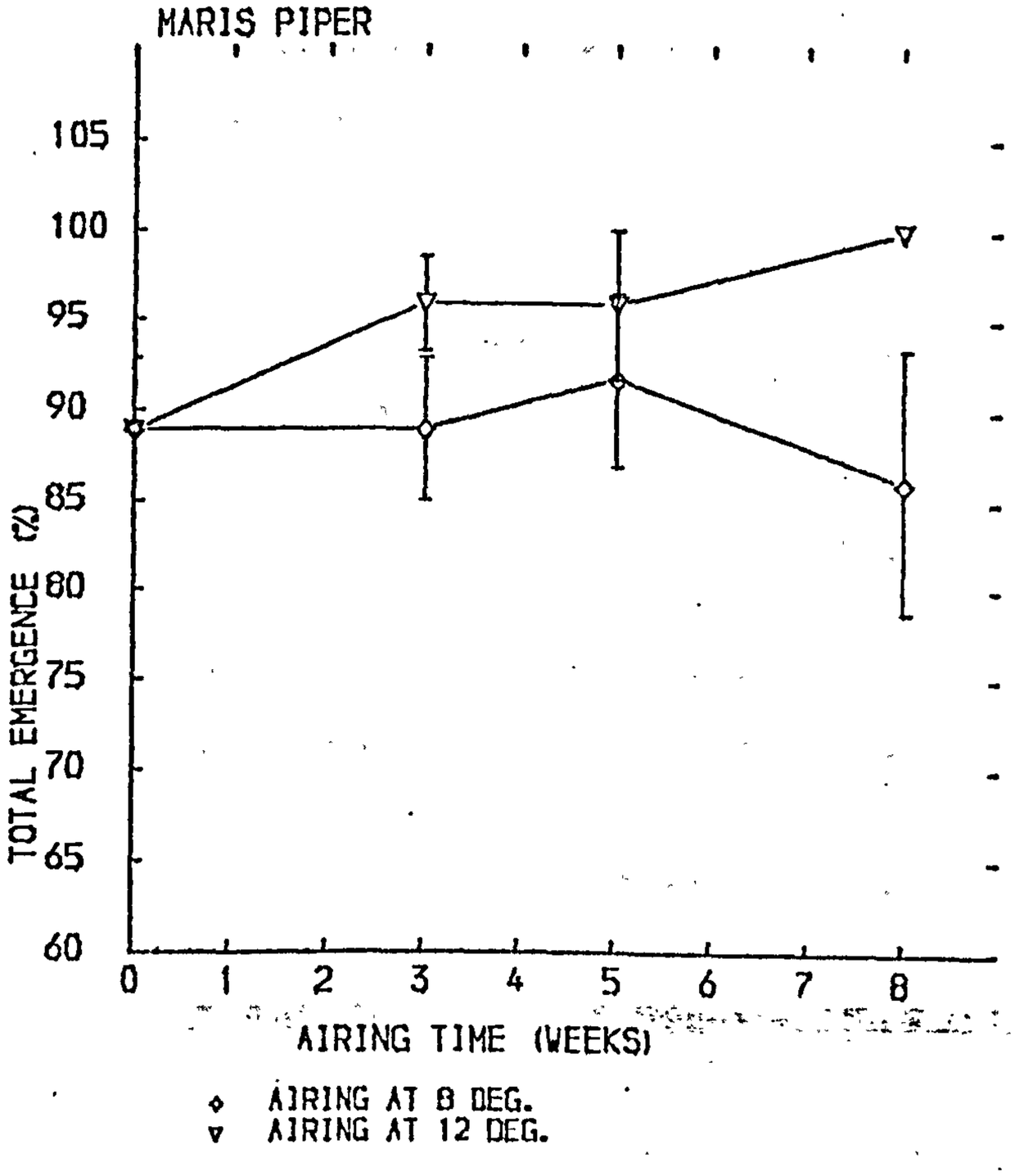
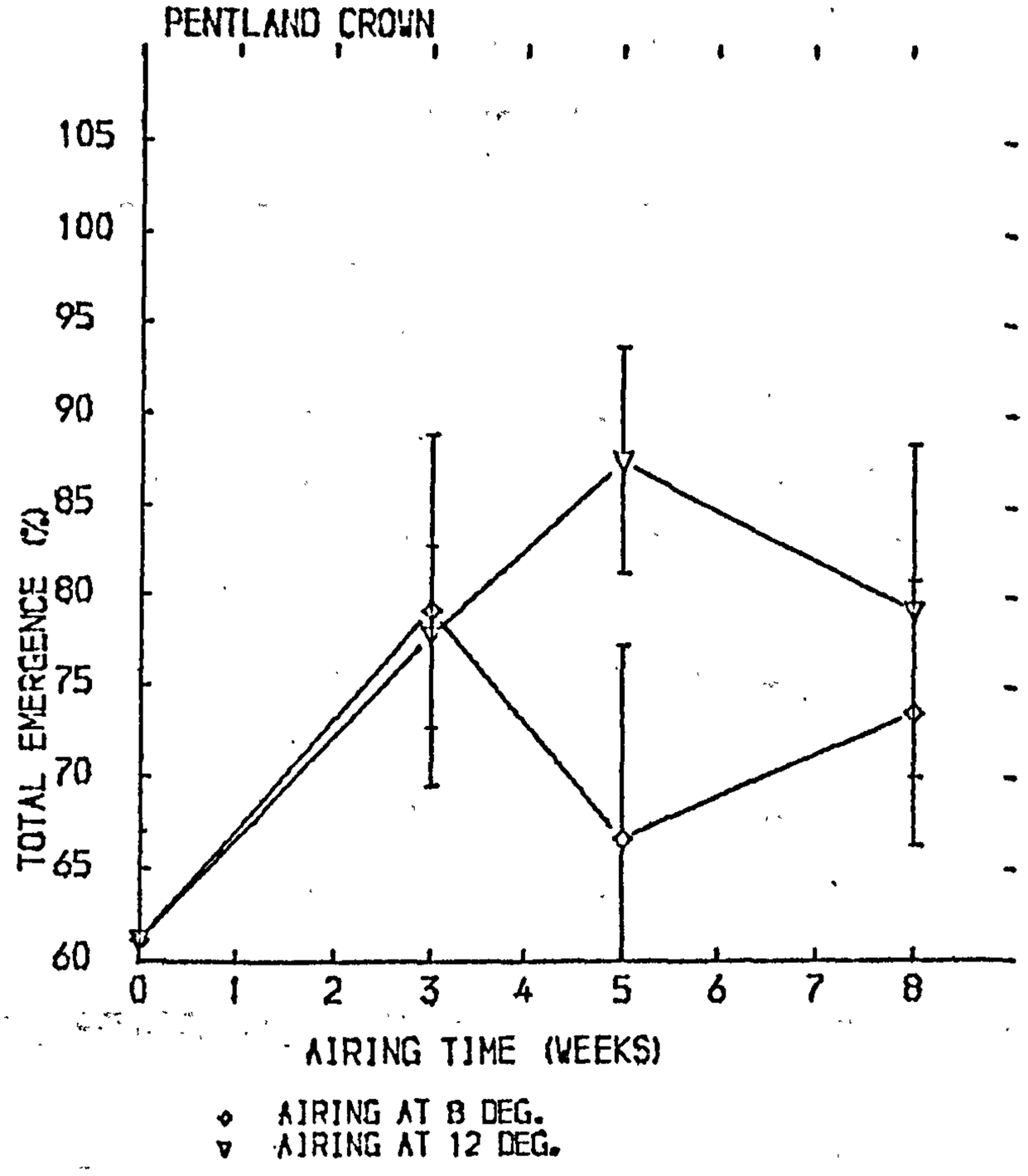
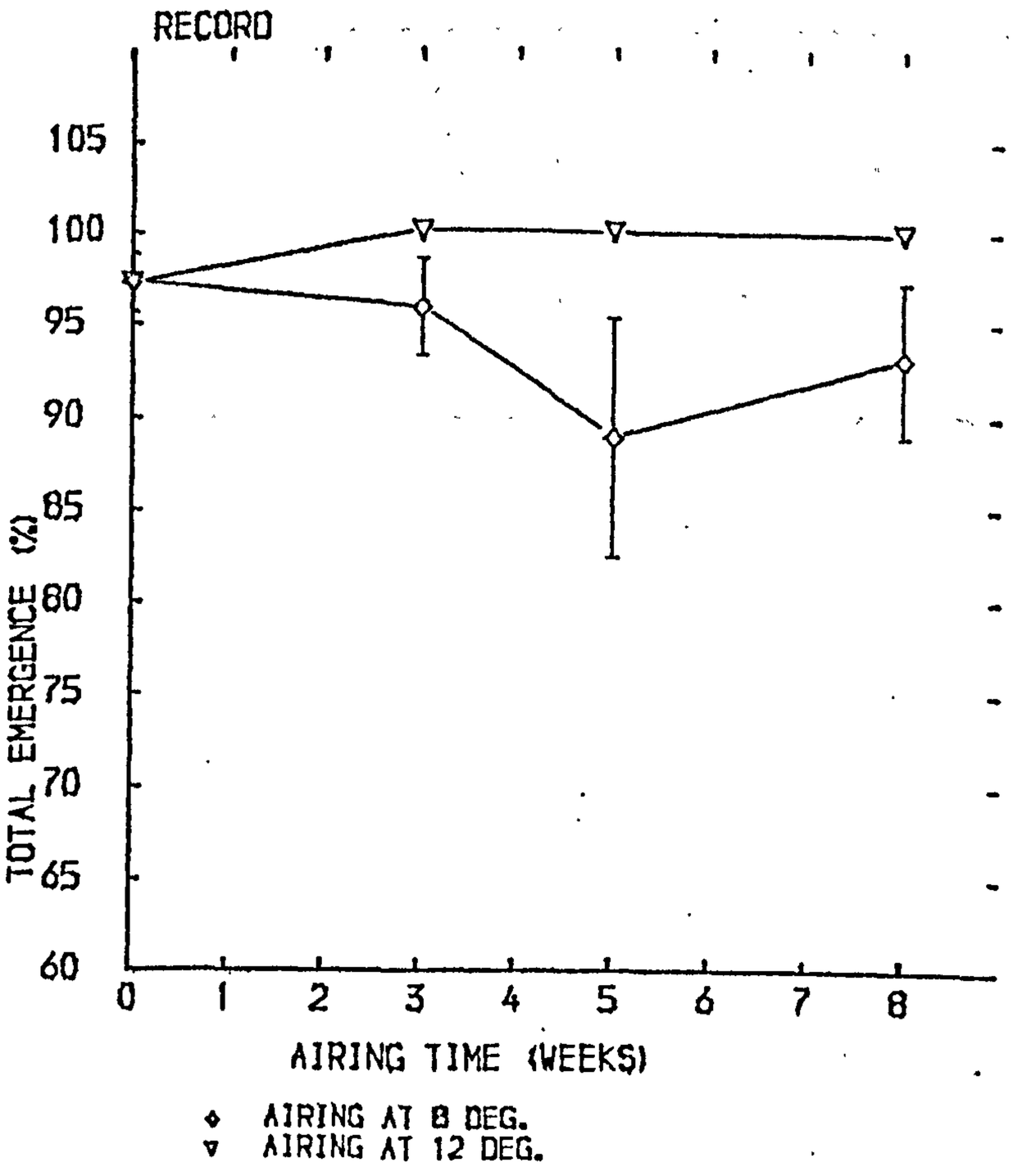


FIGURE 5.3.20 THE EFFECT OF STORAGE CONDITIONS ON TOTAL EMERGENCE







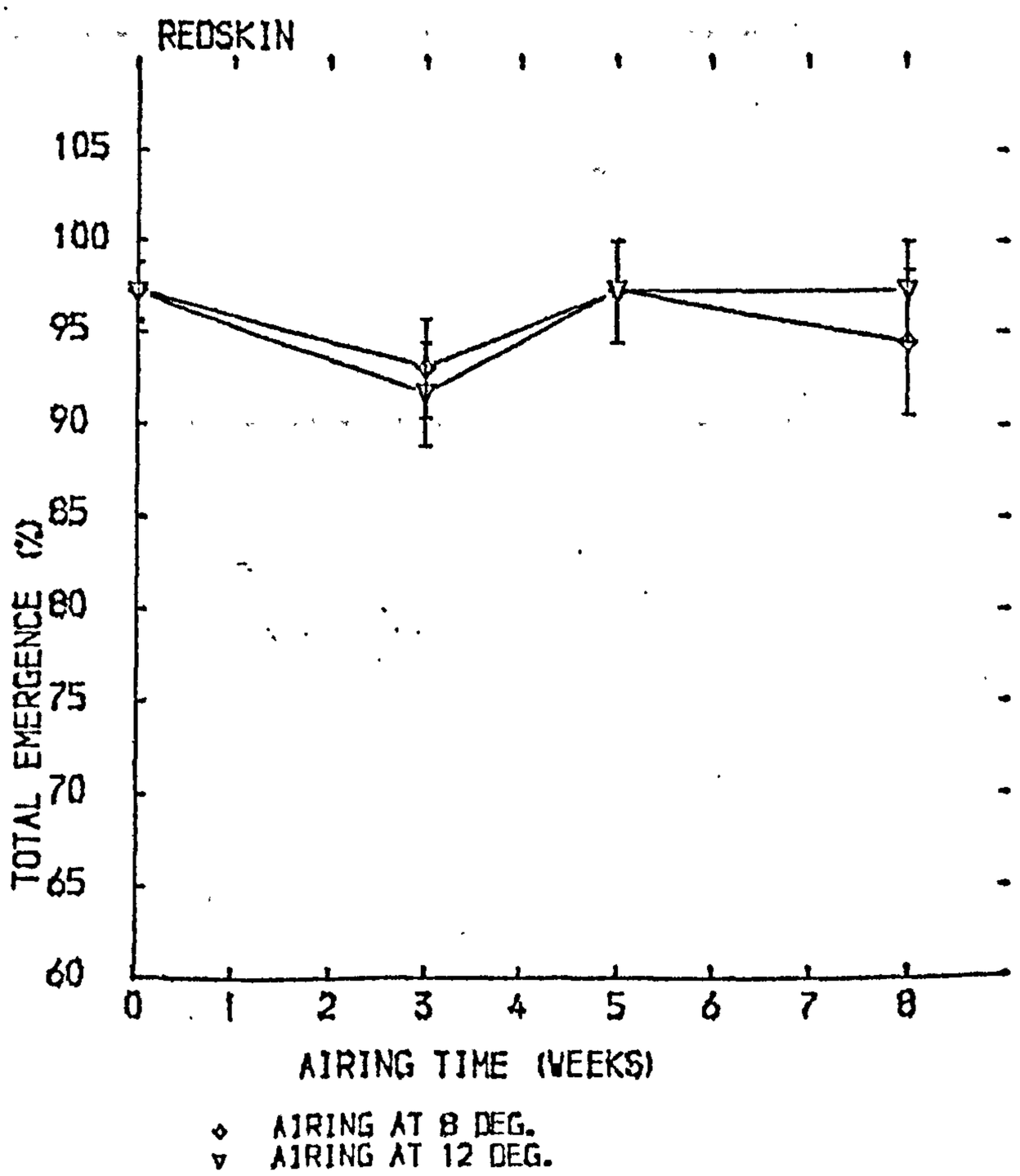


Table 5.3.8. Parameters of linear models of the form $y = \alpha + \beta x$, describing the relationship between airing time (at $8^{o}C$ and $12^{o}C$) and emergence characteristics (y) (1974-75)

Emergence characteristic	Cultivar	Intercept(α)	Slope(β)		Correlation coefficient	
			$8^{o}C$	$12^{o}C$	$8^{o}C$	$12^{o}C$
Mean emergence time (days)	M.Piper	45.6	-0.81	-1.08	-0.61**	-0.64**
	P.Crown	44.5	-0.63	-1.32	-0.50*	-0.71**
	Record	40.7	-0.00	-0.74	-0.00	-0.71**
	Redskin	42.3	-0.63	-0.91	-0.59**	-0.61**
Time to 50% emergence (days)	M.Piper	47.3	-1.25	-1.46	-0.63**	-0.57**
	P.Crown	48.2	-0.79	-1.77	-0.43*	-0.70**
	Record	40.3	0.04	-0.79	0.03	-0.67**
	Redskin	43.9	-0.65	-1.01	-0.49*	-0.57**
% Total emergence	M.Piper	89.9	-0.24	1.31	-0.08	0.56**
	P.Crown	66.2	1.10	2.41	0.20	0.46*
	Record	98.0	-0.69	0.32	-0.26	0.52*
	Redskin	95.7	-0.20	0.18	0.11	0.10

* The probability of getting a more extreme value of the correlation coefficient, if the true correlation coefficient is zero, is less than 0.05

** As above but $P < 0.01$

Table 5.3.9 The effect of storage conditions on the subsequent yield and size distribution of tecnazene treated seed potatoes (1974-75) (Cultivars - Maris Piper and Pentland Crown)

Storage conditions		Yield per plot [a]				Size distribution [b]		
Time [c] (weeks)	Temp. (°C)	Large [d] (kg)	Medium [e] (kg)	Small [f] (kg)	Total (kg)	Large (%)	Medium (%)	Small (%)
1. Maris Piper								
untreated	control	15.4	5.4	0.2	21.0	73.3	25.7	1.0
8	8	10.7	7.6	0.6	19.1	56.0	39.8	3.1
5	8	10.2	8.6	0.8	19.6	52.0	43.9	4.1
3	8	8.3	7.6	0.5	16.5	50.3	46.1	3.0
0	8/12	7.9	6.5	0.6	14.9	53.0	43.6	4.0
8	12	11.8	8.7	0.6	21.1	55.9	41.2	2.8
5	12	10.1	8.8	0.7	19.5	51.8	45.1	3.6
3	12	12.6	6.7	0.5	19.8	63.6	33.8	2.5
3f [g]	8	6.0	9.1	0.8	15.9	37.7	57.2	5.0
2. Pentland Crown								
untreated	control	15.4	2.8	0.1	18.3	84.2	15.3	0.5
8	8	13.1	3.8	0.2	17.1	76.6	22.2	1.2
5	8	13.6	3.8	0.2	17.6	77.3	21.6	1.1
3	8	12.5	3.5	0.2	16.2	77.2	21.6	1.2
0	8/12	9.9	3.4	0.2	13.5	73.3	25.2	1.4
8	12	16.4	2.4	0.2	18.8	87.2	12.8	1.1
5	12	11.7	4.6	0.4	16.7	70.1	27.5	2.4
3	12	11.6	4.5	0.3	16.3	71.2	27.6	1.8
3f [g]	8	11.1	3.8	0.3	15.2	73.0	25.0	1.9

a. Mean of 4 replicates.

b. Expressed as a % of total yield.

c. Airing time prior to planting.

d. > 50mm.

e. 32-50mm..

f. < 32mm.

g. Forced ventilation.

Table 5.3.10 The effect of storage conditions on the subsequent yield and size distribution of tecnazene treated seed potatoes (1974-75) (Cultivars - Record and Redskin)

Storage conditions		Yield per plot[a]				Size distribution[b]		
Time[c] (weeks)	Temp. (^{o}C)	Large[d] (kg)	Medium[e] (kg)	Small[f] (kg)	Total (kg)	Large (%)	Medium (%)	Small (%)
1. Record								
untreated	control	14.0	5.0	0.3	19.3	72.5	25.9	1.6
8	8	8.1	8.5	0.6	17.1	47.4	49.7	3.5
5	8	7.9	7.3	0.6	15.8	50.0	46.2	3.8
3	8	7.1	8.9	0.5	16.5	43.0	53.9	3.0
0	8/12	6.5	8.2	0.7	15.3	42.5	53.6	4.6
8	12	12.5	6.9	0.4	19.8	63.1	34.8	2.0
5	12	9.9	7.1	0.5	17.4	56.9	40.8	2.9
3	12	8.7	8.0	0.7	17.4	50.0	46.0	4.0
3f[g]	8	8.2	7.7	0.4	16.3	50.3	47.2	2.5
2. Redskin								
untreated	control	17.4	4.6	0.4	22.3	78.0	20.6	1.8
8	8	14.3	6.2	0.5	20.9	68.4	29.7	2.4
5	8	11.9	6.4	0.5	18.7	63.6	34.2	2.7
3	8	9.9	6.4	0.4	16.7	59.3	38.3	2.4
0	8/12	10.3	5.8	0.4	16.5	62.4	35.2	2.4
8	12	15.7	6.0	0.4	22.2	70.7	27.0	1.8
5	12	11.6	7.5	0.6	19.7	58.9	38.1	3.0
3	12	13.4	6.8	0.4	20.6	65.0	33.0	1.9
3f[g]	8	10.6	6.2	0.5	17.3	61.3	35.8	2.9

a. Mean of 4 replicates.

b. Expressed as a % of total yield.

c. Airing time prior to planting.

d. > 50mm.

e. 32 - 50mm.

f. < 32mm.

g. Forced ventilation.

5.3.10. (Record and Redskin) with their associated storage conditions.

It should be noted that a certain amount of damage was caused to the foliage of some drills by a tractor which accidently sprayed the experimental area whilst spraying the rest of the field against blight. This has increased the variability of the data. Removal of the affected plots proved impossible and more sophisticated statistical approaches were considered unnecessary as the various effects were still apparent, although correlation coefficients were adversely affected.

As with the emergence data an attempt has been made to correlate performance with both residues and storage conditions. The forced airing treatment has again been excluded from the statistical analysis.

Yield and tecnazene residues

To investigate the relationship between tecnazene residues at planting and the seven variables, a regression search was conducted amongst various transformations as previously described. To economise on space only those for the four yield variables are reported as the size distribution variables showed very similar patterns.

As previously described for emergence any transformations had to show a consistent improvement over at least three cultivars before it was adopted.

The transformations, resultant parameters and correlation coefficients are shown in table 5.3.11. for all four yield variables.

Using these parameters the functions have been plotted and are illustrated in figures 5.3.21. (yield of large tubers), 5.3.22. (yield of seed-sized tubers), 5.3.23. (yield of small tubers) and 5.3.24. (total yield). Treatment means are also indicated to give a visual indication of "goodness of fit".

Although simple linear plots have been used to describe the relationship between tecnazene residues and yield of large tubers there was a suggestion from the regression search that for Maris Piper and Record the true relationship might be slightly concave and that for Pentland Crown and Redskin, slightly convex.

This could perhaps be accounted for by the fact that the latter cultivars have a strong natural tendency, to produce a high

Table 5.3.11. Parameters of linear models of the form $y = \alpha + \beta x$ describing the relationship between tecnazene residues* or transformations thereof (x) and yield characteristics or transformations thereof (y) (1974-75)

Yield characteristic	Transformations		Cultivar	Intercept	Slope	Correlation
	Yield	Residue		α	β	coefficient
Yield of large tubers (kg/plot)	none	none	M.Piper	14.7	-5.45	-0.47***
			P.Crown	16.0	-2.71	-0.30**
			Record	13.5	-5.79	-0.58***
			Redskin	17.6	-6.00	-0.43***
i.e. Yield of large tubers = $\alpha + \beta x$						
Yield of seed-sized tubers (kg/plot)	$\log_{10}$	$\log_{10}$	M.Piper	0.881	0.040	0.42***
			P.Crown	0.538	0.036	0.29**
			Record	0.886	0.051	0.52***
			Redskin	0.802	0.036	0.49***
i.e. Yield of seed-sized tubers = $10^{(\alpha + \beta \log x)}$						
Yield of small tubers (kg/plot)	$\log_{10}$	$\log_{10}$	M.Piper	-0.207	0.143	0.71***
			P.Crown	-0.676	0.082	0.46***
			Record	-0.286	0.069	0.52***
			Redskin	-0.374	0.008	0.06
i.e. Yield of small tubers = $10^{(\alpha + \beta \log x)}$						
Total yield (kg/plot)	none	squared	M.Piper	21.4	-4.00	-0.35**
			P.Crown	18.7	-1.35	-0.24
			Record	18.8	-2.14	-0.26
			Redskin	22.5	-3.88	-0.38**
i.e. Total yield = $\alpha + \beta x^2$						

* Tecnazene residues in mg kg^{-1}

** The probability of obtaining a more extreme value of the correlation coefficient, if the true value is zero, is less than 0.05

*** As above but $P < 0.01$

TEXT BOUND INTO THE SPINE

FIGURE 5.3.22 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND THE YIELD OF SEED-SIZED TUBERS

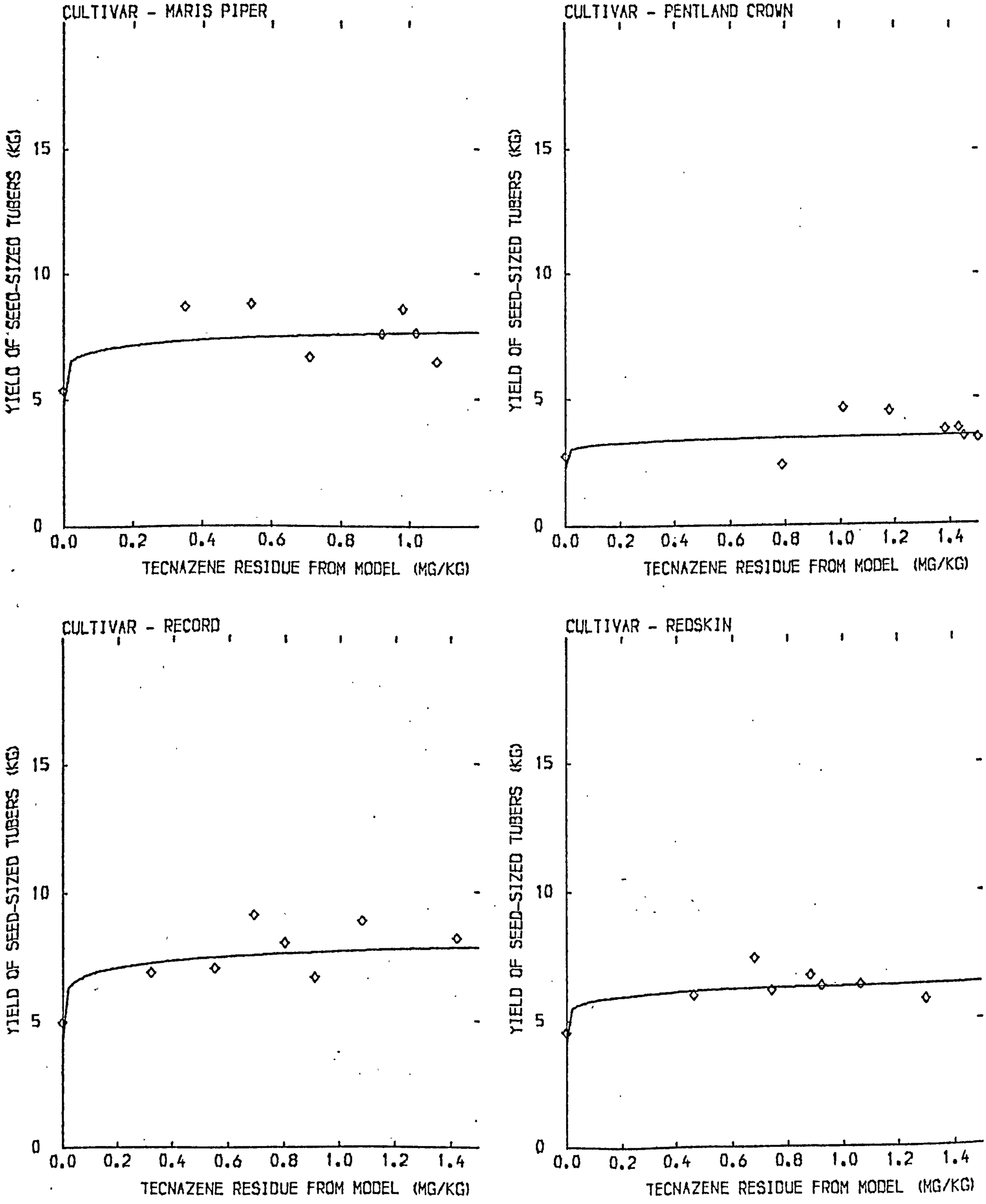


FIGURE 5.3.23 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND THE YIELD OF SMALL TUBERS

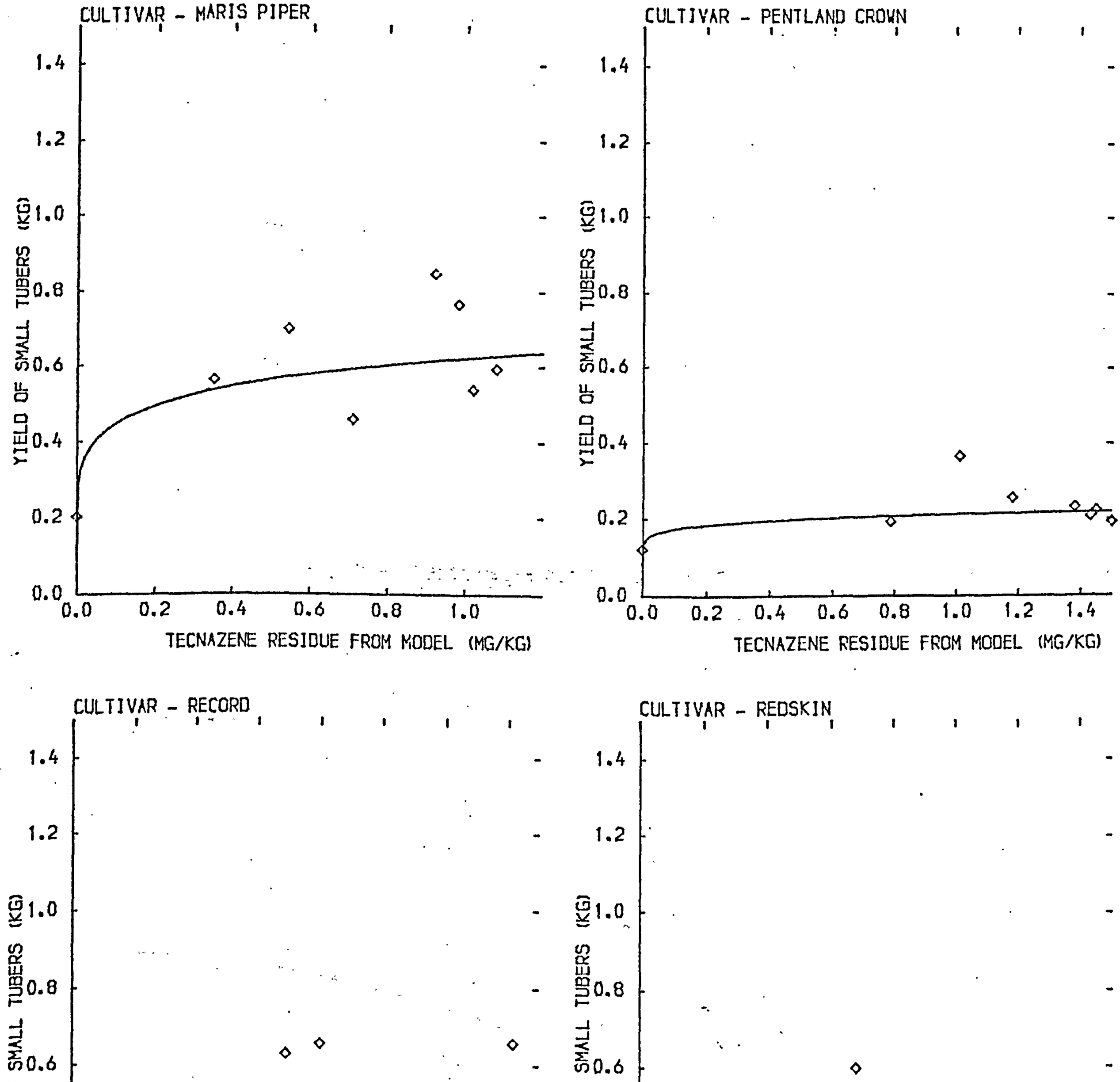
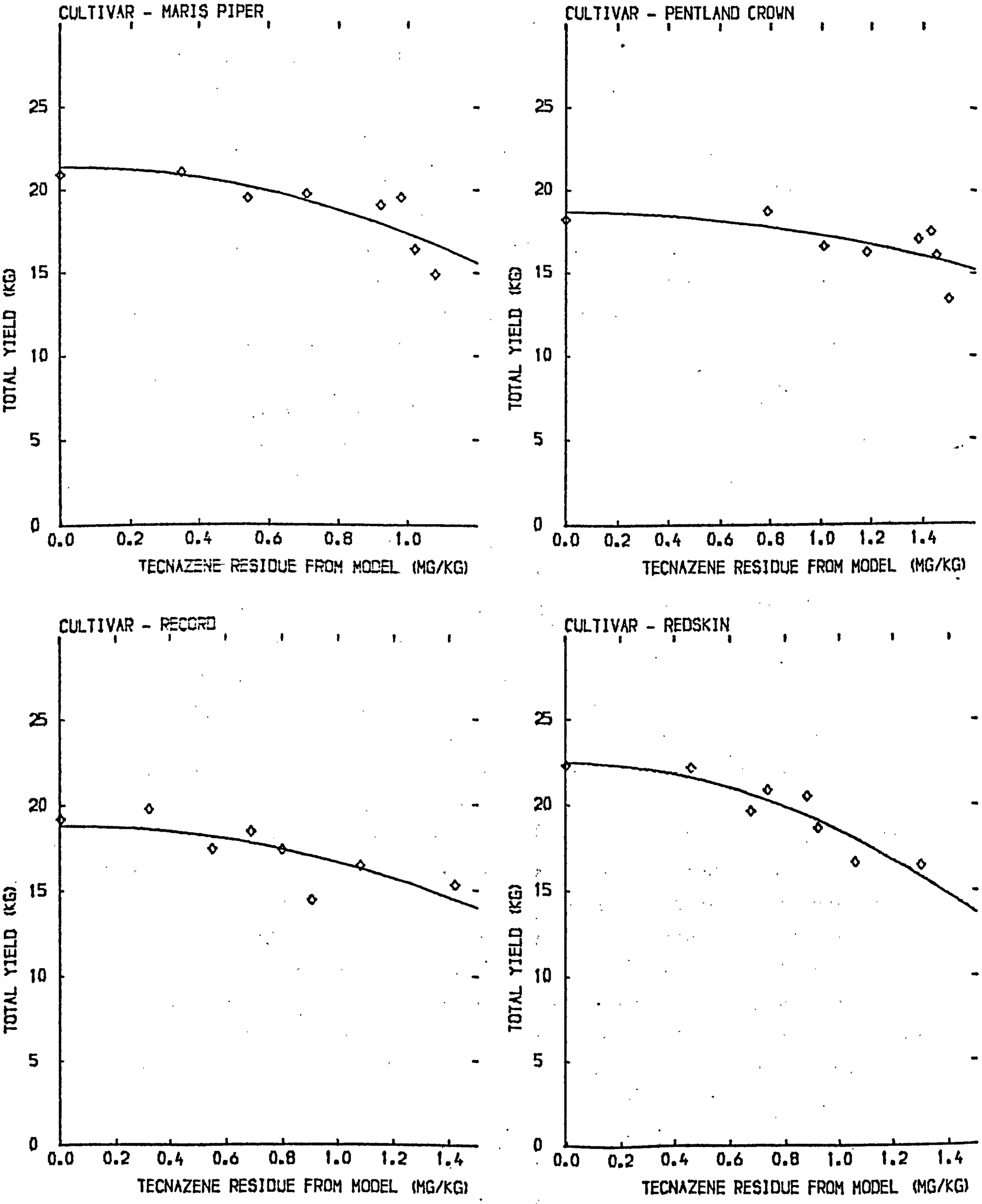


FIGURE 5.3.24 THE RELATIONSHIP BETWEEN TECNAZENE RESIDUES AT PLANTING AND TOTAL YIELD



proportion of large tubers, which is not overcome by low levels of tecnazene residues.

The magnitude of the effect on total yield is surprisingly large as can be seen from the value of the slopes which indicate the reduction in yield associated with a residue of 1mg kg^{-1}.

For yield of seed-sized tubers the log V log relationship produced much improved correlation coefficients. The reason for this is that the yields of such tubers from the untreated controls (residue of 0.0mg kg^{-1}) are substantially below that for even the treated samples with the lowest residue levels. The magnitude of the changes associated with increasing residue levels still further is relatively small although significant. These observations suggest that tecnazene could in fact be used to maximise seed production. In this respect it might be worth investigating various application rates and application times so that seed production could be optimised with the minimum effect on total yield. In situations where the maximum number of tubers is desired regardless of total yield such as in the multiplication of virus-tested stem cutting material tecnazene could be recommended on the basis of the current study.

Similar effects were also found with the yield of very small tubers. The possible influence of this finding on the problem of ground-keepers will be discussed later. It should be noted that these very small tubers, although normally discarded in ware and commercial seed crops are of value in multiplication work.

In the case of total yield the best transformation was found to involve squaring the tecnazene residue i.e. total yield is reduced much more dramatically by large than small residue levels.

Although it is obvious from the information above that different cultivars respond by different amounts to given levels of tecnazene residues, similar models can be constructed to predict the performance of an "average cultivar". In an attempt to summarise the information from this experiment this has been done and the relative performance predicted for such an "average cultivar" is shown in table 5.3.12. for various levels of tecnazene residues.

From this table it can be seen that increasing tecnazene residues are associated with delayed emergence, reduced total emergence,

Table 5.3.12. Predicted performance of seed potatoes* with various levels of tecnazene residues estimated in washed tubers at planting (Based on statistical models covering all cultivars in the 1974-75 experiment)

Performance factor	Tecnazene residue (mg kg^{-1})						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Delay in emergence (days) (MET)	0	2	4	6	8	10	13
% Emergence	100	98	95	93	91	88	86
Relative yield of large tubers	100	92	84	76	68	60	52
Relative yield of seed-sized tubers	100**	127	131	133	134	136	137
Relative yield of small tubers	100**	148	156	161	165	167	170
Relative total yield	100	99	97	92	86	78	69
Relative revenue*** for seed crop	100	108	106	100	99	95	91

* neglecting cultivar effects.

** levels at zero residues based on observed values as when residue level approaches zero, log residue approaches minus infinity. In practise the model works well if assume 0.0 = 0.0001mg kg^{-1}

*** based on a seed to ware price ratio of 3:1 (see text)

reduced yield of large tubers, increased yield of small and seed-sized tubers and reduced total yield. Of special interest to Scottish growers, however, is the observation that revenue from a seed crop would actually increase at low tecnazene residue levels and then decrease at the higher levels. It should be borne in mind however that this experiment was conducted at a ware spacing and was not defoliated until September so the figures should be treated with some caution. They are included only to indicate that the effects discussed above may in fact benefit the grower financially.

Yield and storage conditions

The effect of storage conditions on the four yield variables are shown in figures 5.3.25. (yield of large tubers), 5.3.26. (yield of seed-sized tubers), 5.3.27. (yield of small tubers) and 5.3.28.(total yield). Standard error bars have been included to indicate the variability between plots.

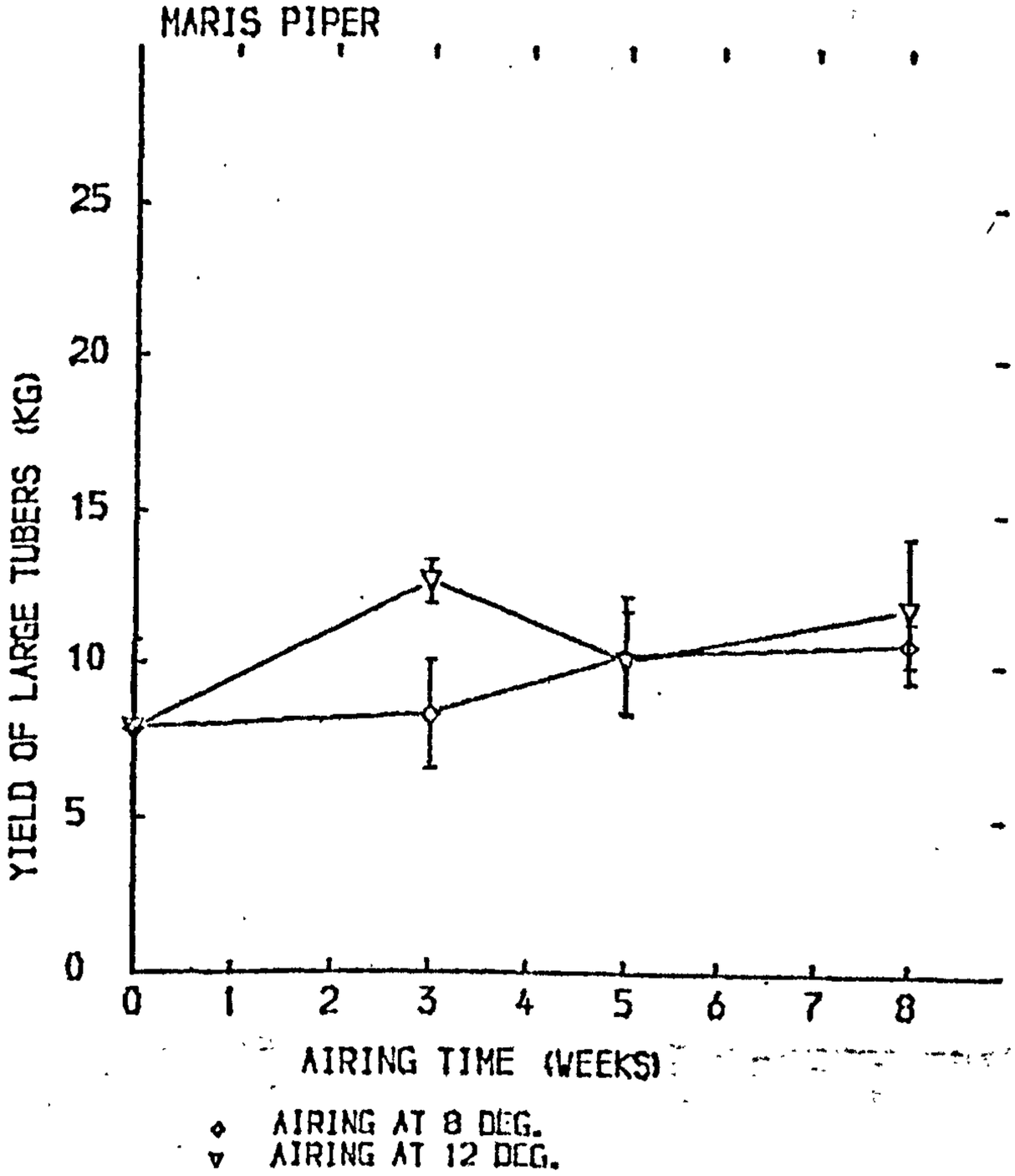
These relationships were further investigated using a regression search. However for the reasons explained earlier for the emergence variable simple linear models were eventually adopted for all four variables. The parameters of these models are reported in table 5.3.13.

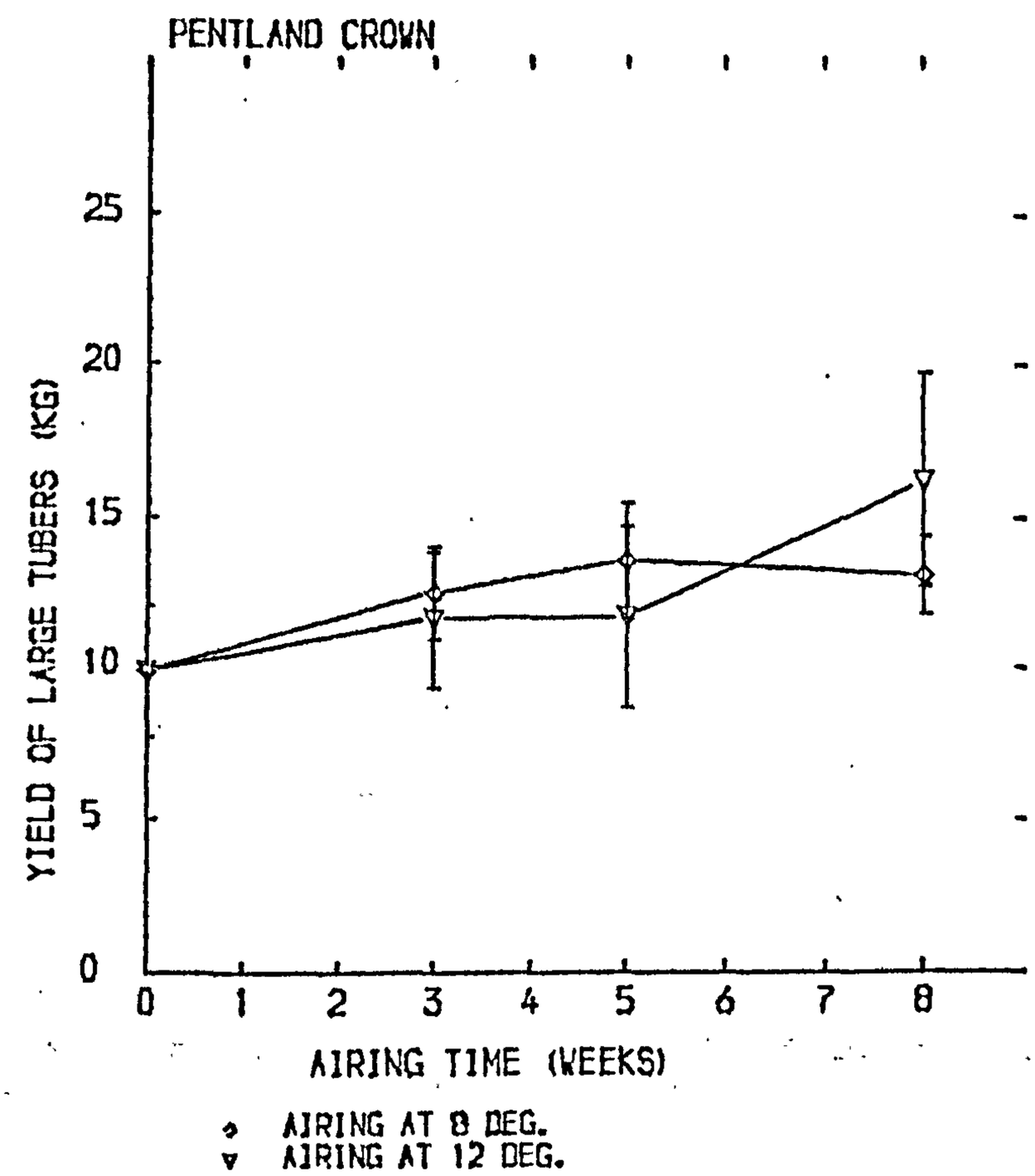
From this table it can be seen that,,in general, storage at $12^{o}C$ produced a substantial positive effect on the yield of large tubers. Airing at $8^{o}C$ increased this factor only very slightly.

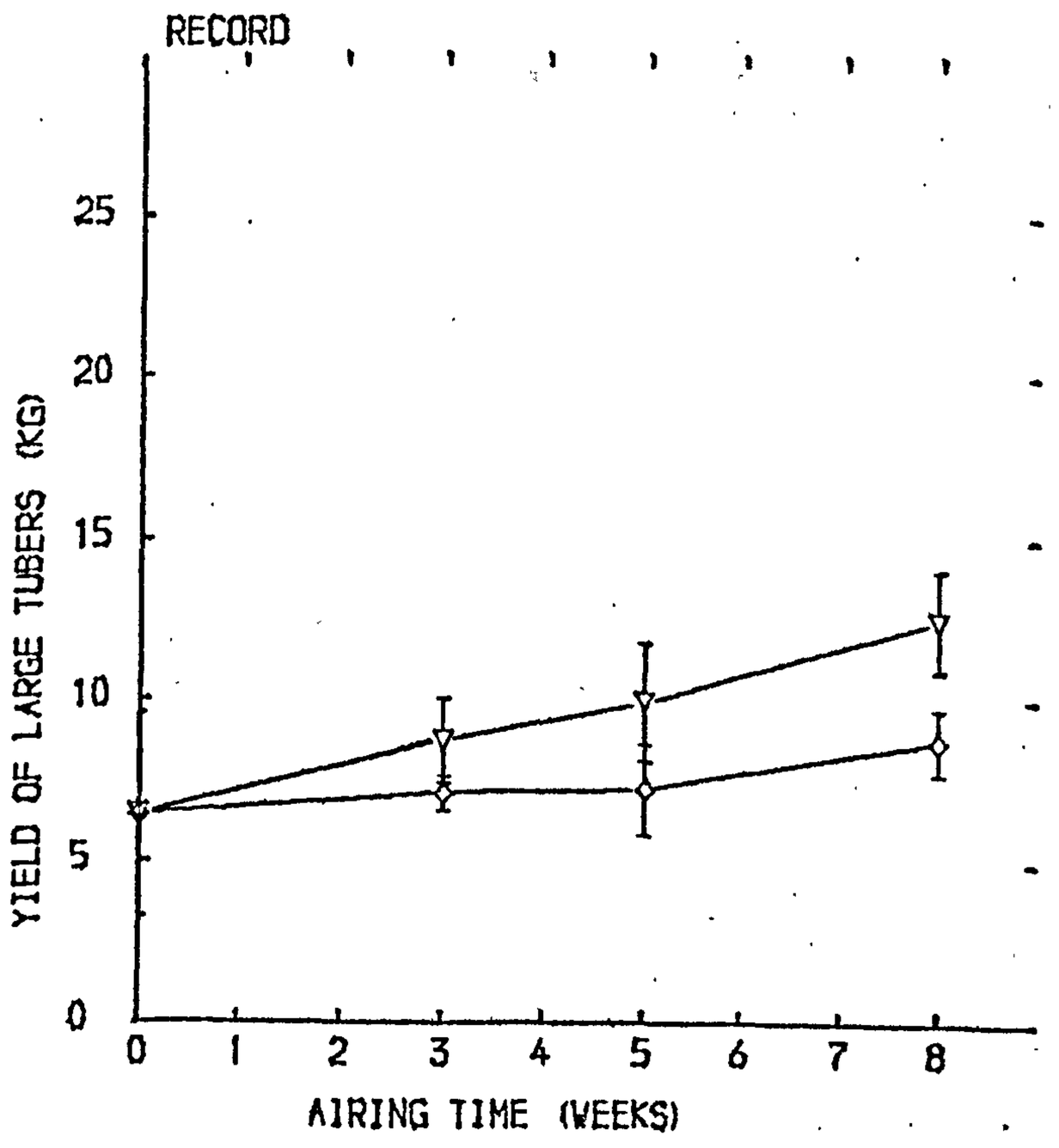
Storage conditions apparently have only a minor effect upon the yield of seed-sized tubers. This is consistent with the earlier observation that whether tecnazene is present or not is more important than the level at which it is present. It would appear therefore that the effects of tecnazene on this factor are permanent and can be only marginally affected by storage conditions. Similar arguments can be applied to the yield of small tubers.

For total yield the model predicts more substantial effects. For all four cultivars airing at $12^{o}C$ will produce a greater yield than airing at $8^{o}C$. For the cultivar Maris Piper, for example, the model implies that 8 weeks airing at $8^{o}C$ would increase the total yield by 30% and that airing for a similar period at $12^{o}C$ would increase yield by 43% to a figure comparable to untreated controls.

FIGURE 5.3.25 THE EFFECT OF STORAGE CONDITIONS ON YIELD OF LARGE TUBERS







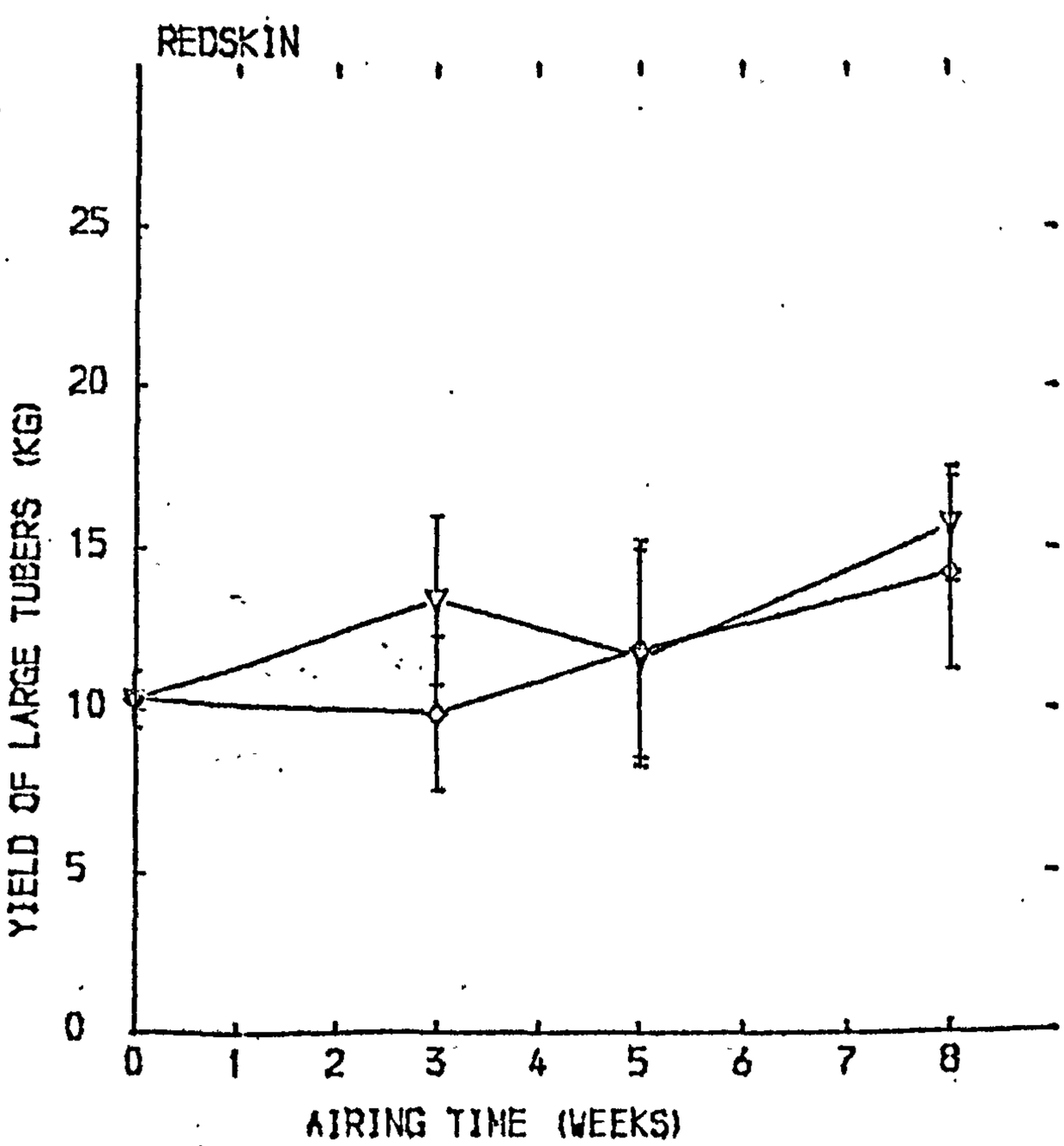
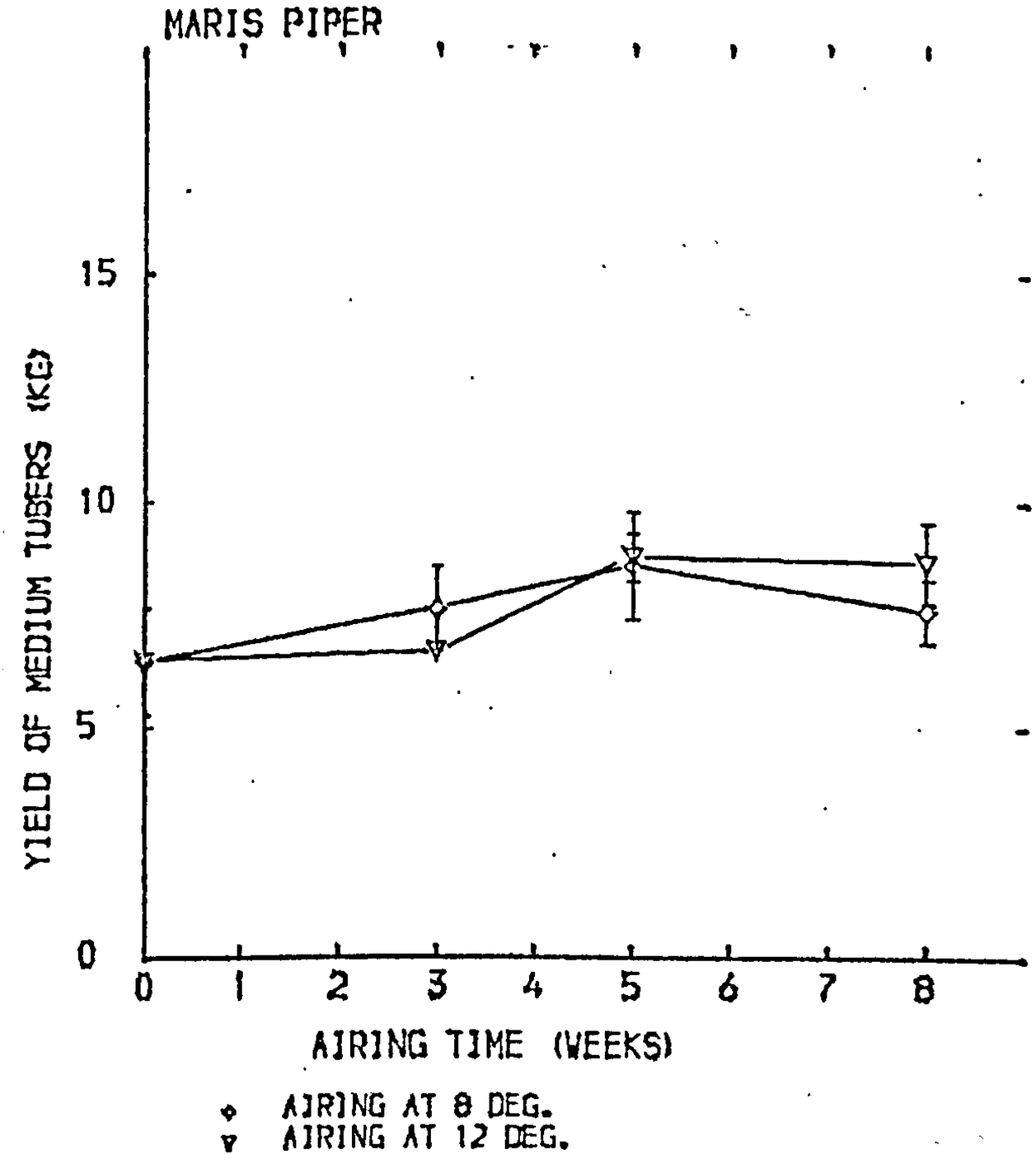
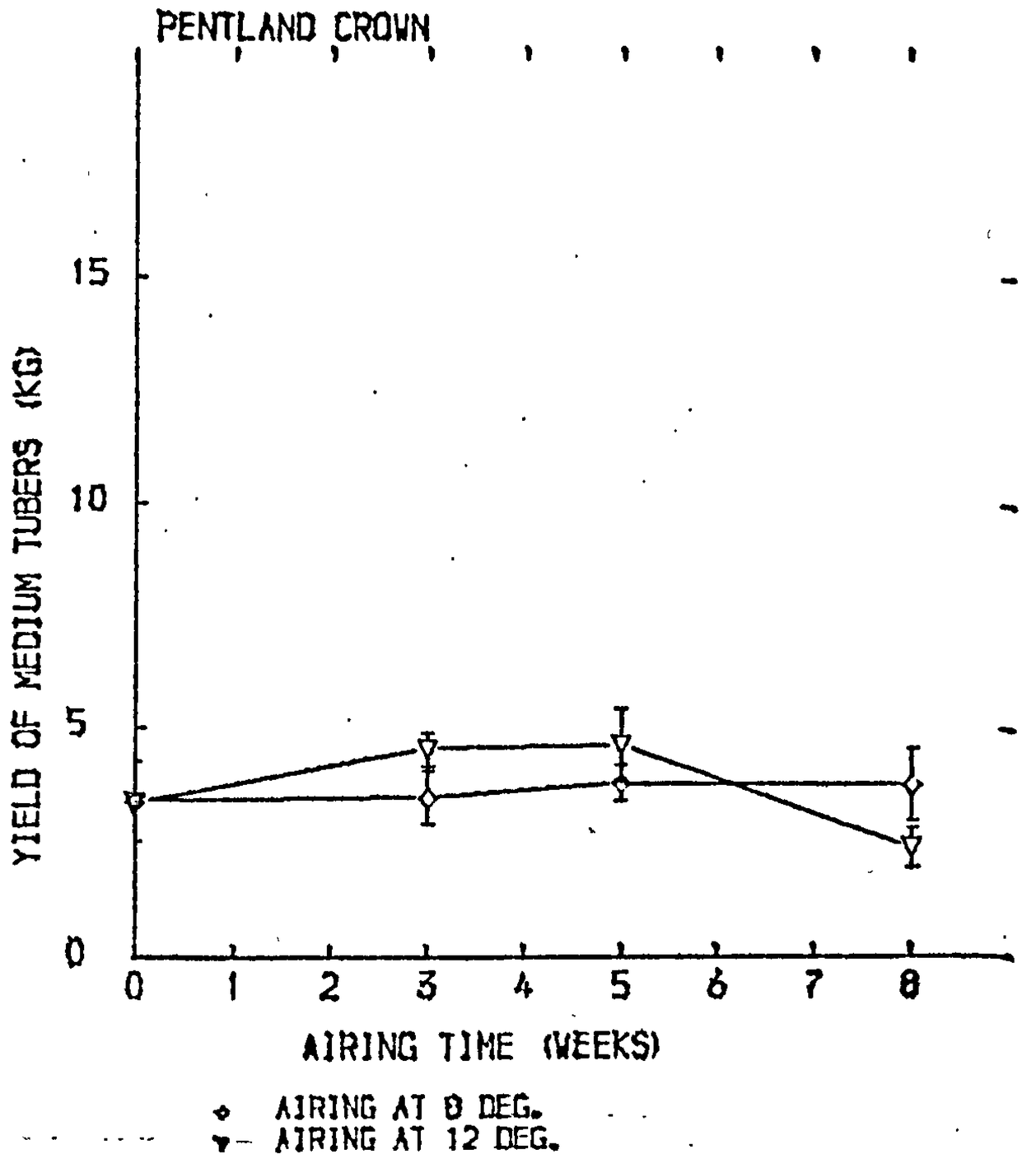
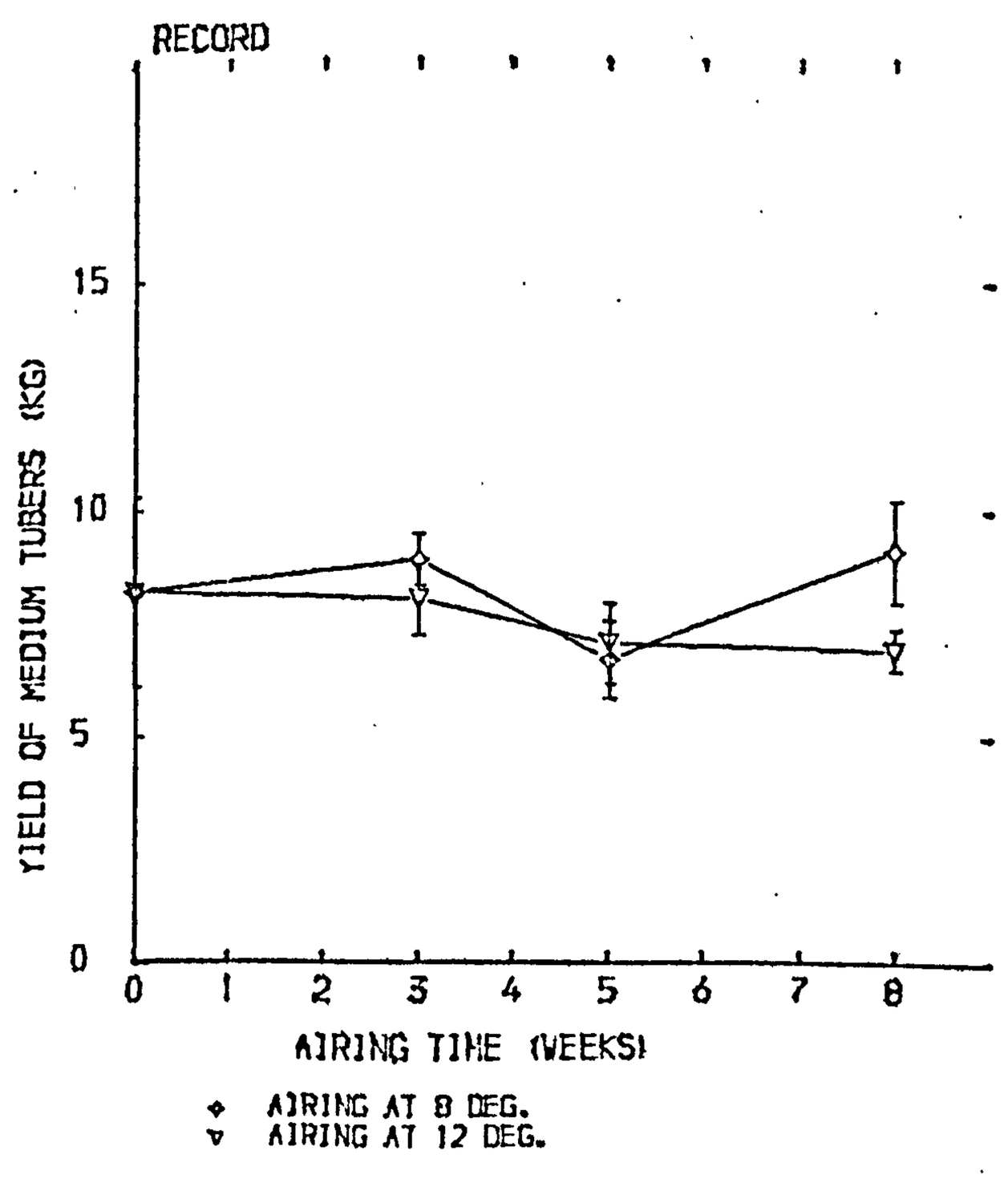


FIGURE 5.3.26 THE EFFECT OF STORAGE CONDITIONS ON YIELD OF MEDIUM TUBERS







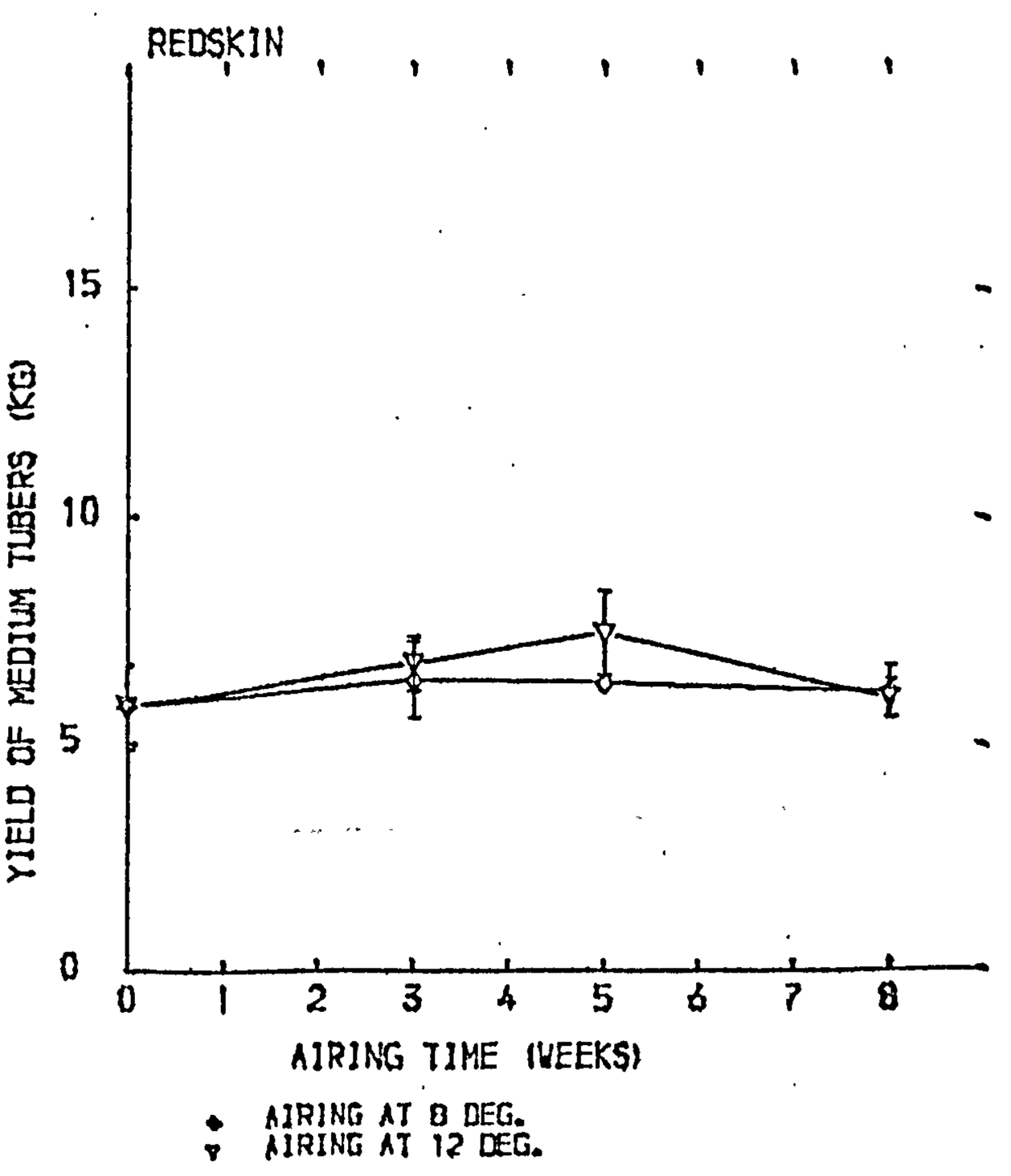


FIGURE 5.3.27 THE EFFECT OF STORAGE CONDITIONS ON YIELD OF SMALL TUBERS

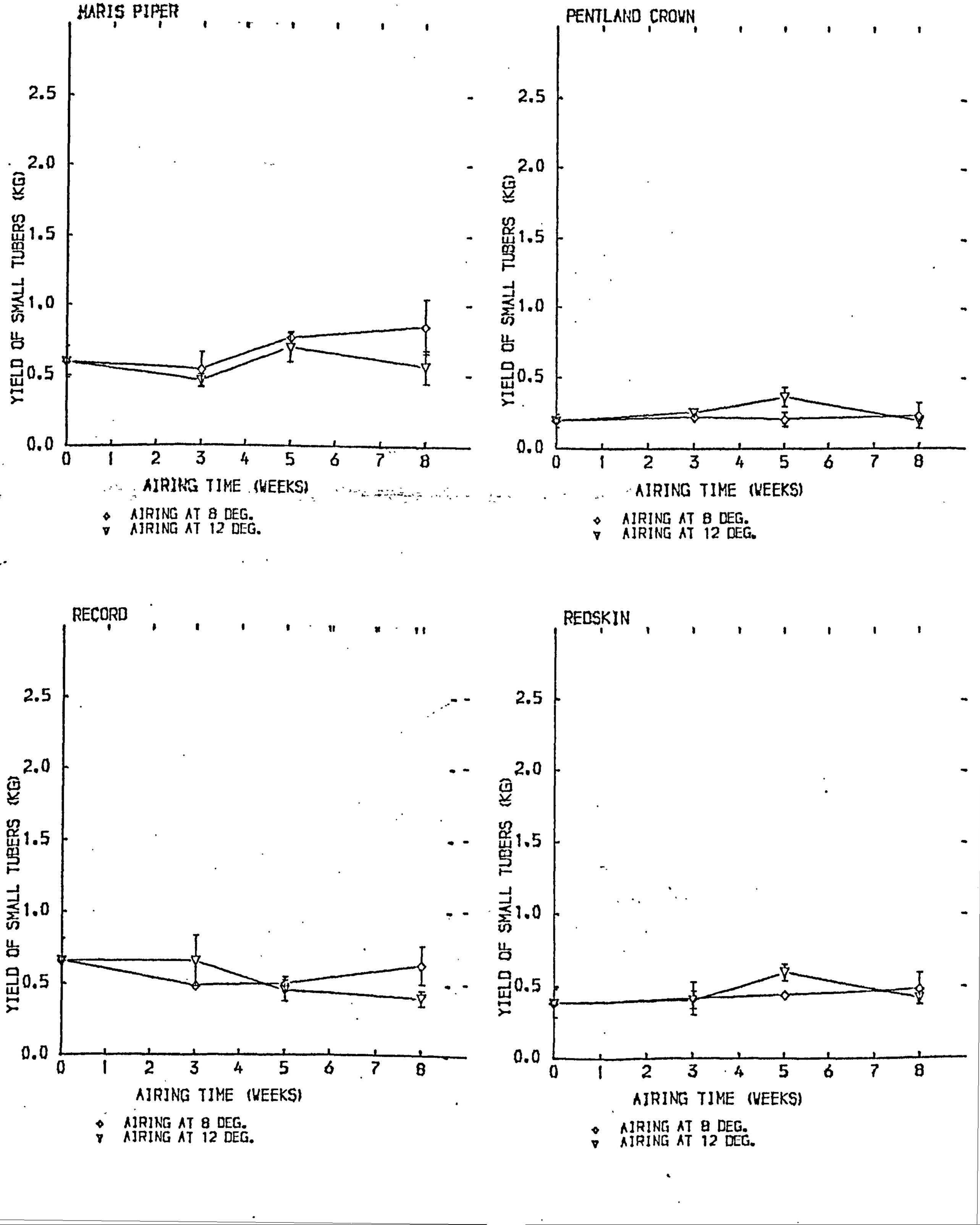


FIGURE 5.3.28 THE EFFECT OF STORAGE CONDITIONS ON TOTAL YIELD

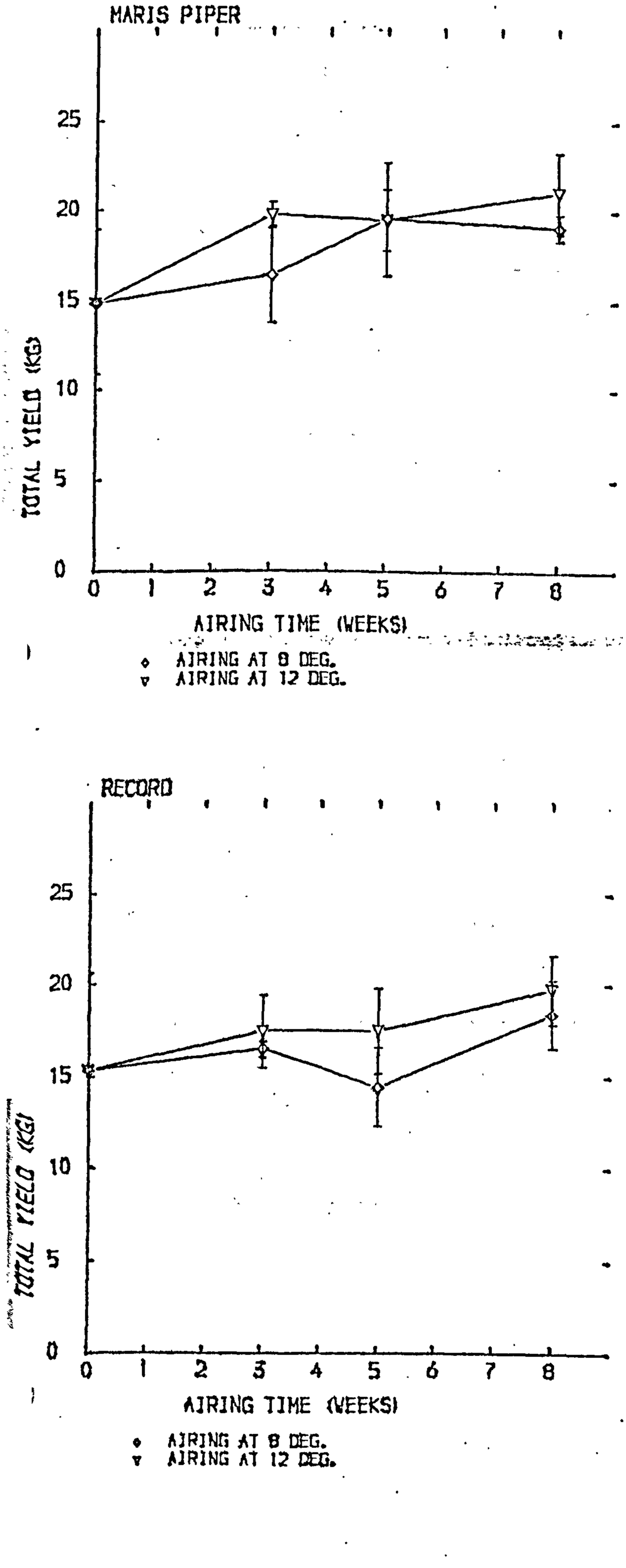


Table 5.3.13. Parameters of linear models of the form $y = \alpha + \beta x$, describing the relationship between airing time (at $8^{o}C$ and $12^{o}C$) and subsequent yield (y) (1974-75)

Yield characteristic	Cultivar	Intercept(α)	Slope(β)		Correlation coefficient	
			$8^{o}C$	$12^{o}C$	$8^{o}C$	$12^{o}C$
Yield of large tubers (kg/plot)	M.Piper	8.4	0.39	0.39	0.31	0.28
	P.Crown	10.0	0.42	0.75	0.36	0.41
	Record	6.4	0.27	0.74	0.24	0.51*
	Redskin	10.0	0.52	0.58	0.32	0.37
Yield of seed-sized tubers	M.Piper	6.7	0.16	0.32	0.23	0.53*
	P.Crown	3.8	0.05	-0.17	0.12	-0.23
	Record	8.2	0.04	-0.18	0.05	-0.24
	Redskin	6.2	0.04	0.04	0.10	0.09
Yield of small tubers	M.Piper	0.55	0.037	0.003	0.42*	0.05
	P.Crown	0.22	0.004	0.003	0.12	0.09
	Record	0.64	-0.003	-0.036	-0.04	-0.43*
	Redskin	0.40	0.013	0.010	0.22	0.21
Total yield (kg/plot)	M.Piper	15.6	0.59	0.71	0.32	0.42*
	P.Crown	14.0	0.47	0.63	0.35	0.37
	Record	15.2	0.31	0.52	0.17	0.27
	Redskin	16.6	0.58	0.64	0.36	0.38

* The probability of obtaining a more extreme value of the correlation coefficient, if the true value is zero, is less than 0.05

4) Mean emergence time and total yield

The relationship between MET and total yield was investigated in the course of this experiment. A regression search revealed no reason to deviate from a simple linear regression, the parameters of which are reported in table 5.3.14.

Table 5.3.14. Parameters of the linear model describing total yield in term of MET

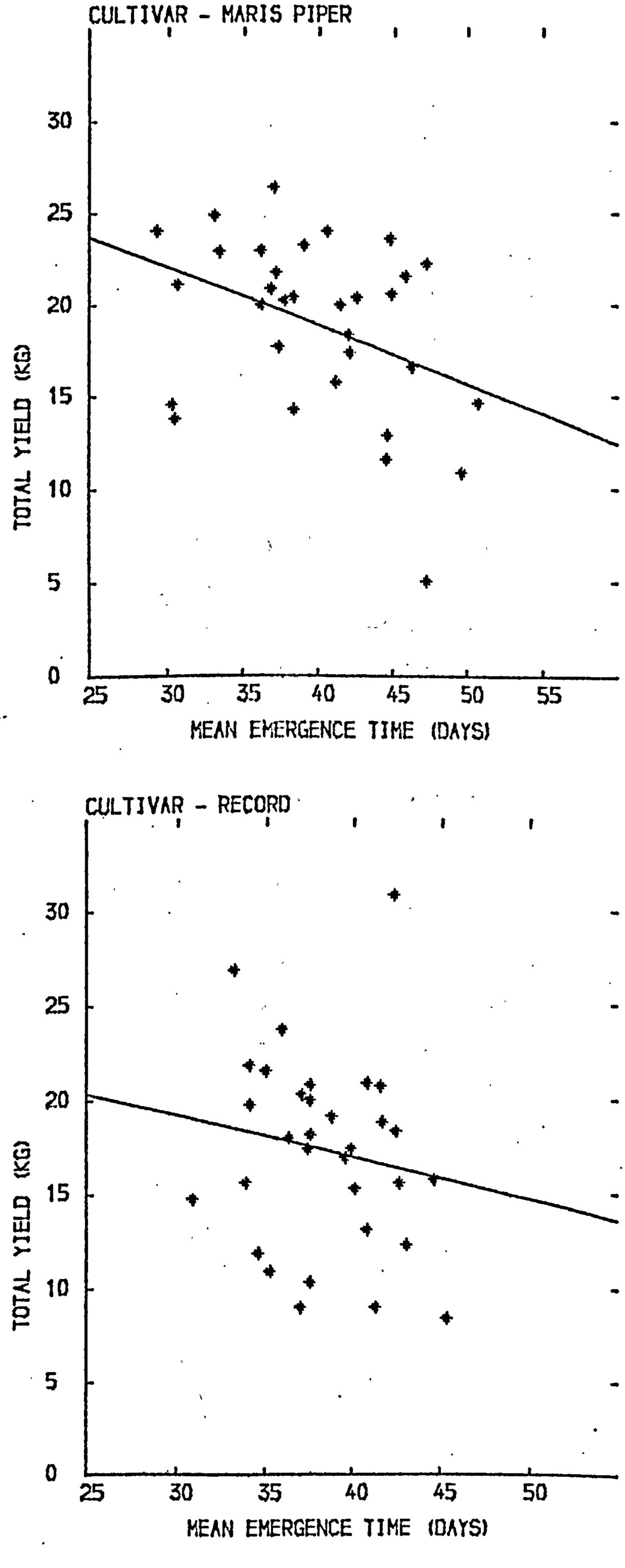
Cultivar	Intercept	Slope	Correlation coefficient
Maris Piper	31.8	-0.32	-0.39**
Pentland Crown	30.6	-0.35	-0.42**
Record	26.0	-0.22	-0.16
Redskin	32.3	-0.33	-0.27

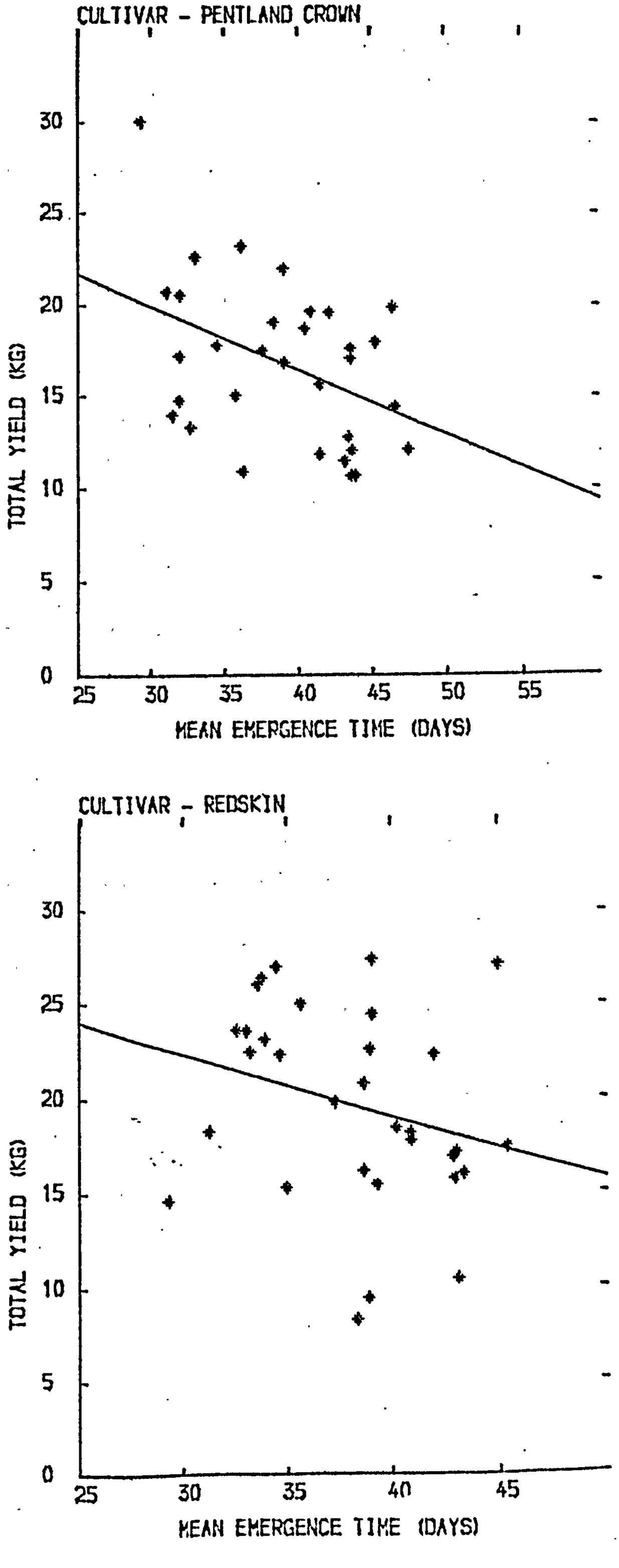
** Significantly different from zero at $P < 0.01$

From these figures it can be shown that, for the cultivar Maris Piper, if emergence is delayed by 5 days from 30 to 35 days the total yield will drop from 22.2 to 20.6 kg/plot - a loss of 7.2%. Similarly for Pentland Crown, Record and Redskin the corresponding losses are 8.7, 5.7 and 7.4% respectively.

However as observed in Section 5.2 the association between delay in emergence and yield reduction is fairly loose. This is further illustrated in the scatter diagrams in figure 5.3.29.

FIGURE 5.3.29 THE RELATIONSHIP BETWEEN MEAN EMERGENCE TIME AND TOTAL YIELD
1974-75 FIELD EXPERIMENT





5.4. THE PERFORMANCE OF TECNAZENE TREATED SEED AFTER CHITTING (1975-76)

5.4.1. Introduction

The aim of this experiment was to examine the recommendations made in Section 5.3.3. under conditions which might be encountered in commercial practice.

This experiment was conducted as part of an integrated programme of research into potato sprout suppressants. To maximise use of facilities, the experiment was therefore designed to fulfil the requirements of the present study and those of an investigation into new sprout suppressant chemicals, by Mr J.L.Beveridge (whose co-operation and assistance is acknowledged), which demanded comparison with tecnazene treated and untreated controls. Because of the scale of these experiments it was not possible to carry out the investigation at two temperatures as in the previous experiment. 10^{o}C was therefore chosen as a suitable compromise. For similar reasons it was not possible to examine a range of airing times. The airing time selected was to be in the range 6-8 weeks as suggested in the previous section, however, because of wet weather at planting time, this was extended to 10 weeks. Despite this and the abnormally dry growing season during 1976 the results are of considerable interest.

The results of a sprout count during the chitting period are also reported as they provide some background information on the mode of action of tecnazene.

In the previous experiment two drills from every plot were considered as guard drills and so discarded. It was therefore decided that the possibility of recording and using the information from these drills should be investigated during the course of this experiment.

5.4.2. Experimental

The experiment consisted of four treatments each involving four 10kg batches of seed potatoes (32 - 50mm)

1) Record - control
2) Record - tecnazene
3) Redskin--control
4) Redskin- tecnazene

Tecnazene (Fusarex) was applied at a rate of 120mg kg^{-1} on 9 December 1975. All batches were then stored at 10°C in good quality cardboard boxes until 2 March when the degree of sprouting was assessed and all existing sprouts removed (as if during a commercial grading procedure). Storage at a lower temperature during this initial period would have been preferred to prevent excessive sprouting of controls, but this was not possible. Sprouting was adequately suppressed in the treated samples. After the sprouts had been removed the tubers were transferred to chitting trays, the contents of each box being divided between two trays. The trays were held at 10°C, with illumination, from vertical fluorescent tubes, and with adequate ventilation, until planting. The number of eyes per tuber producing a sprout length 0.5mm or greater was assessed on thirty tubers from each tray on 15 April 1976.

Planting out

The experimental plot was again situated at Hattrick Farm, Bridge of Weir, Renfrewshire and was approximately 0.5ha of sandy loam soil. Pre-treatment was as described in Section 5.3.2. Tubers were planted by hand on 19 May at 300mm spacing in drills 710mm apart. Each plot consisted of 3 drills 6m long and containing 20 tubers in each drill. The experimental area was protected by guard drills and several non-experimental drills ran the length of the plot to allow tractor spraying.

Experimental design

The experimental design consisted of four independantly randomised blocks each containing the treatments in duplicate. (for reasons concerning the investigation into new sprout suppressants)

Husbandry

Weed control was achieved by applying paraquat just before emergence plus hand-weeding as required. Captafol sprays were applied for blight prevention during August.

Emergence counts

Scores of emergence were taken at 2 day intervals from 3 June till 1 July. All three drills in each plot were individually assessed.

Harvesting and grading

The crop was defoliated using diquat on 6 September and harvested on 29 September. Tubers were collected separately for each of the three drills in each plot and mechanically graded over 52 and 32mm riddles and the yields recorded.

5.4.3. Results and discussion

1) Sprouting

It should be emphasised that the quantity under investigation in this section is not the degree of sprouting during normal storage, but the number of sprouting eyes produced during the chitting period, prior to planting, when illuminated and well ventilated conditions prevailed.

It is also necessary to distinguish between the apical dominance of the sprout and that of the tuber. Although the former was not quantitatively assessed in the current study it was observed that the apical dominance of sprouts is significantly influenced by tecnazene. Brown and Reavill (1954) reported similar effects in that tecnazene encouraged the production of sprouts with a "witches broom" appearance and Brook and Chesters (1959) observed "multiple sprouts" at a large percentage of eyes, both of which can be accounted for by loss of the apical dominance of the sprout. The current investigation concerns the apical dominance of the tuber as estimated by the number of sprouting eyes per tuber.

From the 240 tubers assessed for each treatment, the mean was calculated and is reported in table 5.4.1. ("number of sprouts per tuber").

Table 5.4.1. The effect of tecnazene on the sprouting and emergence behaviour of seed potatoes after chitting (1975-76) (Cultivars - Record and Redskin)

Treatment	Statistic	Number of sprouts per tuber[a]	Emergence characteristics[b]			
			MET (days)	T50 (days)	T75 (days)	Total emergence (%)
Record						
control	mean	2.59	26.3	24.9	29.8	98.1
	S.E.M.	0.07	0.6	0.6	0.6	0.7
tecnazene	mean	2.66	24.6	23.4	27.6	96.9
	S.E.M.	0.09	0.4	0.4	0.6	0.9
	t value	-0.64[c]	2.47[d]	2.31[e]	2.67[d]	1.12[c]
Redskin						
control	mean	4.13	26.9	26.6	31.3	90.8
	S.E.M.	0.11	0.9	0.8	0.7	2.4
tecnazene	mean	3.54	22.8	22.6	26.6	95.8
	S.E.M.	0.16	0.7	0.5	0.9	1.3
	t value	3.02[d]	3.61[d]	4.11[d]	4.33[d]	-1.78[f]

a. Mean of 240 tubers.

b. Mean of 24 replicate drills (8 plots x 3 drills)

c. Not significantly different.

d. Significantly different at $P < 0.01$

e. Significantly different at $P < 0.025$

f. Significantly different at $P < 0.05$

From the cultivar, Record, the difference between the mean of the control and that of the tecnazene treated sample is not significant as determined by a simple t-test. In the case of Redskin the tecnazene sample produced a significantly lower number of sprouting eyes than the controls.

The increase in the number of shoots per planted tuber reported by numerous authors (eg. Brown and Reavill, 1954; Brook and Chesters, 1959; Driver, 1961) must therefore be wholly due to loss of the apical dominance of the sprout. The fact that tuber apical dominance is not lost might indicate that tecnazene acts directly on the sprout and not via the tuber which still retains control of the overall sprouting pattern.

The observed decrease in the case of Redskin can probably be accounted for in terms of retarded sprout growth rather than any positive effect on whole tuber dominance.

2) Emergence

As there can be no difference between outer and centre drills at this stage of growth, data from all three has been used.

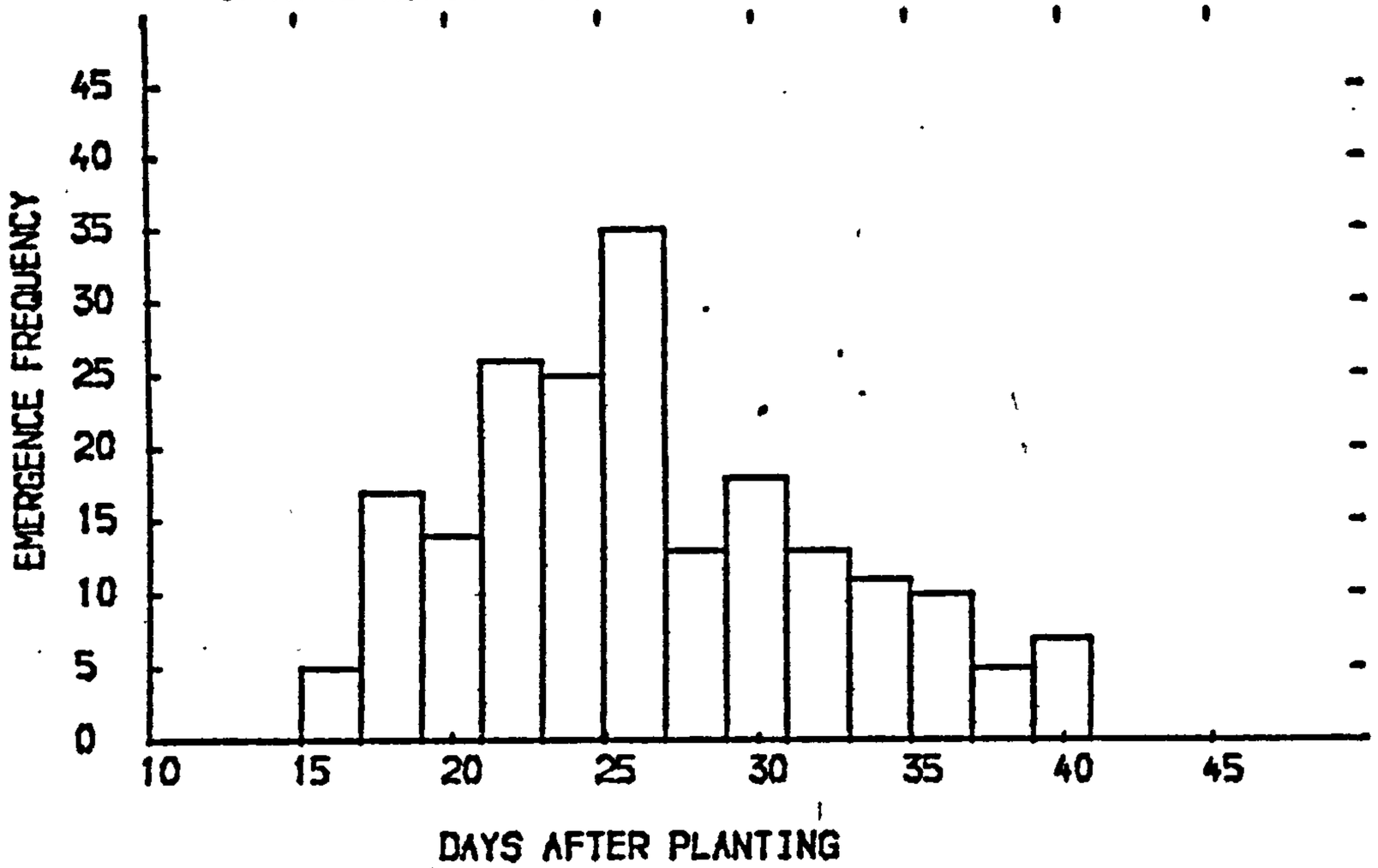
The fact that each treatment appears in duplicate in each block allows the experimental method to be more closely examined. The experiment can be considered as 2 units (A and B) of 4 blocks x 3 drills which have been superimposed on each other. The emergence scores were, therefore, processed for each of these units as described in section 5.3.3., and the resultant emergence profiles are shown for all for treatments in figures 5.4.1. and 5.4.2. for units A and B respectively. Examination shows the agreement between the two is excellent.

The calculated emergence characteristics are shown in table 5.4.1. On this occasion the values from each of the 24 replicate drills have been averaged to facilitate a t-test, rather than pooling the frequency distributions and then calculating the characteristics. The means were then t-tested and the calculated t-statistics are also reported in table 5.4.1., indicating that the mean emergence time (MET), time to 50% emergence (T50) and time to 75% emergence (T75) are all significantly lower for the tecnazene treated sample than for the controls. There is no consistent effect upon total emergence although the difference in the case of Redskin is just significant at the 5% level. The reason for the apparent improvement in emergence times produced by tecnazene is probably the fact that the control sample was delayed because of low vigour, caused by the removal of any sprouts after the initial storage period and the advanced physiological age of the tubers due to the fairly high storage

FIGURE 5.4.1 UNIT A

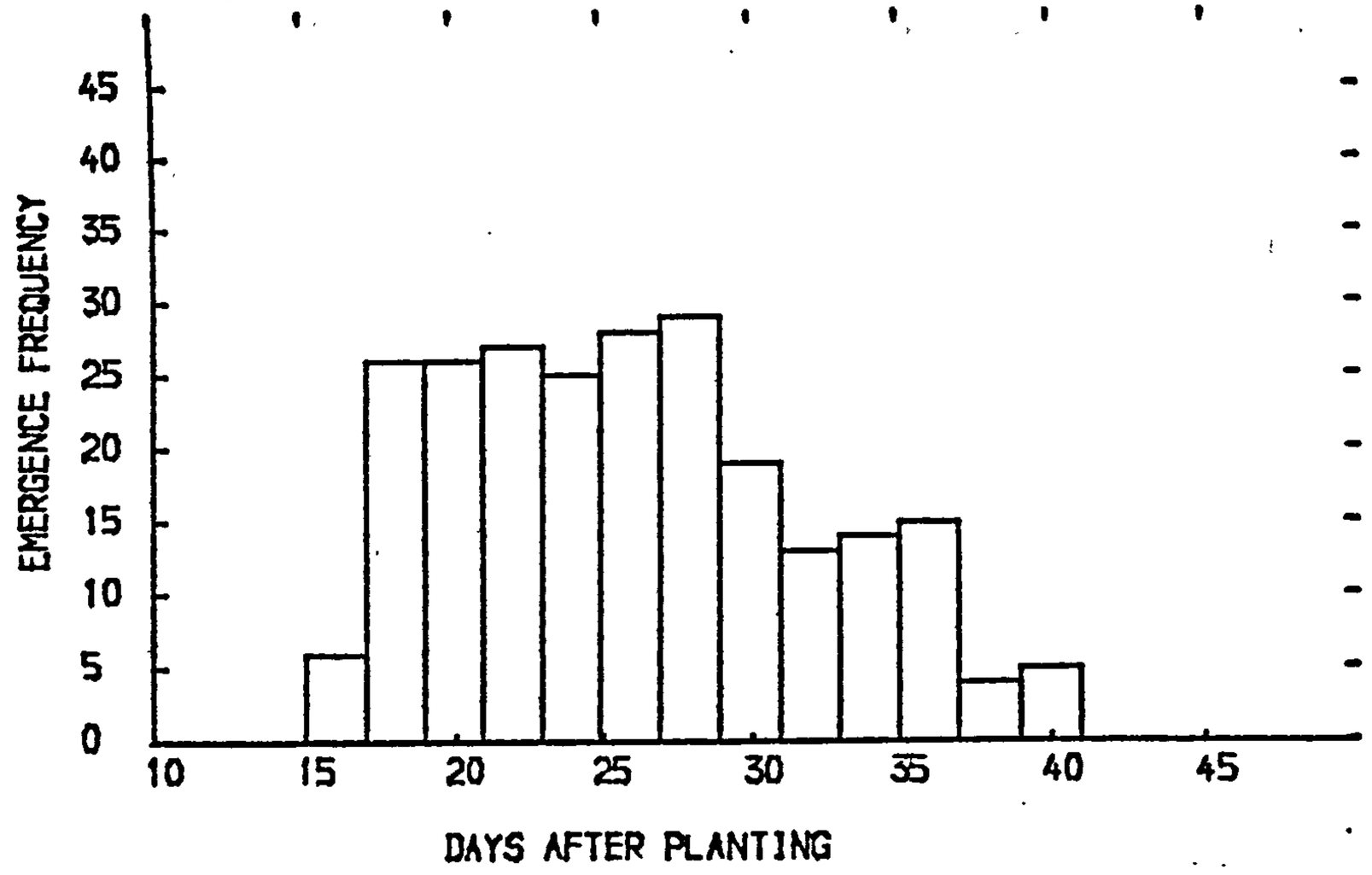
REDSKIN

CONTROL M.E.T=26.9 DAYS T.E=84.5%

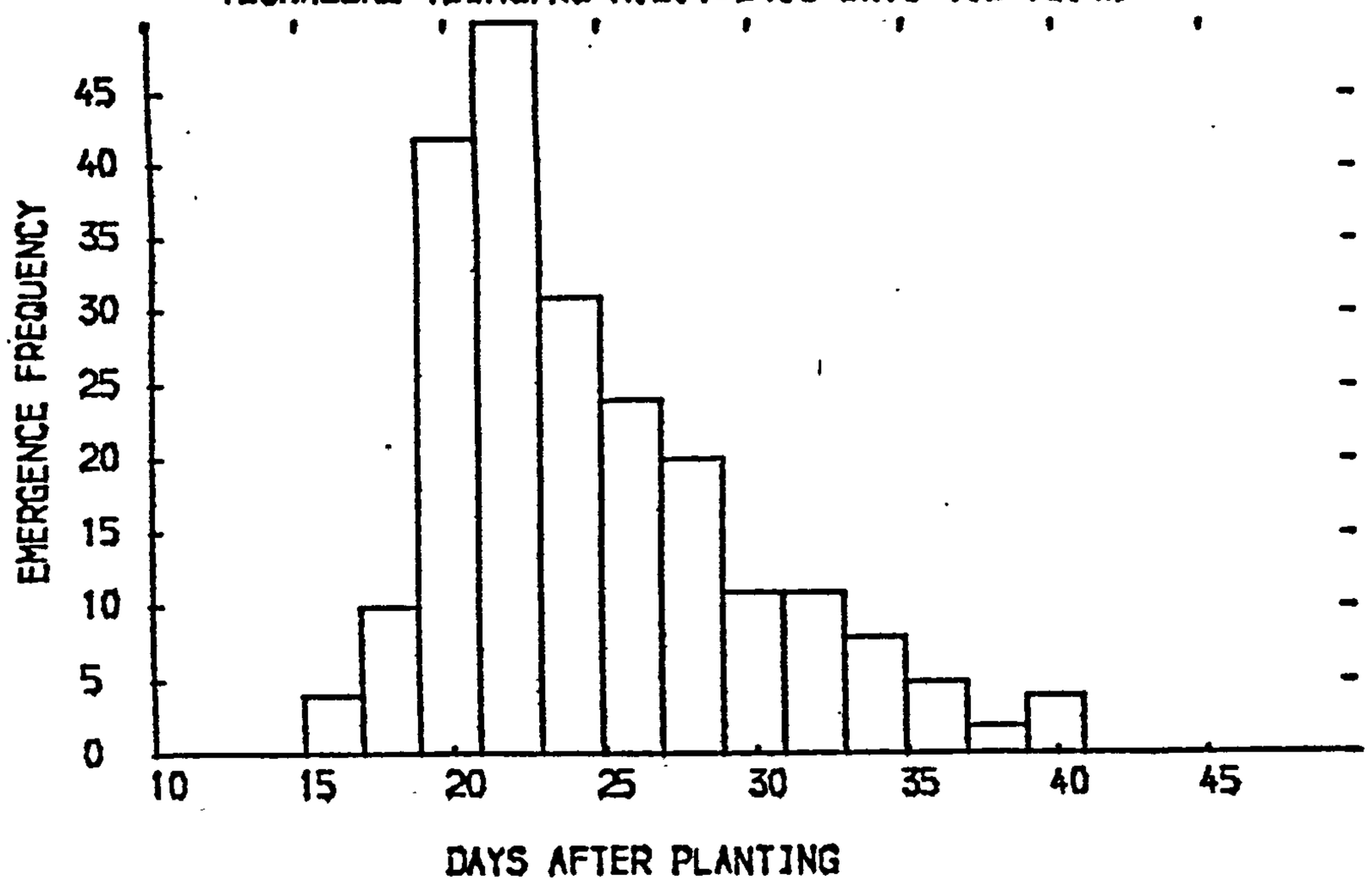


RECORD

CONTROL M.E.T=26.6 DAYS T.E=98.6%



TECNAZENE 120MG/KG M.E.T=24.3 DAYS T.E=95.4%



TECNAZENE 120MG/KG M.E.T=24.6 DAYS T.E=96.6%

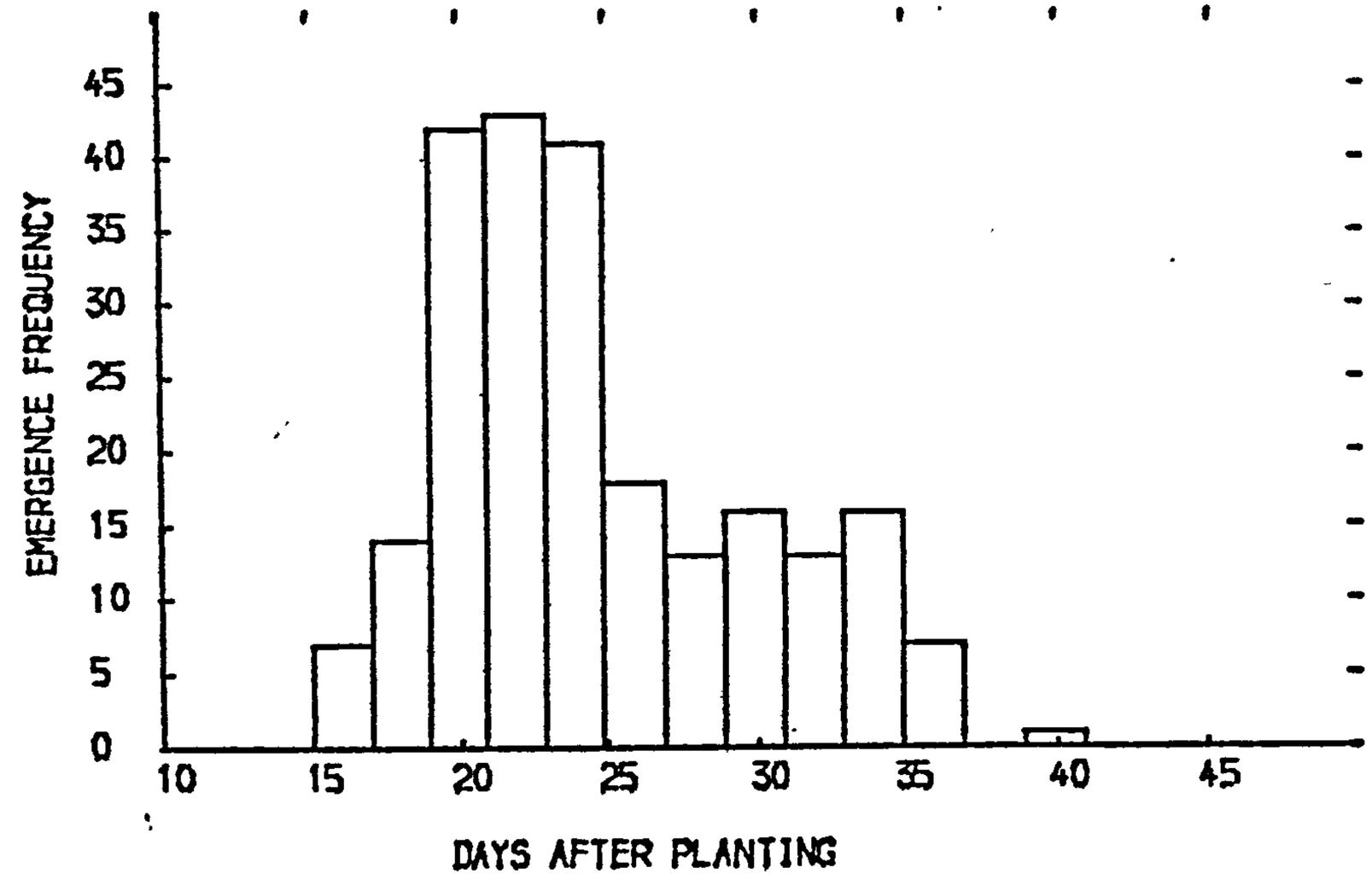
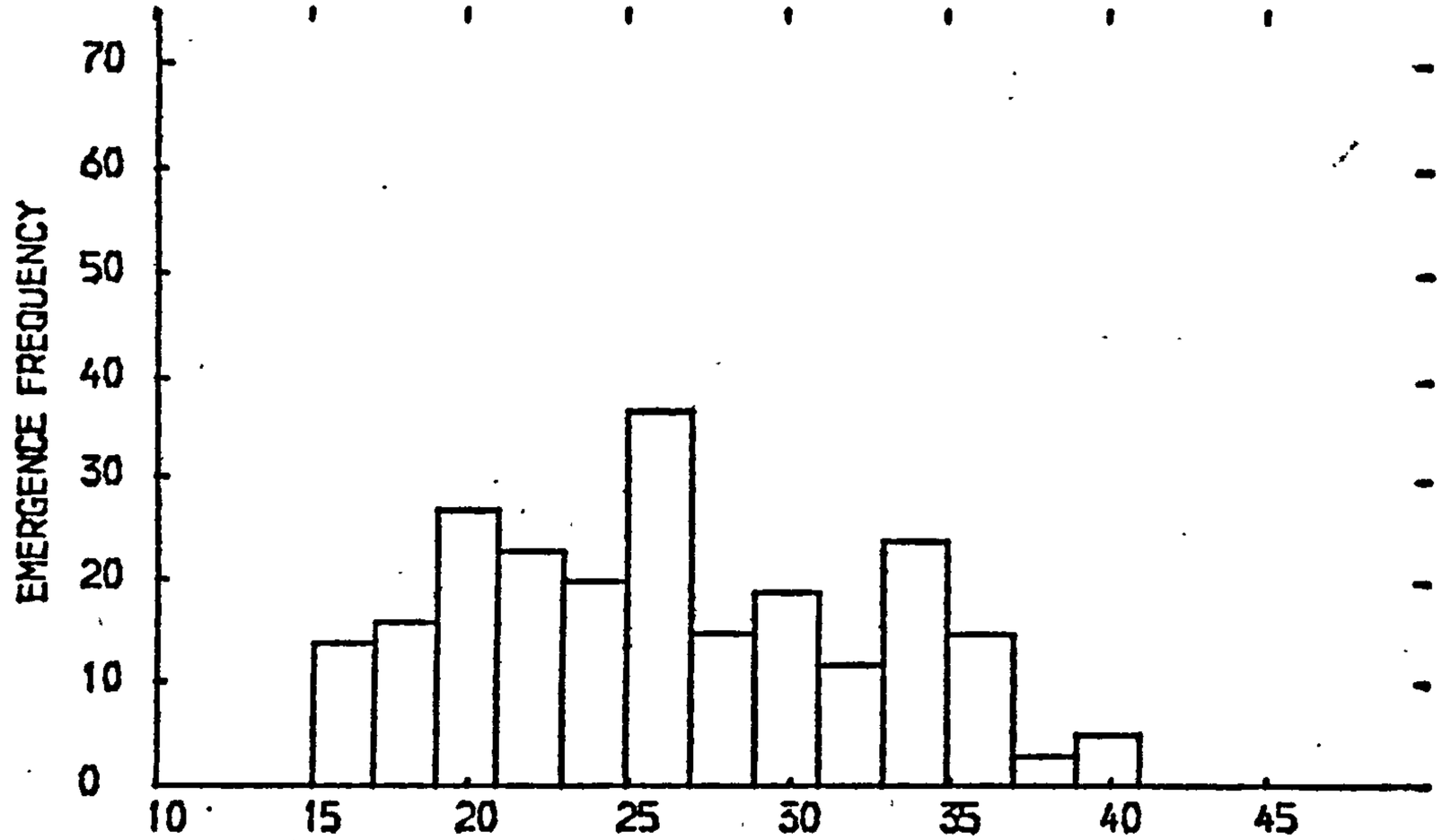


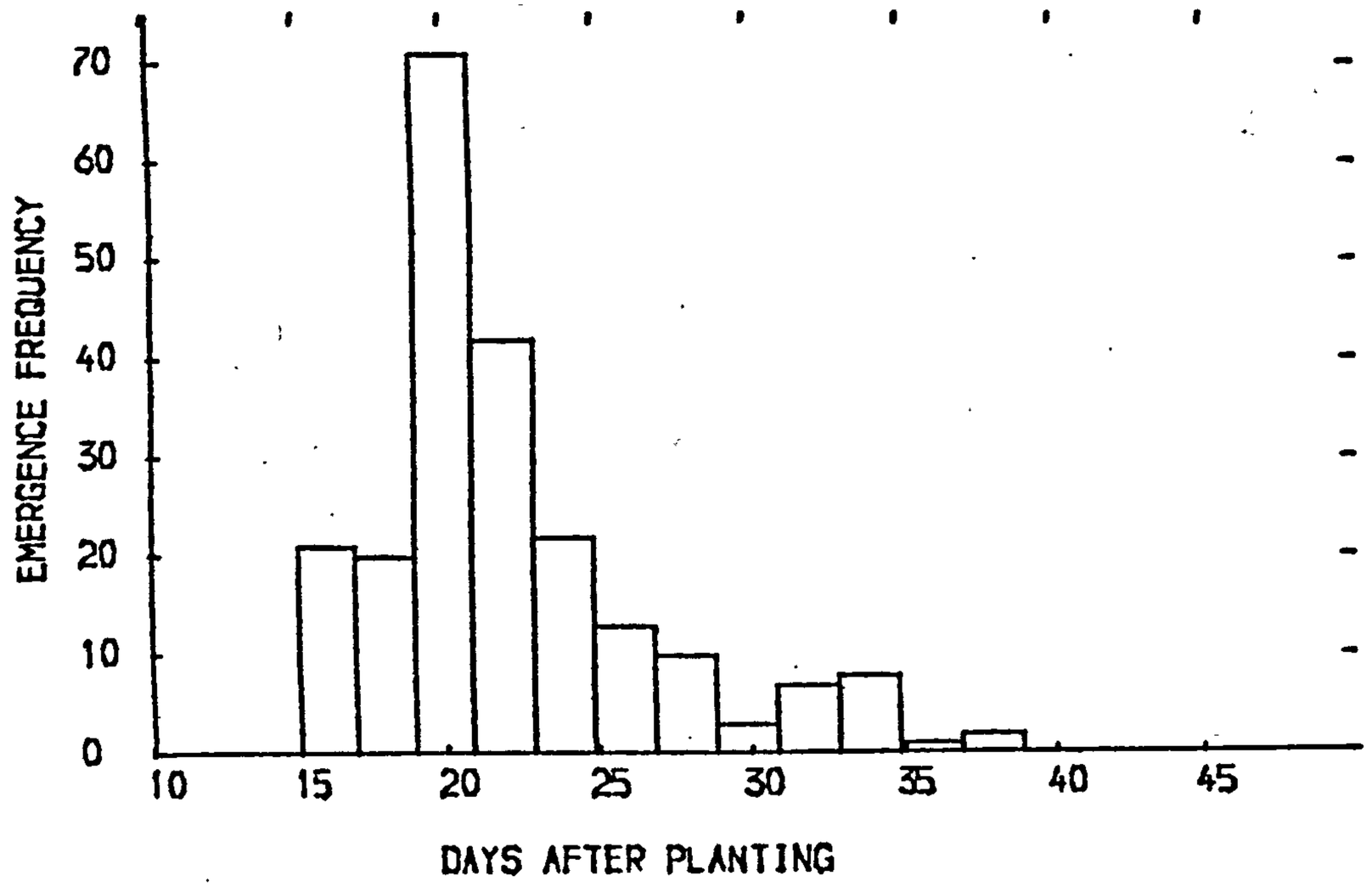
FIGURE 5.4.2 UNIT B

REDSKIN

CONTROL M.E.T=26.5 DAYS T.E=97.1%

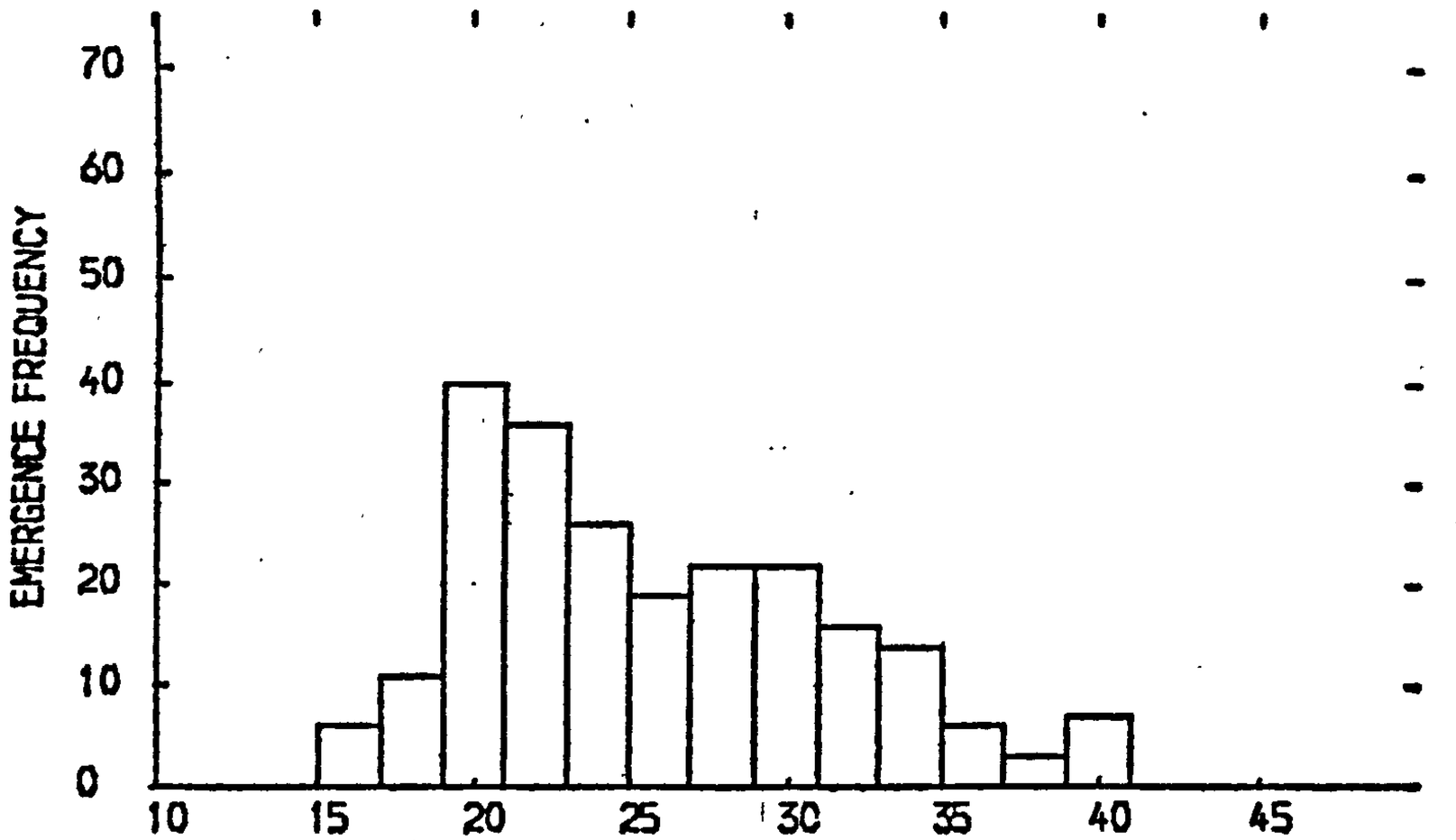


TECNAZENE 120MG/KG M.E.T=21.1 DAYS T.E=96.3%

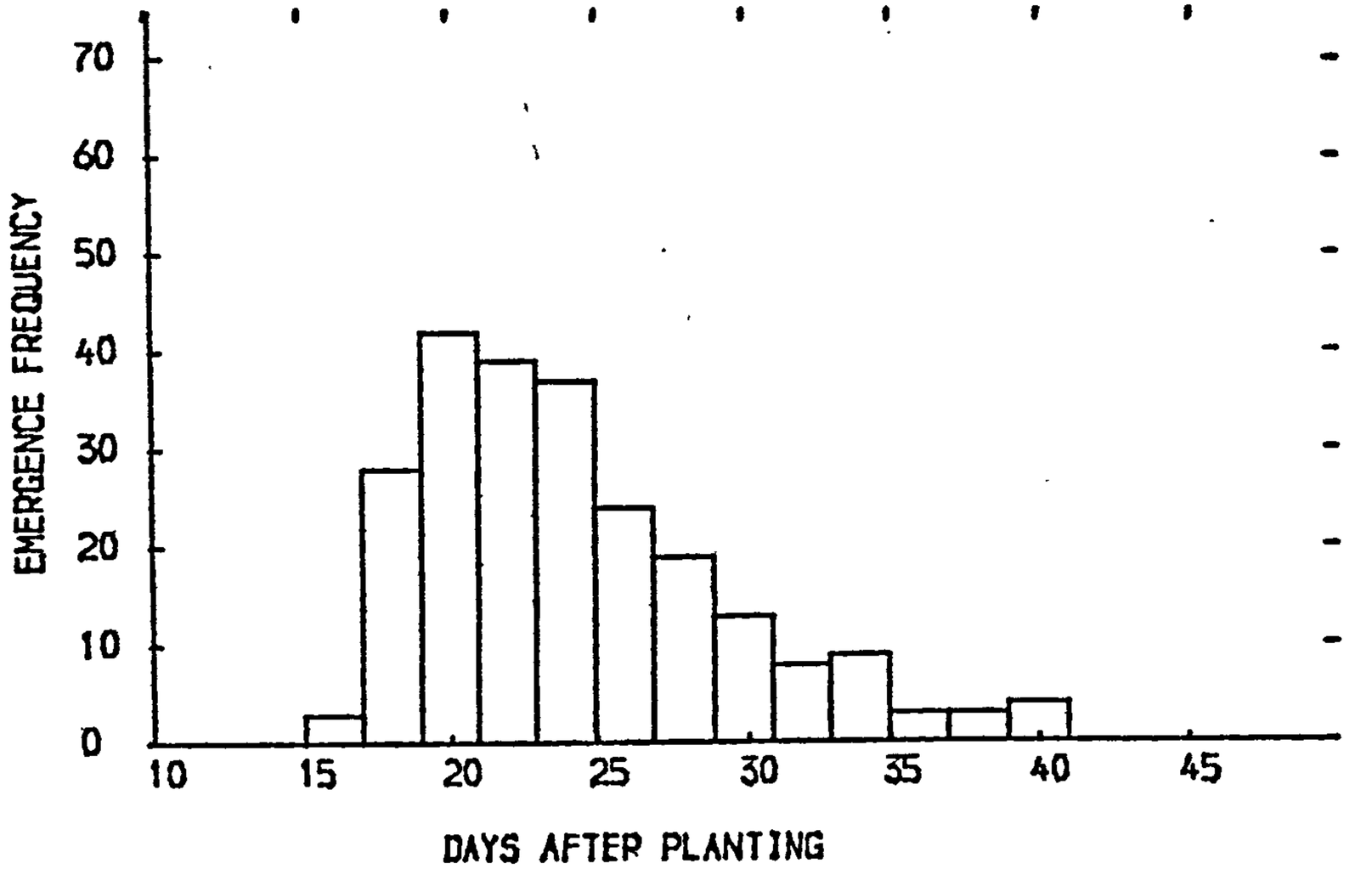


RECORD

CONTROL M.E.T=25.8 DAYS T.E=97.5%



TECNAZENE 120MG/KG M.E.T=24.5 DAYS T.E=97.1%



temperature and extended storage season (Burton, 1966, P.136). It is, nevertheless, interesting to note that given suitable conditions tecnazene treated seed can emerge faster than chitted controls.

3) Yield and size distribution

Close examination of the raw data revealed that the yields obtained from the outer drills of each plot did not differ substantially, or in any systematic way from those obtained from the centre drill. This was further checked by comparing the treatment means with and without the outer drill data, and by t-testing the mean of the centre drills against the means of the outer drills. Further justification for the inclusion of this data is given in Section 5.5.3.

The inclusion of the outer drill data gave a total of 24 replicate drills per treatment as for the emergence data, and thus again allowed analysis using t-tests.

The means of the seven yield and size distribution variables are reported in table 5.4.2. with the results of the t-tests.

In this experiment the total yields have not been dramatically affected. In the case of Record it is slightly lower and for Redskin slightly higher. It is worth noting that if the Redskin figures are corrected for the differing number of emerged plants in control and treated samples the difference becomes non-significant.

The most important finding from this experiment is that despite the long airing period the yield of seed is increased by tecnazene in absolute terms and when expressed as a percentage of the total yield. Thus it might in fact be possible to use tecnazene to increase the yield of seed provided care is taken to air the plants properly before planting. Reduced application rates might also be worth investigating in this respect (see section 5.6.).

Also worthy of note is the yield of very small tubers, many of which would not be harvested during a commercial operation. For both cultivars tecnazene has almost doubled the absolute figure and the percentage in the total yield, and this could lead to a serious ground-keeper problem. This problem may be even more acute than the figures indicate because of the decreased efficiency with which small tubers were lifted. Despite instructions that every tuber, however small was to be harvested some were missed, some escaped through the

Table 5.4.2. The effect of tecnazene on the subsequent yield and size distribution after chitting (1975-76)
(Cultivars - Record and Redskin)

Treatment	Statistic	Yield per drill				Size distribution		
		Large (kg)	Medium (kg)	Small (kg)	Total (kg)	Large (%)	Medium (%)	Small (%)
Record								
control	mean	4.9	4.6	0.30	9.8	49.6	47.2	3.2
	S.E.M.	0.3	0.1	0.02	0.3	2.0	1.8	0.3
tecnazene	mean	2.6	5.0	0.56	8.1	30.4	62.5	7.1
	S.E.M.	0.2	0.2	0.04	0.4	2.0	1.7	0.4
	t value*	6.1	-1.8	-6.0	3.5	6.9	-6.2	-7.8
Redskin								
control	mean	9.1	3.2	0.20	12.5	71.6	26.6	1.8
	S.E.M.	0.6	0.1	0.02	0.6	1.6	1.5	0.1
tecnazene	mean	8.6	5.2	0.39	14.2	59.9	38.2	2.8
	S.E.M.	0.4	0.2	0.02	0.4	1.7	1.8	0.2
	t value*	0.8	-8.0	-7.3	-2.1	5.0	-4.9	-4.2

* Least significant values of the t statistic:
1% level t = 2.39
2.5% level t = 2.00
5% level t = 1.67

holes in the baskets used in the field and some escaped with the soil during the grading operation. With respect to the latter it should be noted that the space between the retaining bars was approximately 18mm.

It would appear therefore that despite the extreme steps taken to remove tecnazene from the seed tubers, the effects of the chemical are still apparent on the size distribution within the total yield, although the total yield itself is little different.

5.5. THE PERFORMANCE OF TECNAZENE TREATED SEED POTATOES AFTER VARIOUS CHITTING PERIODS (1976-77)

5.5.1. Introduction

The aim of this experiment was to further investigate the effect of storage conditions on the performance of tecnazene treated seed during a normal growing season.

Again this experiment was part of a much larger investigation, but on this occasion it was possible to examine various chitting periods, as these were being investigated with respect to the new suppressants of Mr.J.L.Beveridge whose co-operation and assistance is once more acknowledged.

The chitting times chosen were 7 weeks, 4 weeks and 1 week as these times were of the order which might be usefully recommended as they are commercially feasible with respect to seed delivery dates and other commercial constraints.

Unlike the 1974-75 experiment this experiment included a control for every treatment so that tecnazene independent physiological effects could be isolated if necessary from those produced directly by the chemical.

Each drill of every plot was independently assessed to check the conclusions of the work in the previous season (see section 5.4.3.).

5.5.2. Experimental

The experiment consisted of ten treatments for each of two cultivars, of which only six treatments were eventually planted out, the remainder acting as insurance against a posponement of the planting date. The six selected for planting were:

1) 1 week chitting - control
2) 1 week chitting - tecnazene
3) 4 weeks chitting - control
4) 4 weeks chitting - tecnazene
5) 7 weeks chitting - control
6) 7 weeks chitting - tecnazene

Certified seed potatoes (32-50mm) of the cultivars Maris Peer (FS2) and Red Craigs Royal (AA1) were divided into 10kg batches in closed cardboard boxes. Each treatment for each cultivar consisted of 2 such batches.

Tecnazene was applied to treatments 2,4 and 6 as previously described at a rate of 135mg kg^{-1} on 30 December 1976 and all batches were then stored at $10^{o}C$ until they were opened on 14 March (treatments 5 and 6), 5 April (treatments 3 and 4) and 25 April (treatments 1 and 2). Again all tecnazene treatments had effectively prevented sprouting.

Existing sprouts were removed on opening (as if during commercial grading) and the contents of each box transferred to two chitting trays which were then stored at $10^{o}C$ with illumination and free ventilation until planting.

Planting

The experimental plot was again situated at Hattick Farm, Bridge of Weir, Renfrewshire and was approximately 0.25 ha of sandy loam soil. Pre- treatment was as described in section 5.3.2. except that 10% aldicarb granules were applied before planting.

Tubers were planted by hand on 3 May at 230mm spacing (a seed production spacing) in drills 710mm apart. Each plot consisted of 3 drills which in the case of Maris Peer were 4.6m long and contained 20 tubers per drill, and for Red Craigs Royal, because of a slightly larger seed size, plots were 4m long and contained 17 tubers per drill. The experimental plot was protected by guard drills.

Experimental design

The experimental design consisted of four independently randomised blocks with the constraint that alternate plots in both directions were of different cultivars to simplify the geometry of the plan. This had the added advantage that rows and columns were

balanced with respect to cultivar.

Husbandry

Weed control was achieved using paraquat and monolinuron plus hand weeding as required. As the chemicals were applied using a hand sprayer application was delayed to 5% emergence and emerged plants were avoided. Blight control was as previously described.

Emergence counts

Scores of emergence were taken at 2 day intervals from 22 May to 9 July, each drill of each plot being individually assessed.

Harvesting and grading

The crop was mechanically defoliated on 29 September and harvested on 5 October and graded over 52 and 32mm riddles as previously described.

5.5.3. Results and discussion

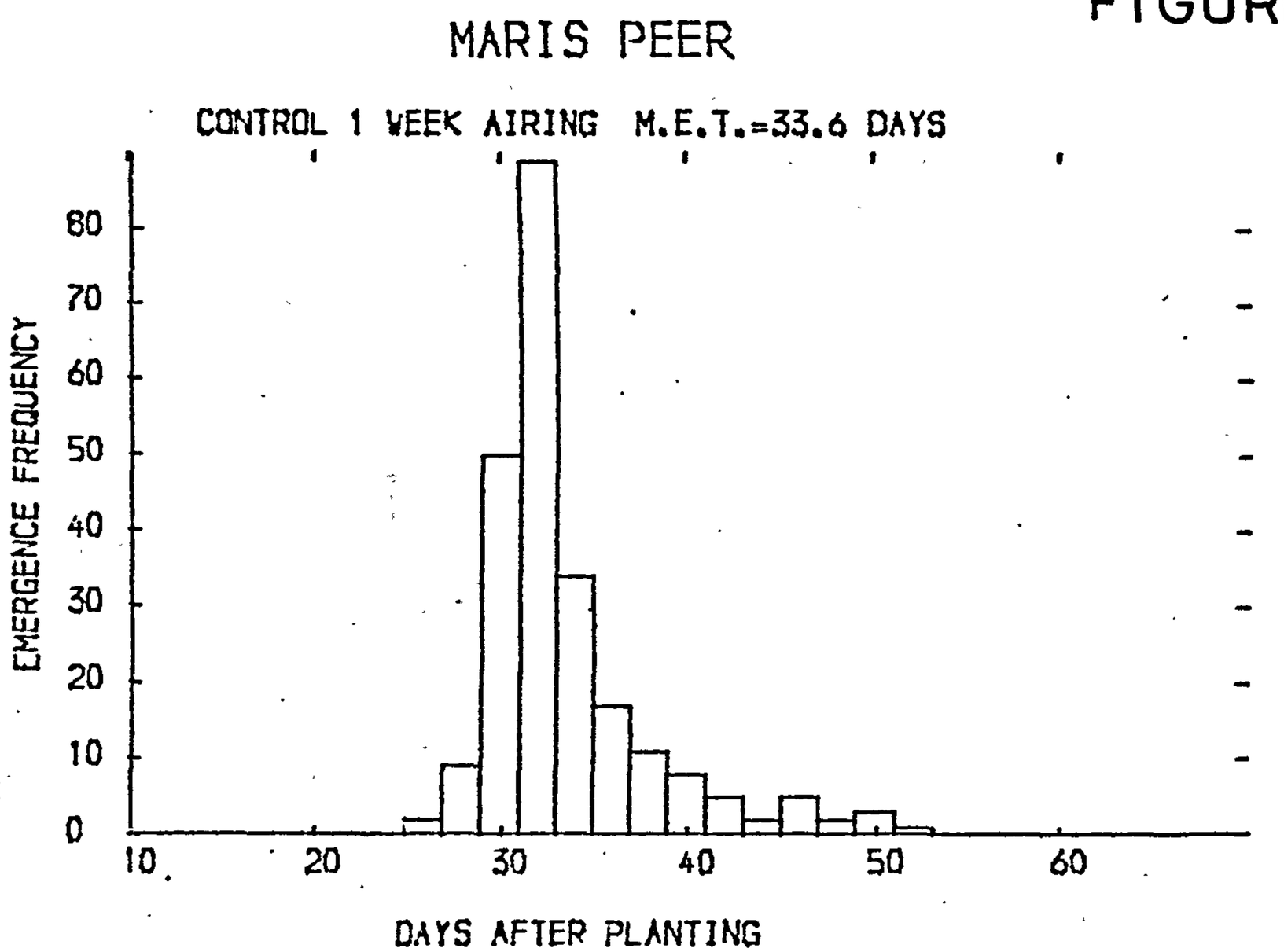
As in section 5.3.3. graphical means have been used widely to indicate the nature of the original data so that various tables are as simple as possible.

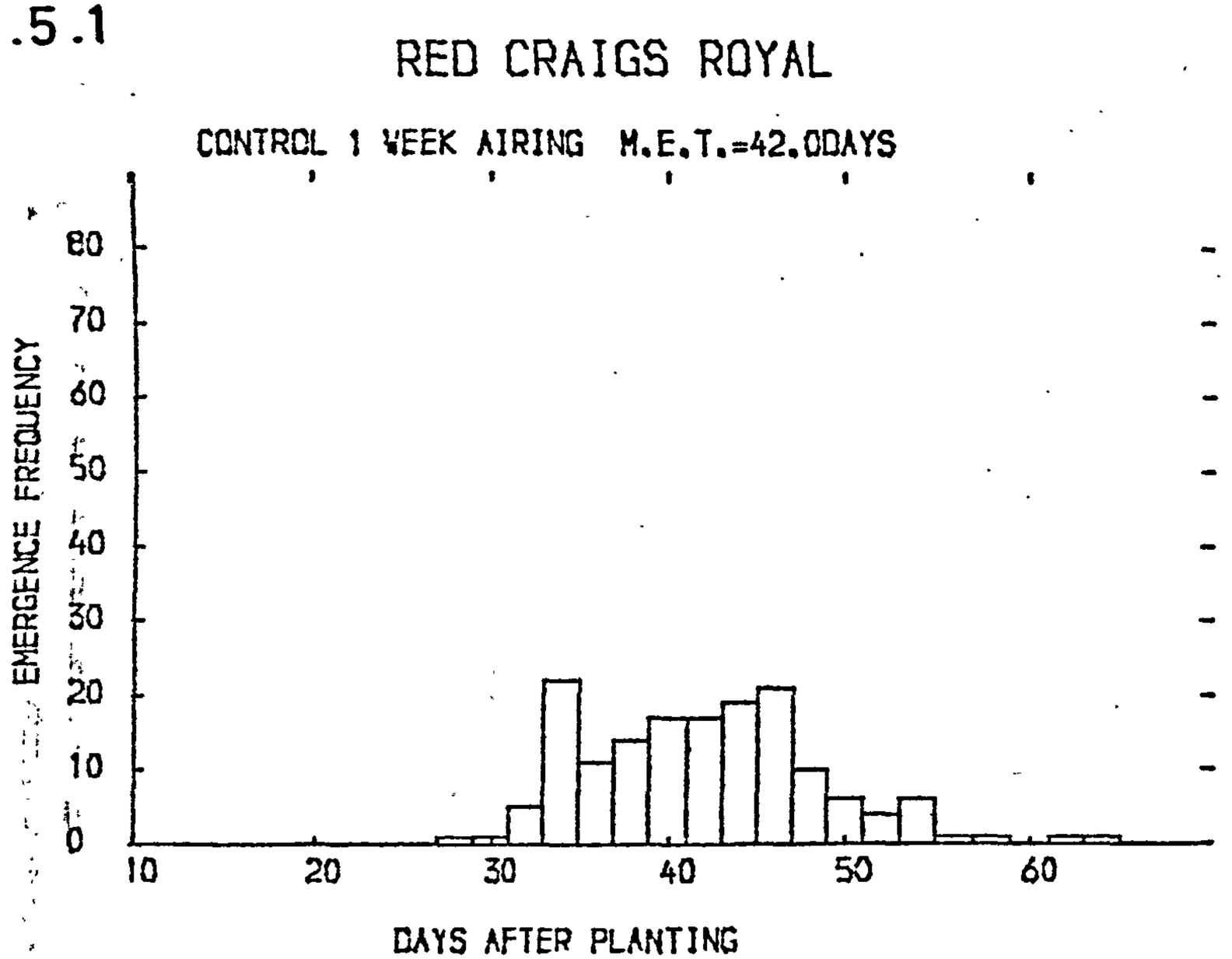
Emergence

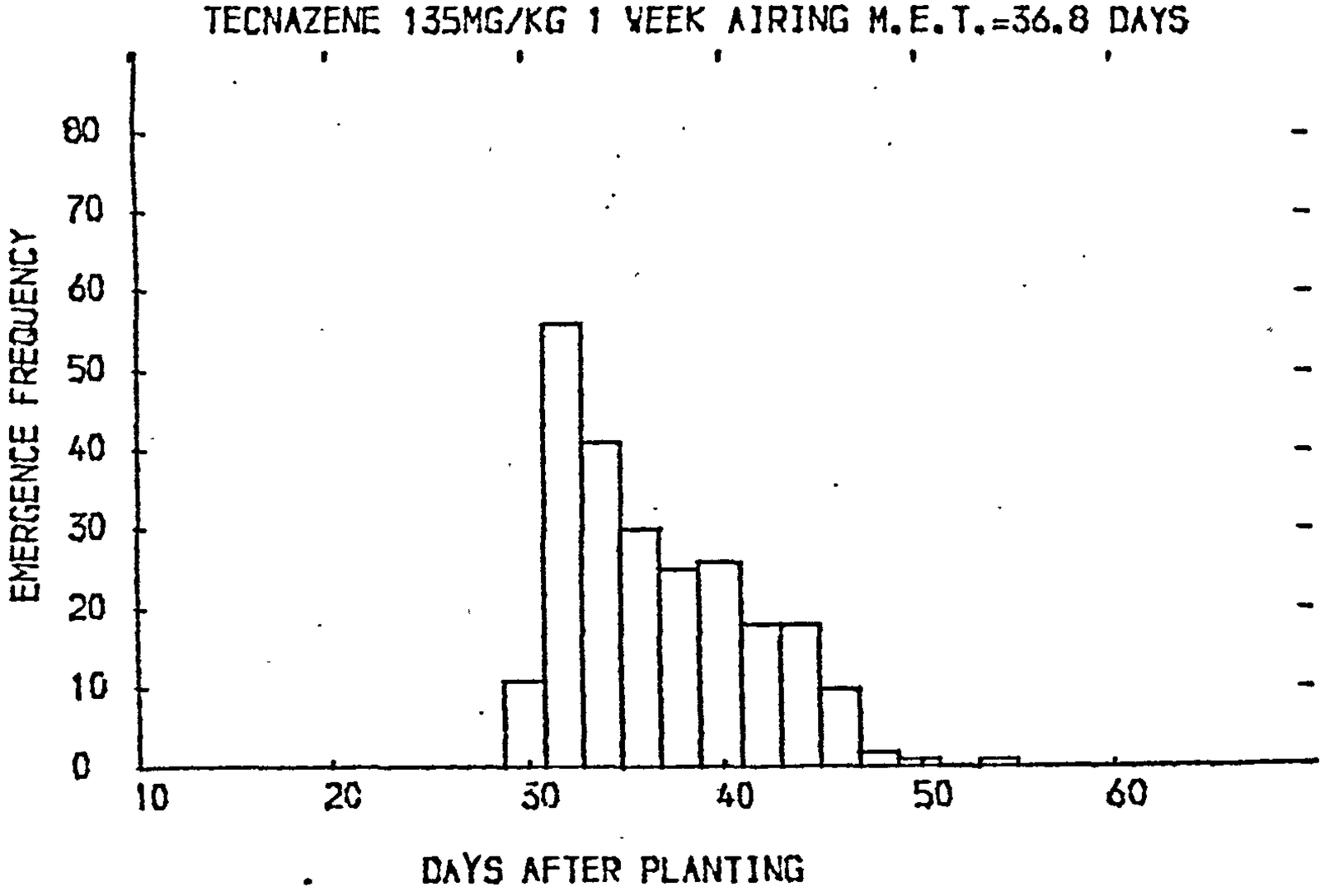
The emergance scores were processed as previously described in section 5.3.3. and the resultant emergence profiles are illustrated in figures 5.5.1. (1 week chitting), 5.5.2. (4 weeks chitting) and 5.5.3. (7 weeks chitting). In this experiment tecnazene treated samples tended to produce more squat emergence profiles. Also obvious are the effects of the prevailing weather conditions. In general it had been noted that in all the experiments dry weather tends to delay emergence and an ensuing wet period will produce an immediate increase in the emergence rate. In this experiment one such wet period commenced on the 40th day after planting and produced increased emergence over the following few days. This is especially apparent in those samples where a large percentage of plants had not emerged before the wet period.

From these pooled frequency distributions the emergence characteristics have been calculated and are reported in table 5.5.1.

FIGURE 5.5.1


MARIS PEER
CONTROL 1 WEEK AIRING M.E.T.=33.6 DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING


RED CRAIGS ROYAL
CONTROL 1 WEEK AIRING M.E.T.=42.0DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING


TECNAZENE 135MG/KG 1 WEEK AIRING M.E.T.=36.8 DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING

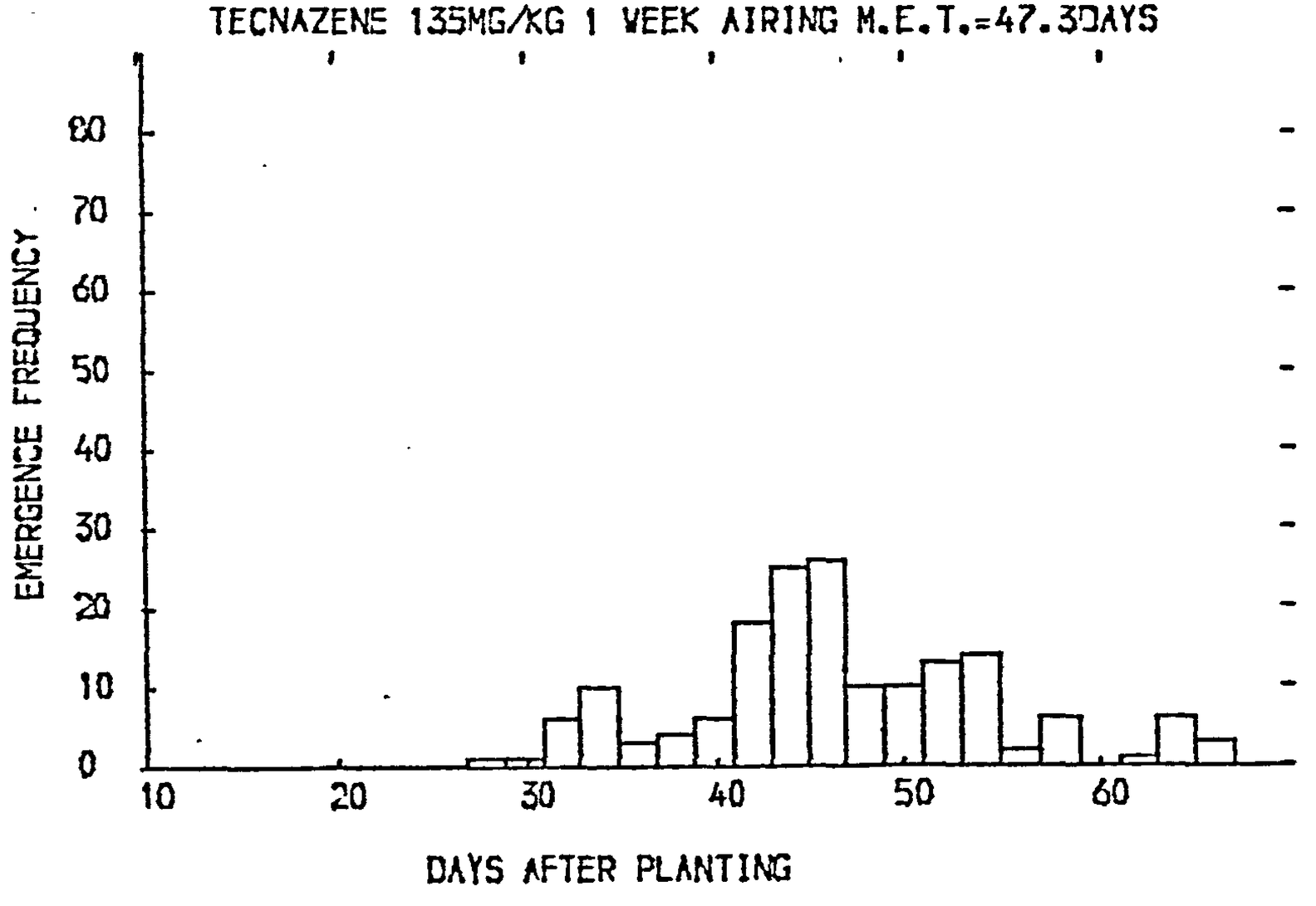

TECNAZENE 135MG/KG 1 WEEK AIRING M.E.T.=47.3DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING

FIGURE. 5.5.2

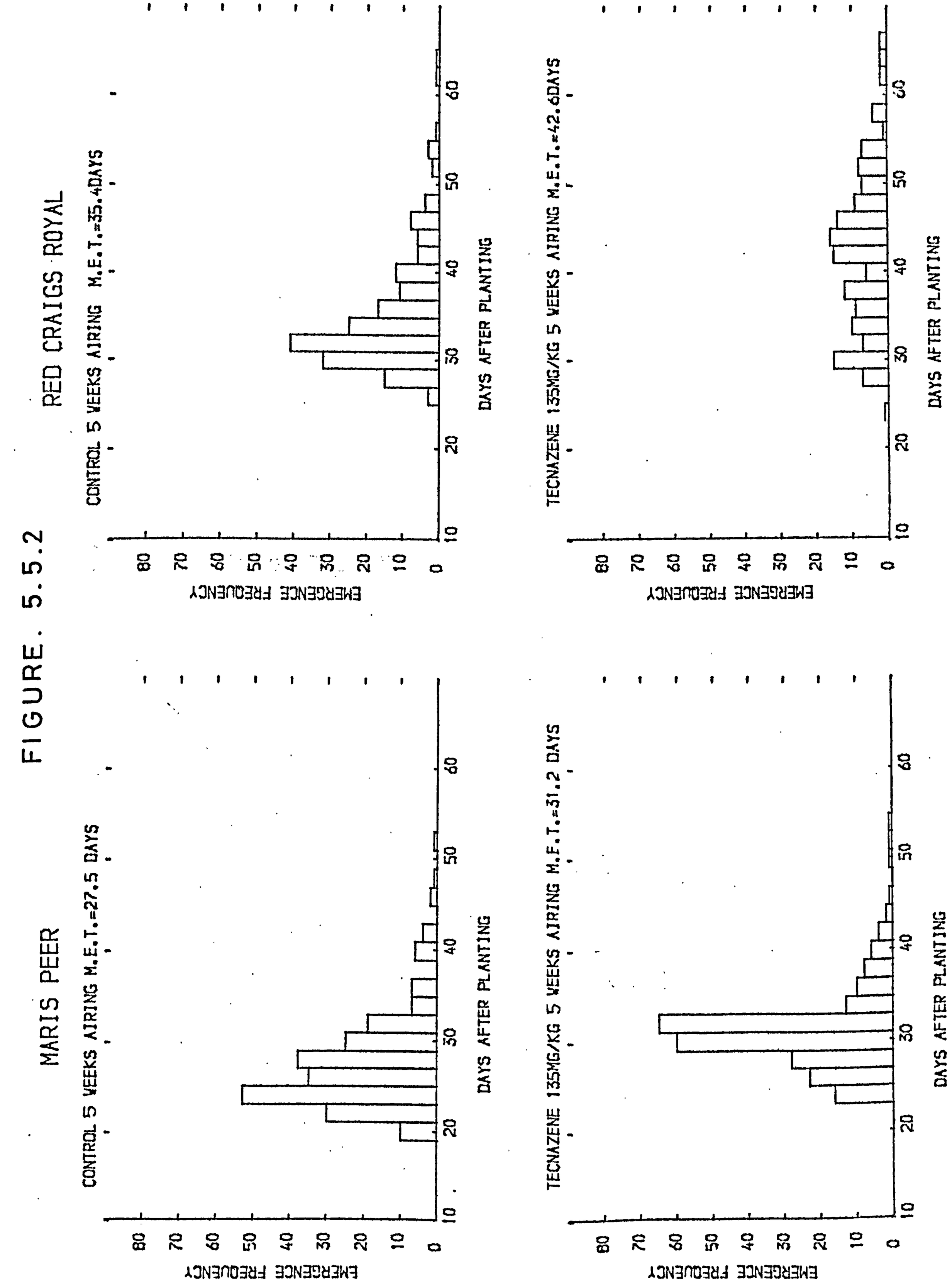
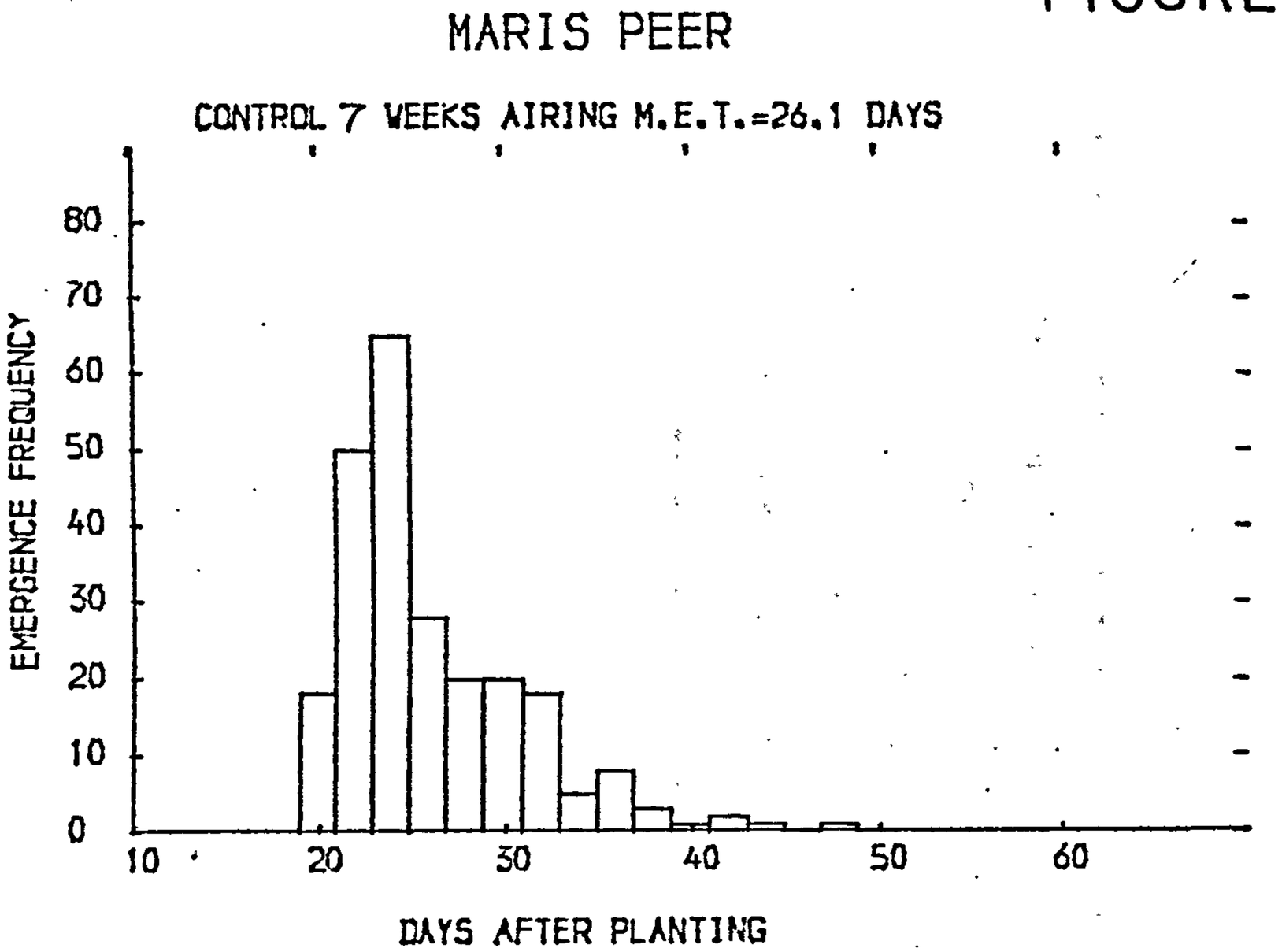
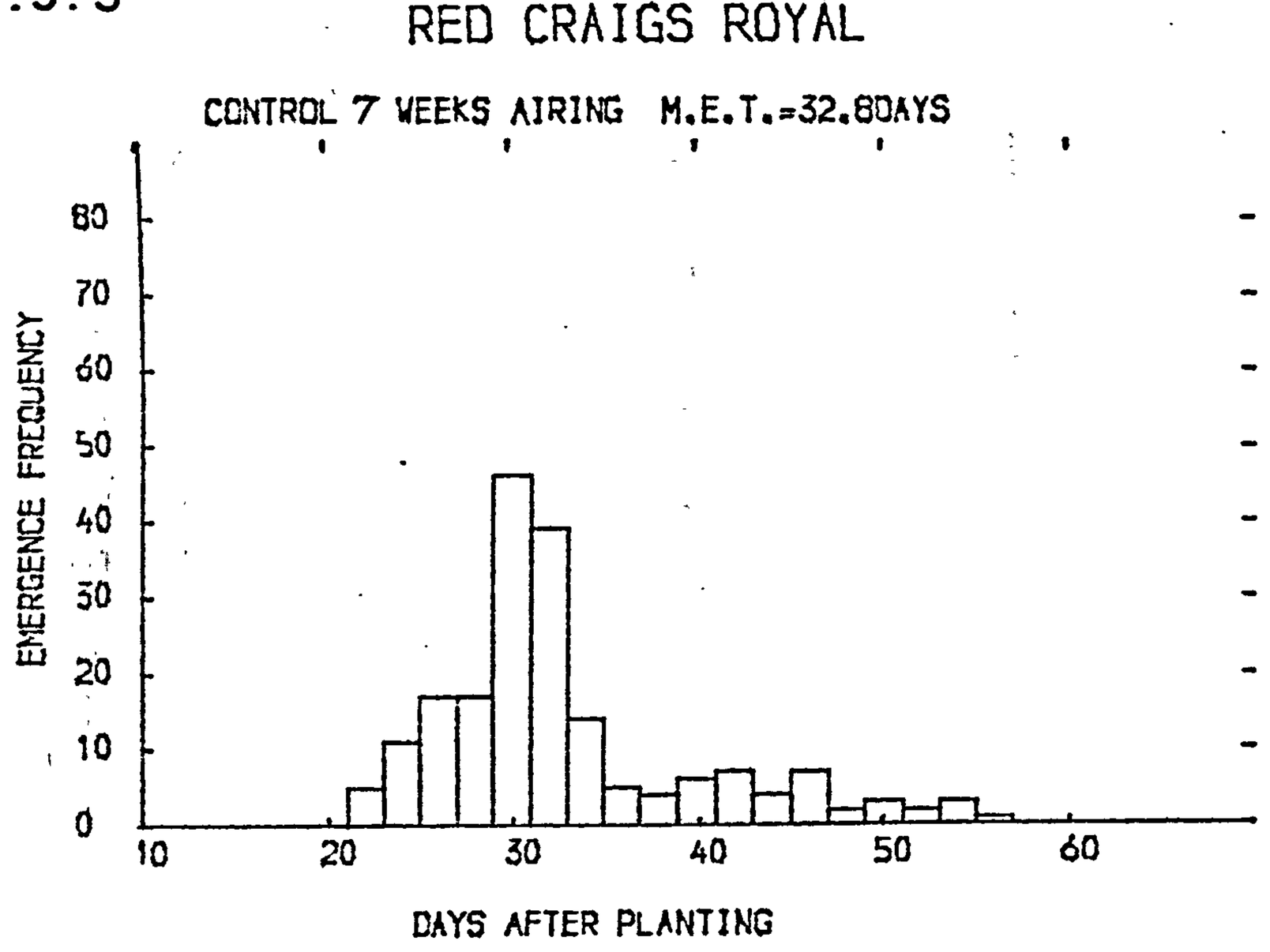

RED CRAIGS ROYAL
CONTROL 5 WEEKS AIRING M.E.T.=35.4DAYS
TECNAZENE 135MG/KG 5 WEEKS AIRING M.E.T.=42.6DAYS
MARIS PEER
CONTROL 5 WEEKS AIRING M.E.T.=27.5 DAYS
TECNAZENE 135MG/KG 5 WEEKS AIRING M.F.T.=31.2 DAYS
EMERGENCE FREQUENCY
DAYS AFTER PLANTING
0
10
20
30
40
50
60
70
80

FIGURE 5.5.3


MARIS PEER
CONTROL 7 WEEKS AIRING M.E.T.=26.1 DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING


RED CRAIGS ROYAL
CONTROL 7 WEEKS AIRING M.E.T.=32.8DAYS
EMERGENCE FREQUENCY
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30
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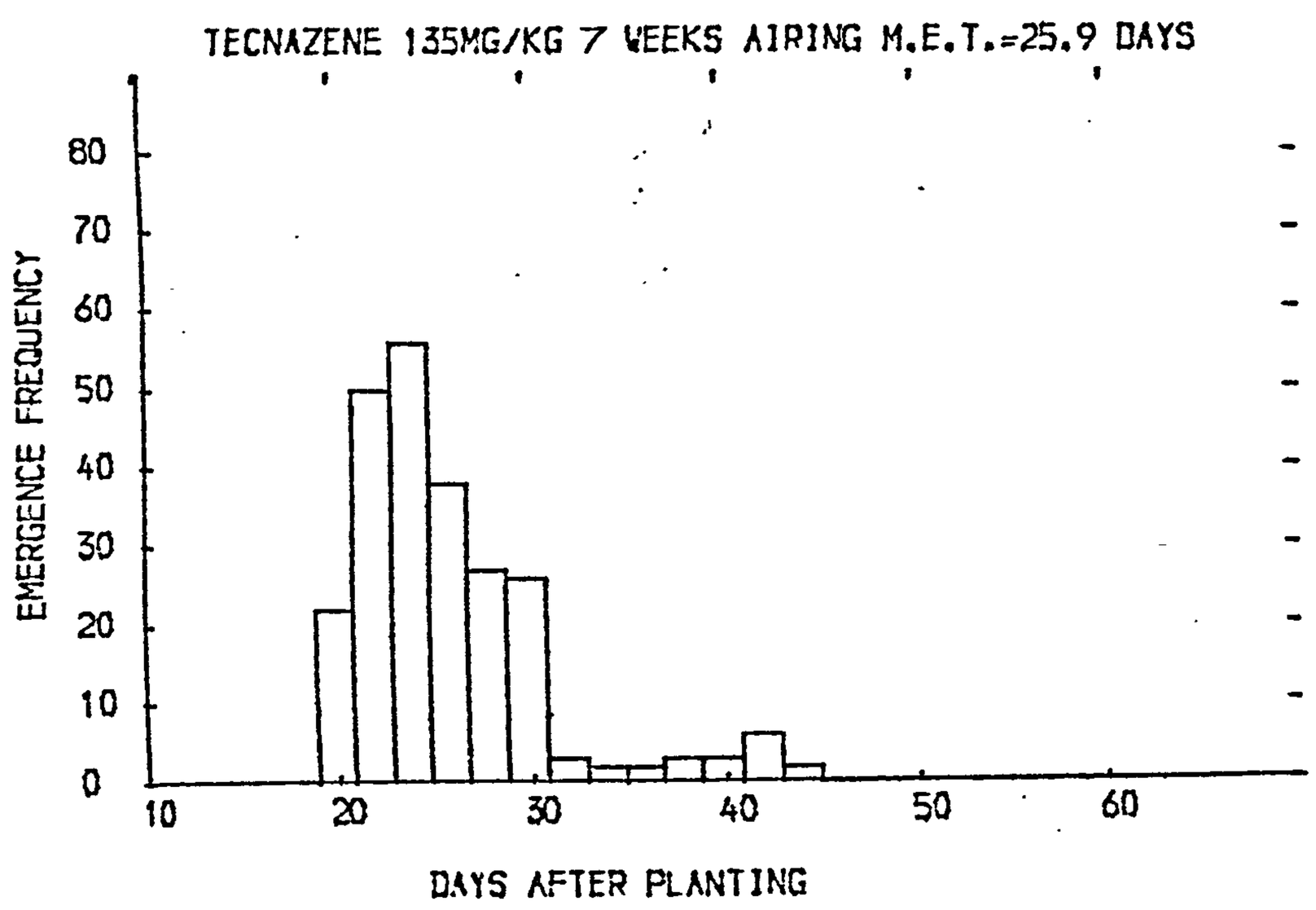
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING


TECNAZENE 135MG/KG 7 WEEKS AIRING M.E.T.=25.9 DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING

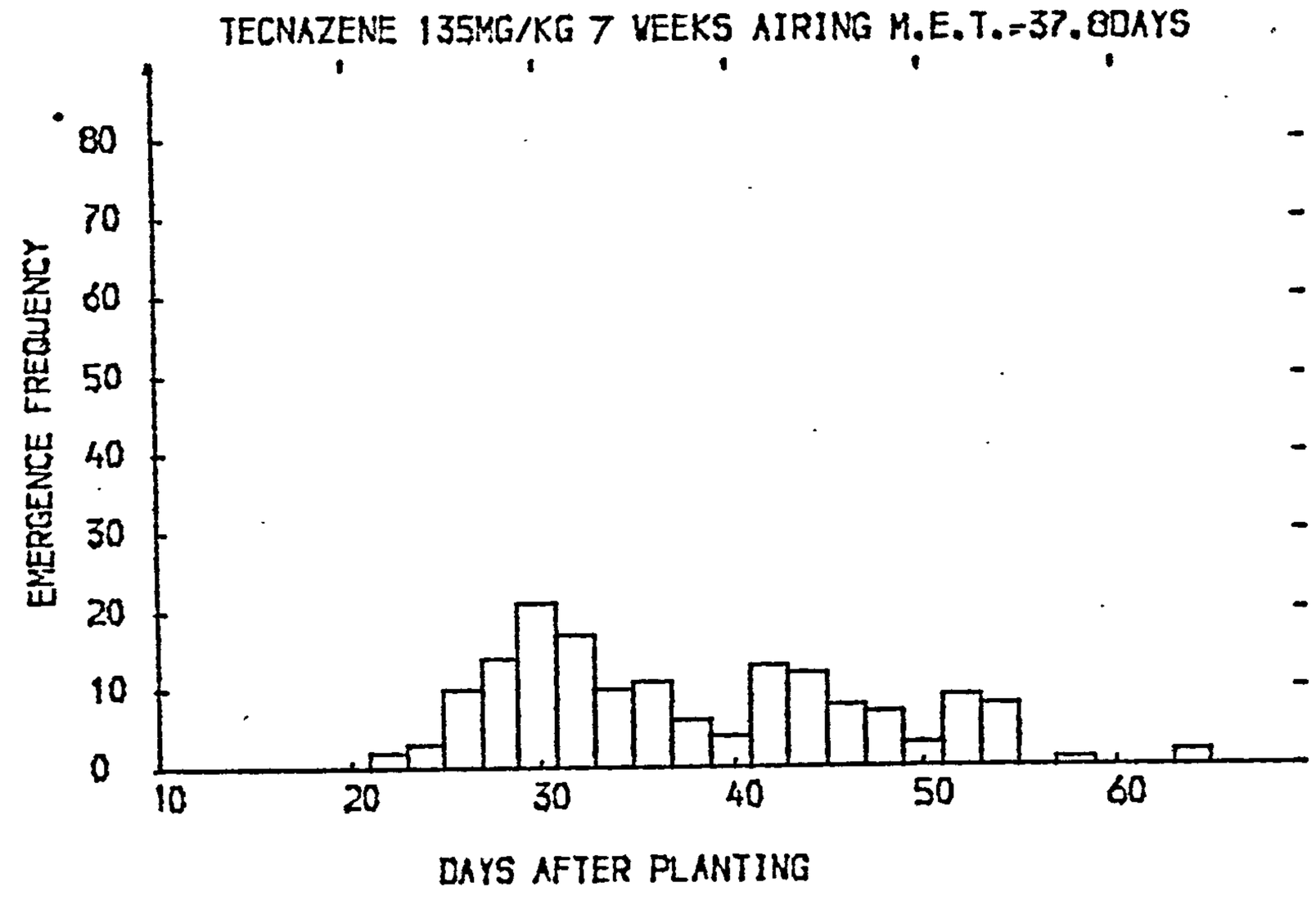

TECNAZENE 135MG/KG 7 WEEKS AIRING M.E.T.=37.8DAYS
EMERGENCE FREQUENCY
0
10
20
30
40
50
60
70
80
10
20
30
40
50
60
DAYS AFTER PLANTING

Table 5.5.1 The effect of storage conditions on the subsequent emergence behaviour of tecnazene treated seed potatoes using data pooled from replicate treatments (1976-77) (Cultivars - Maris Peer and Red Craigs Royal)

Cultivar	Treatment	Chitting time[a] (weeks)	Emergence characteristics			
			MET[b] (days)	T50[c] (days)	T75[d] (days)	Total emergence[e] (%)
Maris Peer	control	1	33.6	32.3	34.8	99.2
	tecnazene	1	36.8	35.8	40.3	99.6
	control	4	27.5	26.5	30.1	99.2
	tecnazene	4	31.2	30.8	32.6	99.6
	control	7	26.1	24.6	28.9	100.0
	tecnazene	7	25.9	24.7	28.0	100.0
Red Craigs Royal	control	1	42.0	44.5	54.7	77.5
	tecnazene	1	47.3	47.4	58.3	80.9
	control	4	35.4	33.9	40.5	92.1
	tecnazene	4	42.6	45.6	66.0	75.5
	control	7	32.8	31.3	36.6	94.6
	tecnazene	7	37.8	41.6	53.8	78.9

a. Airing at 10°C with illumination.

b. Mean emergence time.

c. Time to 50% emergence.

d. Time to 75% emergence.

e. Total emergence as a percentage of tubers planted.

The relationship between chitting period and emergence behaviour is further illustrated in figures 5.5.4. (mean emergence time), 5.5.5. (time to 50% emergence) and 5.5.6. (total emergence). In these diagrams the means of each treatment for replicate drills have been plotted with their associated standard errors thus indicating the variation between individual drills.

Figures 5.5.4. (MET) and 5.5.5. (T50) both indicate that for the cultivar Maris Peer, the substantial difference between the emergence of treated samples and controls at the shorter chitting periods has been removed by the 7 week period. The situation with respect to Red Craigs Royal is however more complex because of the poor total emergence (figure 5.5.6.), and interpretation of these results is postponed until the results have been examined statistically.

The individual drill data was first subjected to an analysis of variance and the resultant F statistics are reported in table 5.5.2.

These figures indicate that cultivar, treatment and chitting time all have a highly significant effect on MET, T50 and total emergence. Of the first order interactions the one for cultivar x treatment is highly significant for all three variables. The interaction with TE is especially important since it is caused by the very poor emergence of the cultivar Red Craigs Royal. Subsequent yield and size distribution data for this cultivar must be treated with caution.

In an attempt to further quantify the relationship between the chitting period and the emergence characteristics (MET, T50 and total emergence) a regression search was conducted amongst various transformations as previously described. Although none of these transformations produced a substantial improvement in the correlation coefficient it is worth mentioning that there were indications that the first few weeks of airing produce a greater effect than subsequent weeks, in that either log x or x raised to a fractional power produced some improvement (see also figures 5.5.4. and 5.5.5.). However as stated earlier, unless any transformation produced an improvement of more than 0.05 in the correlation coefficient then linearity was assumed. It is therefore the parameters of the simple linear model $y = \alpha + \beta x$ which are reported, for MET, T50 and total emergence, in table 5.5.3., with their associated correlation coefficients.

FIGURE 5.5.4 THE EFFECT OF STORAGE CONDITIONS ON THE MEAN EMERGENCE TIME OF TECNAZENE TREATED SEED POTATOES

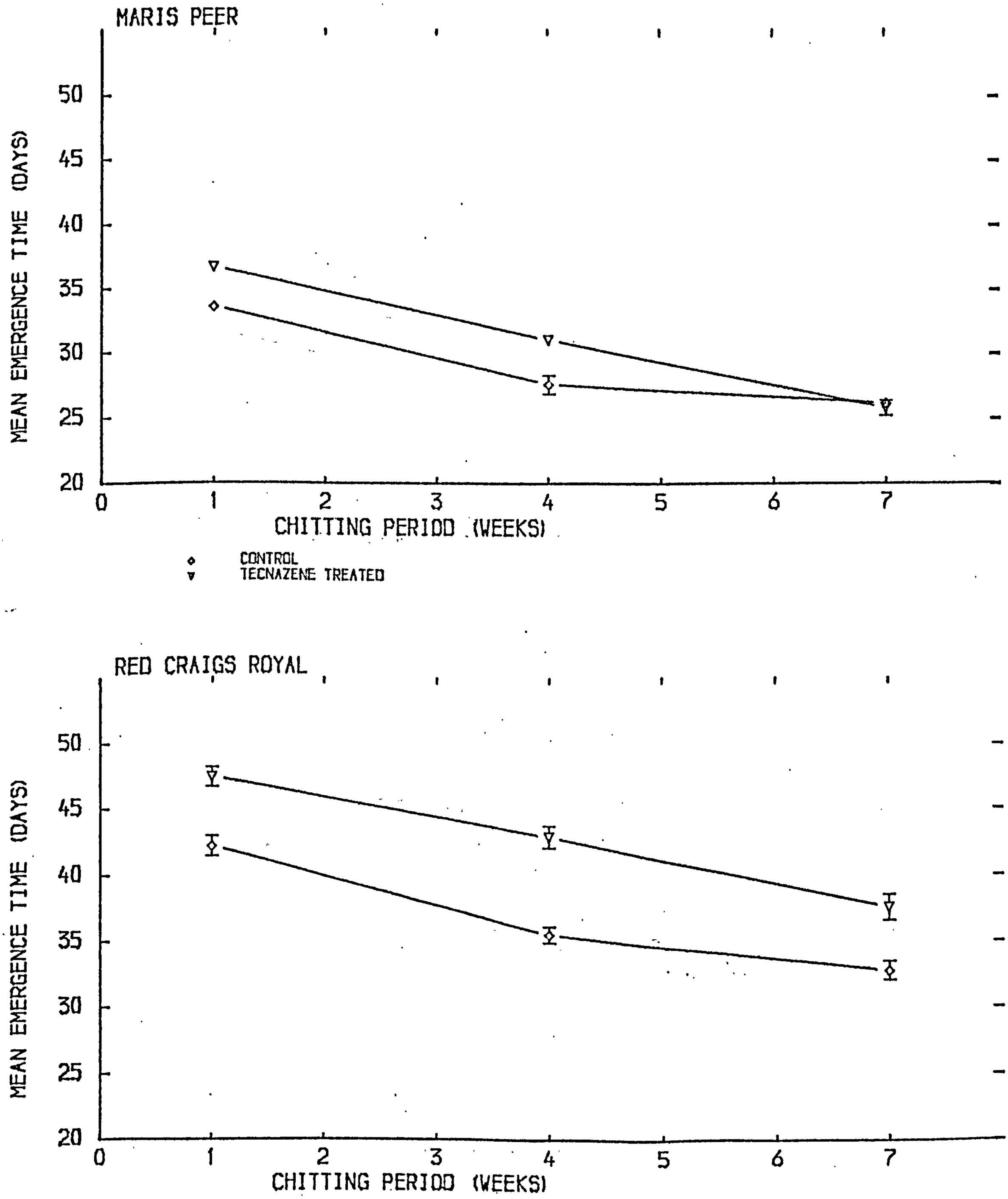


FIGURE 5.5.5 THE EFFECT OF STORAGE CONDITIONS ON THE TIME TO 50% EMERGENCE OF TECNAZENE TREATED SEED POTATOES

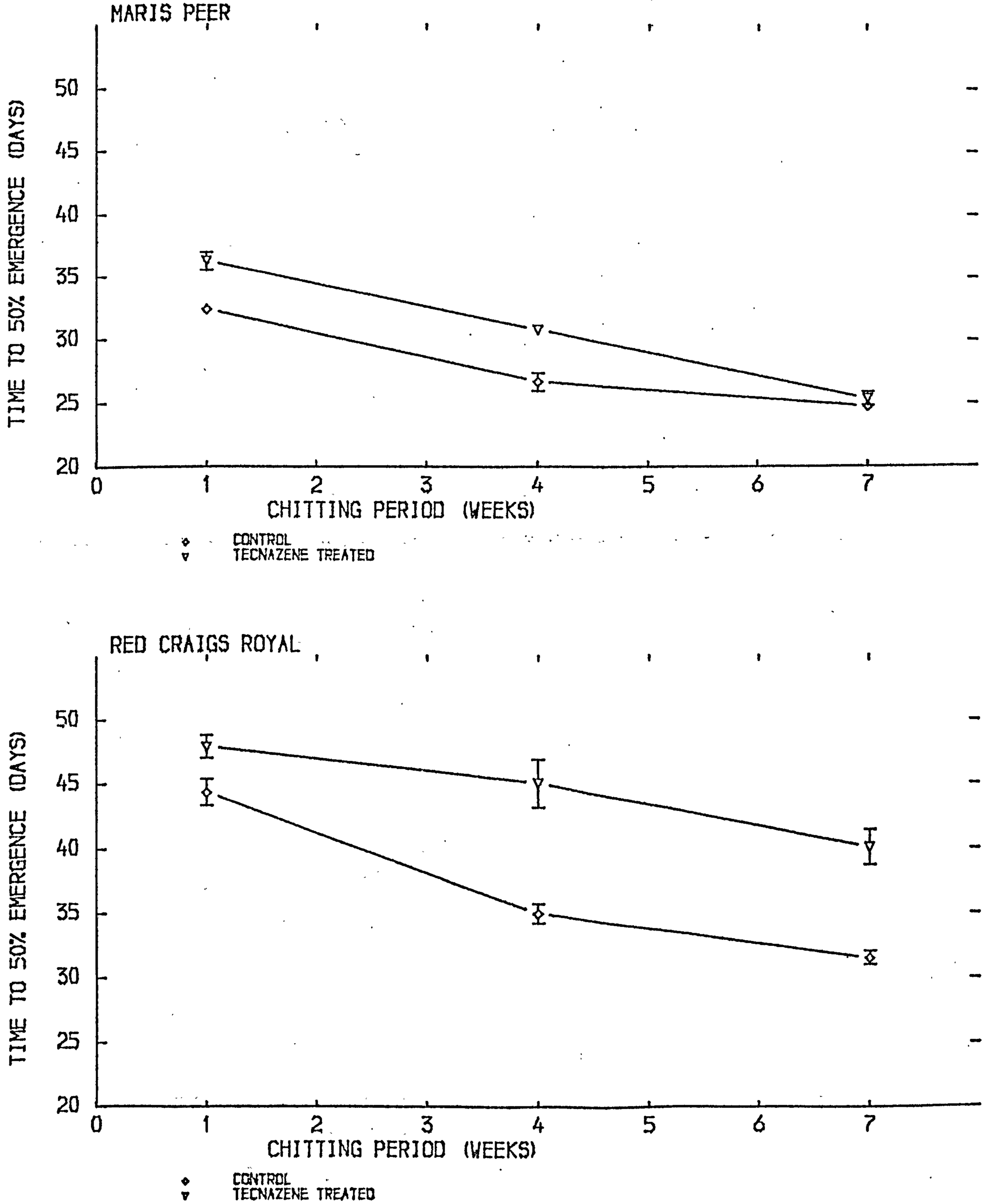


FIGURE 5.5.6 THE EFFECT OF STORAGE CONDITIONS ON THE TOTAL EMERGENCE OF TECNAZENE TREATED SEED POTATOES

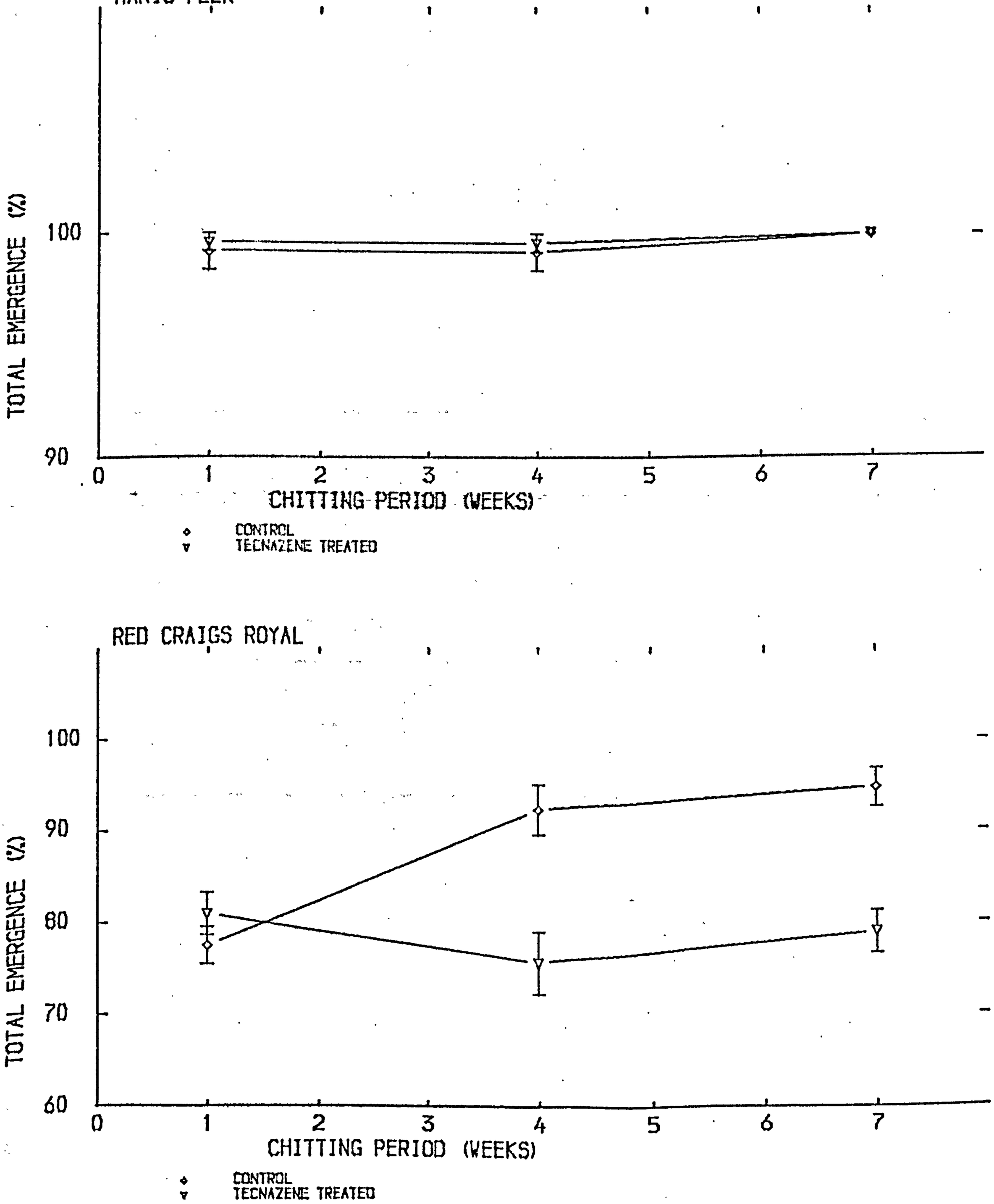


Table 5.5.2. F statistics[e] from an analysis of variance on the effect of tecnazene on the subsequent emergence after various chitting periods (1976-77)

Source of variation	Degrees of freedom[d]	Emergence characteristic		
		MET	T50	Total emergence
Cultivar (C)[a]	1	642.1	476.6	239.9
Treatment (T)[b]	1	112.5	98.7	19.7
Chitting (Ch)[c]	2	209.4	121.8	5.1
C x T	1	22.7	19.1	22.1
C x Ch	2	0.2	0.3	3.8
T x Ch	2	6.0	3.9	9.8
C x T x Ch	2	1.4	5.8	9.4

a. Maris Peer, Red Craigs Royal.

b. Control, tecnazene.

c. 1 week, 4 weeks, 7 weeks.

d. Within replicates = 132 (denominator).

e. Least significant values of the F statistic.

5% level	1% level
F(1,132) = 3.9	F(1,132) = 7.0
F(2,132) = 3.1	F(2,132) = 4.9

Table 5.5.3. Parameters of the linear model ($y = \alpha + \beta x$) describing the relationship between chitting period (x) and the emergence characteristics (y) of the cultivars Maris Peer and Red Craigs Royal (1976-77)

Variable	Cultivar	Treatment	Intercept (α)	Gradient (β)	Correlation coefficient
Mean emergence time	MP	control	34.0	-1.24	-0.83*
		tecnazene	38.6	-1.82	-0.94*
	RCR	control	43.1	-1.56	-0.84*
		tecnazene	49.1	-1.63	-0.81*
Time to 50% emergence	MP	control	33.0	-1.28	-0.88*
		tecnazene	38.1	-1.82	-0.93*
	RCR	control	45.4	-2.13	-0.87*
		tecnazene	49.5	-1.29	-0.55*
Total emergence	MP	control	98.9	0.14	0.14
		tecnazene	99.4	0.07	0.15
	RCR	control	76.7	2.86	0.65*
		tecnazene	79.7	-0.32	-0.09

* The probability of obtaining a more extreme value for the correlation coefficient is less than 0.001 if the true correlation coefficient is zero.

The slopes of these lines indicate that increased chitting periods will improve the mean emergence times of both control and tecnazene treated samples, but the effect is greater in the latter.

Chitting time has no significant effect on the total emergence of the cultivar Maris Peer (see also figure 5.5.6.). However, in the case of Red Craigs Royal a highly significant effect is apparent amongst the control samples. This can be accounted for by the removal of existing sprouts when the samples were placed in chitting trays. Those which were chitted for 4 or 7 weeks were desprouted earlier and retained more of their energy reserves for the growth of new sprouts, and had a substantial period for recovery.

In the case of the tecnazene treated seed of this cultivar there is no relationship between chitting time and % emergence.

Yield and size distribution

The yield data was first carefully examined in various ways to check whether the data from the outer drills of each plot could be included.

1) The computer programme written for the preliminary analysis of yield and size distribution data also prints maps of the experimental area with the appropriate yield or size distribution data inserted in each drill of each plot. Careful examination of these maps revealed no reason for not including the data.

2) Similarly examination of column means for sets of three adjacent drills corresponding to a set of plots revealed no substantial or systematic effects.

3) An analysis of variance incorporating drill effects showed them to be non-significant.

4) The outer drills were classified as being adjacent to a plot with below or above the ground mean for the cultivar and the yields of the two groups expressed as a percentage of the yield of the centre drill were subjected to a t-test which proved non-significant. Thus taking into account treatment effects and locality effects there is no advantage in a drill being adjacent to a low yielding drill.

These findings can be accounted for in that:

1) The nutrient status of soil was high and unlikely to be a limiting factor.

2) The water deficit of the soil during the experiment never gave cause for concern.

3) The cultivars chosen do not produce very large plants so light is unlikely to be a limiting factor.

4) The effects produced by the various treatments were relatively small.

It was therefore concluded that the outer drill could be safely included giving 12 replicate drills per treatment.

From the recorded data the means of the seven yield and size distribution variables were calculated and are reported in table 5.5.4.

All seven variables have been plotted against chitting period in figures 5.5.7. (yield of large tubers), 5.5.8. (yield of seed-sized tubers), 5.5.9. (yield of small tubers), 5.5.10. (total yield), 5.5.11. (%large), 5.5.12. (% seed-sized) and 5.5.13. (% small). Standard error bars have been included to indicate the variability of the data.

The seven variables were also subjected to an analysis of variance and the resultant F statistics are shown in table 5.5.5. These, however, must be interpreted with caution because of the poor total emergence of the cultivar Red Craigs Royal. They do, however, indicate that cultivar and treatment account for most of the variation in the results. Chitting time has a smaller but significant effect on the yield of large tubers, total yield and the size distribution variables. The treatment-chitting period interaction is significant for the yield of large tubers and total yield i.e. chitting period has a different effect on tecnazene treated samples to that produced in the controls.

To further quantify the relationship between chitting period and the seven variables a regression search was conducted amongst various transformations. However as none of these produced the required improvement in correlation coefficient, and as estimations were made at only 3 chitting periods, it was decided to use simple linear regressions, the parameters of which are reported in table 5.5.6. for all seven variables. Again caution is demanded when interpreting the results from the cultivar Red Craigs Royal.

Table 5.5.4 The effect of storage conditions on the subsequent yield and size distribution of tecnazene treated seed potatoes(1976-77) (Cultivars - Maris Peer and Red Craigs Royal)

Treatment	Chitting time (weeks)	Yield per drill[a]				Size distribution[b]		
		Large[c] (kg)	Medium[d] (kg)	Small[e] (kg)	Total (kg)	Large (%)	Medium (%)	Small (%)
1. Maris Peer								
control	1	11.1	3.7	0.1	14.9	74.2	24.9	0.9
tecnazene	1	7.3	7.1	0.5	14.9	48.4	48.2	3.5
control	4	11.6	4.0	0.2	15.9	73.3	25.4	1.4
tecnazene	4	7.2	7.2	0.5	14.9	48.0	48.8	3.3
control	7	12.9	2.8	0.2	15.8	81.1	17.9	1.0
tecnazene	7	9.8	6.5	0.4	16.7	58.5	39.1	2.4
2. Red Craigs Royal								
control	1	9.9	1.5	0.1	11.5	86.5	12.8	0.7
tecnazene	1	10.0	2.8	0.1	12.9	77.4	21.8	0.8
control	4	13.1	2.5	0.1	15.6	83.6	15.7	0.7
tecnazene	4	8.3	3.1	0.2	11.6	72.0	26.3	1.7
control	7	11.8	2.6	0.1	14.5	80.7	18.6	0.7
tecnazene	7	10.3	3.4	0.2	13.9	74.0	24.4	1.6

a. Mean of 12 replicate drills (4 plots x 3 drills).

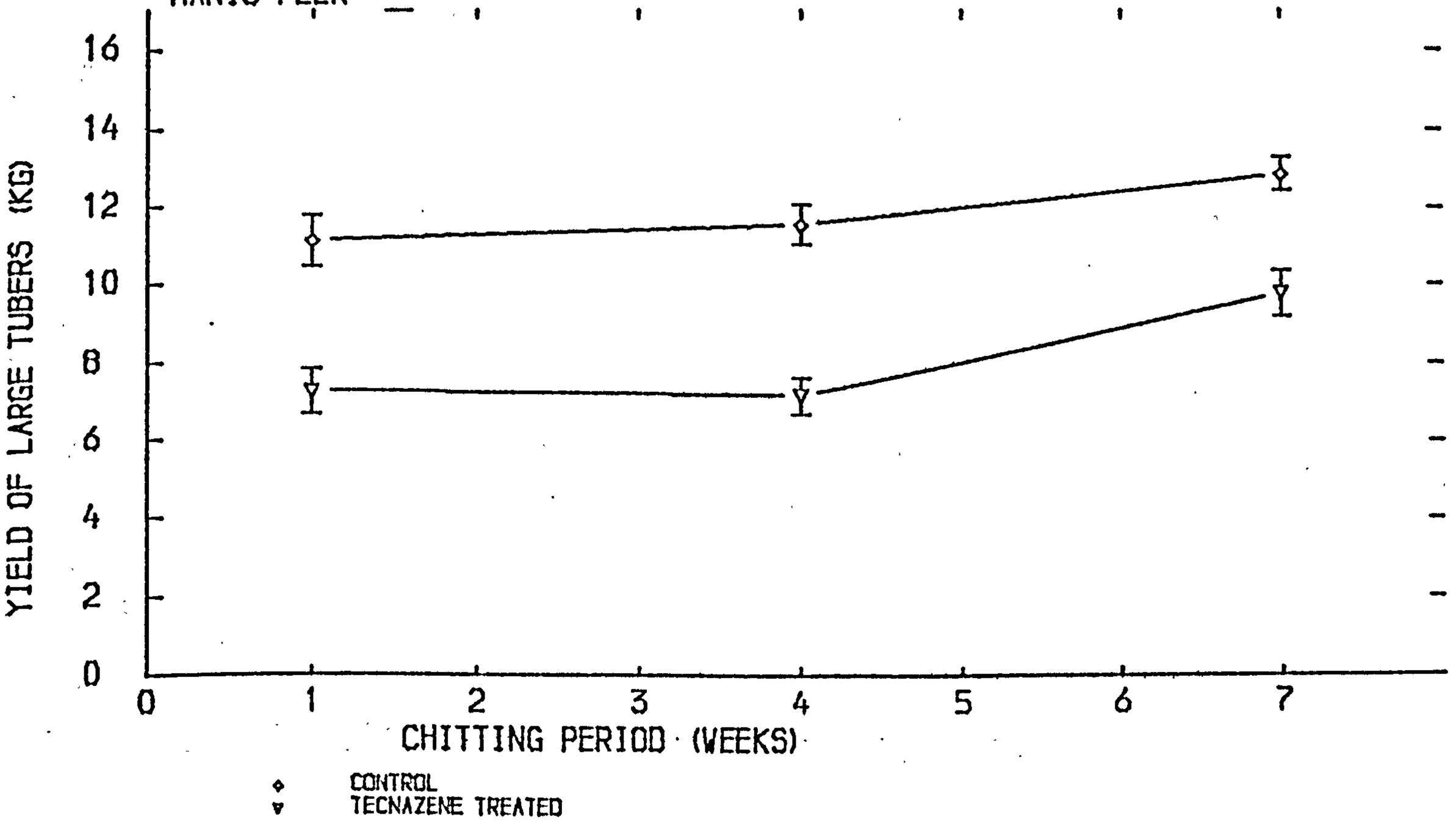
b. Expressed as a % of total yield.

c. > 50mm.

d. 32 - 50mm.

e. < 32mm.

FIGURE 5.5.7 THE EFFECT OF STORAGE CONDITIONS ON THE YIELD OF LARGE TUBERS CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL



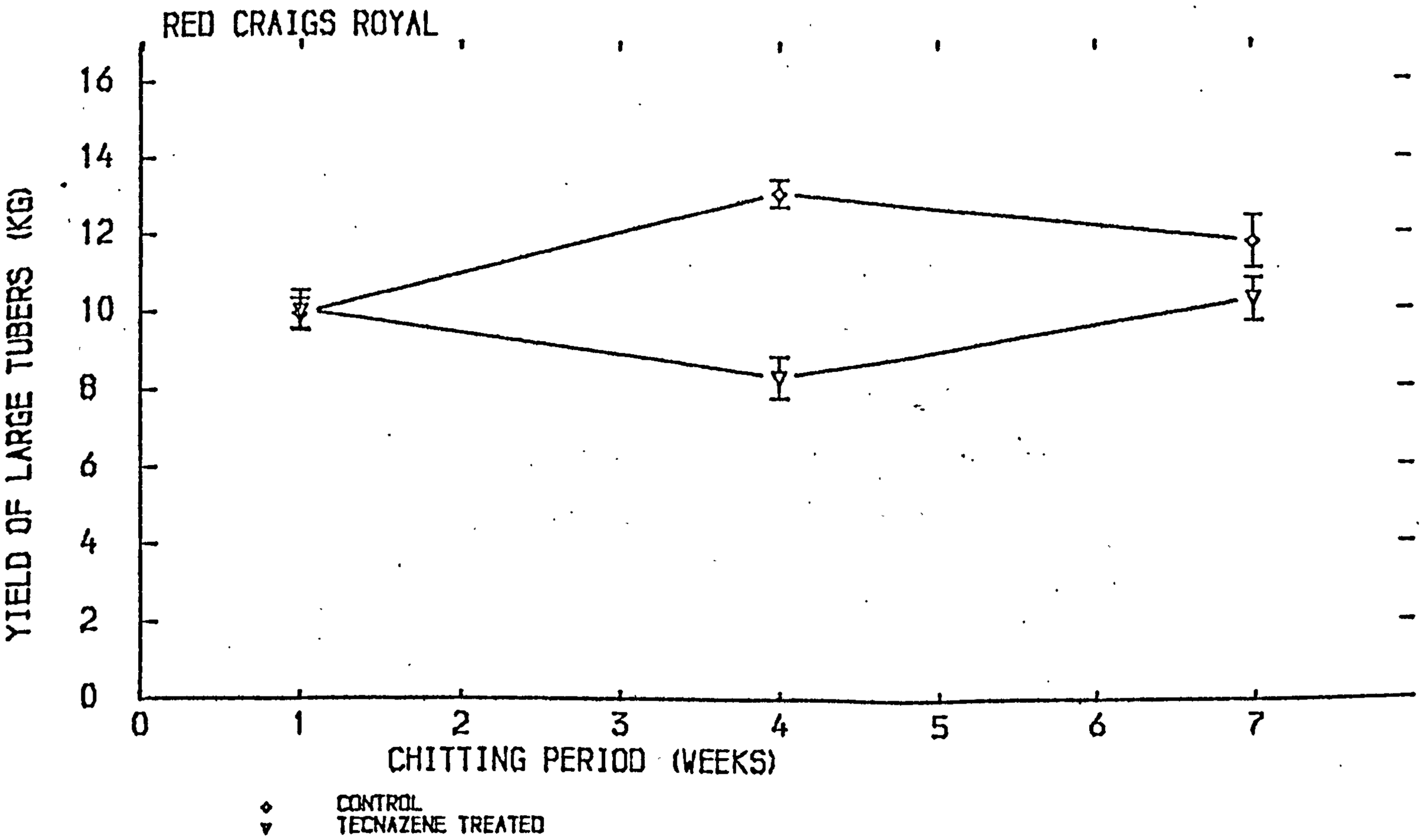


FIGURE 5.5.8 THE EFFECT OF STORAGE CONDITIONS ON THE YIELD OF MEDIUM TUBERS CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

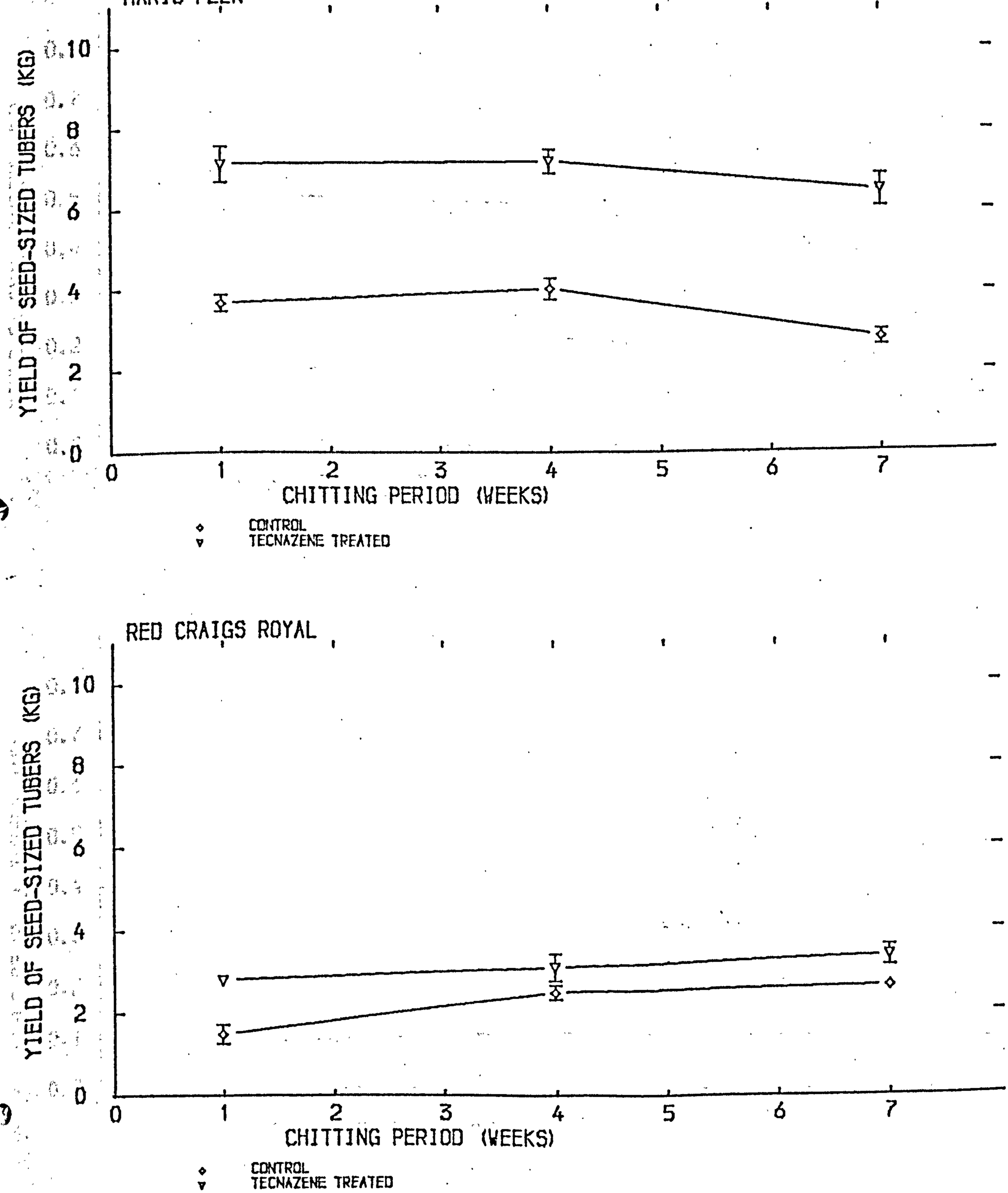


FIGURE 5.5.9 THE EFFECT OF STORAGE CONDITIONS ON THE YIELD OF SMALL TUBERS CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

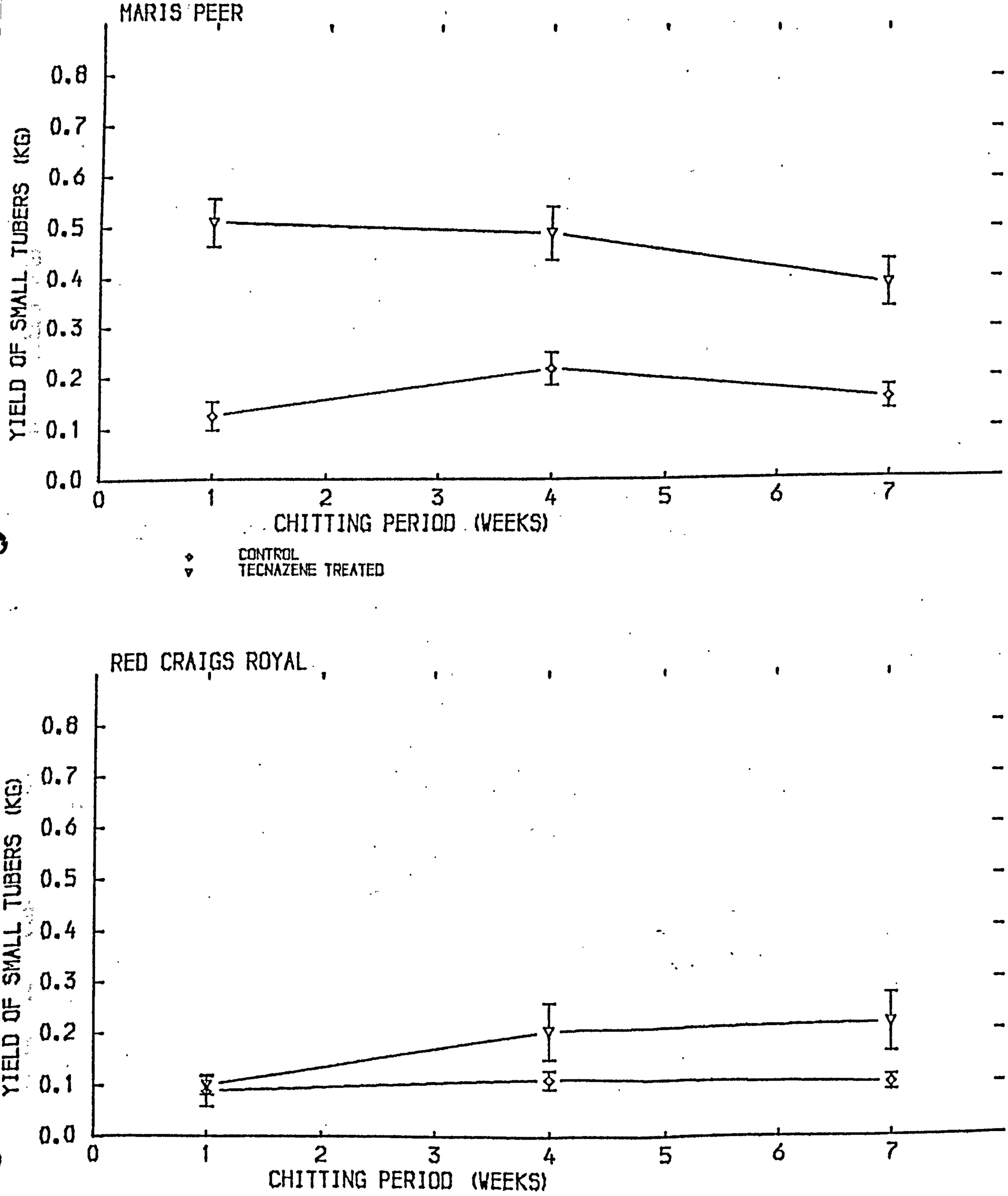


FIGURE 5.5.10 THE EFFECT OF STORAGE CONDITIONS ON THE TOTAL YIELD CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

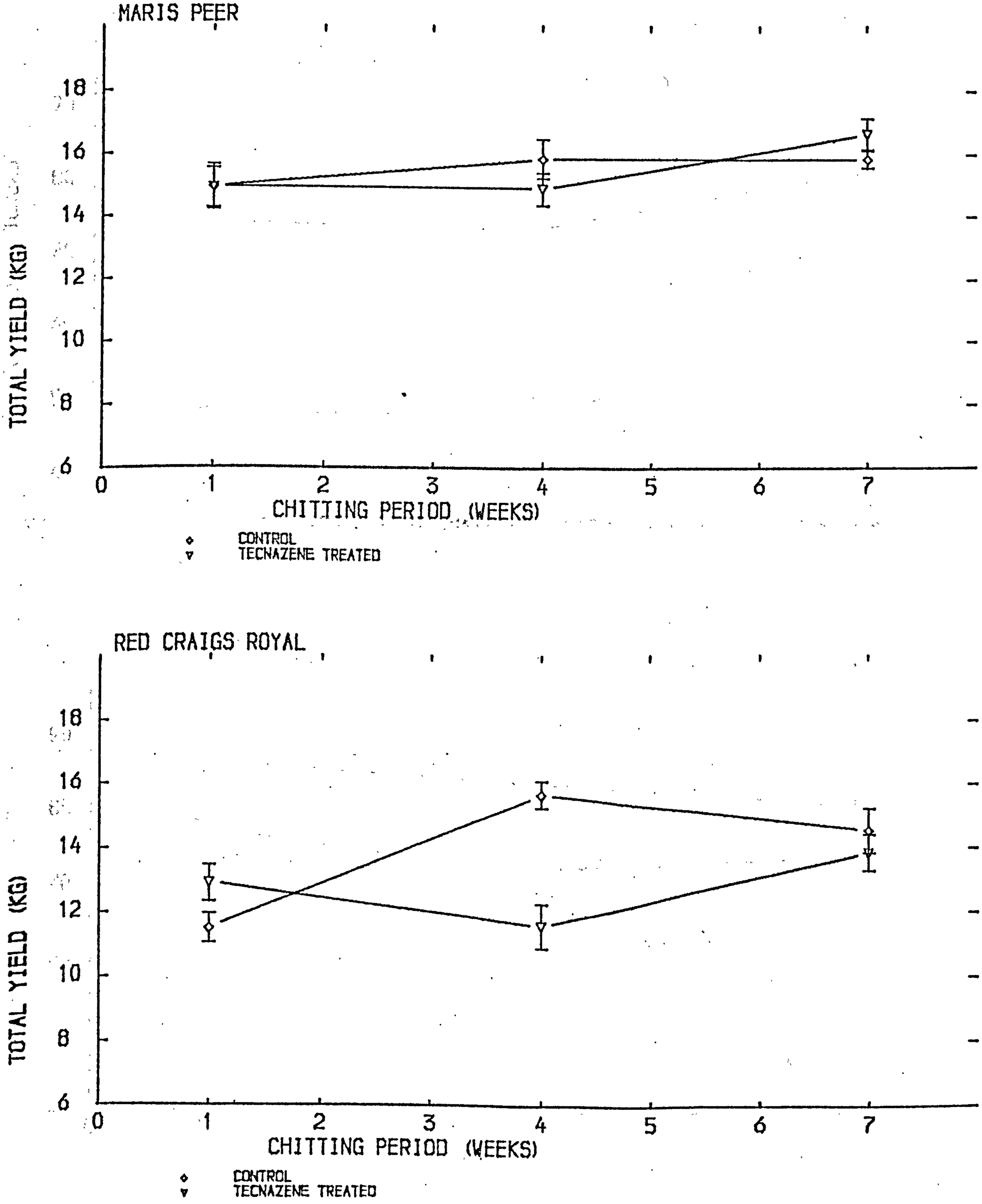


FIGURE 5.5.11 THE EFFECT OF STORAGE CONDITIONS ON THE PERCENTAGE OF LARGE TUBERS. CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

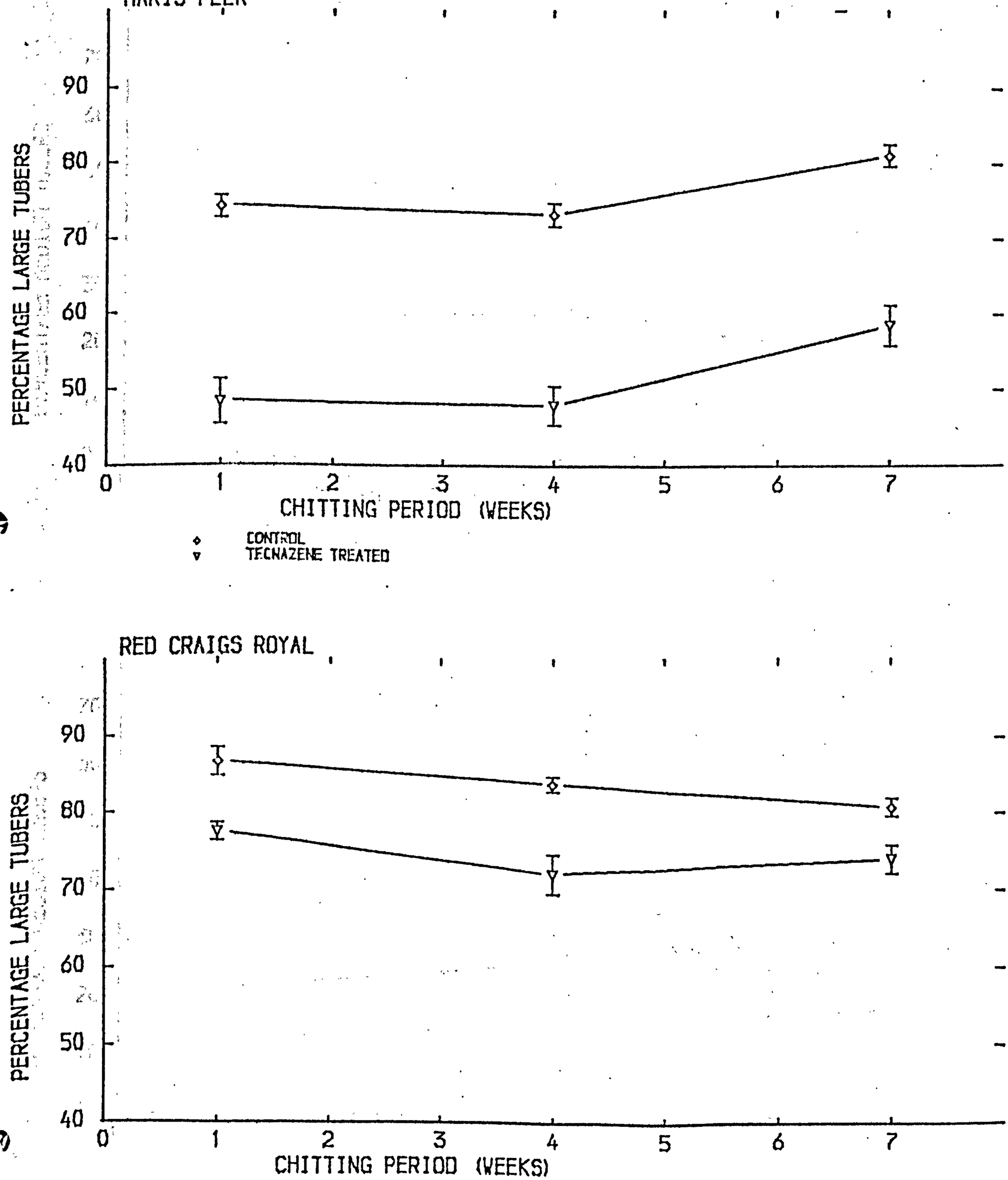


FIGURE 5.5.12 THE EFFECT OF STORAGE CONDITIONS ON THE PERCENTAGE OF MEDIUM TUBERS. CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

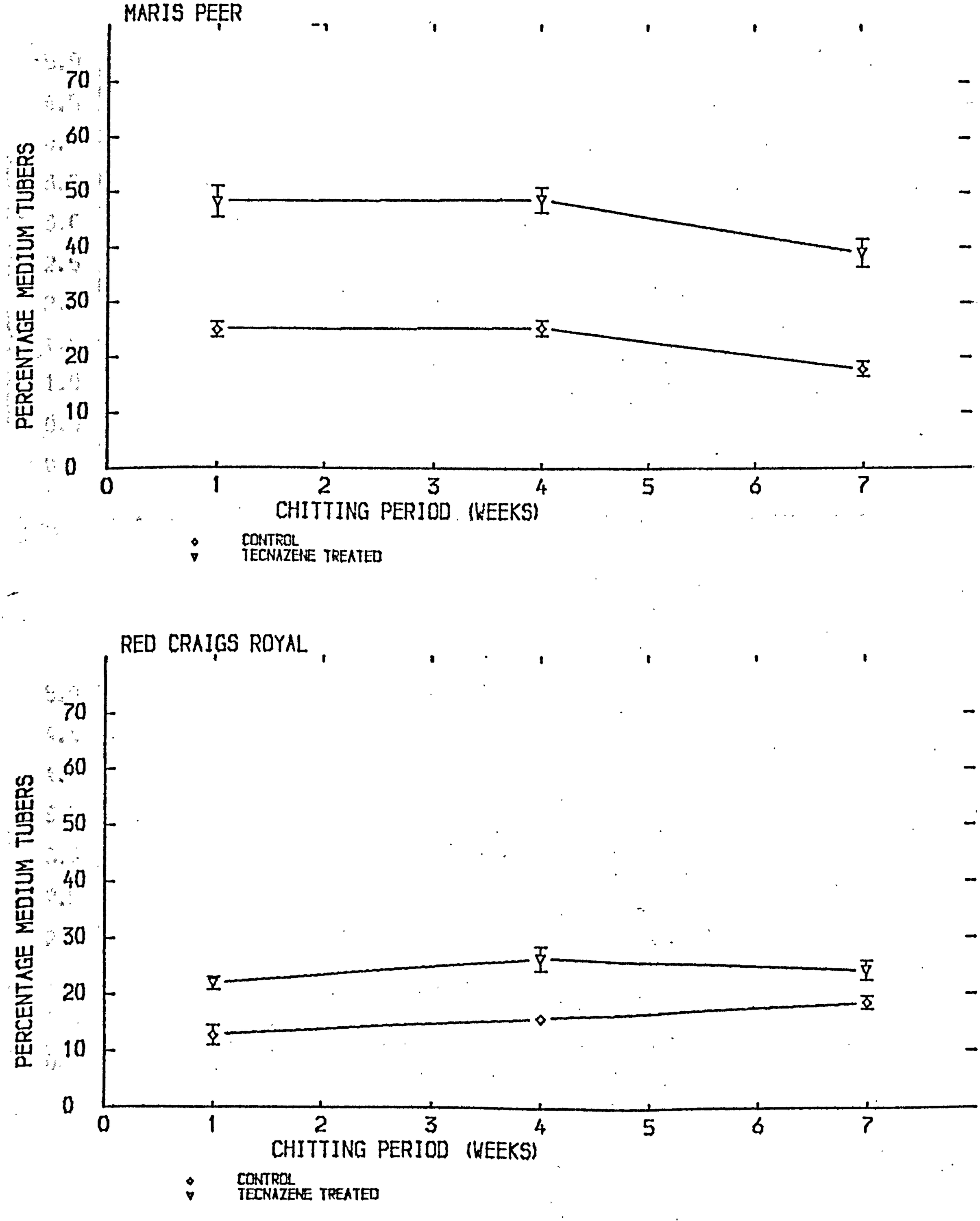


FIGURE 5.5.13 THE EFFECT OF STORAGE CONDITIONS ON THE PERCENTAGE OF SMALL TUBERS. CULTIVARS - MARIS PEER AND RED CRAIGS ROYAL

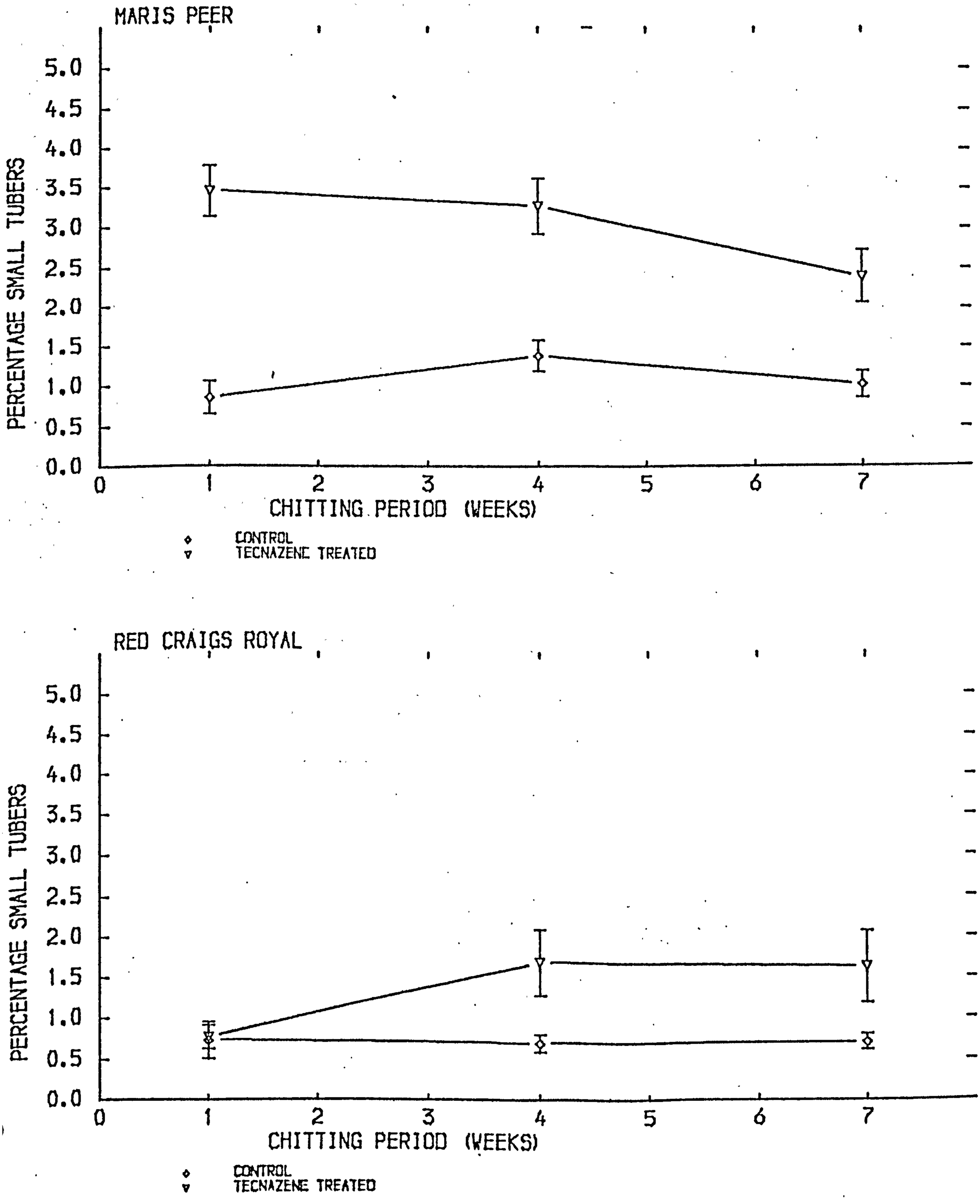


Table 5.5.5 F statistics[e] from an analysis of variance on the effect of tecnazene on yield and size distribution (1976-77)

Source of variation	Degrees of freedom[d]	Yield				Size distribution		
		Large	Medium	Small	Total	%Large	%Medium	%Small
Cultivar(C)[a]	1	3.6	273.6	65.0	44.4	178.2	181.2	43.0
Treatment(T)[b]	1	90.1	188.7	69.6	3.4	221.5	220.8	68.6
Chitting (Ch)[c]	2	9.7	2.9	1.9	8.7	5.0	5.0	1.8
C x T	1	7.8	66.9	25.3	2.6	46.6	45.7	17.3
C x Ch	2	2.3	9.7	2.3	0.8	11.9	12.2	2.7
T x Ch	2	7.4	0.8	0.1	8.9	1.0	1.0	0.3
C x T x Ch	2	4.1	0.5	3.0	4.1	0.2	0.1	4.2

a. Maris Peer, Red Craigs Royal.

b. Control, tecnazene.

c. 1 week, 4 weeks, 7 weeks.

d. Within replicates = 132 (denominator)

e. Least significant values of the F statistic:

5% level	1% level
F(1,132) = 3.9	F(1,132) = 7.0
F(2,132) = 3.1	F(2,132) = 4.9

Table 5.5.6. Parameters of the linear model $y = \alpha + \beta x$ describing the relationship between chitting time (x) and the subsequent yield and size distribution (y) of the cultivars Maris Peer and Red Craigs Royal (1976-77)

Variable	Cultivar	Treatment	Intercept	Gradient	Correlation coefficient
Yield of large tubers (kg/plot)	MP	control	10.7	0.29	0.36*
		tecnazene	6.4	0.42	0.47**
	RCR	control	10.3	0.31	0.37*
		tecnazene	9.3	0.05	0.06
Yield of seed-sized tubers (kg/plot)	MP	control	4.1	-0.15	-0.39**
		tecnazene	7.4	-0.11	-0.20
	RCR	control	11.4	0.19	0.61**
		tecnazene	2.7	0.10	0.27
Yield of small tubers (kg/plot)	MP	control	0.15	0.005	0.13
		tecnazene	0.54	-0.020	-0.29*
	RCR	control	0.09	0.002	0.08
		tecnazene	0.10	0.019	0.29*
Total yield (kg/plot)	MP	control	14.9	0.15	0.19
		tecnazene	14.3	0.29	0.36*
	RCR	control	11.9	0.51	0.50**
		tecnazene	12.1	0.16	0.18
% Large tubers	MP	control	71.6	1.15	0.47**
		tecnazene	44.8	1.69	0.40**
	RCR	control	87.5	-0.96	-0.45**
		tecnazene	76.7	-0.56	-0.20
% Seed-sized tubers	MP	control	27.4	-1.17	-0.49**
		tecnazene	51.4	-1.51	-0.39**
	RCR	control	11.8	0.97	0.48**
		tecnazene	22.5	0.42	0.17
% Small tubers	MP	control	0.98	0.03	0.10
		tecnazene	3.76	-0.18	-0.36*
	RCR	control	0.73	-0.00	-0.02
		tecnazene	0.79	0.14	0.28*

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

From these relationships, the analysis of variance and the diagrams the following conclusions were drawn.

1) The effect of tecnazene treatment on total yield is relatively small under the conditions of this experiment.

2) The effect of tecnazene treatment on the size distribution is substantial, permanent and consistent with the previous experiments. The yield of large tubers was smaller and that of seed-sized tubers was greater, than that from untreated controls, in both absolute and proportional terms.

3) The length of the chitting period has a significant effect upon certain of the variables. The magnitude is less than that produced by treatment but is still considered to be of importance. Using the data from table 5.5.6. it can be shown that for Maris Peer the predicted yield of large tubers for no chitting period is 10.7 kg/plot. Every week of chitting over the range investigated would produce an increase of 0.3kg/plot, giving 12.8kg/plot after 7 weeks.

The corresponding figures for a tecnazene treated sample are 6.4, 0.4 and 9.2kg/plot. When converted to tonnes ha^{-1}, these effects are of obvious commercial significance.

4) The results of this experiment again suggest that tecnazene might be exploited for maximising seed production. It should be noted however that in the case of Maris Peer at least, long chitting periods have a slight negative effect on seed production although this effect is less pronounced than with the untreated controls.

5) As in the previous experiments tecnazene produced a substantial and highly significant increase in the yield of very small tubers. This could, as previously stated, have an influence on the number of ground-keepers. Although long chitting periods will reduce this effect, in the case of Maris Peer at least, no pre-planting treatment which might be commercially feasible could completely overcome the problem.

Mean emergence time and yield

The relationship between MET and total yield was further investigated during the course of this experiment. Once more a linear relationship was adopted, the parameters of which are shown in table 5.5.7. and the resultant plot in figure 5.5.14.

Table 5.5.7. Parameters of the linear model describing total yield in terms of MET

Cultivar	Intercept	Slope	Correlation coefficient
Maris Peer	20.6	-0.17	-0.37**
Red Craigs Royal	23.3	-0.25	-0.57**

** Significantly different from zero at P < 0.01

From these figures it can be shown that, for the cultivar Maris Peer, if emergence is delayed by 5 days from 30 to 35 days total yield will drop by 5.5% and that the corresponding figure for Red Craigs Royal is 7.9%.

5.6. SUMMARY OF THE EFFECTS OF TECNAZENE ON SEED POTATOES AND THEIR SUBSEQUENT PERFORMANCE

5.6.1. Sprouting

1) Throughout this investigation tecnazene has been found to provide efficient control of sprout growth under conditions of limited ventilation.

2) The effect of tecnazene on the number of tubers with sprouts is less dramatic than its effect on sprout length.

3) Tecnazene is more effective on some cultivars than others and it is suggested that further investigations into this subject might prove worthwhile.

4) Pre-planting storage periods at high temperature (12°C) and with adequate ventilation reduce the level of tecnazene residues dramatically but also allow excessive sprouting.

5) It is therefore suggested that pre-planting airing should take place under illuminated conditions. Under these conditions a

FIGURE 5.5.14 THE RELATIONSHIP BETWEEN MEAN EMERGENCE TIME AND TOTAL YIELD 1976-77 FIELD EXPERIMENT.

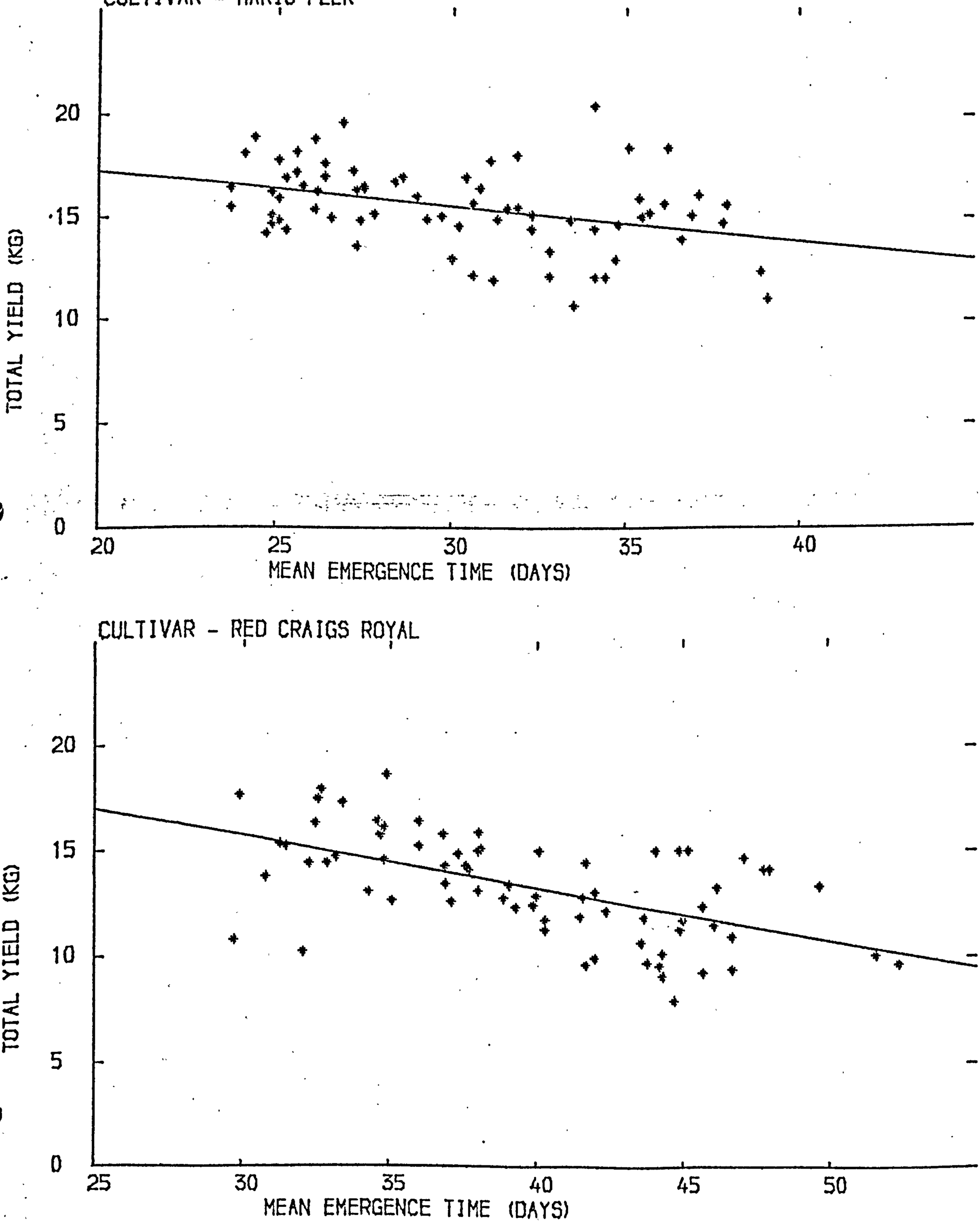
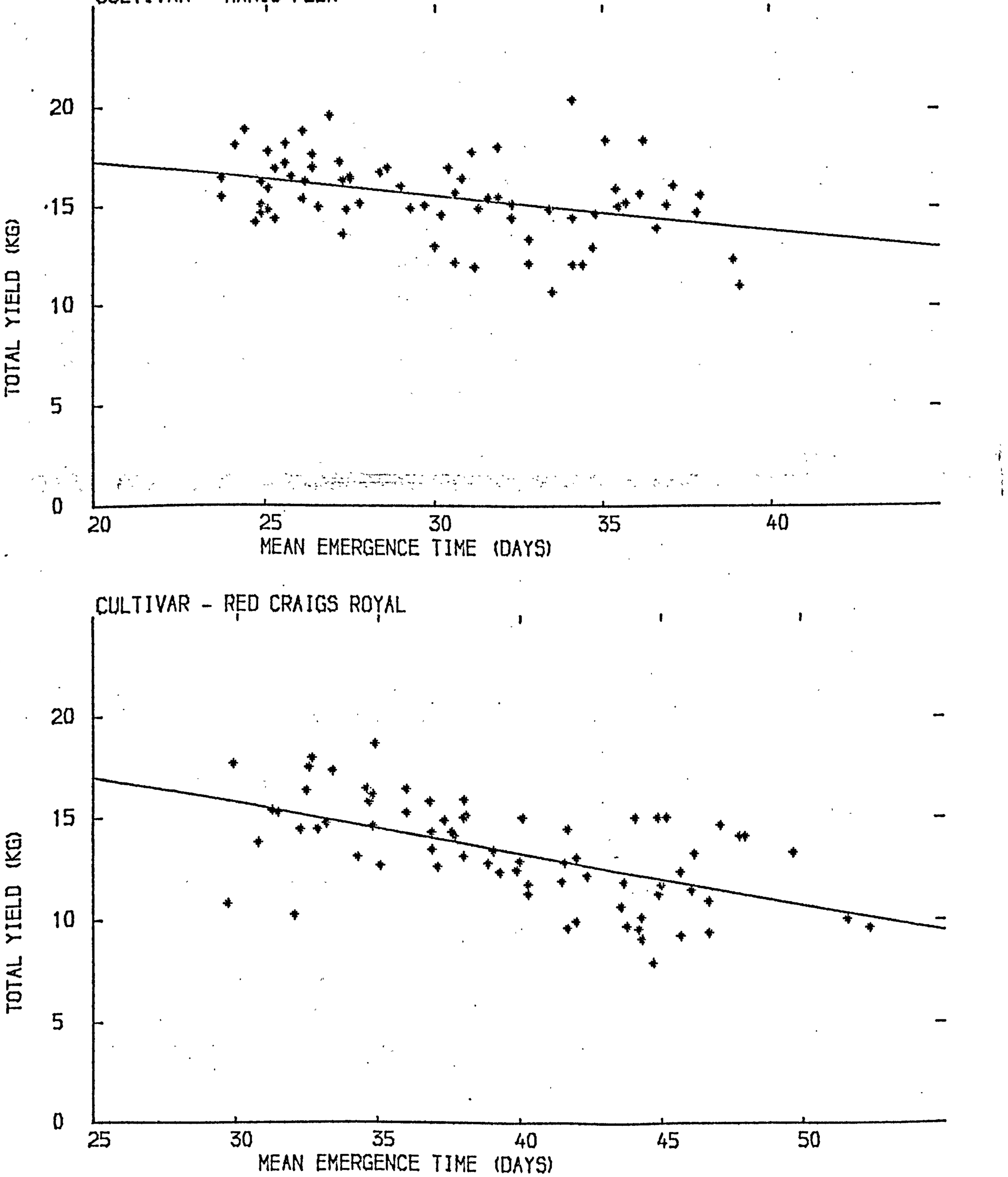


FIGURE 5.5.14 THE RELATIONSHIP BETWEEN MEAN EMERGENCE TIME AND TOTAL YIELD 1976-77 FIELD EXPERIMENT.



period of 6-8 weeks at 10°C appears to be adequate to minimise many of the adverse effects on subsequent performance.

5.6.2. Emergence

1) Tecnazene can cause delayed emergence and also reduced total emergence although the latter is very much cultivar dependant.

2) These deleterious effects can be minimised by using suitable storage conditions.

3) Delayed emergence is associated with a reduction in total yield, each day of delay costing at least 1% of total yield.

5.6.3. Yield and size distribution

1) Tecnazene can under adverse conditions cause severe reductions in yield. Reductions up to 40% have been recorded.

2) Tecnazene can on occasion produce no reduction or even a very small increase as compared to untreated controls. However these observations are usually associated with physiologically very old control samples.

3) The range of yield reductions likely to be encountered under commercial conditions is probably from 0 to 30% when tecnazene is used at the full application rate of 135mg kg^{-1}.

4) Tecnazene has a permanent effect upon the size distribution of tubers within the total yield, treated samples producing substantial more seed-sized and small tubers.

5) It is suggested that tecnazene could be exploited commercially to maximise seed production although further experimental work is required to establish precise application rates if total yield reduction is to be minimised.

6) For multiplication of VTSC and similar stocks it is suggested that the evidence presented in this thesis is sufficient to justify its use, although further information on tuber multiplication rates might prove useful.

7) Tecnazene because of its effect on the number of very small tubers could increase ground-keeper problems. It is therefore suggested that experiments should be conducted under commercial conditions to determine the number of ground-keepers left after harvesting operations.

8) Tecnazene might also be usefully exploited in crops destined for canning as a large percentage of small tubers is desirable in this situation. The results obtained during the 1976-77 experiment with the cultivar Maris Peer might prove useful in this respect.

9) It is recommended that steps should be taken to ensure that the tecnazene residue determined on washed tubers at planting should be 1.0mg kg^{-1} or less to minimise the effect on subsequent performance.

CHAPTER 6

THE EFFECT OF SPROUT SUPPRESSANT CHEMICALS ON THE SUGAR CONTENT OF POTATO TUBERS

6.1. INTRODUCTION AND LITERATURE SURVEY

The sugar content of potatoes has been the subject of much research which has produced a vast literature, a large proportion of which is devoted to studies on carbohydrate biosynthesis for which potato tissue is admirably suited. As the present investigation is primarily concerned with the commercial implications of changes in sugar content no attempt has been made to present a detailed review of this material, but rather to provide relevant and necessary background information for the experimental work described later. The references cited should therefore be regarded as representative, rather than a comprehensive list. Literature concerned specifically with the effect of individual sprout suppressants on sugar content has already been reviewed under the appropriate headings in Chapter 3 and so only a brief summary will be attempted below.

6.1.1. The sugar content of potato tubers

The main carbohydrate in potato tubers is of course, starch accounting for some 65-80% of the dry matter (Schwimmer and Burr, 1967). The soluble sugar content of potatoes is by comparison fairly low, varying (as will be discussed later) from only trace amounts to as much as 10% of the dry matter when it might account for $\frac{1}{3}$ to $\frac{1}{2}$ of the non-starch solids (Barker, 1939).

Of the numerous sugars which have been identified in potato tubers,

glucose, fructose and sucrose are quantitatively the most important, to such an extent that the total sugar content is usually taken as the sum of the three. Similarly reducing power is usually assumed to be derived only from glucose and fructose.

Minor sugars which have been identified include maltose, galactose, xylose, raffinose, melibiose, a heptulose and melezitose (Schwimmer et al, 1954; Schwimmer and Burr, 1967; Urbas, 1968; Kimura et al, 1969; Shaw, 1969). Numerous phosphorylated sugars have also been detected including glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose 1,6 diphosphate and various triose phosphates (Arreguin-Lozano and Bonner, 1949; Schwimmer et al, 1955; Mori et al, 1960; Vecher and Masny, 1966). The levels of these phosphorylated compounds have been discussed by Isherwood (1973; 1976) in relation to starch-sugar interconversion in potato tubers.

Many of the older colorimetric techniques of total sugar analysis produced figures which included some of these compounds if present in the extract. The error involved, however, is likely to be small unless the sugar level of the tuber is very low (Schwimmer and Burr, 1967).

Similarly estimations of reducing power include some of the above compounds and conceivably various non-sugar components such as ascorbic acid, tyrosine, cysteine, glutathione and inositol (Schwimmer et al, 1954).

Because of their quantitative importance glucose, fructose and sucrose have proved of greatest commercial significance and so the current investigation has been restricted to these three compounds.

The potato tuber is not of course a homogenous tissue but consists of different anatomical regions (Artschwager, 1918; 1924; Burton, 1966; Schwimmer and Burr, 1967; Smith, 1968; Reeve et al, 1969). The sugar content of the various regions may differ. For example Dimalla and van Staden (1977) found that soluble sugar levels, especially glucose levels, are higher in the region of the apical bud during the entire storage season. Others have also discussed such variations (Baijal and van Vliet, 1966; Baker, 1969; Edelman et al, 1969).

Isherwood (1976) has taken these distribution studies a stage further to the subcellular level. On the basis of analytical data

from sieved freeze dried starch grains he suggests that most of the glucose, fructose and sucrose exists outside the amyloplast membrane, but that most of the glucose-6-phosphate occurs inside.

Investigations related to the utilisation of potato sugars, however, usually confine themselves to the estimation of sugars in whole tubers and this practice will be adopted in the present study. Variations in distribution must, however, be borne in mind when devising sampling procedures.

6.1.2. The commercial significance of sugar content

Potatoes do not normally taste sweet unless they contain an abnormally high amount of sugar. Such levels may be produced under laboratory conditions but are not commonly found in practice and so the influence of sugars on sweetness is of little commercial significance (Burr, 1966).

Sugars have, however, a significant indirect effect on the flavour, appearance and shelf-life of potato products through their participation in non-enzymic browning reactions during the processing and subsequent storage of both fried and dehydrated products.

When potato tissue is fried the golden colour which develops is due to reactions at the frying temperature between amino acids and glucose, fructose and sucrose (Shallenberger et al, 1959). These non-enzymic browning or "Maillard" reactions and their inhibition by sulphur dioxide have been studied in vitro and extensively reported by H.S. Burton and his co-workers. They have shown the development of the golden colour to be a multi-stage process involving degradation of sugars to α,β unsaturated compounds which react with α amino compounds to produce brown-coloured conjugates. Co-polymerisation then takes place to produce more deeply coloured material. The rate of production and the chemical nature of the end products are dependent on the initial reactants and their relative abundance (Burton et al, 1962a; 1962b; 1962c; 1963a; 1963b; 1963c; Burton and McWeeny, 1963, 1964). The products of these reactions also contribute towards the aroma and flavour of fried potato products (Burr, 1966).

As amino nitrogen is normally present in excess the colour limiting factor is normally the sugar content (Sweetman, 1930), and

more specifically at normal frying temperatures it is the content of reducing sugars which is most closely correlated with colour development (Denny and Thornton, 1940; 1941; Hoover and Xander, 1961). Fitzpatrick et al (1965) have shown that as much as 85% of the reducing sugars present may be lost during crisp manufacture. Sucrose only participates in these reactions if the frying temperature is unusually high (Denny and Thornton, 1940).

It is therefore generally accepted that under normal conditions the content of reducing sugars has the greatest influence on the colour of fried products, and if excess reducing sugar is present then the colour which develops after frying to the correct texture will be too dark. If frying time is reduced then texture will suffer. Burton (1966) has reviewed numerous methods of minimising this problem during the manufacturing process, the most commercially acceptable being a simple leaching process to remove the offending sugar. The best solution, however, is to use only potatoes with a suitable sugar content.

The ideal figure for reducing sugar lies in the range 100-300mg/100g fresh weight and is given by one author as 180mg/100g. Below 100g/100g the colour may be too pale. The optimum values for crisp and chip production are similar although slightly higher levels may be acceptable for chip production (Burton, 1966, Talburt and Smith, 1967, Smith,1968, Burton and Wilson, 1970).

Similar non-enzymic browning problems are also encountered during the manufacture of dehydrated potatoes (Burton, 1945). However, in this case there is the added problem that browning reactions continue during the storage of the product especially at temperatures above 25°C (Gooding et al, 1956). Preparation of dehydrated products has been extensively reviewed by Burton (1966), Talburt and Smith (1967), and Smith (1968). It is generally accepted that optimum levels for dehydrated products are similar to those for fried products.

6.1.3. Factors which affect the sugar content of potatoes

In the potato tuber there exists a dynamic equilibrium between starch and glucose, fructose and sucrose levels. The biochemistry of these starch-sugar and sugar-sugar interconversions and the involve-

ment of various phosphorylated intermediates have been extensively studied (Recondo and Leloir, 1961; Leloir et al, 1961; Edelman, 1963; 1969; Rees and Duncan, 1972; Isherwood, 1973; 1976; Kennedy and Isherwood, 1975; Pollock and ap Rees, 1975).

The precise position of the equilibrium between the various metabolites is of great commercial significance and is determined substantially by environmental factors, particularily temperature. The physiology of the potato tuber has therefore been extensively studied and has been reviewed by Burton (1966).

The material below has therefore been confined to a brief outline of the more important factors which influence potato sugar levels and are likely to affect the experimental work described later.

The influence of pre-harvest factors on sugar levels

Any factor which affects the growing plant is likely to affect the sugar content of the developing tuber and may affect the sugar level at harvest either directly or indirectly through its influence on maturity (Hughes, 1974).

High levels of nitrogenous fertilizers will increase levels of reducing sugars (Hope et al, 1960) whilst high levels of potassium may have the opposite effect (Welte and Müller, 1966).

Low soil temperature at harvest time may produce increased reducing sugar levels (Kissmeyer-Nielsen and Weckel, 1967; Walkof, 1970).

Burton and Wilson (1970) have shown that latitude can also affect sugar levels. The further north potatoes are grown, the higher the sucrose and reducing sugar levels at maturity.

The factor, however, which has most effect on sugar levels at harvest and subsequently during storage is the maturity of the tuber at lifting (Appleman and Millar, 1926; Singh and Mathur, 1938; Burton, 1965; Sowokinos, 1973). Generally, freshly harvested immature tubers have a much higher content of sucrose than mature tubers. Burton (1965) found that immature tubers of the cultivar Majestic contained 1000mg/100g of sucrose whereas mature tubers contained only 60mg/100g. The corresponding figures for reducing sugar content were 200mg/100g and 550mg/100g respectively, although Sowokinos (1973) found that glucose and fructose levels also decline as the tuber matures. On

lifting, immature tubers undergo a rapid readjustment of sugar levels and much of the sucrose is converted to fructose and glucose (Burton, 1965).

The other major factor in determining sugar levels is cultivar (Denny and Thornton, 1940; Burton, 1965).

Little information is available on the effects of fungal or viral disease on sugar levels.

Post-harvest influences on sugar content

The most important single influence on sugar levels in mature tubers during the storage period is temperature and since Müller-Thurgau (1882) first discovered that storage temperatures of below $6^{o}C$ increased sugar content the various effects of temperature have been the subject of many investigations. For convenience the main conclusions of this research are described below under individual headings although in practice more than one of these processes may be occurring at the same time.

a) Cold temperature accumulation

Cold temperature accumulation of sugars is a gradual process occurring at temperatures of less than $10^{o}C$, resulting in a maximum sugar concentration after several weeks (Barker, 1932). Barker found that the lower the temperature the higher the maximum value and the longer it takes to reach this value.. At -1,1,3 and $10^{o}C$ he determined maximum values of 6700,3400,900 and 200mg/100g respectively. The same author has also reported that previous storage history has an influence on this process (Barker, 1939). Both reducing sugars and sucrose accumulate but not necessarily in the same proportion (Denny and Thornton, 1940; Arreguin-Lozano and Bonner, 1949; Treadway et al, 1949; Burton, 1965; Tishel and Mazelis, 1966; Burton, 1969; Jarvis et al, 1974; Samotus et al, 1974a). It is generally accepted, however, that reducing sugars and fructose in particular accumulate faster than sucrose.

Different cultivars show markedly different tendencies to accumulate sugars under conditions of cold storage (Denny and Thornton, 1941; Watada and Kunkel, 1955; van Vliet et al, 1961; Burton, 1965; Jarvis et al, 1974; Samotus et al, 1974a).

The reasons for low temperature accumulation remain obscure although it has been suggested that high concentrations of soluble sugars render many plants more resistant to injury under freezing conditions.(Heber and Satarius, 1964; Mayland and Cary, 1970).

b) Reconditioning

Potatoes which have become excessively sweet at low temperatures may be de-sweetened or reconditioned by storage for several weeks at a higher temperature, usually 15-20^{o}C (Müller-Thurgau, 1882; Huelin and Barker, 1939). The rate of sugar loss and the extent to which it will proceed is cultivar dependent (Samotus et al, 1974b).

c) Senescent sweetening

Prolonged storage at high temperatures may also lead to an accumulation of sugar. As it is associated with advanced physiological age it is generally known as senescent sweetening and is substantially irreversible (Huelin and Barker, 1939; Barker, 1946). The higher the storage temperature in the range 7.5-20^{o}C the earlier senescent sweetening will develop, although there is considerable variation between cultivars (Burton et al, 1959).

Both reducing sugars and sucrose accumulate although in most instances the latter is the major product (Burton et al, 1959; van Vliet and Schriemer, 1963; Verma et al, 1974).

Senescent sweetening is related to sprouting (Huelin and Barker, 1939; Barker, 1946), and van Vliet and Schriemer (1963) and Burton (1965) found good correlations between sweetening and the amount of sprout growth. The mobilisation of carbohydrates during sprouting has been further discussed by Edelman and Singh (1966), Edelman et al (1969), and Dimalla and van Staden (1977).

Aspects of the physiology and biochemistry of these temperature - influenced changes have been reviewed by various authors (Burton,1966; Heinze, 1966; Smith, 1968; Burton, 1973; Burton, 1974; Hughes, 1974).

d) Relative humidity

The effects of relative humidity on sugar content are somewhat controversial. Sparks (1973a) found that lowering relative humidity from 95% to 85% results in an increased content of reducing sugars, whilst Pätzold (1974) repeatedly found that storage at very low humidity

(60-70%) produced lower concentrations of reducing sugars than at 95%. The latter author also reports that the opposite was true for sucrose concentrations.

6.1.4. The effect of sprout suppressant chemicals on sugar content

The effect of sprout suppressant chemicals on sugar content is a subject of some apparent disagreement. Many authors have reported little or no effect, others increases and still others reductions in reducing sugar and sucrose levels.

Much of the apparent disagreement can be accounted for by oversimplification and misinterpretation by review authors. Indeed the first paper published on the subject (Denny et al, 1942) has been completely misunderstood by Schwimmer and Burr (1967).

Despite such rather confusing reviews of the subject the main effects of the most widely used chemicals, maleic hydrazide, chlorpropham and propham are slowly emerging and have been discussed under the appropriate headings in Chapter 3. These findings will be considered again during the discussion on the present experimental work in section 6.3.

The effect of tecnazene on sugar content, however, has not been widely investigated. Gooding and Hubbard (1956) reported that tecnazene had no effect on reducing sugars in clamp stored tubers, yet when their results are examined carefully they indicate that reducing sugar levels were substantially lower than the controls in the later part of the storage season.(24% lower at the final sampling date in mid-May). Gooding et al (1956b) found that the lowest sugar content obtained from a series of commercial samples was from a tecnazene treated store. Unfortunately no untreated control was available.

Because of the lack of conclusive evidence on the effect of tecnazene on sugar levels the present study was initiated. Other chemicals were included in the later stages for comparison.

6.2. A PRELIMINARY INVESTIGATION INTO THE EFFECT OF TECNAZENE ON SUGAR CONTENT

6.2.1. Introduction

The purpose of this preliminary investigation was primarily to develop suitable analytical methods, gain experience and useful background information for the design and completion of the more elaborate experiment described later.

It was considered that these aims could be satisfied by using material from the residue experiment of 1974-75 which has already been described in Chapter 4.

The analytical method

There are basically two techniques for estimating the sugar content of potatoes and other plant tissue these being a) methods which employ spectrophotometry as a detection method and b) chromatographic techniques.

a) Spectrophotometric methods

Spectrophotometric methods can be subdivided into those based on estimation of a colour produced by chemical reaction, and those which employ enzymes and estimate either product or co-enzyme.

The former have been historically very important in potato research and are still widely used. For example the Somogyi (1952) technique of estimating reducing power and the Roe (1948) technique of estimating fructose, can be usefully exploited to give estimations of glucose, fructose and sucrose in potato tubers (Jarvis et al, 1974). These techniques, however, suffer from lack of specificity and demand a high degree of technical skill to produce reproducible figures. Their main advantage is their rapidity and this can be extremely important in industrial situations. Various methods of estimating reducing power in such situations have been discussed by Lindsay (1973).

Some of the problems of specificity have been overcome by the use of enzymes and modern enzymic methods are now used by some research laboratories (Isherwood, 1973). The unstable nature of enzymes however demands constant monitoring and replacement.

b) Chromatographic techniques

Of the many existing chromatographic techniques only gas chromatography gives the required resolution and precision, although future developments in detection equipment for high pressure liquid chromatography may prove useful. GC also offers possibilities for simultaneous estimation of other compounds besides glucose, fructose and sucrose.

As GC facilities were readily available and as the technique satisfies the criteria mentioned above this was the technique selected for the proposed experiments.

GC, however, demands that compounds must be volatile at reasonable temperatures and so suitable derivatives of sugars must first be prepared. Of the variety of derivatives available the O-trimethyl-silyl ethers are the simplest to prepare and are most popular. Their synthesis and chromatography have been extensively discussed by Sweeley et al (1963) and since then a considerable literature has amassed on the GC of carbohydrates. Shaw (1969) reports that even then there were over 150 papers on the subject. Most of this literature, however, depends heavily on the original work of Sweeley and his co-workers.

Several authors have reported modifications of the method of Sweeley et al specifically for the analysis of potato sugars (Kimura et al, 1969; Rumpf, 1969; Shaw, 1969). The method adopted below is based to varying extents on these papers although several modifications were found necessary to suit the requirements of the present investigation.

It is advantageous to include an internal standard in the reaction mixture and several have been suggested in the literature. Davison and Young (1969) recommend myo-inositol for plant extracts but unfortunately potatoes contain significant amounts of this compound (Shaw, 1969; Kimura et al, 1969). The use of ∝- D glucoheptulose has also been reported but its retention time is similar to that of myo-inositol (Kline et al, 1970). Sorbitol and mannitol have also been used but are eluted in the already crowded glucose region and may be incompletely resolved (Martin and Eib, 1968; Kimura et al, 1969). Varns and Shaw (1973) suggest that stearic acid is ideal for for potato extracts. However during the development work for the

present programme major problems were encountered with this compound during the analysis of a large number of unpurified potato extracts. Stearic acid appears to be preferentially adsorbed by column residues and its use had to be abandoned. Shaw (1969) partially purified his extracts before analysis.

Gehrke and his co-workers have used a number of polyaromatic compounds as internal standards (Gehrke and Ruyle, 1968; Gehrke and Lakings, 1971). Three of these compounds anthracene, phenanthrene and flurene were tested and phenanthrene was found to have a suitable retention time, appearing between β glucose and sucrose. This compound was therefore used in the preliminary investigation described below, although ideally an internal standard with properties more similar to the compounds being analysed would have been preferred to allow constant monitoring of reagents and equipment. Phenanthrene is also highly toxic.

6.2.2. Experimental

Samples were taken on the 9 April 1975 from all batches in the 1974-75 residue experiment described in Chapter 4. Six healthy tubers were randomly selected from from each batch and an 8mm diameter core was taken from each, about half-way between the ends of the tuber, and avoiding any eyes. The 6 cores (approx. 25g) were placed in a sealable plastic bag, weighed then dropped into liquid nitrogen where they were kept until extraction.

The cores were then transferred to a blender with 150cm^3 methanol, allowed to heat up for a few minutes then blended at high speed for 2 minutes. The homogenate was then filtered through a fluted Whatman No.1 filter paper into a 250cm^3 volumetric flask, and the blender washed with 2 x 30cm^3 portions of methanol which in turn were used to wash the residue in the filter paper. The flask was then made up to 250cm^3 with methanol, mixed and 1cm^3 aliqots of the mixture pipetted into each of two 2 dram vials (1 dram = 1.177g) and dried over P_2O_5 for 24 hours in a vacuum desicator. If smaller vials are used then when high vacuum is applied the sample may be lost due to bubble formation. This does not happen with the larger surface area of the 2 dram vial.

Derivatisation

The silylating reagent consisted of 20cm^3 hexamethyldisilazane (HMDS), 10cm^3 trimethylchlorosilane (TMCS), 30mg phenanthrene made up to 100cm^3 with AR pyridine which had been dried over KOH pellets.

1.0cm^3 of this reagent was added to each sample, the vial sealed with a teflon-lined screw cap and shaken until the sugars had dissolved leaving only a finely divided precipitate of ammonium chloride. Samples were stable for several days at room temperature provided they were not exposed to light for long periods.

Standards

Stock solutions of glucose, sucrose and citric acid were prepared by dissolving 500mg of the pure compound in approximately 10cm^3 of de-ionised water in a 100cm^3 volumetric flask. 24 hours later after anomerisation had occurred (where appropriate) the solutions were made up to 100cm^3 with AR methanol giving a concentration of 5mg cm^{-3}.

10cm^3 aliquots of these solutions were mixed and diluted to 100cm^3 with AR methanol to give standard sugar mixtures containing 0.5mg cm^{-3}.

Derivatives of individual sugars could then be prepared by drying down 0.05cm^3 of stock solution . Standard mixtures of sugars were prepared by drying down 0.5cm^3 of the standard mixture, and derivatising as for samples.

Chromatographic conditions

The gas chromatograph used was the Packard 419 described previously. It was fitted with two 2m x 6mm O.D. glass columns containing 5% OV17 on Gas Chrom Q prepared as described in Chapter 4. The carrier gas was nitrogen at a flow rate of 40cm^3 min^{-1} and the F.I.D.s were supplied with H_2 at 25cm^3 min^{-1} and O_2 at 120cm^3 min^{-1}. The injection port was held at 230^{o}C (higher temperatures produce degradation) and the detector at 285^{o}C. Linear temperature programming involved a 1min inital hold at 130^{o}C rising by 6^{o}C min^{-1} to 260^{o}C for a final hold of 10min, a 3min cooling period and 2.5min stabilisation before the next injection. The chromatograph was operated in independent mode so that two samples could be analysed simultaneously.

2-4µl aliquots of the reagent mixture were injected using a Hamilton series 700 syringe. Peak areas were estimated by triangulation.

Peak identification

Peaks were identified by comparison with standard chromatograms of individual compounds and with published data.

From the typical chromatogram illustrated in figure 6.2.1. it can be seen that OV17 does not completely resolve the two fructose peaks. Similar findings with silicone based columns have been reported by Sweeley et al (1963) and Shaw (1969). The larger of the two peaks is β-fructopyranose and the smaller β-fructofuranose and the ratio of their areas is the same as that found in aqueous equilibrium solution, the smaller amounting to some 15% of the total (Shaw, 1969). A more polar polyethylene glycol based column will resolve the two but is not suitable for mixtures containing disaccharides because of high column bleed. As the ratio of the two peaks is constant estimations were based on the larger β-fructopyranose form.

The OV17 column did resolve the two forms of glucose and in the standard solutions the ratio of ∝ D glucopyranose to β D glucopyranose was the expected aqueous ratio of 40:60 and was stable. However as previously reported citric acid was eluted with the ∝ D glucopyranose peak and so all estimations were made on the β D glucopyranose peak (Varns and Shaw, 1973).

Calculation

As the method produced a linear response for each of the three important peaks simple proportionality could be used.

From the standard chromatograms the relative weight response (RWR) of each of the sugars was calculated from the expression:

$$RWR = \frac{\text{area of sugar peak}}{\text{weight of sugar in vial}} \times \frac{\text{weight of internal standard in vial}}{\text{area of internal standard peak}}$$

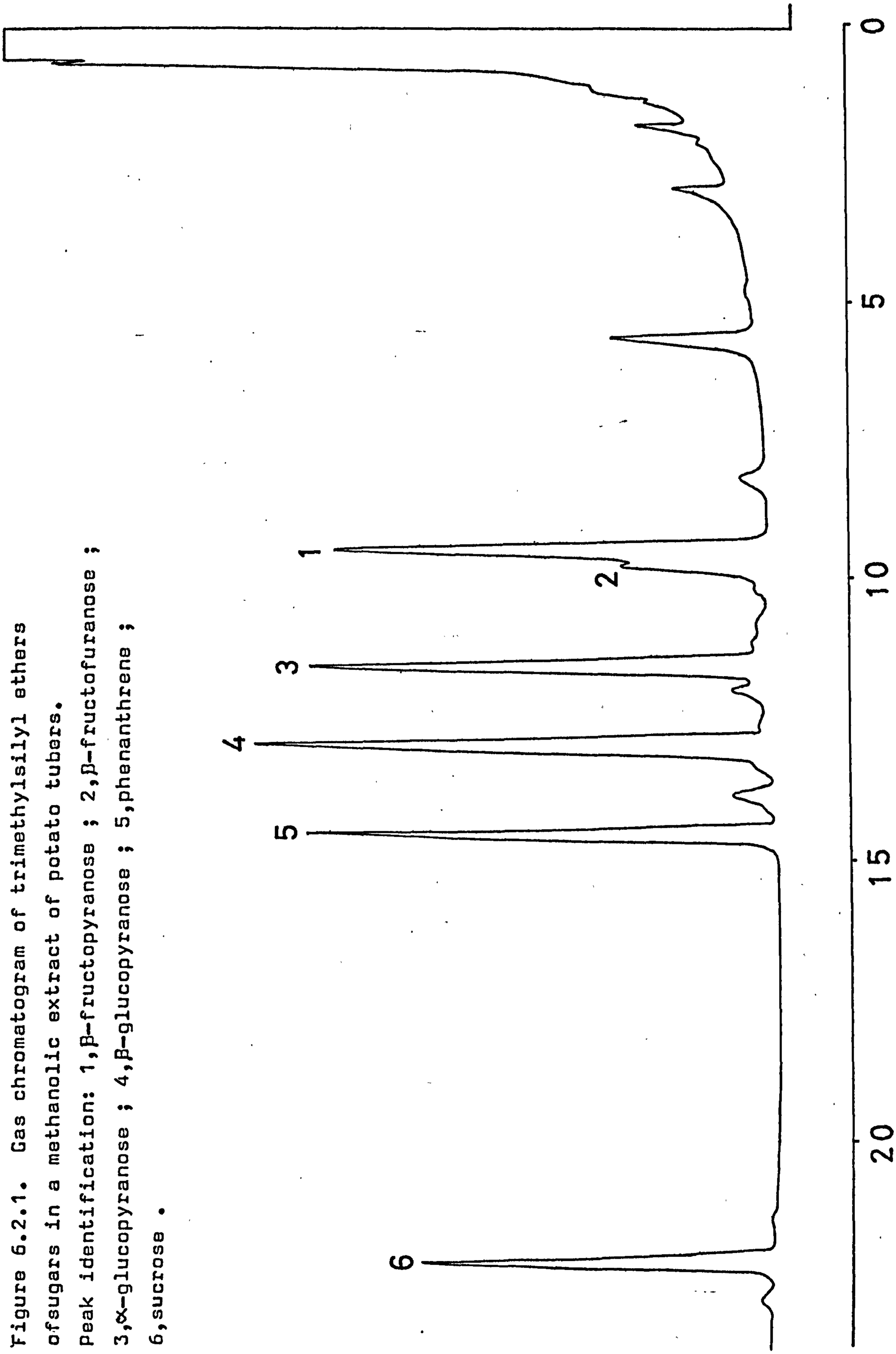


Figure 6.2.1. Gas chromatogram of trimethylsilyl ethers ofsugars in a methanolic extract of potato tubers.
Peak identification: 1,β-fructopyranose ; 2,β-fructofuranose ; 3,α-glucopyranose ; 4,β-glucopyranose ; 5,phenanthrene ; 6,sucrose .

then for a sample chromatogram:

$$\text{weight of sugar in vial} = \frac{\text{area of sugar peak}}{\text{RWR}} \times \frac{\text{weight of internal standard}}{\text{area of internal standard}}$$

From this figure the concentration in the original potato tubers is easily calculated.

6.2.3. Results and Discussion

The first point to emerge during the calculations was the high variability of the data. This was somewhat disappointing as great care had been takenduring the development of the derivatisation and chromatographic procedures to ensure good reproducibility. Standard errors of the mean based on replicate injections of the same sample were less than $\pm$3% for all sugars. Similarly replicate analysis of the same extract produced errors of less than $\pm$7% over an extended period. Although it is felt that these figures could be improved by use of a more satisfactory internal standard they cannot account for the high variability in the results. Re-extraction of residues after filtering indicated that the extraction procedure was not at fault. The source of this high variability must therefore be the earlier sampling and sub-sampling stages, and is probably due mostly to the very small sample used.

The fructose, glucose and sucrose concentrations determined are reported in table 6.2.1. (Maris Piper and Pentland Crown) and table 6.2.2. (Record and Redskin) with their associated storage treatments.

Despite the high variability several points are still apparent.

1). The untreated controls which had been stored at 8^{o}C and were freely sprouting contained higher amounts of all three sugars than equivalent treated samples.

2). Samples which had been tecnazene treated, stored at 8^{o}C then transferred to 12^{o}C contained the lowest amount of all three sugars, as might be predicted from the effects of reconditioning discussed earlier.

Table 6.2.1. Sugar levels in tecnazene treated and untreated tubers subjected to a variety of storage conditions.
Cultivars - Maris Piper and Pentland Crown.

Cultivar	Storage conditions		Sugar level (mg/100g fresh wt.)*			
	Airing time (weeks)	Temperature (^{o}C)	Fructose	Glucose	Sucrose	Total
Maris Piper	- untreated control -		340.8	398.5	96.7	836.0
	0	8	259.3	320.3	88.4	668.0
	3**	8	204.6	278.6	74.9	558.1
	5	8	234.5	294.3	69.9	598.7
	8	8	227.0	276.4	76.9	580.3
	3	12	213.5	280.7	47.1	541.3
	5	12	218.5	271.8	65.2	555.5
	8	12	212.8	298.7	66.7	578.2
Pentland Crown	- untreated control -		96.7	205.4	127.6	429.7
	0	8	48.2	156.6	127.3	332.1
	3**	8	39.1	161.5	100.4	301.0
	5	8	70.1	186.4	113.7	370.2
	8	8	93.1	200.8	104.2	398.1
	3	12	55.7	135.7	86.7	278.1
	5	12	48.5	140.3	73.8	262.6
	8	12	30.7	112.4	93.6	236.7

* Mean of replicate treatments where appropriate.

** Includes the forced ventilation treatment.

Table 6.2.2. Sugar levels in tecnazene treated and untreated tubers subjected to a variety of storage conditions.
Cultivars - Record and Redskin.

Cultivar	Storage conditions		Sugar level (mg/100g fresh wt.)*			
	Airing time (weeks)	Temperature (^{o}C)	Fructose	Glucose	Sucrose	Total
Record	- untreated control -		262.2	381.6	151.4	795.2
	0	8	129.9	221.9	103.5	455.3
	3**	8	117.4	255.0	110.0	482.4
	5	8	171.5	253.7	135.0	560.2
	8	8	144.0	271.6	128.9	544.5
	3	12	125.6	229.7	87.3	442.6
	5	12	139.6	218.0	92.5	450.1
	8	12	81.7	180.4	86.6	348.7
Redskin	- untreated control -		152.5	289.8	101.3	543.6
	0	8	133.1	258.1	112.3	503.6
	3**	8	118.2	242.0	105.7	465.9
	5	8	120.5	273.8	97.8	492.1
	8	8	117.6	261.7	95.4	474.6
	3	12	68.3	220.9	92.7	381.9
	5	12	100.0	217.4	78.4	395.8
	8	12	93.5	212.9	94.6	400.0

* Mean of replicate treatments where appropriate.

** Includes the forced ventilation treatment.

3). The treated samples stored at 8°C throughout the storage season had sugar levels intermediate between those of the controls and those stored at 12°C for several weeks.

4). The high variability masks any effect which might be due to airing time.

It should be borne in mind, however, before drawing too many conclusions from these results that the control tubers were stored in a polythene tent ventilated from outwith the building and although the temperature was identical to those batches in the rest of the room other factors such as humidity might be involved (see Chapter 4 for experimental details).

These results do not however lend support to the statement by Burton (1966) that "- sweetening occurs to even greater extent if sprouting is suppressed chemically -". On the contrary, they tend to indicate that potato quality may be improved or at least preserved by the use of chemicals such as tecnazene.

6.3. AN INVESTIGATION INTO THE EFFECTS OF VARIOUS SPROUT SUPPRESSANT TREATMENTS ON SUGAR CONTENT DURING A STORAGE SEASON

6.3.1. Introduction

Based on the experience gained during the preliminary investigation described in section 6.2. the current investigation wasundertaken during the 1977-78 storage season, and incorporates various measures to overcome the deficiencies of the previous experiment.

On this occasion sugar levels were estimated throughout the storage season and besides control and tecnazene treated samples, chlorpropham and a hand-desprouted treatment were included to allow easier comparison with published results. A new chemical, on which no information was available, dimethylnaphthalene (DMN) was also included (Beveridge et al, 1978).

First, however, each stage of the analytical procedure was critically examined and improvements made where possible.

The analytical method

a) Sugar oximes as alternative derivatives

As the existing analytical method was very slow the possibilities of using alternative derivatives which could be analysed more rapidly were investigated.

The existing method produces twin peaks for the different anomeric forms of glucose which require slow temperature programming for their complete resolution. Sweeley et al (1963) reported that the multiple peak problem could be overcome by first converting the sugars to their corresponding oximes and then forming the O-TMS ethers of these oximes. A modification of his procedure was therefore tried.

Derivatives were produced by adding to the residue of 1cm^3 of potato extract, 0.5cm^3 of a 10mg cm^{-3} solution of hydroxylamine hydrochloride in pyridine, stirring for 30min at 70-75^{o}C, cooling then adding 0.5cm^3 of silylating reagent containing internal standard (see later).

The resultant derivatives yield only one peak per sugar, but during these investigations it was found that to adequately resolve the glucose and fructose oximes and yet still elute the sucrose peak in a reasonable time, a temperature programme was still required, although a slightly faster programme could now be used.

The method, however, involves an extra pipetting step and a two stage reaction which was found to be time consuming and may lead to extra error. This approach was therefore abandoned in favour of a modification of the existing method, although with improved technical resources the oxime method would probably be the method of choice.

b) Alternative reagents

Various alternative reagents were considered and tested. Both N,O-bis trimethylsilylacetamide (BSA) and trimethylsilylimmidazole (TMSI) were found to produce good results and no precipitate of ammonium chloride which can block syringes. TMSI is a particularly useful reagent because of its tolerance of moisture. However as syringe blocking was not a serious problem and these reagents are approximately ten times more expensive than HMDS yet offer no other

significant improvements, neither was adopted.

c) The internal standard

Phenanthrene as was stated earlier is not the ideal internal standard for sugar analysis as it is chemically so dissimilar to the compounds being analysed. It may also be carcinogenic.

Phenyl β-D glucopyranoside does possess enough structural similarity and is now commercially available for use as an internal standard for sugar analysis. As it is eluted in the vacant area between glucose and sucrose it proved admirably suited to the present purpose and was therefore adopted.

d) Detector contamination

In the previous investigation it was found that repeated injections of 2-4µl of the reaction mixture very quickly reduced detector sensitivity because of contamination with silica.

Various authors have suggested that this problem can be avoided by removing excess reagent and solvent in a stream of nitrogen and taking up the residue in a known amount of hexane or other suitable solvent (Shaw, 1969).

However, preliminary investigations indicated that this reduced the reproducibility of the technique probably due to :

1) the extra pipetting step.

2) ease of contamination with moisture which may lead to hydrolysis of the derivatives.

3) volatility of some components (Gehrke and Ruyle, 1968).

This approach was therefore abandoned in favour of much smaller injections (1µl or less) of the reaction mixture. This modification was facilitated by the higher sugar contents of the solutions produced by modifications in the extraction technique described later.

e) The reaction vessel

Because of the amount of shaking required to achieve complete derivatisation of the dried methanolic extracts new $3cm^3$ reaction vials with magnetic stirring bars ("Reactivials" - Pierce Chemical Co.) were tried and found to greatly enhance the dissolution of the sugar residue, especially if solutions were warmed to 50^oC for a few minutes after addition of the reagent.

f) Sampling and extraction

As the main source of error in the previous experiment was due to sampling too little tissue from too few tubers various other procedures were investigated.

Firstly it was decided on the basis of a) experience with the previous experiment b) results from the residue experiments and c) reference to the literature, that the original sample should be approximately 1kg of tubers.

Extraction of the whole sample would be ideal but enormous quantities of methanol would be required (75 litres on each sampling date), and filtration would present a major problem.

As this was not acceptable some form of sub-sampling was necessary. Masceration of the 1kg sample then sub-sampling (as discussed for residue analysis) was ruled out because of enzyme activity.

Slicing into liquid nitrogen, grinding the frozen slices with a mortar and pestle and sub-sampling the homogenous powder is the method used in some laboratories. This method,however, consumed large quantities of liquid nitrogen and is too hazardous to be applied routinely to 30 samples per day.

Burton (1965) used much slower freezing at $-20^{o}C$ presumably in a deep-freeze. However as it takes approximately 6 hours to freeze in this way, again, the possibility of enzyme action cannot be ruled out.

The method finally adopted therefore, was a modification of the previous coring technique to give larger and more representative samples. This in turn demanded modification to the extraction method to cope with the extra material.

6.3.2. Experimental

The experiment involved 3 cultivars, 5 storage treatments and 2 replicates in a full factorial design, sampled on 7 dates throughout the storage season.

Tubers (32-52mm) from all three cultivars (Desiree, Maris Peer, and Red Craigs Royal) were harvested on 5 October from a 1977 field experiment and were once grown from certified seed, and had identical cultural histories.

They were stored in sacks at 10-15°C until 18 November 1977 when they were transferred to 10kg boxes and treated.

After treatment all batches were stored in a constant temperature room, at 8°C and approximately 85% relative humidity, in a randomised design.

Treatments

The five treatments were:

1) Control - no treatment.

2) Hand-desprouted - no chemical but tiny sprouts were removed manually on the 3rd and 5th sampling dates.

3) Tecnazene treatment - 45g of a 3% formulation (Fusarex) were applied to each 10kg batch as previously described giving an application rate of 135mg kg^{-1}.

4) Chlorpropham treatment - 2g of pure chlorpropham were dissolved in 50cm^3 acetone giving a stock solution of 40mg cm^{-3}. 5cm^3 aliqots of this solution were added to 25g batches of alumina in screw-capped jars, the acetone allowed to evaporate, and the mixture shaken for 1 hour. Each batch was sufficient to treat 10kg of tubers giving an application rate of 20mg kg^{-1}.

5) Dimethylnaphthalene (DMN) treatment - 1.0g of a 99.9% mixture of DMN isomers were weighed into 25g batches of alumina in screw-capped jars, the mixture shaken for 1 hour then used to treat 10kg of tubers giving an application rate of 100mg kg^{-1}.

Sampling

The first samples were taken on 1 December 1977 then subsequently at three week intervals until 15 April 1978 when it was judged that the untreated material was of no commercial value. Photographs were taken of representative samples from each batch on the final sampling date.

Each sample consisted of 12-14 healthy tubers and was sub-sampled by removing two 8mm cores from each tuber, the second core being taken by pushing the cork borer into the tuber immediately adjacent to the cavity left by the first, at an angle such that the borer emerged immediately adjacent to the cavity but having traversed it on the way through the tuber. This prevents over-

sampling of the centre of the tuber.

Approximately 150g of cores were collected from each sample, placed in sealable plastic bags, weighed, and immediately frozen in liquid nitrogen where they were stored until they were extracted.

Extraction

The frozen sample was transferred to a 400cm^3 beaker and 200cm^3 of methanol added. The temperature was allowed to rise to -10^{o}C and the contents transferred to a blender using 50cm^3 of methanol to rinse the beaker. If the 150g sample was blended straight from liquid nitrogen the blades of the blender tended to shatter.

After blending at high speed for 2 min the homogenate was filtered through a 90mm Whatman No. 1 filter paper under reduced pressure. The blender and filter pad were washed with 2x50cm^3 portions of methanol.

The combined filtrate was made up to 500cm^3 with methanol, mixed and two 10cm^3 samples placed in screw-capped vials and stored at -18^{o}C until required for analysis.

Derivatisation and chromatography

1cm^3 aliquots from each of the two stored samples of extract were pipetted into 3cm^3 "Reactivials" and dried as previously described. Teflon coated stirring bars were placed in each and 0.5cm^3 of silylating reagent added, the mixture warmed for a few minutes to 50^{o}C then allowed to cool for 2 hours with continuous stirring.

The silylating reagent contained 25cm^3 HMDS, 10cm^3 TMCS and 100mg of phenyl β D-glucopyranoside diluted to 100cm^3 with AR pyridine which had been dried over KOH pellets.

0.5-1μl aliquots of the reaction mixture were then injected into the gas chromatograph. Chromatographic conditions were as previously described excepting that a slightly different programme was used involving an initial hold for 2 min at 125^{o}C rising by 6^{o}C min^{-1} to 275^{o}C with a final hold of 10min.

Stock solutions, and standard mixtures were prepared as previously described and derivatised as above.

Peak height and width at half height were measured and a computer programme was written to calculate results and errors

from these raw measurements.

Errors

The method was thoroughly checked for overall errors and their sources.

1) The relative weight responses for the three sugars with respect to phenyl β D glucopyranoside determined over 5 replicates were:

1. fructose 0.951 ± 0.011 (1.2%)
2. glucose 0.835 ± 0.006 (0.7%)
3. sucrose 1.098 ± 0.022 (2.0%)

These figures must, of course, be determined for each column and checked at regular intervals, although in the present investigation they were found to be constant for a particular column over a period of several weeks.

2) Errors between injections. The standard error of the mean expressed as a % of the mean was calculated over 5 replicate injections of the same solution and found to be:

1. fructose ± 3.2%
2. glucose ± 2.9%
3. sucrose ± 2.5%

3) Errors between analysis. The S.E.M. expressed as a % of the mean was calculated over 5 replicate analysis of the same extract and found to be:

1. fructose ± 6.4%
2. glucose ± 2.9%
3. sucrose ± 3.6%

4) Errors due to analysis + sampling. Five separate extracts from 1kg samples were made as described above. The S.E.M. expressed as a % of the mean was calculated after analysis and found to be:

1. fructose ± 10.3%
2. glucose ± 9.6%
3. sucrose ± 4.6%

Retention times

Under the chromatographic conditions described the retention times of the various peaks are:

1.	fructose	8.6min
2.	∝ glucose + citric acid	10.0min
3.	β glucose	11.1min
4.	phenyl β D glucopyranoside	16.3min
5.	sucrose	17.9min

6.3.3. Results

The duplicate analytical figures from each extract were averaged to produce a mean figure for each batch at each sampling date. The figures for duplicate boxes have been further averaged to produce a treatment mean and these figures are reported in tables 6.3.1. (Desiree), 6.3.2. (Maris Peer), and 6.3.3. (Red Craigs Royal) for fructose, glucose, sucrose and total sugar concentration.

The results have also been illustrated graphically in figures 6.3.1. to 6.3.9. Both forms of representation were considered essential to allow easy comparison of absolute values since the graphs necessarily have different scales.

Photographs of representative samples of the various treatments are to be found in plates 6.3.1. - 6.3.3.

A typical chromatogram of a potato extract is illustrated in figure 6.3.10.

6.3.4. Discussion

In general, replicate analysis of the same extract produced excellent agreement as might be predicted from the error figures given above. Much of the remaining error is accounted for in the estimation of peak dimensions.

The results from duplicate treatments were satisfactory although it is felt that the sampling stage could be improved still further, perhaps by analysing a $\frac{1}{4}$ or $\frac{1}{8}$ part of every tuber. It is also suspected that sampling only tubers in a narrow weight range might improve reproducibility eg. 125 - 150g.

However the overall variability has been much reduced compared

Table 6.3.1. The effect of various sprout suppressant treatments on sugar levels* during storage at 8°C.
Cultivar - Desiree.

Treatment	Sugar**	Time after treatment (weeks)***						
		2	5	8	11	14	17	20
Control	F	83.1	84.2	84.4	86.9	86.7	121.5	138.6
	G	157.7	133.9	119.3	110.3	118.6	164.7	229.1
	S	144.8	133.4	139.1	137.5	139.3	181.2	226.3
	T	385.6	351.5	342.8	334.7	344.6	467.4	594.0
Hand-desprouted	F	78.1	79.3	76.5	87.2	81.1	96.8	130.1
	G	158.2	138.7	111.5	106.8	109.1	132.7	156.3
	S	140.5	139.3	145.5	139.8	118.3	150.4	163.4
	T	376.8	357.3	333.5	333.8	308.5	379.9	449.8
Tecnazene	F	90.9	78.4	80.6	77.0	82.4	81.4	93.4
	G	156.2	138.8	121.4	94.9	104.6	120.4	124.6
	S	139.7	133.9	140.7	138.2	115.8	136.8	156.0
	T	386.8	351.1	342.7	310.1	302.8	338.6	374.0
Chlorpropham	F	83.3	77.5	89.9	88.8	72.8	84.8	82.4
	G	152.1	132.2	129.2	117.6	93.0	110.7	103.1
	S	138.8	134.2	136.3	127.6	114.8	146.3	173.8
	T	374.2	343.9	355.4	334.0	280.6	341.8	359.3
DMN	F	86.6	80.0	82.7	84.0	90.9	92.8	95.2
	G	161.6	130.1	117.0	106.3	97.8	99.1	98.8
	S	140.9	131.4	129.1	130.3	110.3	142.7	153.6
	T	389.1	341.5	328.8	320.6	299.0	334.6	347.6

* All sugar levels in mg/100g fresh weight.

** F = fructose, G = glucose, S = sucrose, T = total = F + G + S.

*** Tubers were harvested on 5 Oct. 1977, stored at 10 - 15°C, treated on 18 Nov. and placed in storage at 8°C.

Table 6.3.2. The effect of various sprout suppressant treatments on sugar levels* during storage at 8°C.
Cultivar - Maris Peer.

Treatment	Sugar**	Time after treatment (weeks)***						
		2	5	8	11	14	17	20
Control	F	83.7	83.4	91.6	90.6	96.8	186.4	247.3
	G	207.8	217.7	173.5	172.6	239.4	307.2	332.2
	S	107.0	106.8	119.7	143.5	162.1	239.4	260.4
	T	398.5	407.9	384.8	406.7	498.3	733.0	839.9
Hand-desprouted	F	84.4	88.9	97.7	112.2	105.1	180.6	237.8
	G	205.1	223.4	212.8	201.1	274.3	295.3	281.3
	S	119.2	115.6	117.0	124.4	135.7	181.2	206.9
	T	408.7	427.9	427.5	437.7	515.1	657.1	726.0
Tecnazene	F	88.0	77.3	91.7	77.1	73.3	74.5	106.7
	G	221.2	196.5	199.1	165.4	157.3	153.2	187.2
	S	115.9	114.7	106.5	100.4	104.6	112.5	139.8
	T	425.1	388.5	397.3	342.9	335.2	340.2	433.7
Chlorpropham	F	83.0	86.2	95.7	88.7	71.9	83.6	108.1
	G	228.9	233.3	228.3	204.5	184.3	213.0	203.3
	S	120.1	118.0	116.0	103.2	108.4	129.3	130.6
	T	432.0	437.5	440.0	396.4	364.6	425.9	442.0
DMN	F	83.9	85.9	97.9	72.9	68.5	73.9	100.3
	G	216.5	198.0	197.2	176.7	137.2	174.3	201.1
	S	117.3	106.3	103.7	101.7	97.4	120.8	133.3
	T	417.7	390.2	398.8	351.3	303.1	369.0	434.7

* All sugar levels in mg/100g fresh weight.

** F = fructose, G = glucose, S = sucrose, T = total = F + G + S.

*** Tubers were harvested on 5 Oct. 1977, stored at 10 - 15°C, treated on 18 Nov. and placed in storage at 8°C.

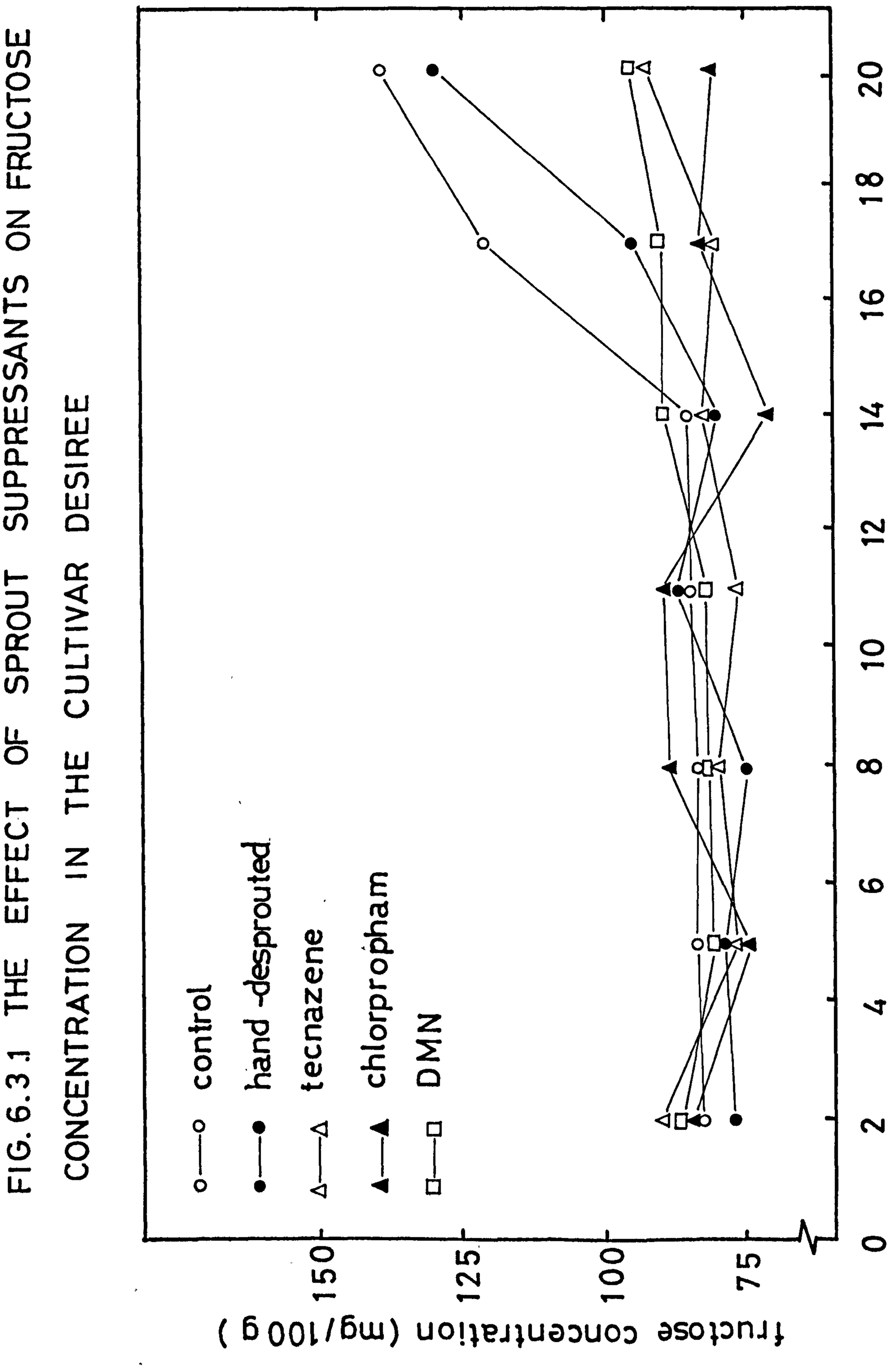
Table 6.3.3. The effect of various sprout suppressant treatments on sugar levels* during storage at 8°C.
Cultivar - Red Craigs Royal.

Treatment	Sugar**	Time after treatment (weeks)***						
		2	5	8	11	14	17	20
Control	F	89.3	87.5	74.2	81.1	89.5	203.1	269.3
	G	309.6	280.0	270.1	250.3	248.1	313.9	430.2
	S	87.4	91.5	98.7	125.0	172.3	215.5	249.5
	T	486.3	459.0	443.0	456.4	509.9	732.5	949.0
Hand-desprouted	F	86.8	80.7	89.1	89.9	91.0	154.5	167.5
	G	303.7	304.2	267.3	260.7	303.4	337.9	380.7
	S	93.2	82.0	99.6	105.5	107.8	141.3	185.1
	T	483.7	466.9	456.0	456.1	502.2	633.7	733.3
Tecnazene	F	85.4	79.1	79.5	60.8	54.4	80.9	101.0
	G	302.8	290.1	259.7	248.3	220.0	249.7	302.5
	S	89.1	97.2	84.2	83.7	87.5	108.2	169.0
	T	477.3	466.4	423.4	392.8	361.9	438.8	572.5
Chlorpropham	F	77.4	72.5	70.9	71.9	51.6	88.4	100.7
	G	325.8	305.2	254.4	244.9	219.7	238.7	289.5
	S	89.2	91.0	91.1	91.3	83.4	104.8	129.2
	T	492.4	468.7	416.4	408.1	354.7	431.9	519.4
DMN	F	86.5	68.3	75.1	68.0	50.9	67.6	88.4
	G	314.9	289.2	271.4	233.6	201.4	232.9	284.1
	S	93.5	88.6	83.2	84.6	76.9	112.2	115.3
	T	494.9	446.1	429.7	386.2	329.2	412.7	487.8

* All sugar levels in mg/100g fresh weight.

** F = fructose, G = glucose, S = sucrose, T = total = F + G + S.

*** Tubers were harvested on 5 Oct. 1977, stored at 10 - 15°C, treated on 18 Nov. and placed in storage at 8°C.


THE EFFECT OF SPROUT SUPPRESSANTS ON FRUCTOSE
CONCENTRATION IN THE CULTIVAR DESIREE
FIG. 6.3.1
control
hand -desprouted
tecnazene
chlorpropham
DMN
fructose concentration (mg/100g)
150
125
100
75
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

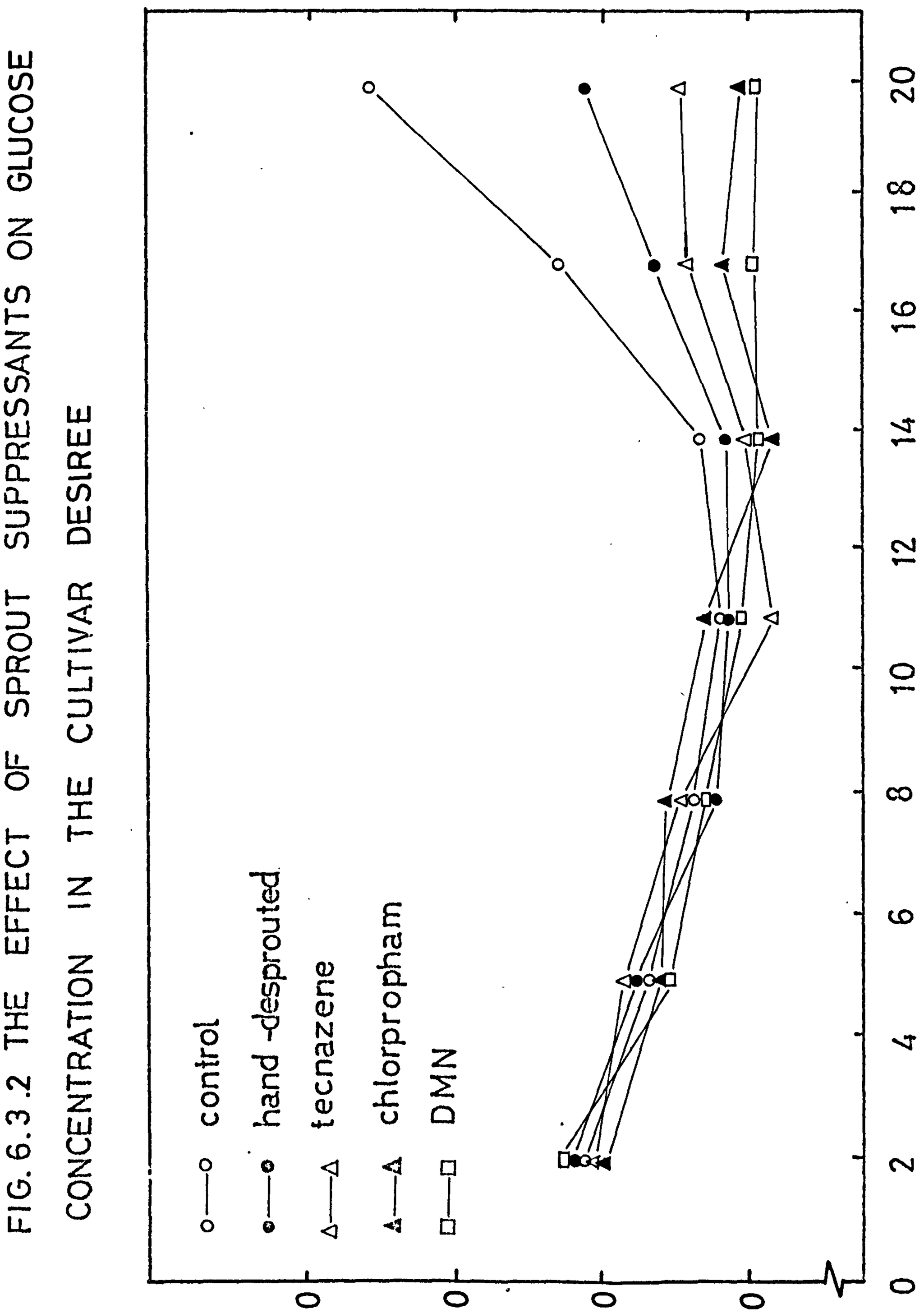


FIG. 6.3.2 THE EFFECT OF SPROUT SUPPRESSANTS ON GLUCOSE
CONCENTRATION IN THE CULTIVAR DESIREE
o—o control
•—• hand -desprouted
Δ—Δ tecnazene
▲—▲ chlorpropham
□—□ DMN
glucose concentration (mg/100 g)
250
200
150
100
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

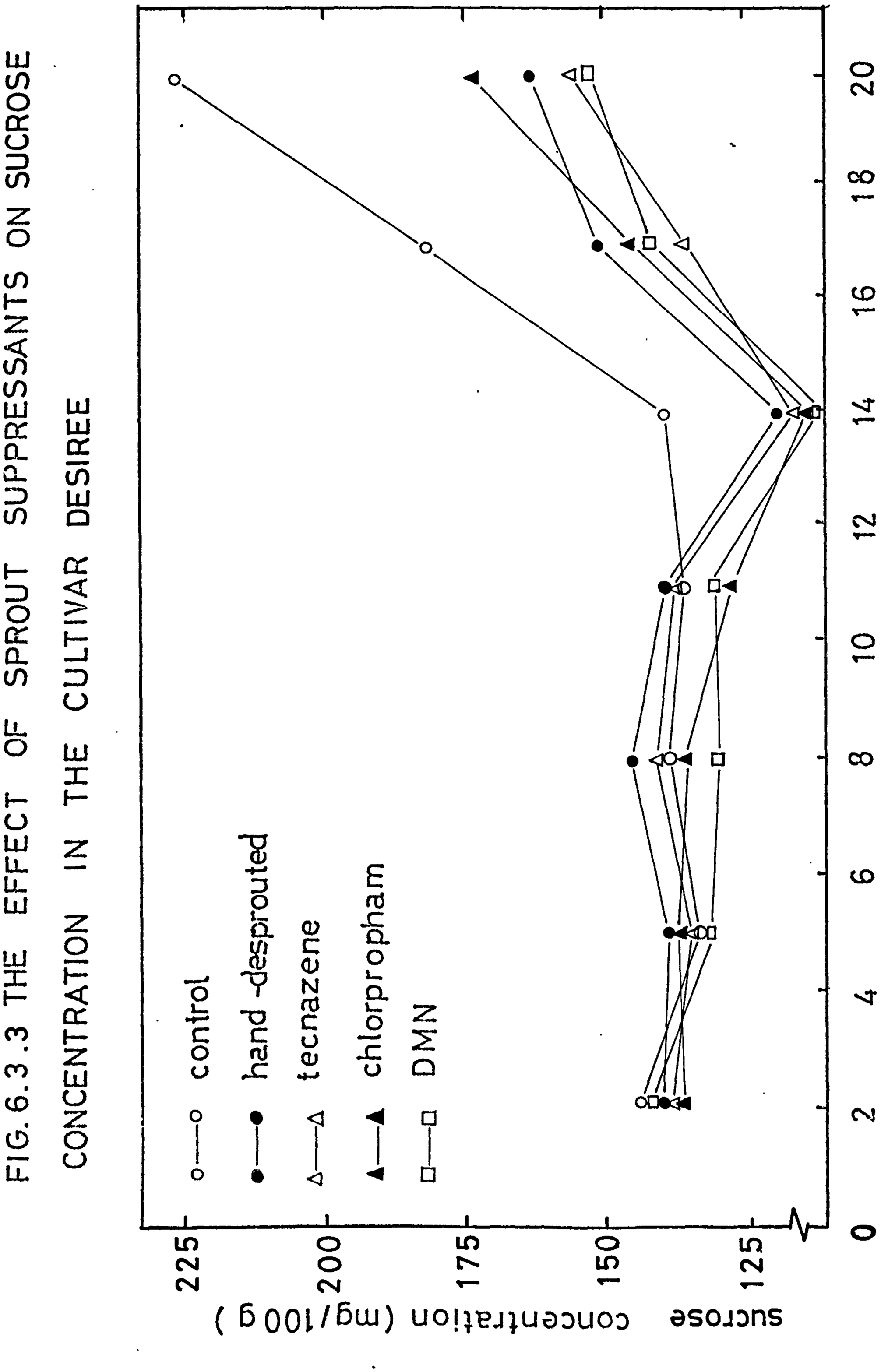


FIG. 6.3.3 THE EFFECT OF SPROUT SUPPRESSANTS ON SUCROSE
CONCENTRATION IN THE CULTIVAR DESIREE
o—o control
●—● hand -desprouted
Δ—Δ tecnazene
▲—▲ chlorpropham
□—□ DMN
sucrose concentration (mg/100g)
225
200
175
150
125
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

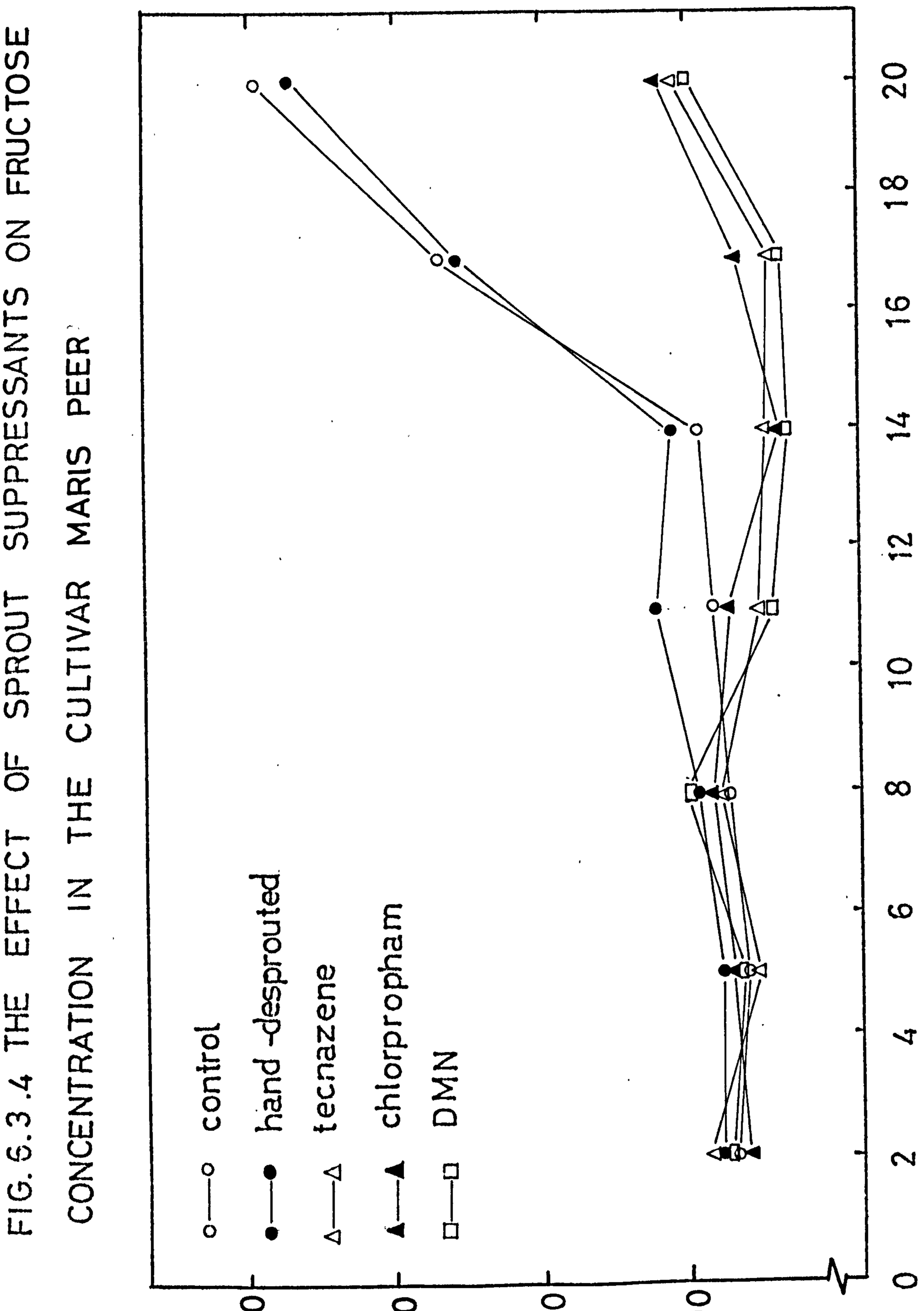


FIG. 6.3.4 THE EFFECT OF SPROUT SUPPRESSANTS ON FRUCTOSE CONCENTRATION IN THE CULTIVAR MARIS PEER
o—o control
●—● hand -desprouted
△—△ tecnazene
▲—▲ chlorpropham
□—□ DMN
fructose concentration (mg/100 g)
250
200
150
100
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

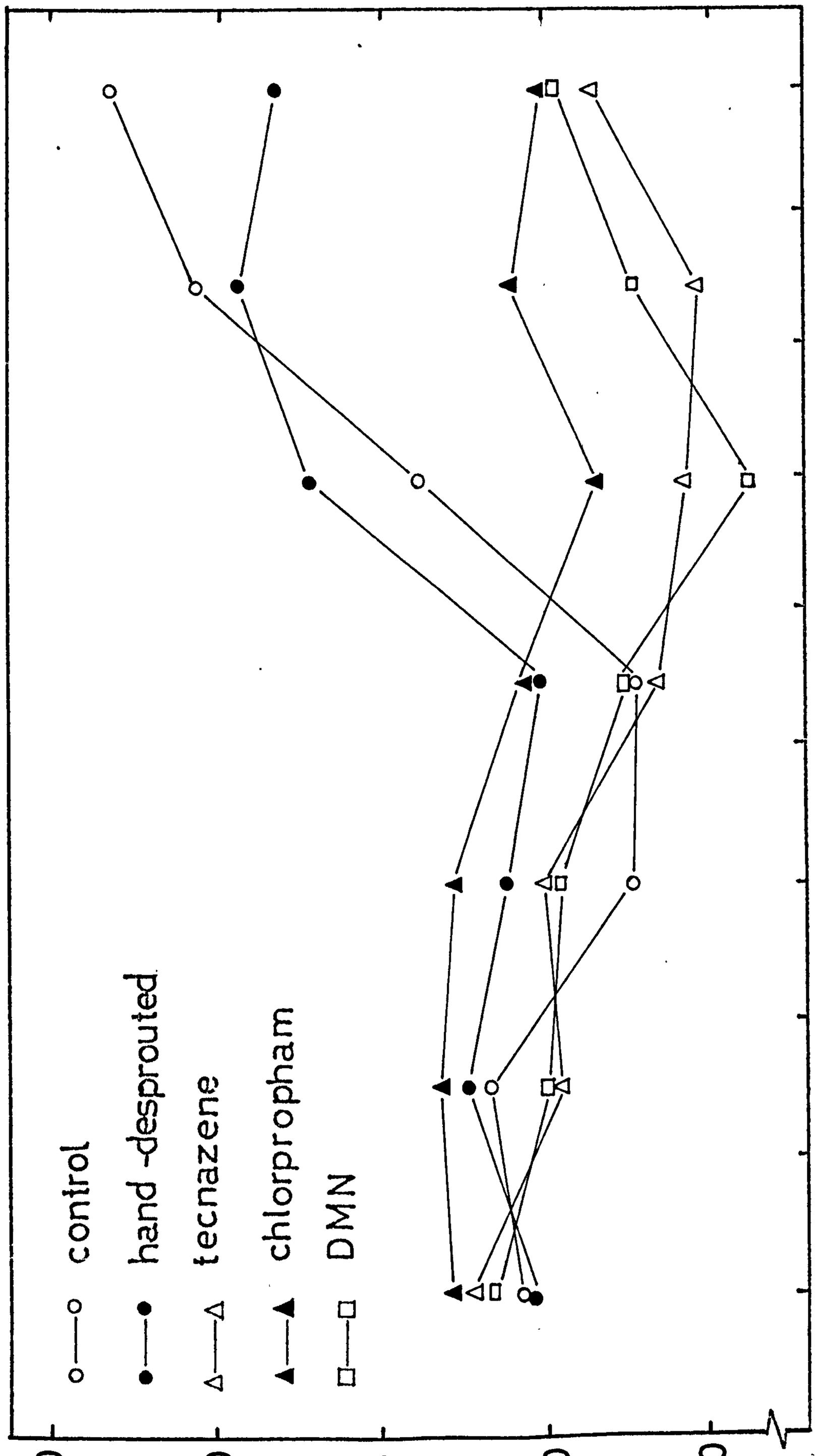


FIG. 6.3.5 THE EFFECT OF SPROUT SUPPRESSANTS ON CONCENTRATION IN THE CULTIVAR MARIS PEER

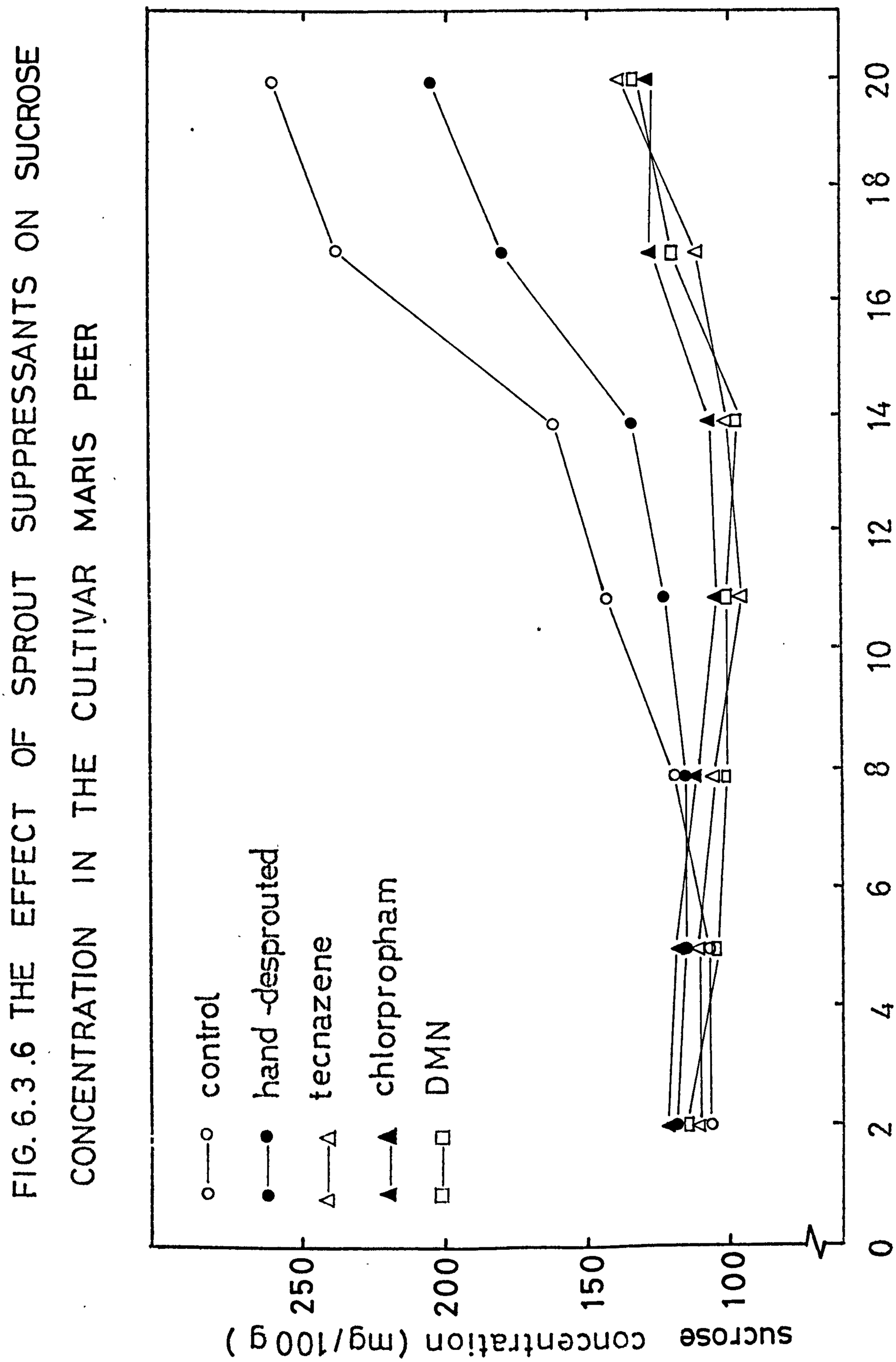


FIG. 6.3.6 THE EFFECT OF SPROUT SUPPRESSANTS ON SUCROSE CONCENTRATION IN THE CULTIVAR MARIS PEER

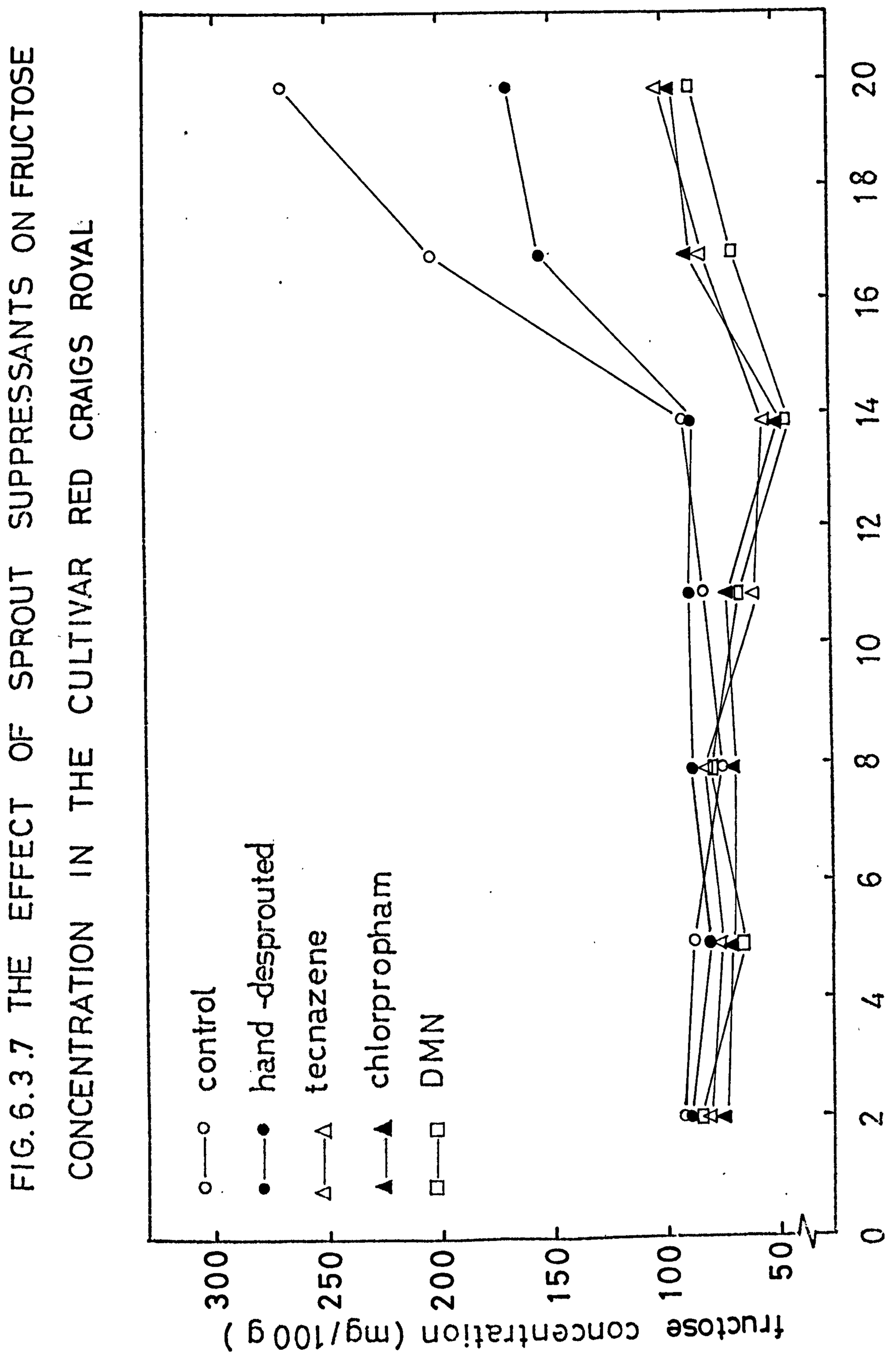


FIG. 6.3.7 THE EFFECT OF SPROUT SUPPRESSANTS ON FRUCTOSE CONCENTRATION IN THE CULTIVAR RED CRAIGS ROYAL
fructose concentration (mg/100 g)
300
250
200
150
100
50
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment
o—o control
•—• hand -desprouted
Δ—Δ tecnazene
▲—▲ chlorpropham
□—□ DMN

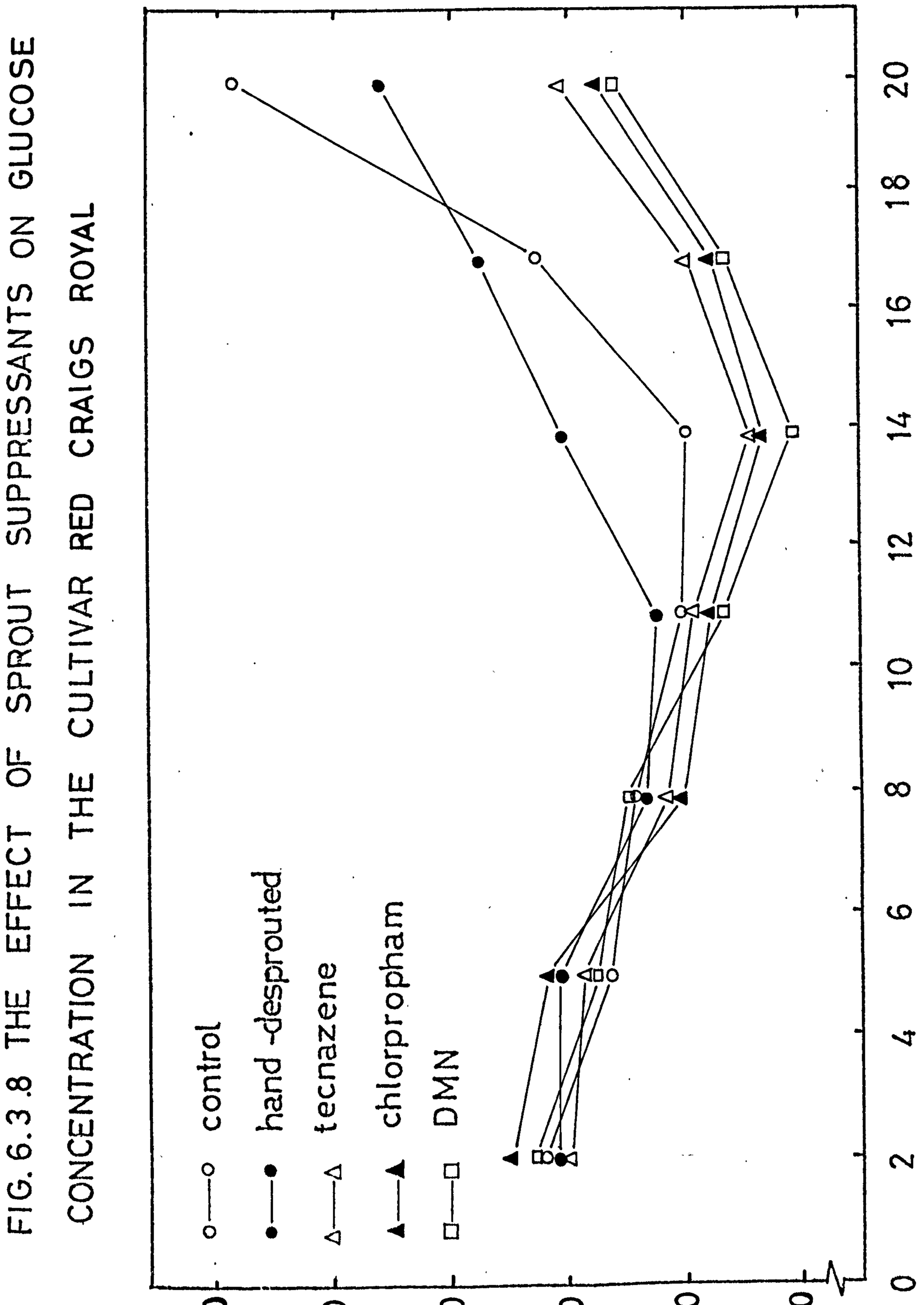


FIG. 6.3.8 THE EFFECT OF SPROUT SUPPRESSANTS ON GLUCOSE
CONCENTRATION IN THE CULTIVAR RED CRAIGS ROYAL
o—o control
•—• handpicked -desprouted
Δ—Δ tecnazene
▲—▲ chlorpropham
□—□ DMN
glucose concentration (mg/100g)
450
400
350
300
250
200
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

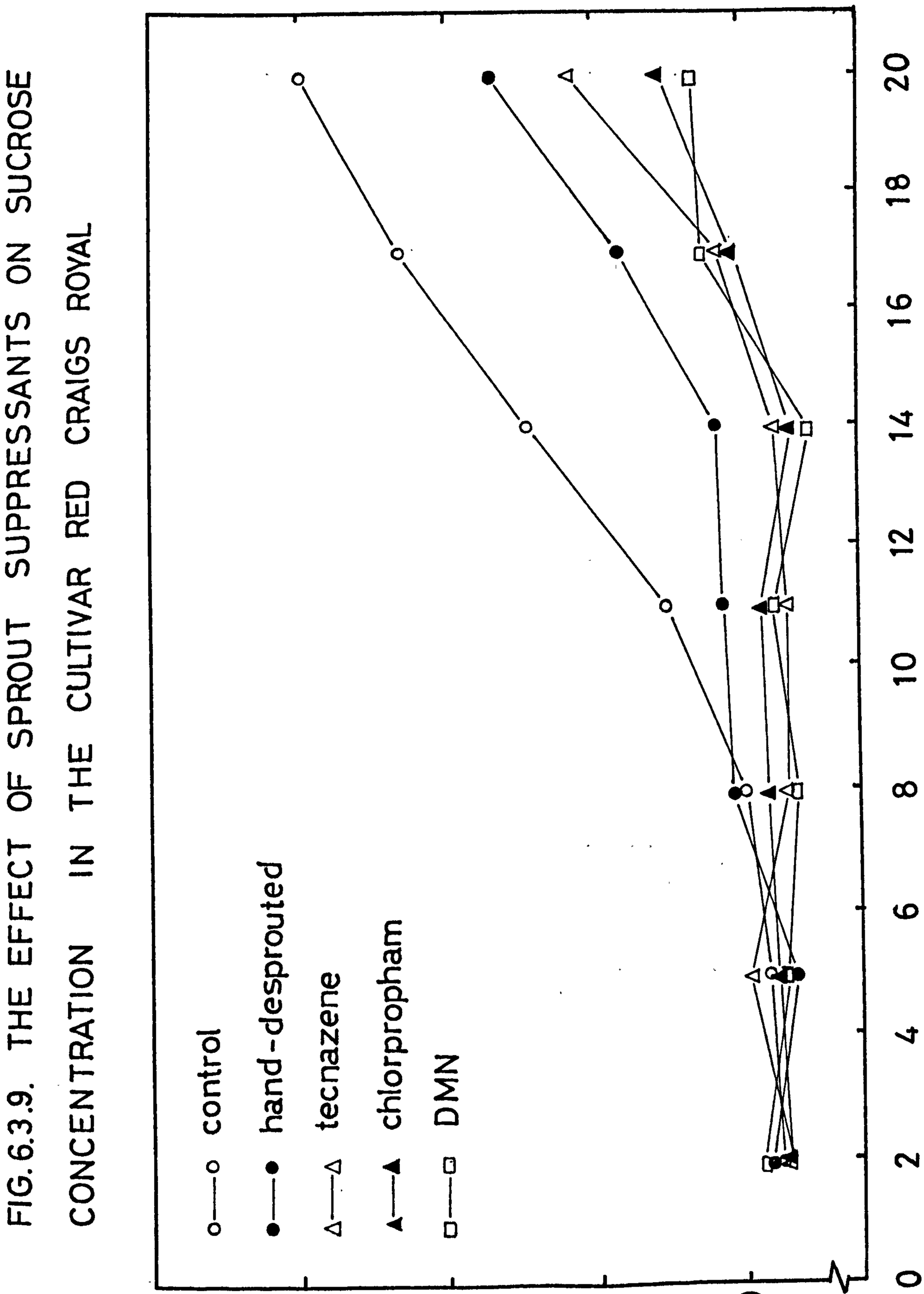
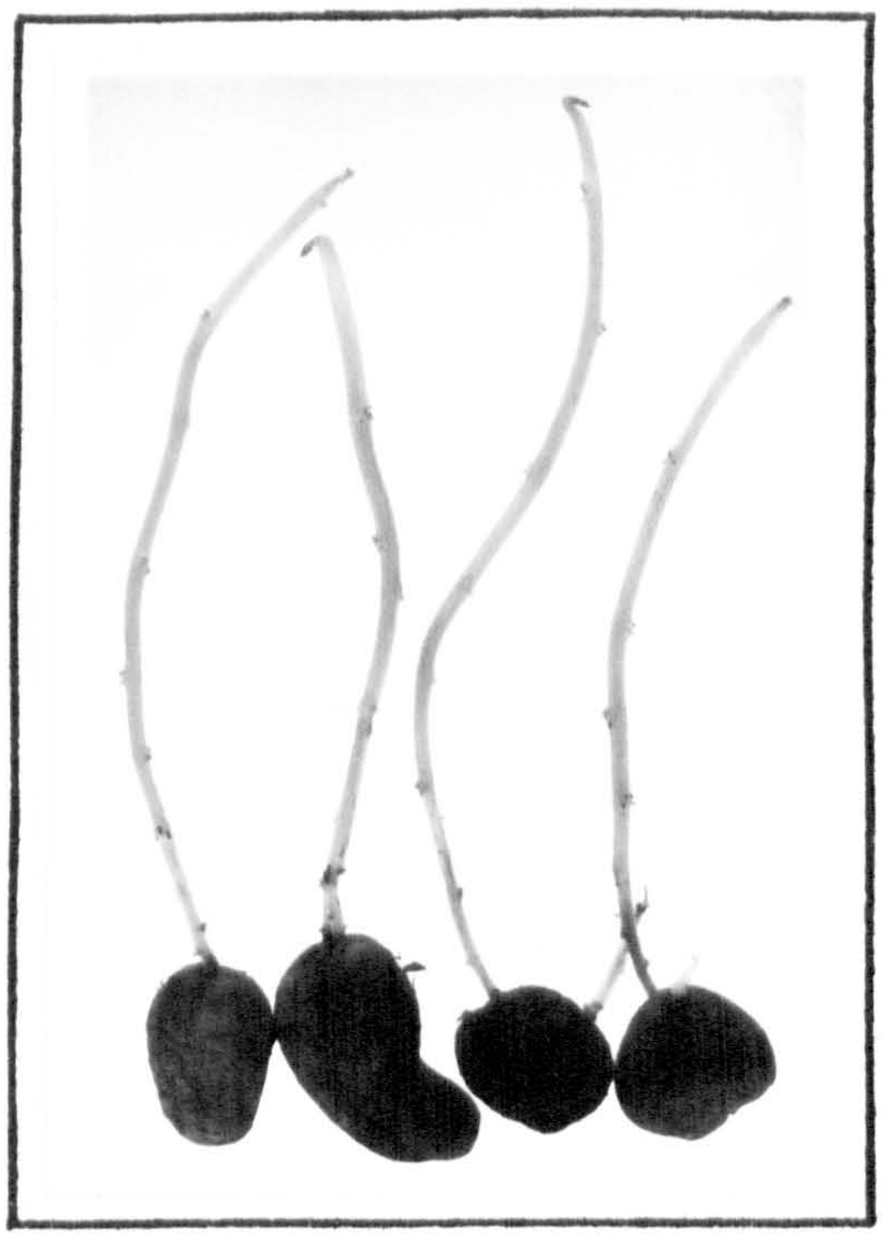
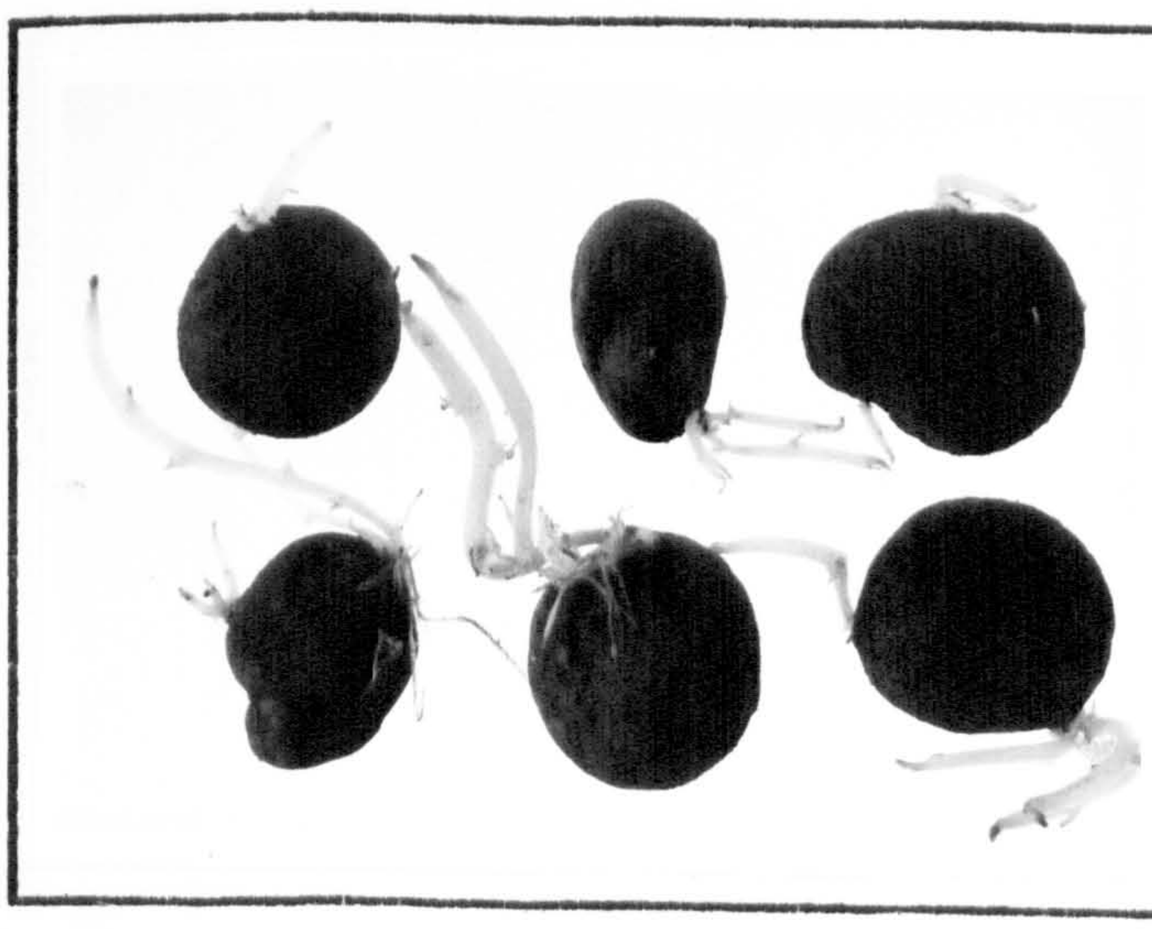


FIG. 6.3.9. THE EFFECT OF SPROUT SUPPRESSANTS ON SUCROSE
CONCENTRATION IN THE CULTIVAR RED CRAIGS ROYAL
o—o control
●—● hand-desprouted
△—△ tecnazene
▲—▲ chlorpropham
□—□ DMN
sucrose concentration (mg/100g)
250
200
150
100
0
2
4
6
8
10
12
14
16
18
20
weeks after treatment

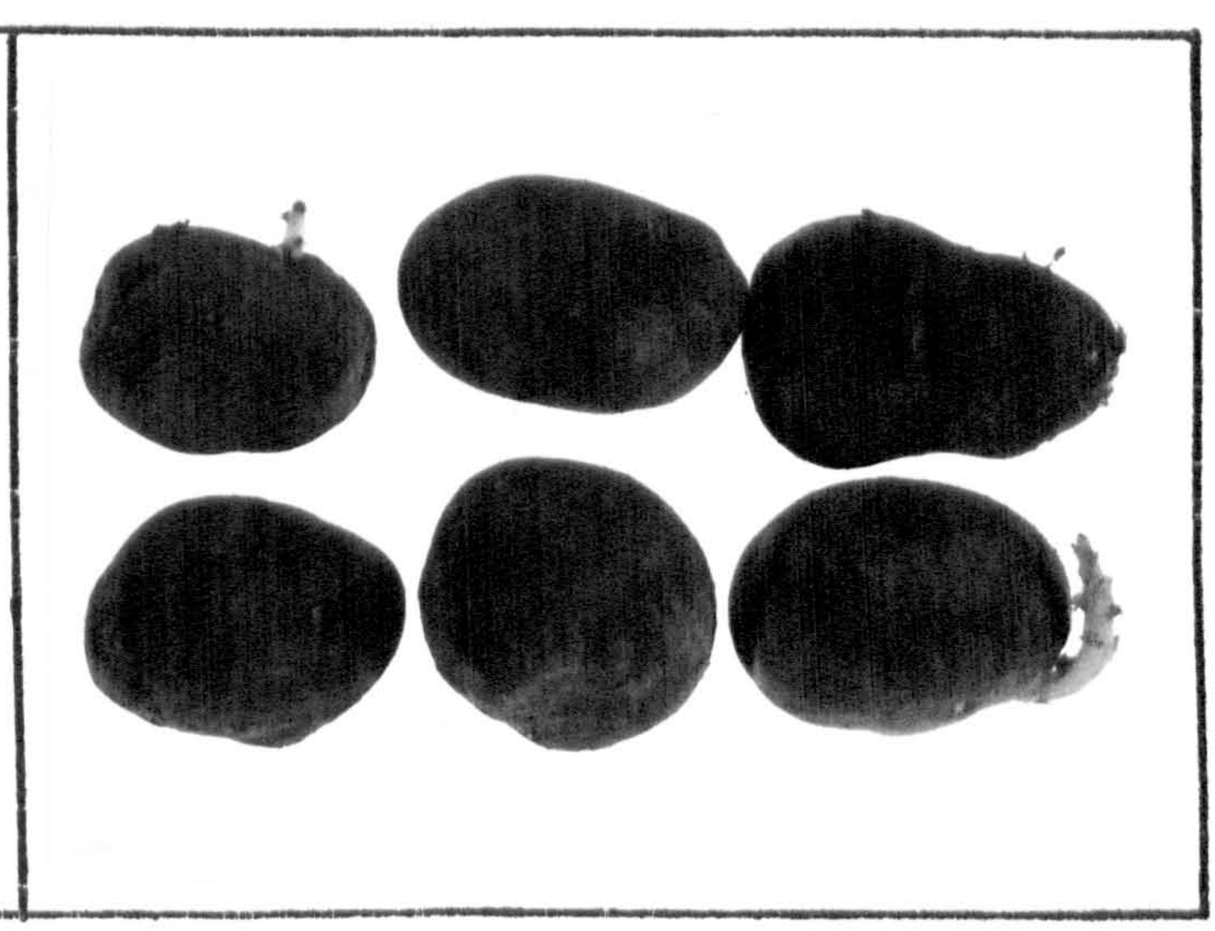
Plate 6.3.1. Tubers of the cultivar Desiree stored for 20 weeks at 8°C after treatment with sprout suppressant chemicals.



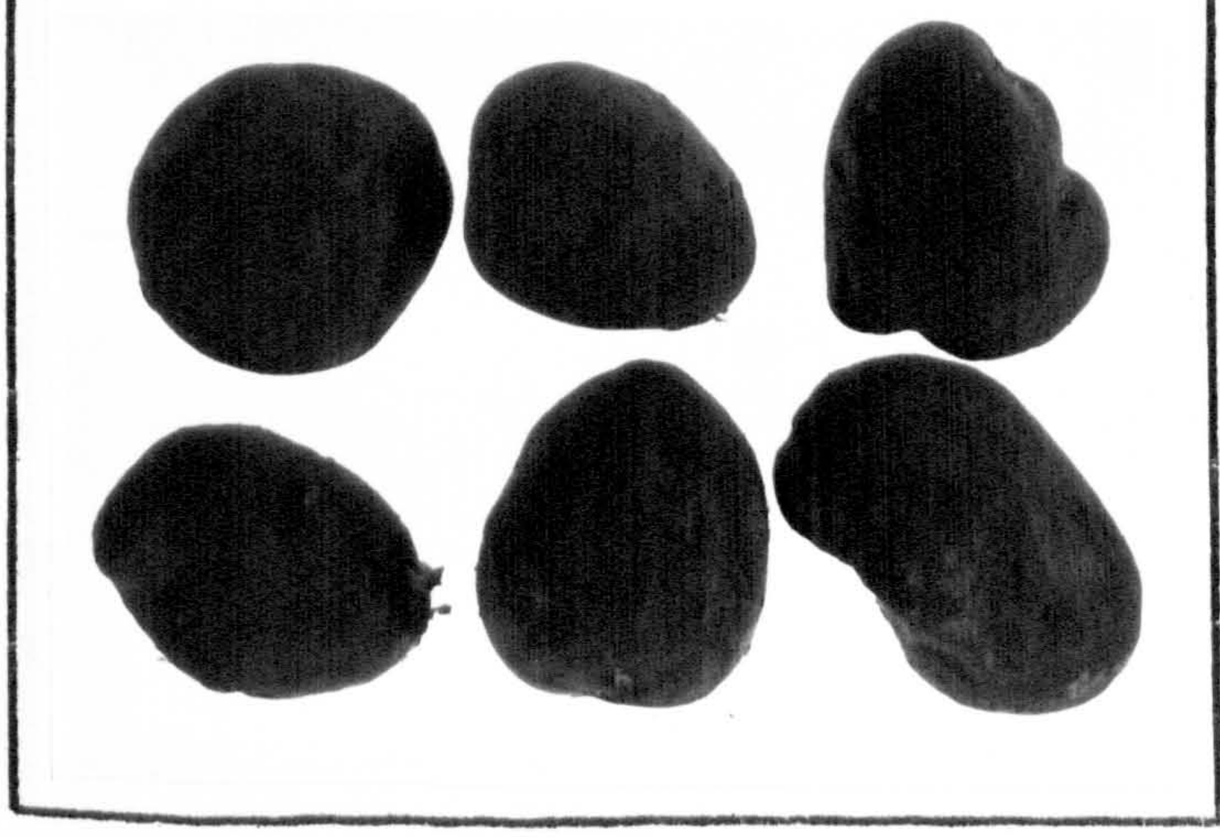
CONTROL



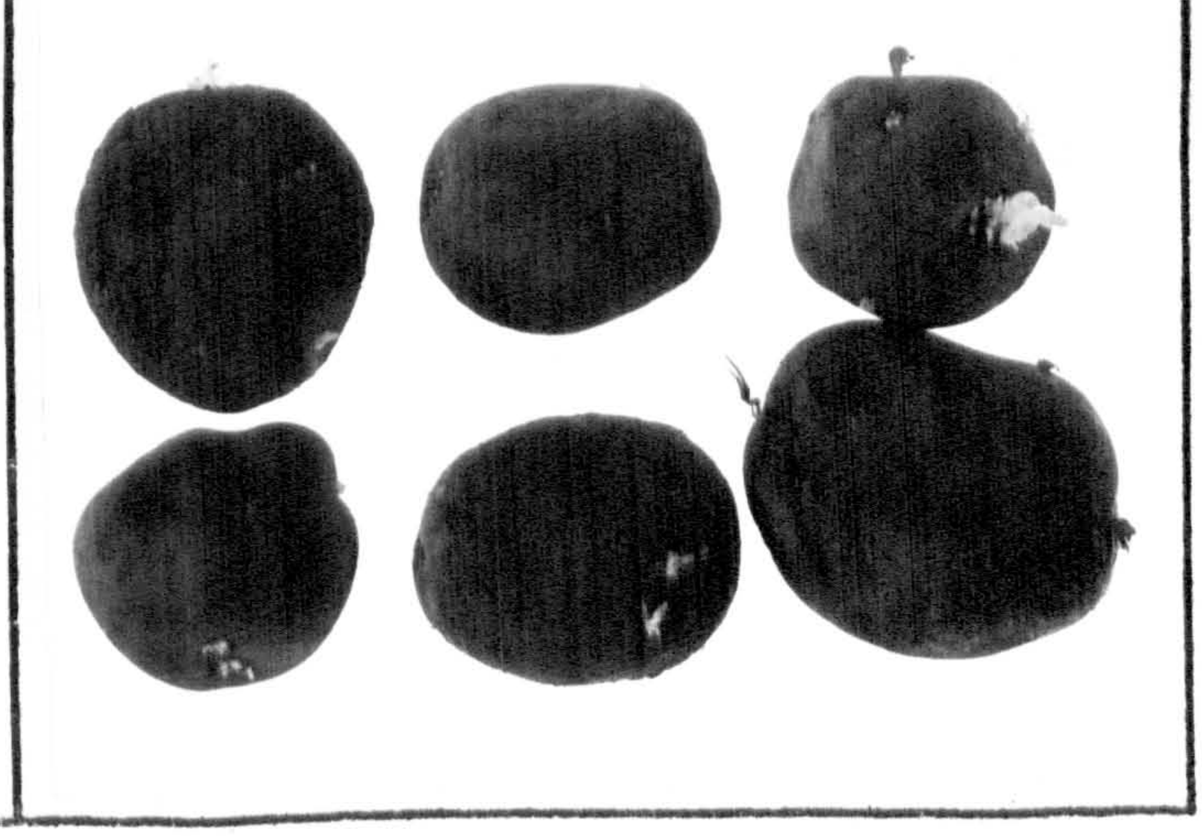
HAND-DESPROUTED



TECNAZENE

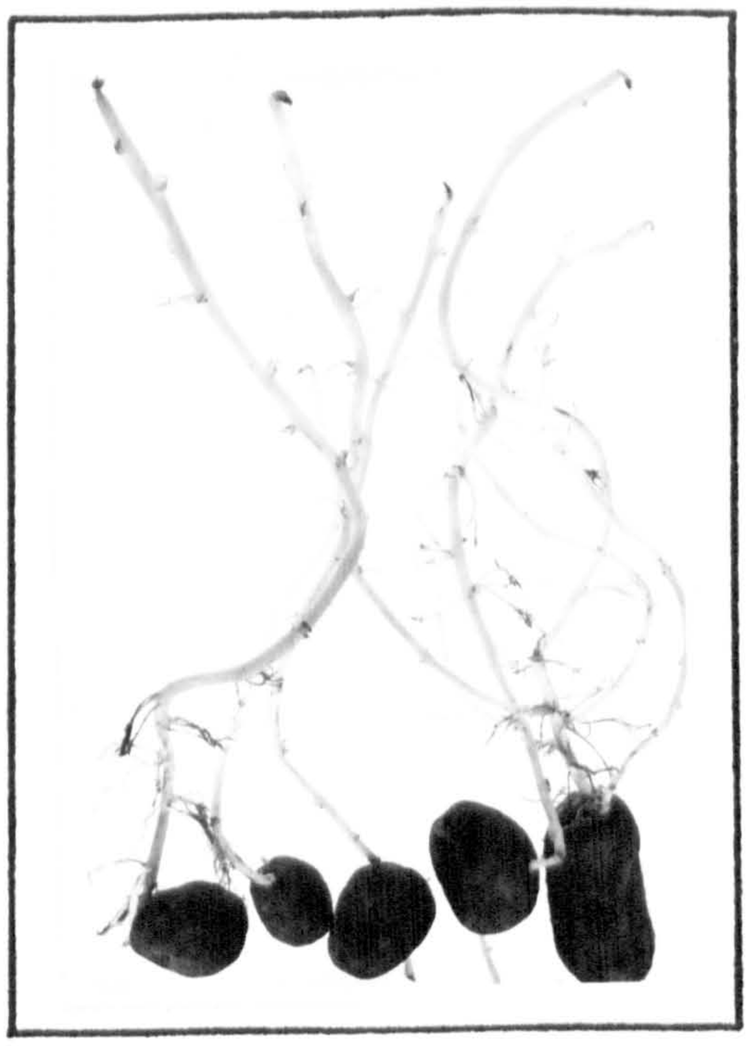


CHLORPROPHAM

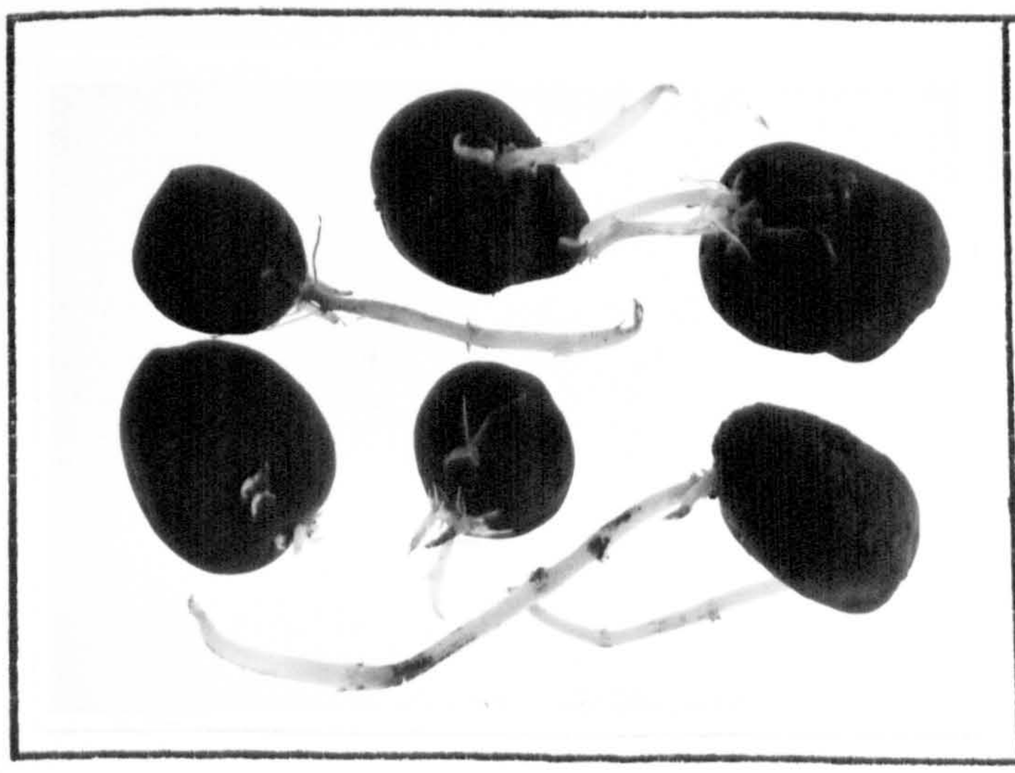


DMN

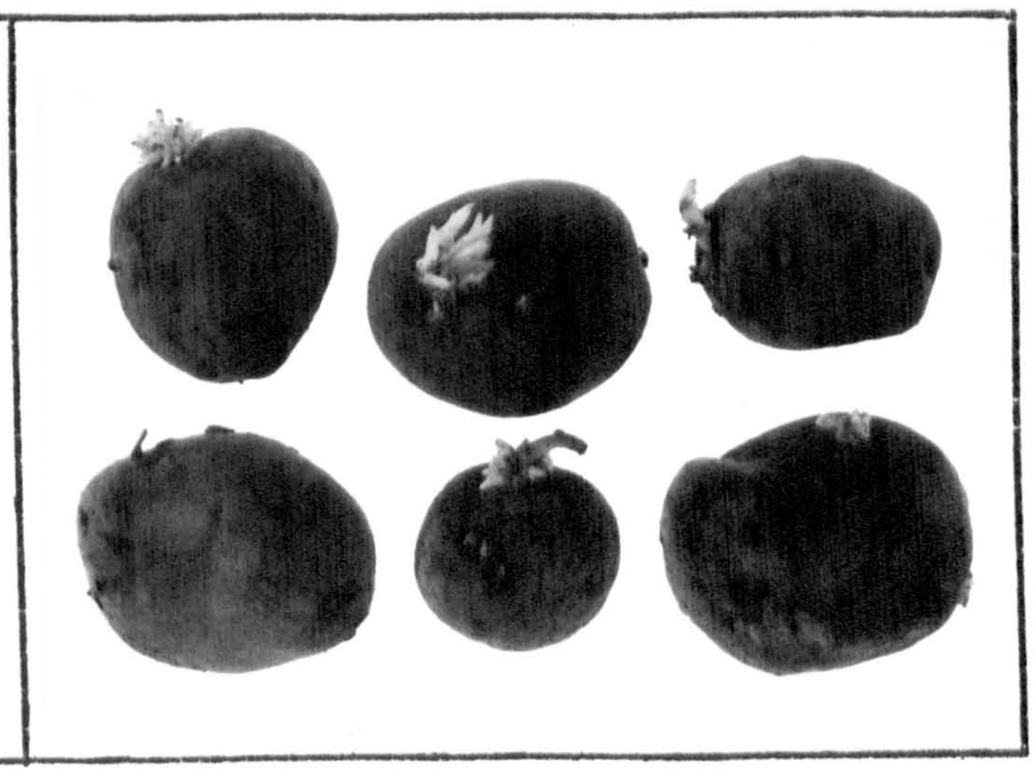
Plate 6.3.2. Tubers of the cultivar Maris Peer stored for 20 weeks at $8^{o}C$ after treatment with sprout suppressant chemicals.



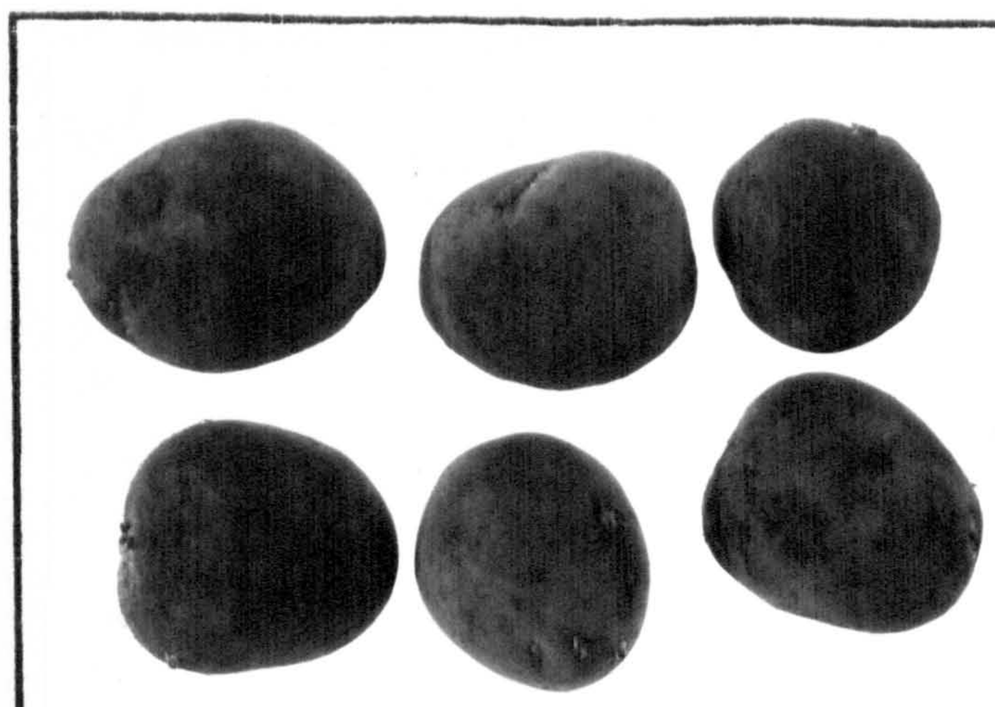
CONTROL



HAND-DESPROUTED



TECNAZENE

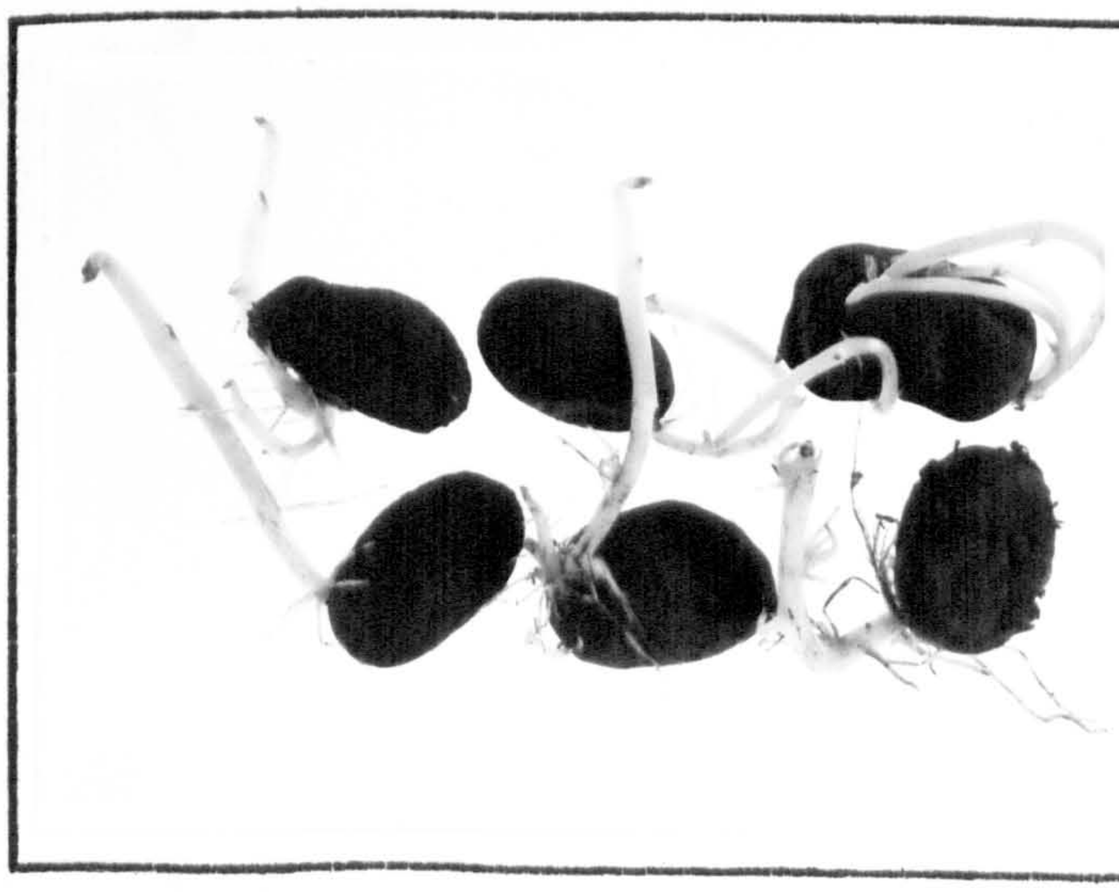


CHLORPROPHAM

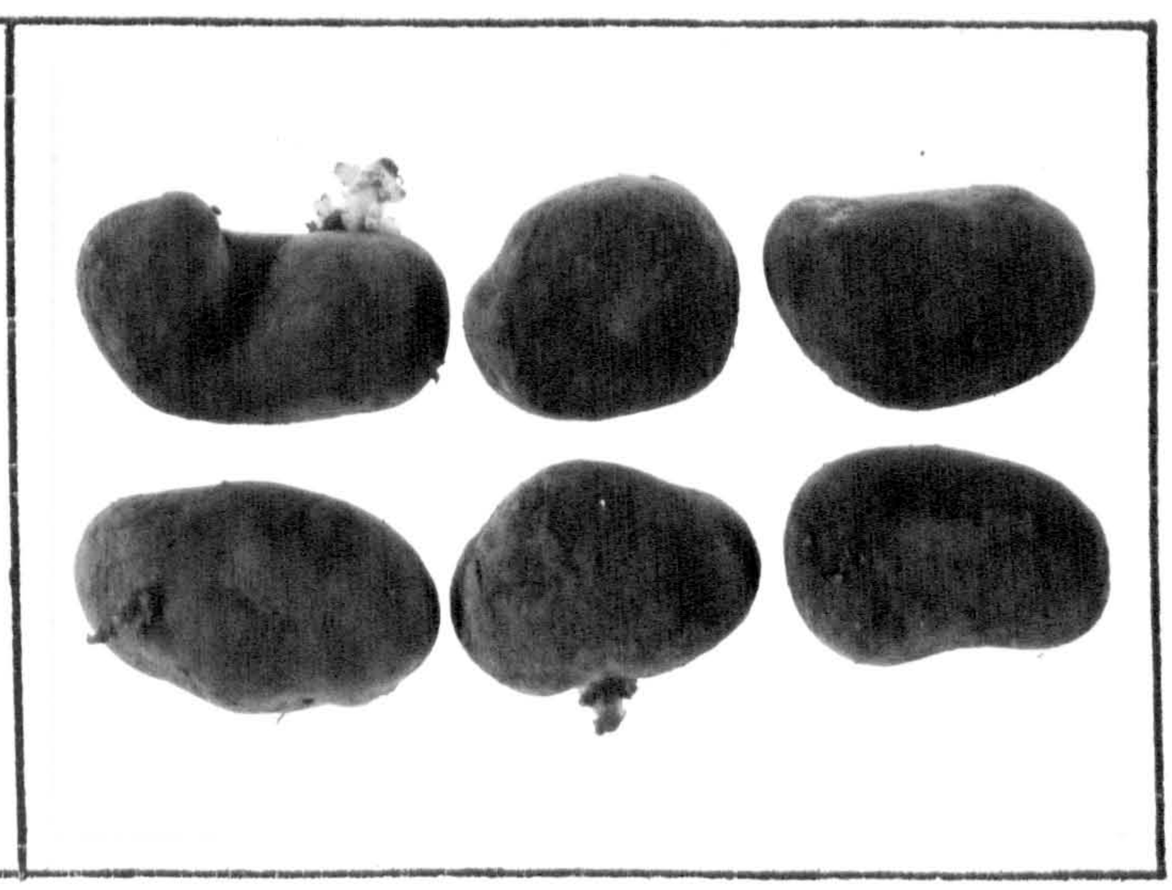
DMN

Plate 6.3.3. Tubers of the cultivar Red Craigs Royal stored for 20 weeks at 8°C after treatment with sprout suppressant chemicals.

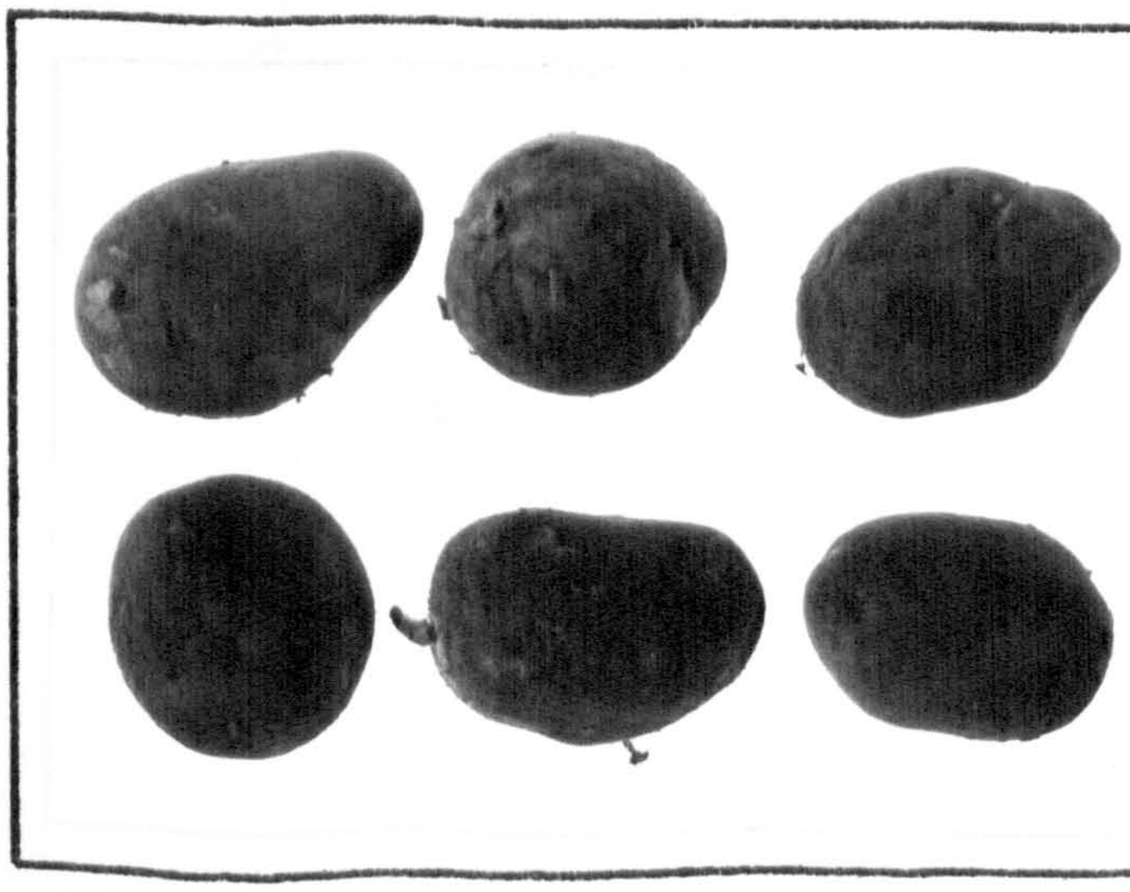
CONTROL



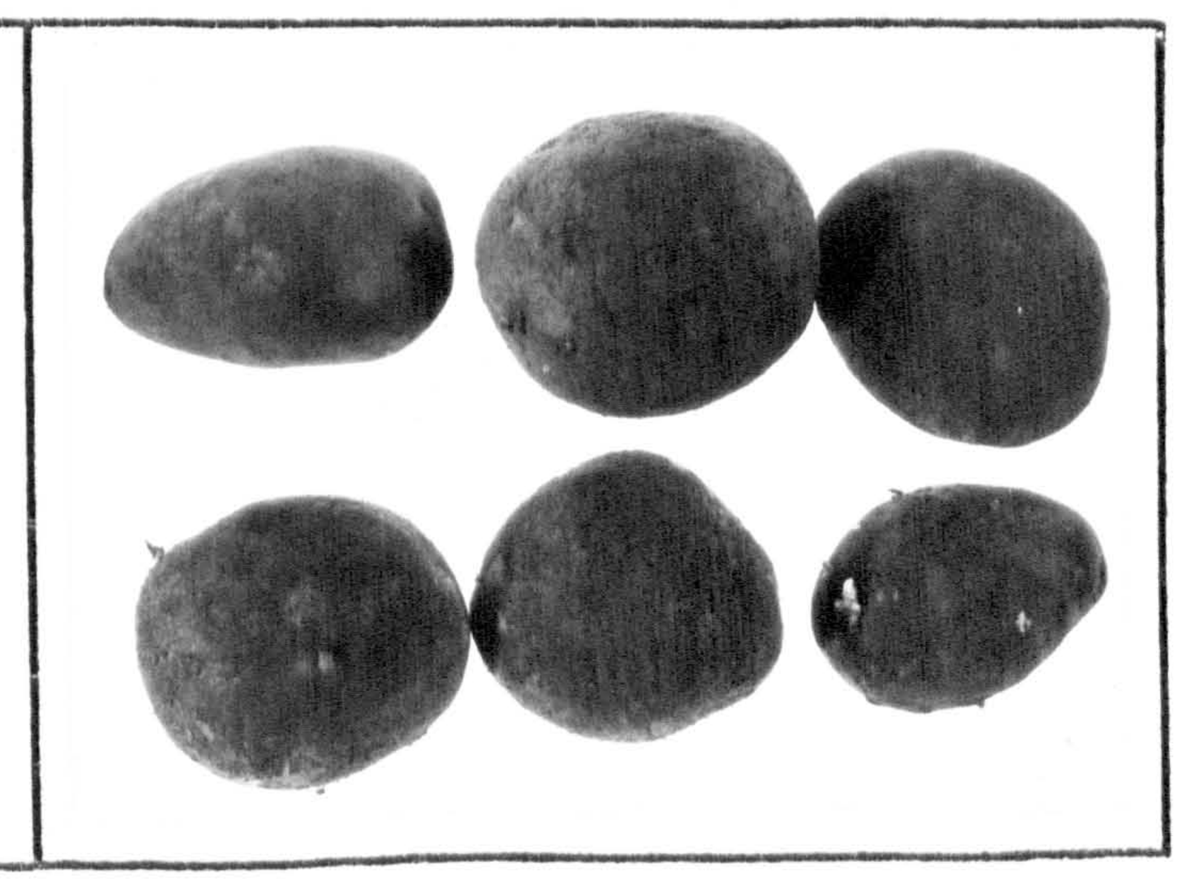
HAND-DESPROUTED



TECNAZENE



CHLORPROPHAM



DMN

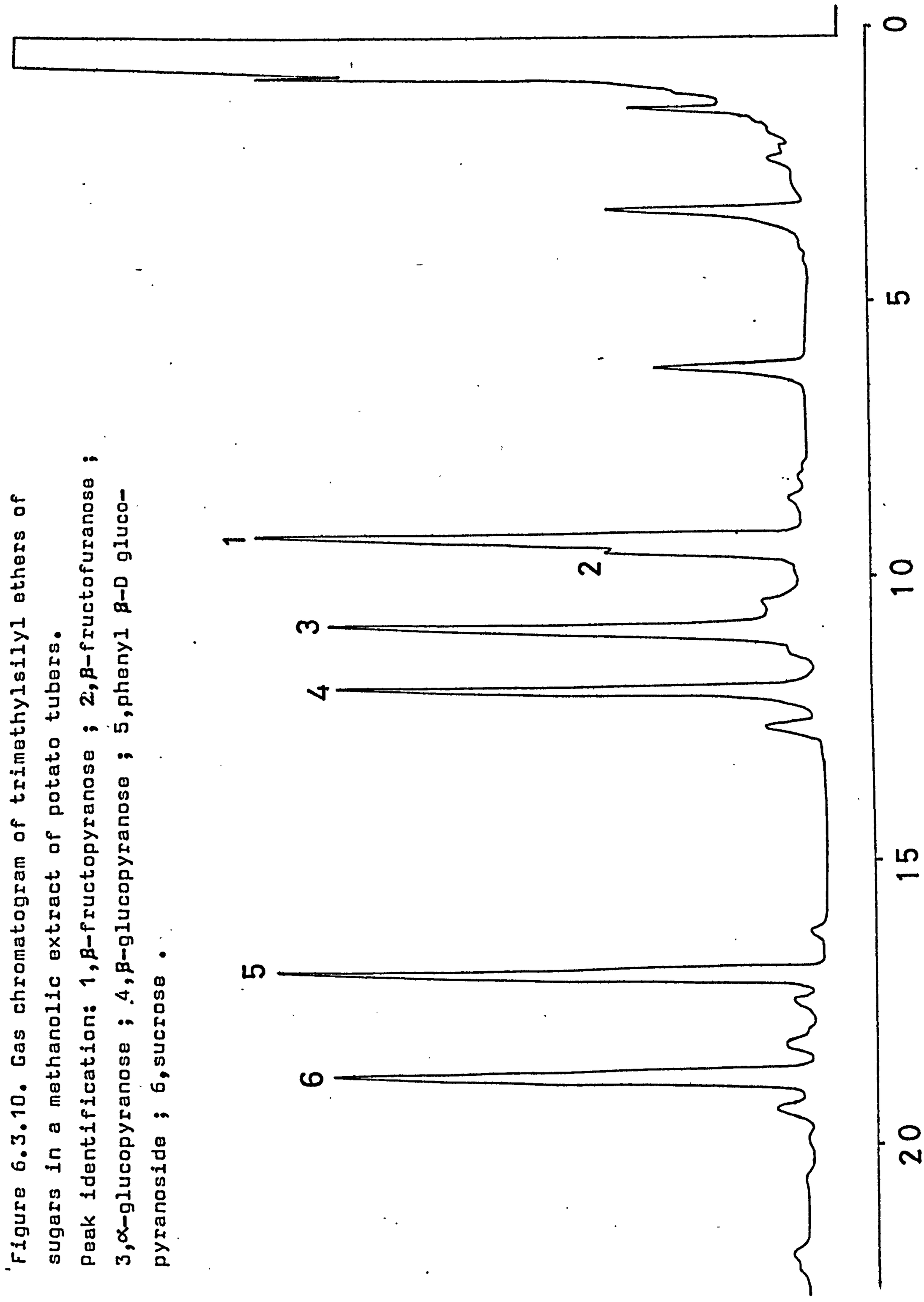


Figure 6.3.10. Gas chromatogram of trimethylsilyl ethers of sugars in a methanolic extract of potato tubers. Peak identification: 1,β-D gluco-3,α-glucopyranose ; 2,β-fructofuranose ; 3,β-fructopyranose ; 4,β-D gluco-pyranoside ; 5,phenyl β-glucopyranose ; 6,sucrose.

with the previous experiment, and fairly conclusive trends are apparent.

1) During the first 10 weeks of the experimental storage period sugar levels showed only marginal changes. There are indications of a slight decrease in fructose, glucose and sucrose concentrations in most instances. This is especially marked for glucose in the cultivar, Red Craigs Royal (see figure 6.3.8.).

2) During the early period there was no consistent differences between any of the treatments. Apparently chemical sprout suppressants have little effect on sugar content in non-sprouting tubers stored at 8°C. This may account for the numerous reports that sprout suppressants have no effect on sugar levels whatsoever (eg. Zaehringer et al, 1966; Kennedy and Smith, 1953). On the otherhand it should be noted that Moll (1968) found that chlorpropham reduced the respiration rate and reducing sugar content in non-sprouting tubers at 2°C and concludes that chlorpropham cannot be regarded merely as an antimitotic agent acting only on the buds, but seems to influence the metabolism of the tuber tissue in its entirety.

3) During the second ten week period there was a tendency for most treatments in all three cultivars to accumulate fructose, glucose and especially sucrose.

4) The accumulation of all three sugars usually occurs later and to a much lesser extent in the chemically treated tubers than in the untreated and hand-desprouted batches. This is in general agreement with the results obtained in the preliminary investigation with tecnazene and with the effects of chlorpropham demonstrated by Moll (1968) at 7 and 12°C, Rumpf (1972) at 10°C, Patzold (1974) at 7 and 10°C, and Baijal and van Vliet (1966) at 10°C. Similar effects can also be produced by maleic hydrazide provided it is applied early enough (Paterson et al, 1952; Salunkhe et al, 1953; Payne and Fults, 1955; Moll, 1973).

5) The accumulation of sugar is associated with the degree of sprouting as can be clearly seen by comparing the sugar levels with the photographs in plates 6.3.1. - 6.3.3. This is in accordance with the findings of van Vliet and Schriemer (1963) and Burton (1965).

6) The levels of all three sugars were lower in the hand-desprouted treatment than in the untreated material contrary to the findings of Patzold (1974) and Isherwood and Burton (1975). However it should be noted that there are substantial differences in experimental methods between these authors and the present investigation. Patzold (1974) desprouted weekly by mechanical damage in a revolving drum, and Isherwood and Burton (1975) removed sprouts manually at weekly intervals and stored at a much higher temperature. In the current investigation tubers were only desprouted twice with the minimum disturbance possible on each occasion.

7) The particular chemical applied appears to be of little consequence providing that it is effective - a finding also reported by Schipper (1975). The experimental results indicate that tubers treated with tecnazene, whose effectiveness was diminishing by the end of the experiment (see plates 6.3.1. - 6.3.3.), contained the highest total sugar levels of the three chemicals. DMN and chlorpropham both effectively inhibited sprouting and have approximately equal total sugar levels.

8) No evidence was found in the present experiment to support the theory that chemical sprout suppression caused sugar to accumulate to a greater extent than in untreated controls as reported by van Vliet and Schriemer (1963), Burton (1965) and Isherwood and Burton (1975). The results of the last paper describing the effects of chlorpropham can probably be accounted for by the high storage temperature as similar findings were also reported by Moll (1968) at the same temperature. The reasons for the difference in effect at higher temperatures is still unexplained. The other two experiments both involved nonanol which may of course have some specific effect due to its high phytotoxicity. However, van Vliet and Schriemer (1963) also included chlorpropham and their finding that this compound increased sucrose accumulation at $10^{o}C$ in the cultivar Bintje remains unsupported.

9) The magnitude of the difference between chemically treated and control tubers in tendency to accumulate sugar was sufficient to be of great commercial significance. Chemical sprout suppressants can therefore preserve processing quality when used on tubers stored at $8^{o}C$.

Conclusion

This experiment has shown that tecnazene, chlorpropham and DMN when applied under conditions resembling those of standard commercial practice and involving harvest at maturity, conditioning at 10 - 15°C for a wound healing period, chemical treatment and storage at 8°C, have little effect in the short term but during storage for several months these chemicals reduce the accumulation of fructose, glucose and sucrose to an extent likely to have a significant effect on processing quality.

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