SOME PHYSIOLOGICAL EFFECTS OF THE HERBICIDE BROMACIL (5-BROMO-3-SEC-BUTYL-6-METHYLURACIL) ON *ASPARAGUS OFFICINALIS* L.

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ABSTRACT

The root-absorbed, photosynthesis-inhibiting herbicide bromacil (5-bromo-3-sec-butyl-6-methyluracil) was applied in sand culture to tissue-cultured 18-month-old Mary Washington 500W clone of Asparagus officinalis L. grown under controlled environmental conditions.

Dose-response characteristics were determined and ED_{20} and ED_{50} values computed by regression analysis for several parameters for asparagus plants exposed to a single application of 0, 2, 4, 8, 16, 32, 64, 128, 256, and 512 p.p.m. bromacil in non-draining pots. The results of this initial broad spectrum studies revealed a drastic decline in visually assessed foliage damage score, shoot growth and root fresh weight, and an increase in shoot death at relatively low concentrations. Good dose-response characteristics were obtained, and time-course data showed that the rate and severity of effects increased with increasing dose. The ED_{50} values 18 days after treatment were: visually assessed damage score, 2.7 p.p.m.; shoot growth, 25 p.p.m.; shoot death, 4.6 p.p.m.; and root fresh weight, 2.1 p.p.m.

A catalogue of colour plates showing visual phytotoxic effects was compiled. The injury symptoms observed were: yellowing of cladophyll tips followed by bleaching with the effects extending towards the base, cladophyll tipping and progressive cladophyll death leading to shoot death.

Equal increment dose-response experiments were conducted at 0, 2, 4, 6, and 8 p.p.m. bromacil, using a portable fluorometer (Model SF-10) to obtain fluorescence emission measurements. The results showed a dramatic decline in the initial rise in fluorescence yield from the cladophyll tips 156 hours after treatment. The ED_{50} value was computed to be 2.3 p.p.m. Fluorescence emission measurements from cladophyll tips from excised shoots placed in bromacil solution at the same concentrations showed a dramatic decline in fluorescence yield within 17 hours indicating that uptake and translocation was more rapid without the roots.

No significant changes in chlorophyll a, chlorophyll b and total chlorophyll concentrations, as determined by 80% acetone extraction technique, were evident in the samples in which a dramatic decline in fluorescence yield

occurred.

The results of this study, conducted under controlled environmental conditions, showed that the asparagus clone tested readily absorbed bromacil through its roots and translocated it to the foliage causing severe initial damage to the photosynthetic apparatus followed by detrimental effects on other parameters such as shoot growth, root fresh weight and shoot death. Even at a bromacil concentration of 2 p.p.m. the asparagus plants were found to susceptible to herbicide damage.

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INTRODUCTION

During the past two or three decades we have seen a widespread acceptance of herbicides in agriculture, horticulture and forestry, and a rapid introduction of new chemicals and application techniques. In New Zealand some 123 different formulations and mixtures are now commercially available (O'Connor,1984) and the estimated expenditure on herbicides for 1984 was $8.4 million (Popay,1984).

In horticulture, a number of soil-applied herbicides have been widely used in most parts of New Zealand. One of these is bromacil. Bromacil (5-bromo-3-sec-butyl-6-methyluracil) is used for the long-term selective weed control of many annual and perennial weeds on asparagus plots in New Zealand. The herbicide is marketed under the trade name "Hyvar X" which contains 800g/kg (80%) of the active ingredient, bromacil. The manufacturer recommends that rates up to 3kg/ha of "Hyvar X" be applied on asparagus which has been established for at least 12 months, as a pre-emergence broadcast treatment before the harvesting season commences.

Since bromacil is a persistent, root absorbed broad spectrum herbicide and asparagaus is a perennial crop, the possibility exists for crop damage due to accumulatory effects. It is known that herbicides act differently under differing conditions. The soil texture (sand,silt,clay), amount of organic matter,climate (precipitation, temperature), all have a bearing on the effectiveness, residual life and safety of the chemical. In New Zealand many people have expressed doubts concerning the safety of bromacil on asparagus (Franklin, 1983).

Recommendations for soil-applied herbicides like bromacil are usually based on field tests carried out over a period of 2-3 years on a range of soil types, chosen to ensure that the conditions experienced will include the extremes encountered in commercial usage. In practice, reliability of the results, especially in respect to crop safety, is very dependent on environmental factors, especially rainfall. More recently, pot tests with plants grown in sand have been developed by researchers at the Weed Research Organisation at Oxford in England to provide more reliable information on crop tolerance to herbicides (Clay

& Davidson, 1978). Pot test tolerance studies on the effects of bromacil on asparagus, conducted under controlled environmental conditions, have not been reported.

The objectives of this research project are to study, under rigorously controlled conditions and using sand culture techniques developed at the Weed Research Organisation, the tolerance of asparagus to bromacil in terms of dose-response relationships. This study will be in three parts:

Part I: In vitro clonal propagation of asparagus plants for use in Part II and Part III of the project.

Part II: A broad spectrum experiment to investigate a wide range of herbicide concentrations to obtain a full range of responses and to determine the ED_{20} and ED_{50} phytotoxic limits. (ED_{20} or ED_{50}= "Equivalent Dose 20 or 50 ": herbicide concentration that causes 20% or 50% plant growth response compared to the untreated control. ED_{20} or ED_{50} values can be derived from dose-response curves).

Part III: Equal increment dose-response experiments around the ED_{20} and ED_{50} limits to determine the speed of action, degree of response and tolerance to increase in herbicide concentration.

CHAPTER 1

CROP: Asparagus officinalis L.

1.1 INTRODUCTION

The asparagus plant from which edible shoots , called spears, are harvested is Asparagus officinalis L. subspecies officinalis and belongs to the Liliaceae family [Tutin et al. (1980) cited in Robb (1984)]

As a popular vegetable delicacy asparagus has been a plant of economic importance from the Greek and Roman Empires (Thompson & Kelly, 1952; Luzny, 1979). Asparagus is believed to have its origins in the Orient and the eastern part of the Mediterranean. Crusading troops returning from the Near East introduced the crop to the European sub-continent (Luzny,1979). Thus, asparagus has been known in the regions of Central Europe from very early times. Even in the year 304 A.D. it was such an expensive vegetable that the emperor Diocletian was compelled to regulate prices by rules (Wricke, 1979).

The 18th century is viewed as an important period in the widespread cultivation of asparagus in France, Germany and other parts of Europe (Luzny, 1979). Today asparagus is ranked as one of the top horticultural crops of economic importance in a number of countries in different continents. Some 23 countries have been listed by Heath (1983) as producing white or green asparagus spears and the estimated world output for 1979 was 432,000 tonnes. The five largest producers in 1979 were:

U.S.A.	112,000 tonnes
Taiwan	102,000 tonnes
France	49,000 tonnes
Italy	40,000 tonnes
Spain	35,000 tonnes

In New Zealand asparagus is one of the few vegetables which is currently attracting widespread interest. In the 1982/83 and 1983/84 N.Z. Summary of Research on Vegetable Crops (Wood, 1984) there were

more reports on asparagus than any other single vegetable crop. In March 1983 the N.Z. Asparagus Council , a growers' council was formed (McLeod, 1983). In May 1983 some 130 persons attended an Asparagus Growers Short Course organised jointly by the Department of Horticulture and Plant Health at Massey University and the Levin Horticultural Research Centre. In the last few years significant increases in plantings have occurred in different parts of New Zealand; from an estimated 630 hectares in 1979 to 3000 hectares in 1983 (Wood, 1983). The expected dramatic increase in total crop production in N.Z. is shown in Figure 1.1.

1.2 RELEVANT BOTANICAL BACKGROUND

Asparagus officinalis L. is a herbaceous perennial plant which can thrive for 15 years or more without the need to replant after each harvest (Haynes, 1983). It takes about 4 years before it reaches full harvest (McLeod, 1983).

The natural habitat of asparagus appears to be on coastal sand dunes above the high tide mark as the plant has been found growing wild in such areas. The plant thus requires very good drainage and for optimal production an open-textured, deep, well aerated, free draining soil. Pure sands or free draining fine sandy loam are often recommended as most suitable for asparagus production (Haynes, 1983).

The underground portion of the asparagus plant is known as the 'crown'; the foliage as the 'fern'. The crown consists of many unbranched fleshy storage roots attached to the closely spaced basal internodes of an underground stem called a 'rhizome'. Fibrous feeding roots which extract water and nutrients from the soil, are attached to the storage roots. The storage roots store carbohydrates to maintain growth and spear (young unexpanded shoot) production in spring. In a mature plant the storage roots are up to 6mm thick and can grow up to 1m in length in a growing season and extend for over 2m (Robb, 1983).

In seedling asparagus, the rhizome extends along a longitudinal axis as new buds progressively develop at the base of the youngest stem. As the plant ages some of the lateral buds at the base of the older stems develop and rhizome growth in another direction occurs (Blasberg,

1932). The older non-functional parts of the stem decay (Tiedjens, 1926). Figure 1.2 shows rhizome growth, root and shoot development from bud clusters.

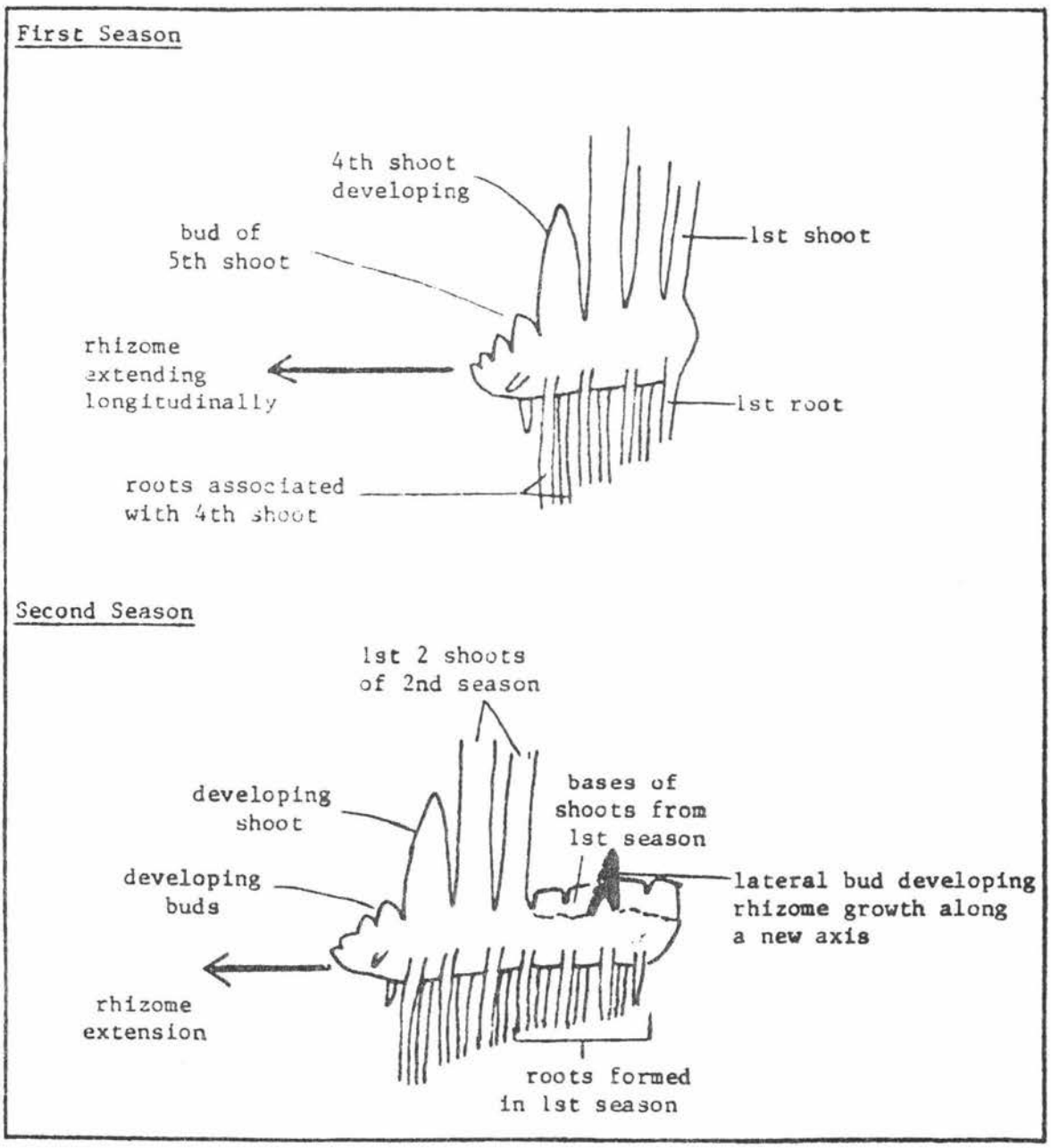


Figure 1.2: Rhizome growth, root and shoot development from bud clusters (Robb, 1983).

The aerial portion of the asparagus plant begins its development from an underground bud which rapidly elongates to form a succulent aerial stem with a compact head of short branches covered by scales. This structure is the asparagus spear of commerce and is capable of growth rates as high as 30cm per day (Meyer et al., 1960). The pattern of bud break is controlled by apical dominance within each bud cluster on the underground stem (Kretschmer and Hartmann, 1979) but it can be influenced by environmental factors such as temperature and soil moisture, the carbohydrate content of the root, and the age and variety of the plant (Robb, 1984)

An asparagus crop must be harvested regularly to ensure that the maximum number of marketable spears are produced. In temperate climates, spears are normally harvested from mid-spring to early summer after which the shoot is allowed to develop and flower. The topgrowth senesces in autumn and the plant remains dormant during winter. Spear growth is strongly dependent on temperature and increases linearly between 7°C and 31°C. The rate of vegetative growth is greater during the day than during the night (Blumenfield et al. 1961). A distinct zone of maximum growth is situated just below the spear tip and is more sensitive to changes in temperature than the rest of the spear (Culpepper & Moon, 1939). Consequently, spear emergence is influenced more by air temperature than by soil temperature (Bouwkamp & McCully, 1975).

Bud size has a major effect on the diameter of the spear. Larger buds usually produce larger spears (Blasberg, 1932).However, a decrease in spear diameter occurs as the season progresses even from large buds if several large spears have previously been removed from the crown (Tiedjens, 1926). This may be due to a decrease in carbohydrate reserves (Robb, 1984). The main period of bud formation is during the fern growth following harvest. The number of buds which form is genetically controlled, and not dependent on the environment or the amount of carbohydrate available (Blasberg, 1932). However, the size of the bud is dependent on the amount of fern growth and hence the amount of carbohydrates available (Tiedjens, 1926).

The foliage of mature asparagus has a fern like appearance and consists of many shoots. Each shoot consists of a central stem which supports many fine, needle-like branches or cladophylls. The leaves of

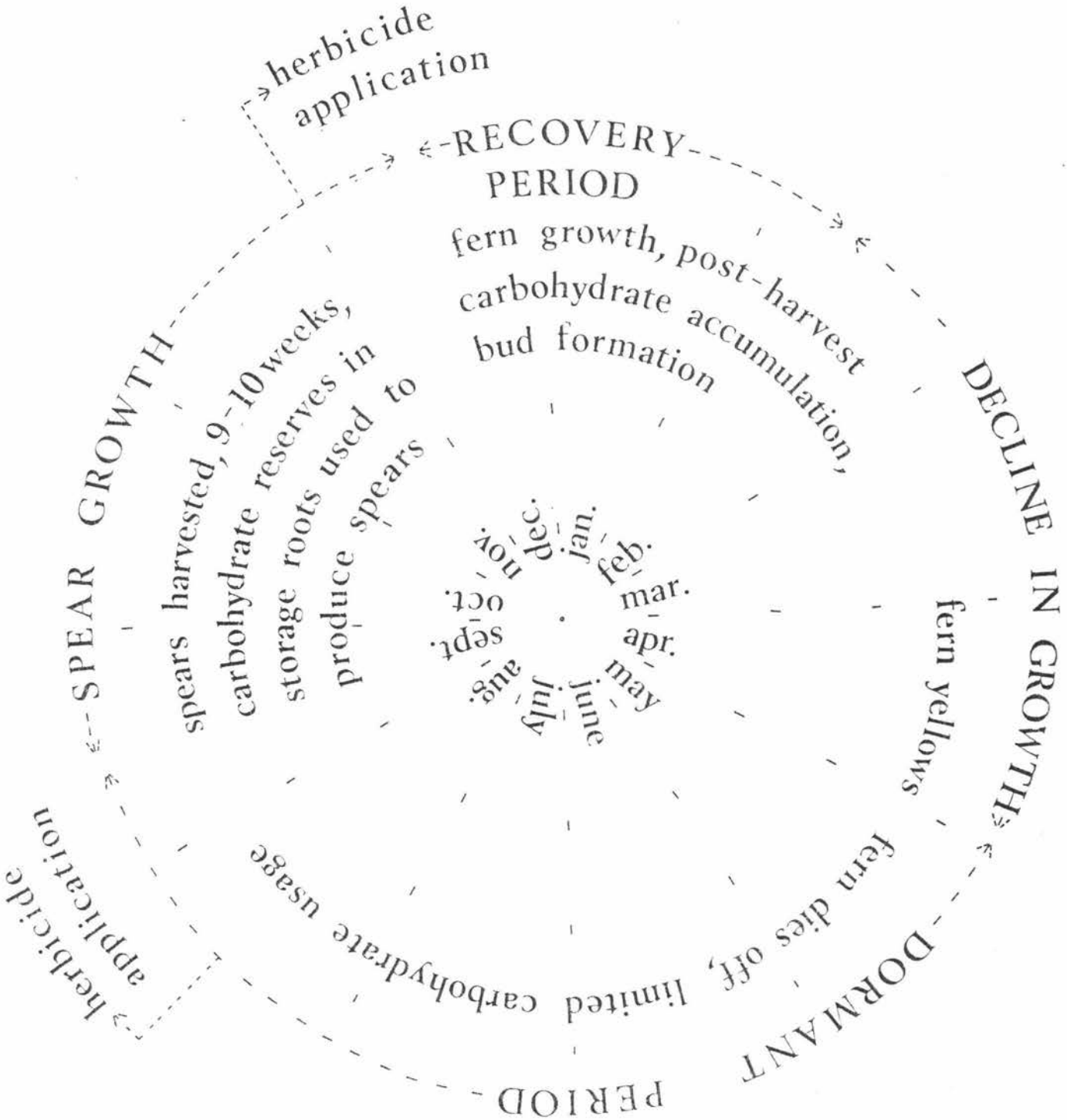


Figure 1.3: Main events in the life cycle of Asparagus officinalis L. in New Zealand.

Asparagus root systems have been shown to have a high incidence of mycorrhizal infection (Powell,1983). Since asparagus does not have fine roots or long root hairs , Vesicular-arbuscular(VA) mycorrhizal fungi greatly increase P absorption from the soil. This mycorrhizal effect is going on in most asparagus plantations (Powell,1983). The effect of mycorrhizal fungi on asparagus was demonstrated in New Zealand initially in some sterilised field soil plots at the Rukuhia Horticultural Research Area and then repeated in pot trials at Ruakura. These studies showed that the plants have an absolute need for mycorrhizal fungi to absorb phosphate efficiently (Powell, 1983). However, the demand for phosphorus by asparagus seems to be low; 14 units Olsen P (Prasad & Bussell,1984).

The actual fertiliser requirements of asparagus have not been clearly defined. Fertiliser field trials conducted in New Zealand have given widely differing results with no correlation between fertiliser application and yield or plant composition. Haynes(1983) states that on highly fertile sandy loams no response to fertiliser may occur for many years whereas on pure sands fertiliser applications are essential. A relatively high lime ($CaCO_3$) level and soil pH in the range of 6 to 6.8 is usually recommended (Haynes, 1983). Haynes also points out that Ca deficiency can cause a loss of vigour, browning and death of young shoots. Recent research by Prasad and Bussell (in Wood,1984) indicates that a soil level of 8 units potassium is adequate and that some substitution of potassium by sodium can occur, but there is no synergistic effect. The high salt-tolerance of asparagus has been attributed to an ability to control the absorption of sodium, thus preventing an imbalance in the roots of the nutrients absorbed (Shimose,1973 cited in Robb,1984).

During autumn senescence, most of the nitrogen, phosphorus and potassium, and trace elements such as magnesium, manganese and calcium are removed from the fern by translocation and by leaching (Carolus,1962).Most of the nutrients required to support harvesting in spring are available from root reserves although some absorption of nitrogen, calcium and sulphur can occur. A shortage of nitrogen decreases fern growth, thus decreasing photosynthetic capacity and crown size. A smaller crown produces less spears and thus yield is reduced (Robb,1984).

Cultivated asparagus is diploid (n=10). Sex of asparagus is generally accepted as being inherited as a simple Mendelian factor (Benson,1982) with female plants being homozygous (XX) and male plants heterozygous (XY) (Rick & Hanna,1943); with a small number of andromonoecious plants (XY and YY) occurring (Sneep,1953).[An asparagus andromonoecious plant is basically a male plant with some female characteristics].However, there is some research evidence to indicate that a heteromorphic chromosome pair may be involved (Marks & Barbour,1973). Marks (1976) proposed a model in which sex expression is controlled by a bipartite supergene with female plants being double recessive and male plants double heterozygotes. In this model hermaphrodite and neuter plants are produced when crossing over occurs in the male parent. Robb(1984) states that it is yet to be clearly established whether asparagus has a heteromorphic chromosome pair.

Asparagus is normally cross-pollinated and bears small, bright yellow insect-pollinated liliaceous flowers. Staminate plants have flowers with well developed stamens but mostly rudimentary pistils containing degenerated ovules. Pistillate plants have flowers with well developed pistils but collapsed anthers lacking pollen (Lazarte & Palser,1979). Occasional flowers on andromonoecious males are bisexual (Robbins & Jones,1925). One asparagus plant produces approximately 400 solitary flowers, borne two per node, one at each side of a cladophyll. All flowers on any one shoot of a plant are not at the same stage of development; they mature acropetally (Lazarte & Palser,1979). On pistillate plants small bright red berries containing up to 6 seeds develop (Robbins & Jones,1925).

There is ample evidence in the literature to indicate that staminate plants are more vigorous, have higher yield and greater longevity than pistillate plants (see review by Robb,1984). Further, biochemical differences also exist between staminate and pistillate plants making the staminate more drought tolerant than pistillate plants (Sivtsev & Sizoz,1971 cited in Robb,1984). These superior qualities of the male plant has led to the selection of male plants after flowering but before transplanting in Taiwan (Hung,1979) and has generated some interest in the development of all-male hybrid cultivars, such as Lucullus from West Germany and 22-8 X 56 from New Jersey (Nichols,1983). Davies (1981) suggested using tissue culture methods to bulk up the parents of high yielding all-male hybrids. Robb and

Seelye (pers. comm.) have been investigating this possibility at the Levin Horticultural Research Station using a Mary Washington 500W clone (MW500W). Up to the present in New Zealand most asparagus beds have been established with crown transplants although there has been a recent increase in establishment with seedling transplants (Wood, 1983).

The brief review on Asparagus officinalis L. undertaken here highlights a number of relevant points. Firstly, asparagus is fast developing into a major horticultural crop in New Zealand with a lucrative export potential. Secondly, the asparagus plant has some somewhat unusual modifications and specialisations, such as cladophylls, salt and drought tolerance,and is relatively deep rooted with a large storage capacity for carbohydrates. Although a number of researchers have turned their attention to the anatomical, physiological, genetic and cultural aspects of this plant there is a paucity of information on the effects of herbicides on this crop. The main aim of this project is to address one of the problems growers in New Zealand are currently facing; the effects of soil-applied herbicides like bromacil on the crop. Dose-response experiments, conducted under carefully controlled conditions, would enable us to assess the possible effects of residual herbicides like bromacil on the crop.

CHAPTER 2

HERBICIDE: BROMACIL (5-bromo-3-sec-butyl-6-methyluracil)

2.1 HISTORY

Bromacil is manufactured by E.I du Pont de Nemours and Co., Delaware, U.S.A. It was introduced for commercial application in 1963 (Fletcher & Kirkwood, 1982). Bromacil is the most active member of a family of highly effective herbicides, the substituted uracils (Gardiner, 1975). Other members of this family are terbacil,isocil,lenacil and DP-733 (Ashton & Crafts, 1973). These compounds have the combined properties of high phytotoxicity and low mammalian toxicity (Gardiner, 1975).

2.2 CHEMICAL FORMULAE

The molecular formula for bromacil is $C_9H_{13}BrN_2O_2$. The structural formula of bromacil is given in Figure 2.1.

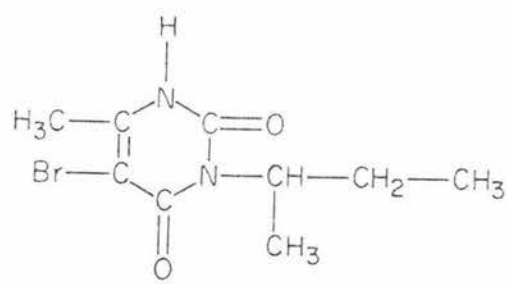


Figure 2.1 Structural Formula of Bromacil

2.3 PROPERTIES

Bromacil is a white, odourless crystalline solid with a relatively low water solubility of 815 p.p.m. at 25°C. In " Hyvar X " it is formulated as a wettable powder. It is temperature stable up to its melting point of 158-159°C. Its rate of volatilisation is very low;

the vapour pressure at 100°C is reported to be 0.8 X 10^{-3} mm of Hg (Pease & Deye, 1972). Bromacil has relatively low soil adsorption characteristics with a K value at 25°C, in silt loam , of 1.5. The K value is the ratio at 25°C of parts per million of herbicide adsorbed by 25g of soil at the point where 1 p.p.m. is in an equilibrated 30 cm^3 aqueous phase. The higher the K value, the stronger is the adsorption by soils. Diuron, a substituted urea , for example, in the same soil type has a K value of 5.Bromacil is non-corrosive, noninflammable, is stable in water and has a low mammalian toxicity with an LD_{50} of 5200mg/kg in rats.(LD_{50}="Lethal Dose 50%": dose required to kill 50% of the test animals; expressed in milligrams of the compound per kilogram of body weight).

2.4 PERSISTENCE AND DEGRADATION IN SOILS

Gardiner (1975) in his review on substituted uracils points out that a number of researchers have studied the rate of disappearance of bromacil in soil. The reported rate of disappearance varied considerably with geographical location. For example, Tucker & Phillips (1970) found that in citrus orchards in Florida with fine sand, where a total of 100lb of bromacil had been applied at a rate of 20lb/acre annually over a period of five years, approximately 1lb was still present in the soil after the last application. Migchelbrink (1971) found a higher percentage of herbicide residue in the soil in Oregon soils.

The only published experiments conducted under field conditions using radiolabelled bromacil were by Gardiner et al. (1969). Results from this study showed that ^{14}C residues of bromacil decreased by 50% after 5 to 6 months in silt loam soil and under the conditions studied. The loss of ^{14}C activity from the samples was presumed to be due to the evolution of $^{14}CO_2$. The assumption was based on laboratory studies on a similar soil type in enclosed systems equipped with gas traps (Gardiner et al., 1969). No evidence for volatile ^{14}C fragments other than $^{14}CO_2$ was found.

Gardiner et al. also reported that even after field exposure of up to 1 year, about 90% of the total activity in the soil extract was from

bromacil in its intact from. However, although the bulk of the ^{14}C activity was from the parent herbicide, positive evidence for hydroxylated bromacil metabolites as minor residues in the soil was obtained. Bromacil metabolites tentatively identified in soil by TLC procedures were 5-bromo-3-sec-butyl-6-hydroxymethyluracil and 5-bromo-3- (2-hydroxy-1-methylpropyl)-6-methlyuracil; products of the oxidation of the 6-methyl group and the 3-sec-butyl group of bromacil, respectively.

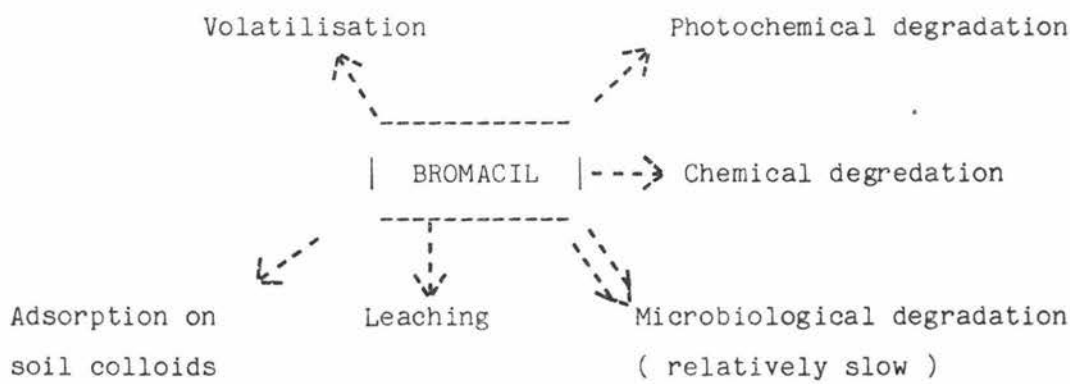
Several researchers have noted that detectable amounts of bromacil can leach from soils if applied at high enough rates (Gardiner,1975). However, due to its relatively low water solubility, leaching does not substantially account for the loss of bromacil from soils. Photodecomposition is another route by which herbicide residues can be dissipated near the soil surface. Jordan et al.(1965) studied the effects of ultra-violet light on bromacil. They observed that the greatest changes and losses occurred under far ultra-violet (240-260 nm) and the least under near ultra-violet irradiation (320-450 nm). About 70% of the bromacil was recovered after 500 hr of exposure to near ultra-violet, which is the closest to natural sunlight. This work plus several field tests indicate that bromacil is not severely affected by sunlight (Gardiner, 1975).

Although, as reported earlier, bromacil has relatively low soil adsorption characteristics, Haque and Coshow (1971) examined the adsorption of bromacil from aqueous solutions onto certain mineral surfaces and found that humic acid surfaces adsorbed considerably more of the chemical than illite, montmorillonite, silica gel, and kaolinite surfaces. Rhodes et al. (1970) studied the mobility and adsorption of bromacil using a soil-TLC technique and classified bromacil between class 3 and class 4 in a scale ranging from class 1, essentially immobile materials, to class 5, the very mobile. Thus, bromacil is neither immobile or excessively mobile and has an intermediate capacity for downward movement into root zones.

Microbiological degradation is believed to be the major route by which bromacil is inactivated in the soil. Soil Diptheroids, Pseudomonas and Penicillium species have been shown to be able to degrade bromacil (Klingman & Ashton, 1975). In the study by Torgeson and Mee (1967), for example, a soil isolate of Penicillium paraherque Abe was

particularly active in the degradation of bromacil. In this study, no herbicidal effects on buckwheat were detected 28 days after treatment with 12.5 lb/acre (about 12 p.p.m.) of bromacil in sterile soil innoculated with P.paraherque. Buckwheat was sensitive to bromacil at 1 p.p.m. in a standard bioassay. However, from the results of studies mentioned earlier on the persistence of bromacil in the soil and the relative importance of other degradation processes, it would seem that microbiological degradation of bromacil is a slow process under field conditions.

The possible degradation mechanisms and their relative importance for bromacil inactivation in the soil as ascertained from the studies in the literature can be summarised as follows:



Key: - - -> relatively minor route

major route

Figure 2.2 (adapted from Fletcher & Kirkwood, 1982)

2.5 UPTAKE, TRANSLOCATION AND METABOLISM OF BROMACIL BY PLANTS

Bromacil is readily root absorbed and xylem translocated to the leaves (Hilton et al.1964). Studies on the comparative mobility of labelled bromacil have shown that it displays rapid and free mobility in the apoplast only (Crafts, 1959). Since bromacil is apparently unable to cross the plasmalemma barrier, transmission across the cortex occurs via the cell walls (apoplast) to the Casparian strip. It is believed to continue the apoplastic migration through the Casparian strip by being sufficiently fat soluble to dissolve in the suberized layer to diffuse through it (Ashton & Crafts, 1973). The Casparian strip is impervious to the polar water molecules.

Since bromacil remains in the apoplast and moves only in the acropetal direction (Shriver & Bingham, 1973) it is believed that bromacil is dependent on the transpiration stream for distribution to the foliar parts of the plant (Ashton & Crafts, 1973). Thus, one would expect the effectiveness of uptake of this herbicide to be related to the transpiration rate. Shriver and Bingham (1973) did find that the absorption of 2-^{14}C-bromacil from solution and translocation to shoots was directly related to the transpiration rate in orchardgrass(Dactylis glomerata L.`Virginia Common') and Kentucky bluegrass (Poa pratense L. `Merion'). Such environmental factors as soil moisture, temperature, light intensity, wind speed and humidity could therefore influence uptake and translocation to the tops of plants. Schreiber et al. (1972) also found that uptake and distribution of soil applied bromacil was enhanced in wheat (Triticum aestivum L.) by increased soil moisture.

When foliage applied, bromacil seems to have limited phytotoxicity. For example, Hurtt (1971) examined the effects of certain adjuvants (0.2% Tween 20, 0.2% WK and 10% AL 411-F, singly or blended) on the foliar absorption of bromacil by Black Valentine beans. The low phytotoxicity of foliar-applied bromacil noted was attributed to limited foliar absorption.

Gardiner et al. (1969) determined the uptake, distribution and metabolism of bromacil in 2-year-old Hamlin orange (Citrus sinensis L. `Osbeck') scions grafted on sour orange (Citrus aurantium L.). The

trees were grown for 4 weeks in sand watered with nutrient solution containing 10 p.p.m. bromacil-2-^{14}C. They reported that less than 5% of the ^{14}C material was taken up by the plants; of this amount, approximately 83% of the absorbed herbicide remained in the roots, and 17% moved into the stems and leaves. Three radio-active compounds were extracted from the roots,stems and leaves in the relative ratio of 10:5:1 respectively. The first two compounds were tentatively identified by TLC Rf values as bromacil and a major hydroxy metabolite, 5-bromo-3-sec-butyl-6-hydroxymethyluracil. A minor metabolite was not identified.

5-bromo-3 sec-butyl-6-hydroxymethyluracil was also found to be the principal metabolite in the study by Jordan and Clerx (1981) in Pineapple sweet orange (Citrus sinensis L.`Osbeck' ) and Cleopatra mandarin (Citrus reticulata L. `Blanco'). A minor metabolite was tentatively identified as 5-bromo-3-(3-hydroxy-1-methylpropyl) -6-methyluracil. Similarly, hydroxy metabolites were detected in extracts from bromacil treated orchardgrass and Kentucky bluegrass, particularly in the latter which is relatively tolerant to the herbicide (Shriver & Bingham, 1973). The metabolites detected from Shriver & Bingham's study included 5-bromo-3-(2-hydroxy -1-methylpropyl)-6-methyluracil and traces of 3-sec-butyl-6-methyluracil and 5-bromo-3-sec-butyl-6-hydroxymethyluracil.

The Jordan and Clerx (1981) study also showed that the more susceptible Pineapple sweet orange absorbed twice as much ^{14}C from bromacil, and accumulated three times as much in the leaves than did Cleopatra mandarin. The amount of conjugated metabolites formed was the same in the roots of the two cultivars, but twice as much formed in the leaves of Cleopatra mandarin as in the leaves of Pineapple sweet orange. Jordan and Clerx concluded that this suggests that the tolerance of citrus cultivars to bromacil is at least partially attributable to low uptake and distribution and to metabolism and conjugation.

Recovery of radioactivity from root-applied 2-^{14}C-bromacil removed by plants in the Shriver and Bingham (1973) study showed that in both grass species studied (orchardgrass and Kentucky bluegrass) shoots contained more radioactivity than roots, unlike the Gardiner et al. (1969) findings for the two citrus cultivars. Both roots and shoots were found to contain metabolites of 2-^{14}C-bromacil. The more

resistant Kentucky bluegrass leaves contained only 21% intact bromacil whereas orchardgrass leaves had 67% intact herbicide after 7 days of treatment with 1 p.p.m. 2-^{14}C-bromacil. The results of these tolerance studies suggest that metabolites are less phytotoxic than intact bromacil, and known phytotoxicity of metabolites supports this concept (Shriver & Bingham, 1973).

Gardiner et al. (1969), based on their findings and the information available on the reductive pathway of pyrimidine degradation (Schulman, 1961), suggested that one mode of degradation of bromacil in plants (and soils) proceeded through hydroxylation of side-chain alkyl groups presumably followed by ring opening and then complete metabolism to carbon dioxide, ammonia and hydrobromic acid. However, the route of hydroxylation possibly varies between species. The major metabolite of bromacil in orange plants was found to be 5-bromo-3-sec-butyl -6-hydroxymethyl uracil (Gardiner et al.1969), whereas in grasses, 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil was the major metabolite identified (Shriver & Bingham, 1973).

2.6 MODE OF ACTION AND PHYTOTOXIC RESPONSES TO BROMACIL

Bromacil has been found to be a potent and specific inhibitor of photosynthesis. Hoffmann et al. (1964) working with spinach (Spinacia oleraceaL.) chloroplasts found that bromacil was a potent inhibitor of the Hill reaction. A 50% inhibition of the Hill reaction (oxygen evolution) was found at 0.5 p.p.m. and complete inhibition at 1.6 p.p.m. Bromacil was also found to be a strong Hill reaction inhibitor of isolated tomato chloroplasts (Hogue, 1967).The effect of bromacil on the growth of Euglena (Euglena gracilis Klebs) after incubation for 10 days in the light and dark was also studied by Hoffmann et al.(1964). In the light, 2-5 p.p.m. of bromacil caused a 50% inhibition of growth compared with no inhibition in the dark at 40 p.p.m. and 16% inhibition at 100 p.p.m.. 4 p.p.m. of bromacil blocked the oxygen production of illuminated cells within 10 minutes of addition. Although the inhibition was rapid and complete, it could easily be reversed by washing the inhibited cells with a buffer. Three

buffer washings of Euglena cells treated with 4 p.p.m. bromacil removed the inhibition caused by the herbicide and restored normal oxygen evolution in the light. No effect on respiration was found as measured by endogenous oxygen in the dark even at a relatively high herbicide concentration of 50 p.p.m.

Couch and Davis (1966) found that 1 p.p.m. of bromacil reduced $^{14}CO_2$ fixation in corn (Zea mays L.) and soybeans (Glycine max L. 'Merr') but had no effect on $^{14}CO_2$ fixation in the dark. Jordan et al. (1966) also found that bromacil did not affect growth of tobacco (Nicotiana tobacum L.) callus tissue in the dark at concentrations below 10^{-3}M, indicating that bromacil was not toxic in the dark at concentrations comparable to those normally used for selective weed control.

Although bromacil is classed as an electron transport inhibitor which causes a block along the oxygen-liberating side of photosynthesis (Ashton & Crafts, 1973), little is known about the exact site of biochemical action or the point(s) of attachment of the herbicide molecules to appropriate receptors at or near the active centres in the chloroplasts. Edidence from the study by Hoffmann et al. (1964), that free reversibility of inhibition exists, indicates that weak bonds such as hydrogen bonds may be involved. Hoffmann (1971) proposed that the anti-photosynthetic effect of bromacil is a result of a block in the pathway between the chloroplast and oxygen evolution (the OH side), causing an accumulation of a phytotoxic product, possibly a reactive free radical.

Bromacil at low concentrations was found to retard senescence in tomato leaf discs maintained in light (Hogue & Warren, 1966) and of maize leaves (Hiranpradit and Foy, 1973). This effect was interpreted as a consequence of partial inhibition of photosynthesis.

Shriver and Bingham (1973) using orchardgrass and Kentucky bluegrass found that suppression of net photosynthesis occurred in both species following soil treatment with rates equivalent to 0, 0.14, 0.28, and 1.12 kg/ha. Photosynthesis in Kentucky bluegrass recovered to control levels at the lower rates by 6 days. However, orchardgrass did not show this recovery in photosynthesis. Water soluble carbohydrate analysis of leaf tissue at the termination of the experiment showed that Kentucky bluegrass had a higher carbohydrate level than

orchardgrass.This would allow Kentucky bluegrass to recover after inactivation of the herbicide.(It was mentioned earlier that bluegrass had a greater capacity to physiologically inactivate bromacil by metabolism compared to orchardgrass). These results were similar to those observed by Van Oorschot (1965) working with flax (Linum usitatissimum L.) a moderately resistant species. Hilton et al.(1964) also found that exogenously supplied carbohydrates partially nullify toxicity symptoms in intact barley plants. Swann and Buchholtz (1966) reported carbohydrate depletion in the rhizomes of quackergrass (Agropyron repens L.) by the action of bromacil.

Although bromacil has clearly been demonstrated to inhibit the Hill reaction and this factor is undoubtedly related to its herbicidal activity, Hoffmann (1971) points out that this anti-photosynthetic effect alone is not sufficient to account for the total phytotoxic action of bromacil. Hewitt and Notten (1966) found that bromacil inhibits the induction of nitrate reductase in leaf tissues. Inhibition was severe at concentrations of 1µg/ml in the infiltrating solutions. Hiranpradit and Foy (1972) also found that the treatment of soybeans with bromacil (0-0.045 p.p.m.) increases the level of nitrogen and phosphorus in the shoots. In treated shoots, accumulation of nitrate nitrogen, protein nitrogen, nucleic acid nitrogen and amino acids occurred.

Ashton et al. (1969) studied the structural modifications of oats induced by bromacil. Total root elongation was inhibited and inhibition increased with concentration through the series 10^{-6}M, 10^{-5}M, 10^{-4}M, 5 X 10^{-4}M, and 10^{-3}M; growth at the latter concentration was almost nil. Inhibition was almost entirely restricted to the terminal 0.5mm, just behind the meristem and the region of greatest elongation in untreated roots. Treated roots were swollen or necrotic and collapsed with necrosis present in meristem, procambium and epidermis. Precocious vacuolation was seen in cells of the root tip and inhibition of cell wall formation resulted in multinucleate cells. In the leaves, bromacil inhibited development of the chloroplast grana and fret system; the loculi of the grana and fret vesicles swelled progressively and the chloroplast envelopes were modified; there appeared to be a loss of membrane integrity.

Shriver and Bingham (1973) found that although bromacil had no effect

on the germination of orchardgrass and Kentucky bluegrass, 5 p.p.m. caused chlorosis, inhibition of shoot growth and few secondary leaves emerged within 25 days when applied in a water-culture system. 1 p.p.m. was the minimum concentration which reduced shoot growth of bluegrass. 0.125 p.p.m. , the lowest concentration tested, gave 90% reduction in growth of orchardgrass after 15 days. A more gradual reduction in bluegrass growth was observed with increasing concentration of bromacil. 1 p.p.m. gave a significant reduction in growth. Visually, root growth of orchardgrass was inhibited by all concentrations of bromacil. Inhibition of bluegrass roots was observed only with concentrations greater than 1 p.p.m.

Differential tolerance to bromacil was also found in two citrus rootstalk cultivars, Cleopatra mandarin and Pineapple sweet orange by Jordan and Clerx (1981). In this study, uniform 8-month old plants were selected from seedbed plantings, and individual plants were transplanted into non-drained containers with sandy loam soil. Trees were watered when necessary to maintain soil moisture at 65% w/w field capacity. Three weeks after transplanting, the soil was treated with 0,1,2,4 and 8 p.p.m. bromacil, which was added to the soil surface in 250 ml of water. Twelve weeks after treatment, visual injury symptoms of chlorosis and necrosis, and fresh weight of roots and tops were recorded. Jordan and Clerx found that Pineapple sweet orange plants were injured more than were Cleopatra mandarin at dosages at 2 p.p.m. or more in the soil. Twice as much bromacil in the soil was required to give the same degree of injury to Cleopatra mandarin. Pineapple sweet orange responded similarly at 2 and 4 p.p.m. bromacil in the soil as the mandarin did at 4 and 8 p.p.m. The Pineapple sweet orange plants were dead at the end of the experiment, whereas mandarin plants were alive, but chlorotic and stunted. The intensity of visual injury symptoms appeared to be about the same in the leaves of sweet orange plants at the 4 p.p.m. and mandarin plants at the 8 p.p.m. treatment.

In summary the review of the properties and activities of bromacil indicates the following main points. Bromacil is a stable compound which is degraded relatively slowly in soils mainly by microbiological action. It has the combined properties of low mammalian toxicity and high phyto-toxicity. It is rapidly root-absorbed and transported acropetally to the leaves. The rate of absorption and translocation appears to depend on the transpiration stream. In the leaves, bromacil

effectively inhibits the Hill reaction. Bromacil has also been shown to affect nitrogen metabolism, growth of roots and shoots, cytological modifications of root cells and chloroplasts, and cause chlorosis and necrosis. Bromacil is relatively slowly metabolised by plants through hydroxylation, the less toxic metabolites formed being mainly the products of the oxidation of the methyl and butyl groups of bromacil. Differential tolerance in cultivars of several closely related species was observed, this being mainly attributable to the inactivation of the herbicide by forming less toxic metabolites.

While a few pot studies have been devoted to the inhibiting and phytotoxic effects of bromacil on a limited number of species , a pot study on the symptoms and toxic effects of bromacil on asparagus has not been reported. However, Rahman and Sanders (1984) did conduct long term field trials at the Ruakura Soil and Plant Research Station to assess the tolerance of asparagus to bromacil by monitoring spear production over three successive seasons.They concluded that no adverse effects were evident in terms of spear production even at twice the recommended rate. The nature of the project reported here is more in line with the pot tolerance studies conducted by Jordan and Clerx (1981).

CHAPTER 3

MATERIALS AND METHODS

3.1 PART 1

3.1.1 Objective: Clonal propagation of *Asparagus officinalis* L.

To minimise the effects of genetic variation in the plants to be used in this project, clonal propagation using *in vitro* techniques was used. This section will report on the procedures used. The stock plants were obtained from the Levin Horticultural Research Station and were all-male Mary Washington 500W clone of *Asparagus officinalis* L.

3.1.2 Procedure for the Establishment of aseptic stock plants

(1) Asparagus spears from high yielding male Mary Washington 500W clone were sterilised by dipping them in 10% Clorox (=0.525 Sodium hypochlorite) for 15 minutes.

(2) The outermost scale of each lateral bud was removed and the buds excised and cultured for 4 to 6 weeks on a Basal medium containing 0.05 to 0.1 p.p.m. NAA (a-naphthaleneacetic acid) and 0.05 p.p.m. Kinetin (6-furfurylamino purine) until shoots developed.

(3) These shoots were then excised and cut into 1-bud segments. The segments were cultured for 10 to 12 weeks in a Basal medium containing 0.1 p.p.m. NAA and Kinetin, until multiple shoots developed.

(4) These plantlets without roots were then placed on a Basal medium containing 0.1 p.p.m. NAA and Kinetin, and were periodically transferred onto fresh medium to promote growth of shoots.

(5) As the plantlets developed vigorous shoots and roots, they became the stock plants which were used as the source of propagants. The time required to develop stock plants was from 6 to 8 months. Plate 3.1 shows a typical jar of stock plants obtained from the Levin Horticultural Research Station.

Plate 3.1

Typical jar of Mary Washington 500W Levin II clone stock plants obtained from J.Seelye, Levin Horticultural Research Station.

3.1.3 Clonal multiplication of MW500W Levin II clone

3.1.3.1 The Basal medium (BM) used contained the following ingredients:

(a) Inorganic salts as prescribed by Murashige and Skoog(1962) but with NH_4NO_3 reduced from 1,650 to 1320 mg/l and KNO_3 reduced from 1900 to 1520 mg/l (refer to Appendix A for details)

(b) Organic substances: 2 p.p.m. glycine, 100 p.p.m. myo-inositol, 0.5 p.p.m. nicotinic acid, 0.5 p.p.m. pyridoxine-HCL, 3% sucrose and 0.7% agar.

(c) Plant growth regulators: NAA and Kinetin were added in various combinations depending on the stage of development of the culture.

The medium was adjusted to pH 5.7 with either 1N NaOH or 1N HCL then autoclaved at 1 kg/cm^2 (15 psi) for 15 minutes. Cultures were maintained at 26°C under 35 $\mu E\ m^{-2}s^{-1}$ from 20-W cool white fluorescent lamps with a 16-hr photoperiod.

3.1.3.2. Clone increase

Lateral buds from the stock plants were used to rapidly increase the clones using techniques developed by Yang(1973,1977,1979) and Yang and Clore (1975). All procedures requiring aseptic conditions were carried out in a laminar flow cabinet.

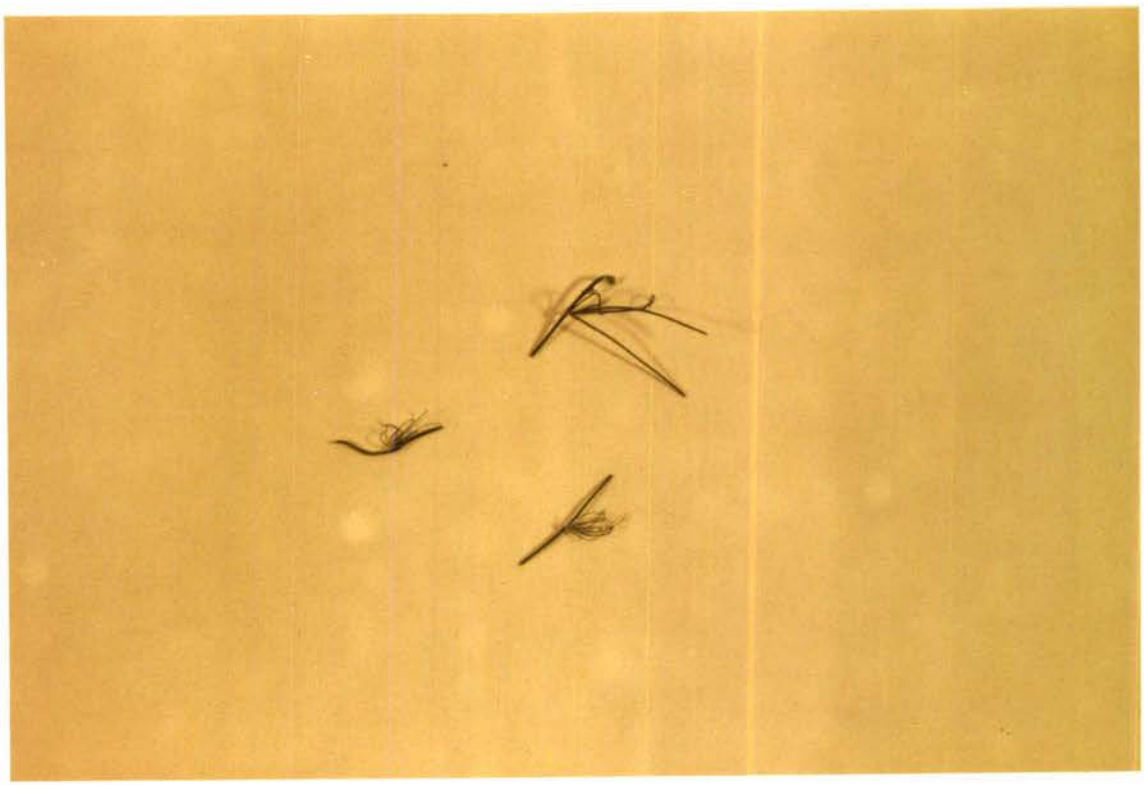
Main stages in clone multiplication

(1) One-bud segments, about 1cm in length, were cut from the aseptic stock plants mentioned earlier.Where possible basal buds and portions of the stem with branch-shoots were used as these have been found to give more complete rooted plantlets (Yang & Clore,1973,1974). Plate 3.2 shows 1-bud nodal segments.

(2) The single-node segments were then transferred to 25 X 200mm culture tubes, containing 20 cm^3 of the Basal Medium supplemented with 0.1 mg/l NAA and 0.1 mg/l Kinetin to promote shoot and root development. The cultures were

Plate 3.2

1-bud nodal segments used in clone multiplication



placed in the culture environment specified earlier. Plate 3.3 shows plantlets after 10 weeks in culture.

(3) Vigorous plantlets with well developed roots were transferred directly from the medium to pots containing a soil mixture of 1 sandy loam:1 peat: 1 sand. They were kept in a glasshouse under intermittent mist for 10 to 14 days to prevent desiccation and promote growth (Yang & Clore,1974; Yang, 1977). Plate 3.4 shows asparagus plants established in soil.

(4) After the shoots started to grow and the cladophylls expanded the young plants were moved to open benches in the glasshouse. Some 180 plants derived from a single staminate parent were clonally propagated using the tissue culture techniques described above.

3.2 PART II

3.2.1 Primary Objective: To conduct broad-spectrum experiments with a wide range of herbicide concentrations to study the dose-response relationships of bromacil and asparagus.

3.2.2 Specific Objectives

(1) To identify the visible symptoms of injury on the foliage as a result of bromacil activity

(2) To study the effect of bromacil based on Visually Assessed Damage Score, Shoot Growth, Shoot Death and Root Fresh Weight as a result of herbicide activity at various concentrations

(3) To compare the effects of bromacil on these parameters by using ED_{20} and ED_{50} values

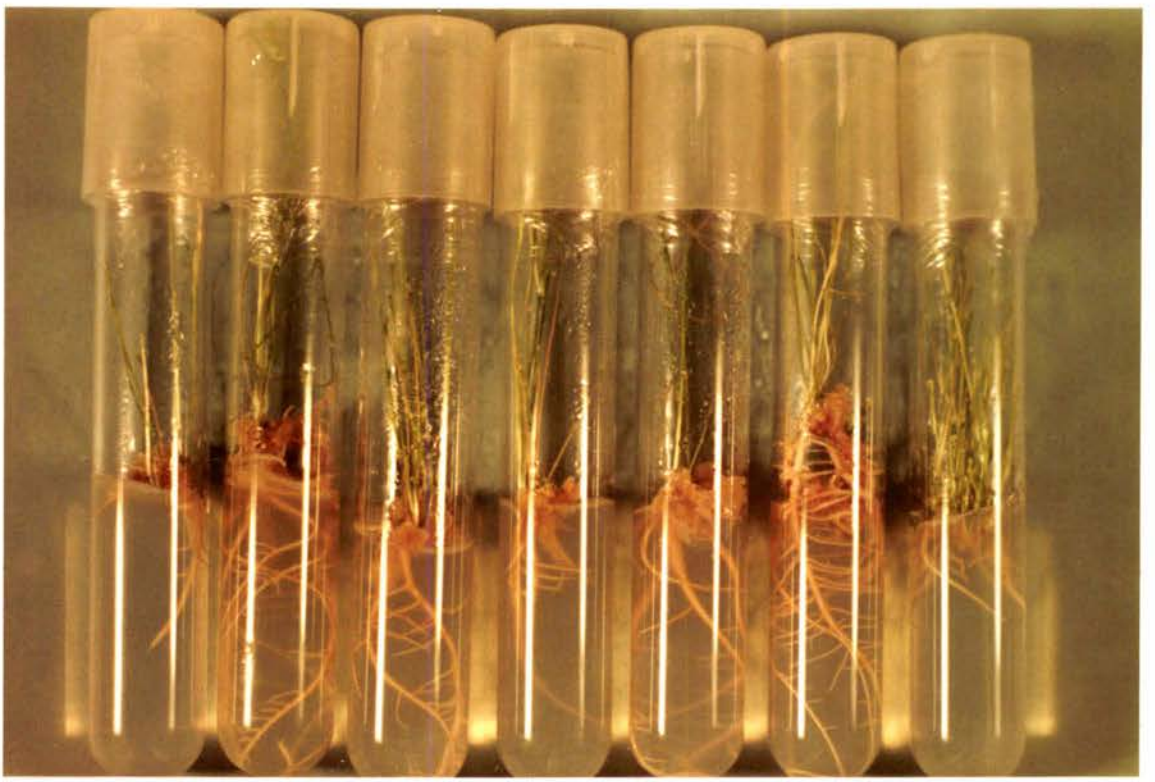
(4) To study the speed of action of bromacil on asparagus, under conditions to be specified, by conducting time-course studies

Plate 3.3

Asparagus plantlets after 10 weeks in culture

Plate 3.4

Asparagus plants established in soil



3.2.3 MATERIALS AND METHODS

One hundred 18-month-old uniformly established plants from Part I were selected. The plants were carefully removed from the soil, roots gently washed to remove soil particles and surface water removed with an absorbent towel.The roots and tops were separated by cutting the stem just above the rhizome and the roots weighed. The roots were repotted in numbered 10cm diameter, semi-rigid, grey plastic pots, lined with clear plastic bags. Each pot was filled with 1.5kg water-washed sterilised sand. Particle size distribution of the sand was approximately <1.2mm, 66.2%; 1.2-2.1mm, 19.4%; 3.3-5mm, 2.9%; and >5mm, 0.6%. All pots were weighed and watered with 1/2 strength Hoagland and Arnon Nutrient solution (refer to Appendix B) to 80% w/w field capacity.

The plants were placed on plant trolleys and wheeled into a D.S.I.R. Climate Room (refer to Appendix C) set at 23°C, 70% relative humidity with a 14-hr photoperiod of about 700 $\mu E\ m^{-2}s^{-1}$. The plants were weighed and watered daily with the Nutrient solution to a soil moisture of 80% of field capacity. Average fluctuations in the soil moisture in the pots were monitored for a 24-hr period and found to be in the range of 60-80% of field capacity.

Two weeks after transfer, prolific topgrowth occurred. All shoots were staked and 50 cm^3 of bromacil, made up to the following concentrations: 0, 2, 4, 8, 16, 32, 64, 126,258 & 516 p.p.m. (refer to Appendix D), was applied using a completely randomised design. Ten plants were used in each of the ten treatments. Nutrient solution was added as needed to attain a soil moisture of 80% field capacity. Plate 3.5 shows plants in the Climate Room on treatment day.

The plants were watered daily with nutrient solution and every three or four days shoot number and height were measured and foliage assessed on a 0 to 10 scale, similar to the one used by Clay and Davidson (1978). All measurements and scoring were carried out by the experimenter. At the end of the treatment period, 18 days after herbicide application, the sand was carefully washed from the roots, excess water removed and total plant weight and root weight were recorded.

Plate 3.5

18-month-old asparagus plants in the Climate Room on treatment day

A photographic record of various toxicity responses was made during the treatment period.

3.3 PART III

From the results obtained from Part II of this project, herbicide concentrations around the ED_{20} and ED_{50} values were selected for equal increment dose-response experiments. The bromacil concentrations selected were 0, 2, 4, 6, and 8 p.p.m.

3.3.1 Objectives:

(1) To determine the effect and speed of action of bromacil on the photosynthetic cladophyll tips by using a fluorometer to measure changes to chlorophyll fluorescence induction curves during the course of the treatment period.

(2) To determine the effect of the various concentrations of bromacil on chlorophyll a , chloropyhll b and total chlorophyll concentrations in the photosynthetic cladophyll tips during the course of the treatment period.

3.3.2 MATERIALS AND METHODS

Fifty, 18-month-old uniformly established plants from Part I were selected and prepared as described in Part II. Bromacil concentrations at the rate of 0, 2, 4, 6, 8 p.p.m. were applied as previously described. Ten plants were used in each of the five treatments.Changes to chlorophyll fluorescence induction curves and to chlorophyll a and b concentrations were determined at 1 to 2 day intervals for a period of 1 week.

3.3.3 EXPERIMENT 1

A miniaturised Kautsky apparatus (solid state fluorometer, Model SF-10) was used to measure changes to chlorophyll fluorescence induction curves in the photosynthetic cladophylls tips.(Refer to Schreiber,

(1975) for background information on the Kautsky apparatus used.) Plate 3.6 shows the Kautsky apparatus used.

Twelve photosynthetic tips, about 2 cm long, were randomly selected and removed for each treatment from plants in the Climate Room after the lights had been turned off for 6 hours. Under subdued green light the dark adapted tips were placed on wet filter paper on an aluminium plate that held 60 samples.The tips and plate were covered with a thin plastic film (`Glad-Wrap') to prevent moisture loss. A solid plastic sheet which contained 60, 3.3 cm diameter holes was placed on top of the plate and held by side-clamps. Plate 3.7 shows the the aluminium plate with the cladophyll tips ready for fluorescence measurements.

The fluorometer sensor was placed in the hole to measure the chlorophyll fluorescence of the underlying plant tissue. By this arrangement, fluorescence measurements were made and induction curves recorded on a chart recorder.The fluorometer was set in a Climate Room held at a constant temperature of 20^{o}C.

3.3.4 EXPERIMENT 2

The photosynthetic samples from Experiment 1 were kept in a dark container under refrigerated conditions to determine chlorophyll a and b concentrations within 12-18 hours.

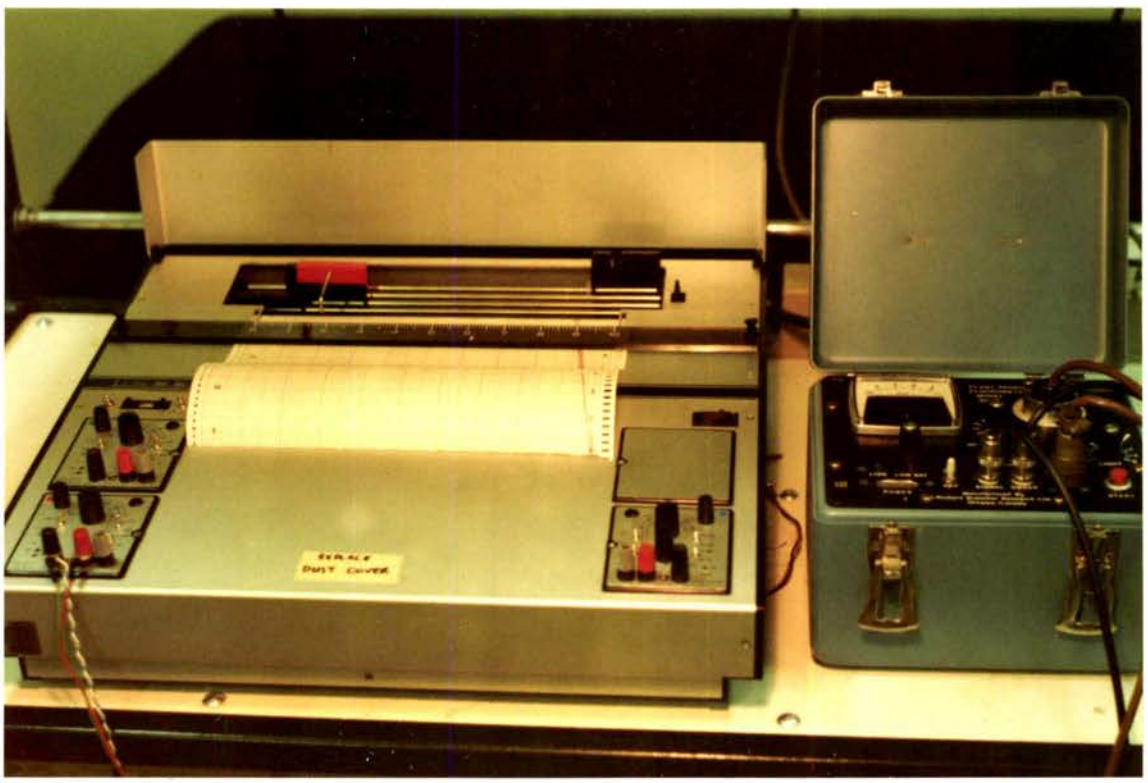
Concentrations of chlorophyll a and b were estimated using the 80% acetone (v/v) method (Sestak et al. 1951). The plant material was accurately weighed and homogenised with 4 cm^3 of 80% acetone in a porcelain mortar and pestle. The slurry was diluted with about 10ml of acetone and filtered through sintered glass under slightly reduced pressure into a 50ml volumetric flask. The residues were washed through the filter funnel with 80% acetone and the filtrate diluted exactly to 50ml and mixed well. Absorbances were measured on a double-beam Varian spectrophotometer (bandwidth 0.1nm) at 663nm, 652nm and 645nm and using 80% acetone as a reference blank. Chlorophyll analysis was carried out immediately after extraction and all extraction work and absorbance readings were taken in a dimly lit room. Calculations were based on the absorption coefficients determined by Mackinney (1941), cited in Goodwin (1976) thus:

Plate 3.6

Miniaturised Kautsky apparatus (Model SF-10) connected to a chart recorder

Plate 3.7

Aluminium plate with cladophyll tips in position for fluorescence measurements




1
2
3
4
5
6
6
7
8
9
6
10

Chlorophyll a (mg/l)= $12.7D_{663} - 2.69D_{645}$

Chlorophyll b (mg/l)= $22.9D_{645} - 4.68D_{663}$

Total Chlorophyll (mg/l)= $20.2D_{645} + 8.02D_{663}$

The total concentration was also checked by measuring the absorbance at 652nm and using the formula determined by Bruinsma(1963) cited in Goodwin (1976) so:

Total Chlorophyll (mg/l) = $1000D_{652}/36$ or $27.8D_{652}$

3.3.5 EXPERIMENT 3

As an extension to Experiment 1 and to ascertain the speed of movement of bromacil in the topgrowth, shoots were cut from untreated plants and placed in flasks containing 50ml of 0, 2, 4, 6, and 8 p.p.m. bromacil solution. Chlorophyll fluorescence induction curves for the cladophylls tips were measured as described for Experiment 1. Plate 3.8 shows asparagus shoots in flasks containing various concentrations of bromacil.

3.4 STATISTICAL ANALYSIS

All data were analysed by using the Minitab and SPSS programmes at the Massey University Computer Centre. The SPSS graphics programme was used for drawing the dose-response curves.

Plate 3.8

Excised asparagus shoots in flasks containing various concentrations of bromacil. Dose from left to right: 0, 2, 4, 6, and 8 p.p.m.

Chapter 4

RESULTS

4.1 PART 11: BROAD SPECTRUM DOSE-RESPONSE EXPERIMENTS

4.1.1 PHYTOTOXICITY OBSERVATIONS

Since visible symptoms of injury are the most important diagnostic criteria for the grower, a catalogue of Colour Plates was compiled to illustrate the progressive changes observed on the asparagus topgrowth and roots due to bromacil damage.

Chlorosis of the cladophylls was the first visible sign of injury (see Plate 4.1). At relatively high bromacil concentrations (32 ppm and above) extensive chlorosis of the cladophylls was observed within 5 days of treatment. The chlorotic areas which started at the tips rapidly became bleached and the effects extended towards the base. Curling and twisting of the cladophylls tips was also evident (see Plates 4.2 and 4.3). The bleaching was followed by necrosis which progressively affected the whole shoot resulting in shoot death. The severity and rate at which this sequence of changes occurred was related to the concentration of the herbicide. Plates 4.4 and 4.5 show representative samples of plants displaying the effects of being exposed to various doses of bromacil 10 days after treatment.

At 2 p.p.m., although some of the cladophylls showed signs of chlorosis and bleaching , this was not generally followed by shoot death. At dose rates of 4 p.p.m. and above the shoots did not show signs of recovery and new shoots emerging were also affected (see Plate 4.3).

Plate 4.6 shows the effects of bromacil on the root systems of two representative samples 31 days after topgrowth was removed and 18 days after treatment. The root systems of the plants treated with a bromacil dose of greater than 2 p.p.m. had very few feeder roots and the storage roots in most cases were hollow.

Plate 4.1

Yellowing of foliage 10 days after treatment with a bromacil dose of 4 p.p.m.

Plate 4.2

Bleached cladophyll tips observed 18 days after treatment with a bromacil dose of 4 p.p.m.

Plate 4.3

Twisting and curling observed on new shoots

Plate 4.4

Representative samples of asparagus plants 10 days after treatment with a single application of bromacil. Dose from right to left: 0, 2, 4, 8 and 16 p.p.m.

Plate 4.5

Representative samples of asparagus plants 10 days after treatment with a single application of bromacil. Dose from right to left: 32, 64, 128, 256 and 512 p.p.m.

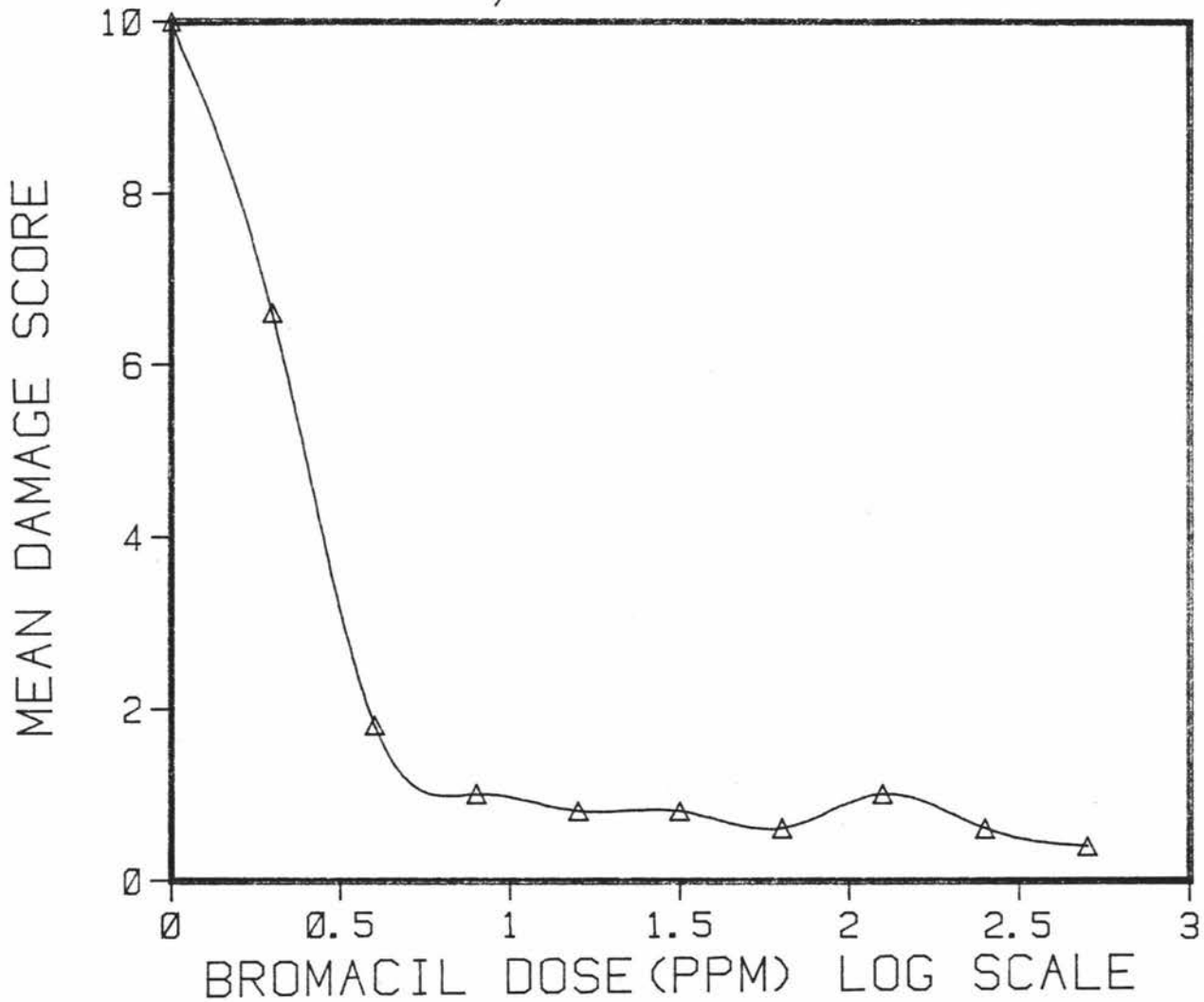

16 ppm
8 ppm
4 ppm
2 ppm
1 ppm


512 ppm
256 ppm
128 ppm
64 ppm
32 ppm

Plate 4.6

Two representative samples of asparagus roots treated with bromacil. Dose from right to left: 0, 4 and 8 p.p.m.

FIGURE 4.1: DOSE RESPONSE CURVE, BROMACIL EFFECTS ON VISUALLY ASSESSED DAMAGE SCORE 18 days after treatment



The visible symptoms as illustrated in the Colour Plates can be summarised as follows:

CHLOROSIS (Yellowing of cladophylls)

BLEACHING (Cladophylls turn white, extending towards base, tip dieback)

NECROSIS (Cladophyll tipping, progressive cladophyll death extending to the base followed by shoot death)

The severity and rate of injury increased with increasing bromacil concentration.

4.1.2 DOSE-RESPONSE RELATIONSHIPS

4.1.2.1 Visually Assessed Foliage Damage (V.A.D.) Score

The severe visual effects of bromacil on the foliage of 18-month-old asparagus plants assessed on a 0-10 scale, 18 days after a single application of bromacil, is shown in Figure 4.1 The visual assessment scale used was as follows:

0 = dead plants

2 = 85% foliage dead

4 = 50% foliage dead

6 = 10% foliage dead

10 = normal plant, indistinguishable from untreated control

The dose-response curve (Figure 4.1) shows a steep, almost linear line reaching a maximum response around 8 p.p.m. At 2 p.p.m. some mild chlorosis and bleaching was observed but rarely followed by shoot death. A mean damage score of 6.6 indicates that less than 10% of the foliage showed signs of injury 18 days after treatment. At bromacil doses of 4 p.p.m. and above severe injury was evident with mean scores less than 2, that is, 85% or more of the foliage was dead.

The regression equation for the near linear phase of the curve (dose range 0 to 8 p.p.m.) was computed to be:

$$Y = 9.62 - 10.6X$$

$$R^2(\text{adjusted for d.f.}) = 91.2\%$$

The ED_{50} values calculated from the regression equation was 2.7 p.p.m.

Time course data for the injury score is presented in Table 4.1.

FIGURE 4.2: DOSE RESPONSE CURVE.
BROMACIL EFFECTS ON SHOOT GROWTH
18 days after treatment

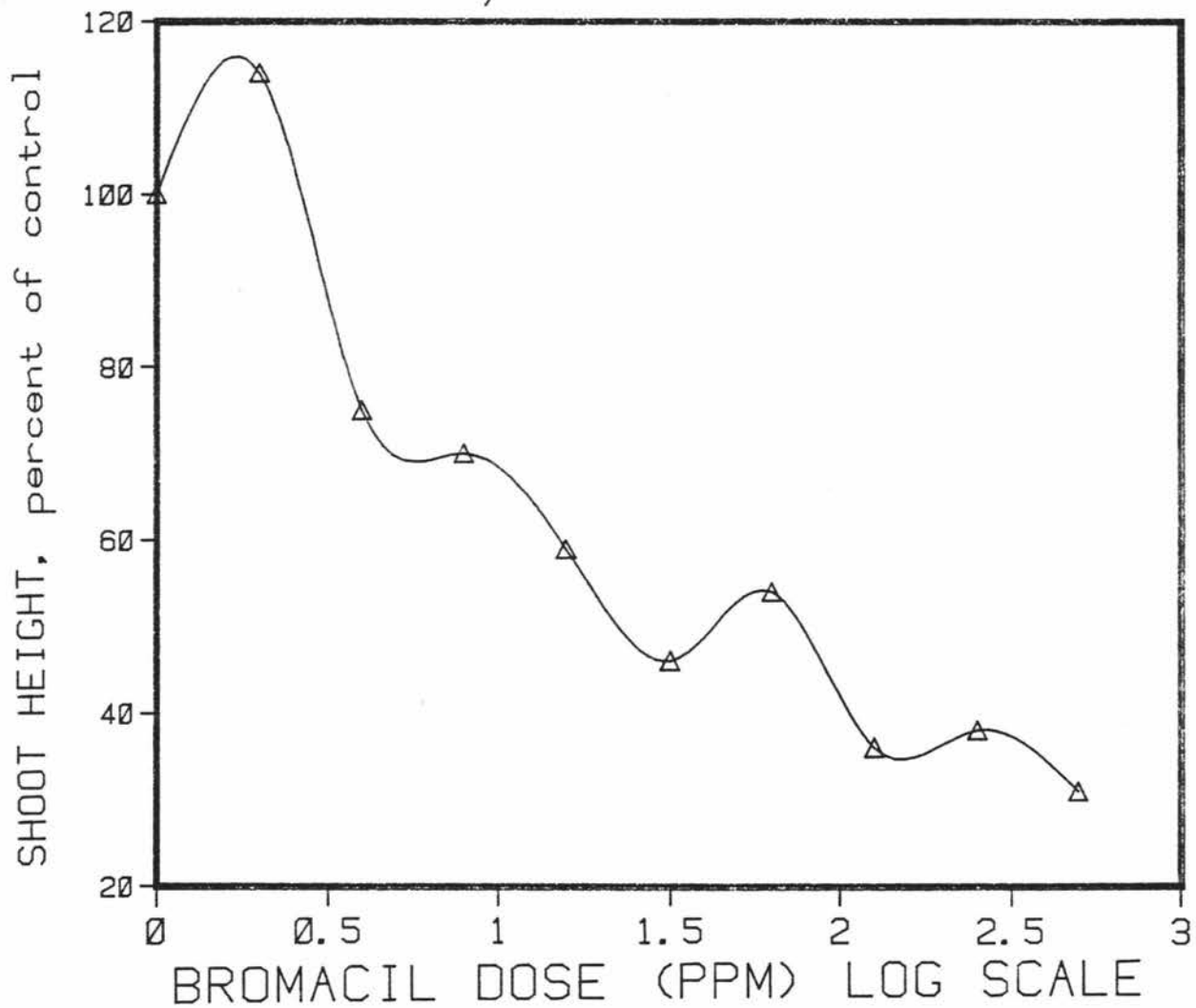


Table 4.1: Time-course data for V.A.D.score

HERBICIDE DOSE (p.p.m.)	MEANS OF VISUALLY ASSESSED DAMAGE SCORE DAYS AFTER TREATMENT					
	0	3	6	10	13	18
0	10	10	10	10	10	10
2	10	10	10	8.8	* 8.2	* 6.6
4	10	9.8	* 8.8	* 4.8	* 3.8	* 1.8
8	10	* 9.2	* 7	* 1.8	* 1.2	* 1.0
16	10	9.8	* 6.8	* 2.8	* 1.6	* 0.8
32	10	9.4	* 7.2	* 3.2	* 1.8	* 0.8
64	10	* 8.2	* 4.4	* 1.8	* 1.6	* 0.6
128	10	* 8.6	* 5.4	* 1.4	* 1.4	* 1.0
256	10	* 7.6	* 4.4	* 1.8	* 1.2	* 0.6
512	10	* 6.6	* 3	* 2	* 1.4	* 0.4

* Significantly different from untreated control at p=0.05

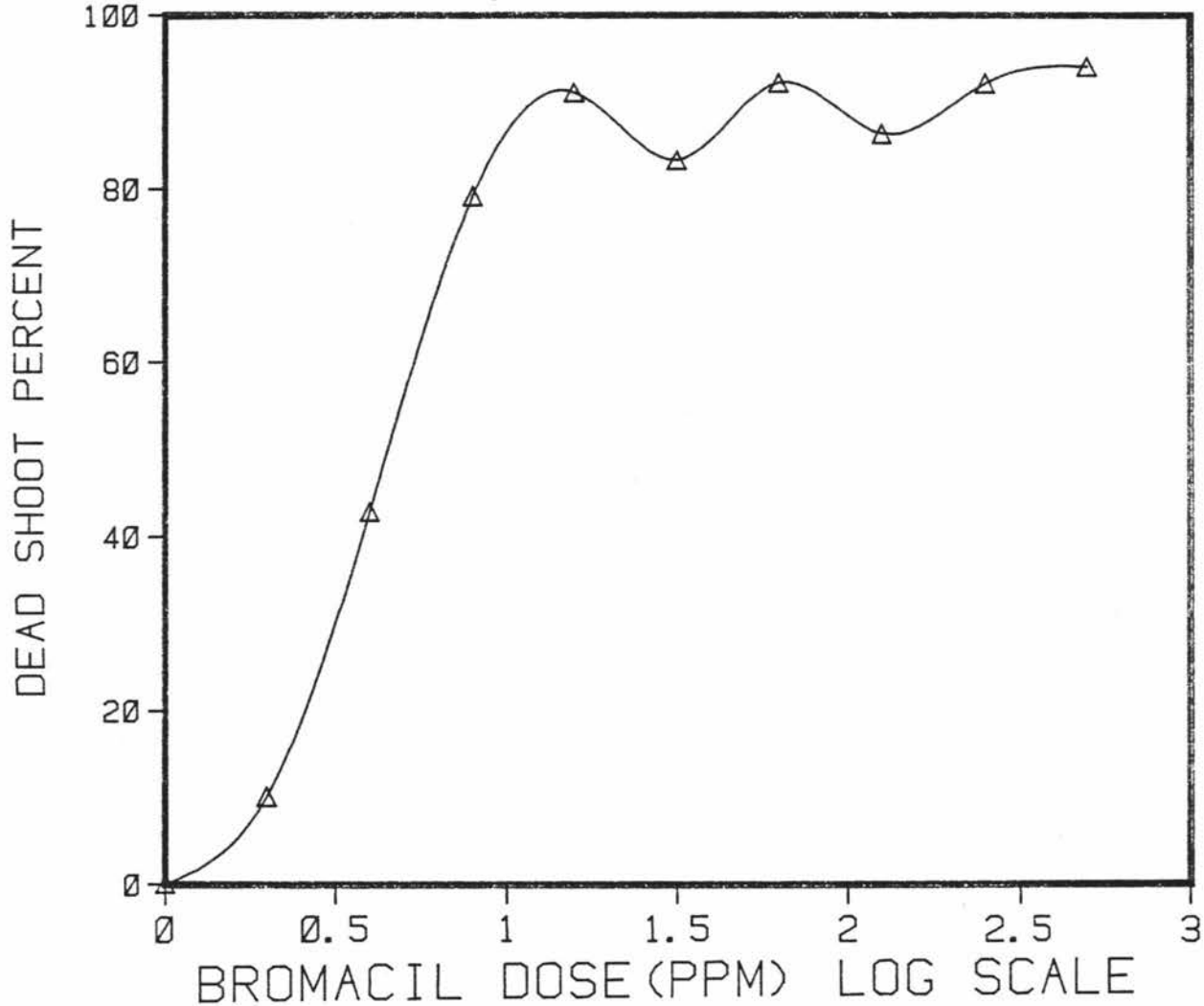
Analysis of the data showed that at 2 p.p.m. significant differences, compared to the untreated control, were apparent in the injury scores 13 days after treatment. At the higher doses, however, significant differences were evident after only 3 to 6 days. Thus, the time at which the injury symptoms appeared was related to the herbicide dose, the higher the dose the quicker the effects appeared.

4.1.2.2 Shoot Growth

The effect of different concentrations of bromacil on shoot growth is presented in Figure 4.2 . Again, clear dose-response relationships are evident showing direct proportional relationships between bromacil dose and inhibition of shoot growth and elongation. The regression equation for the data from 0 to 32 p.p.m. was:

$$Y = 109 - 42.1X$$

FIGURE 4.3: DOSE RESPONSE CURVE.
BROMACIL EFFECTS ON SHOOT DEATH
18 days after treatment



R^2(adjusted for d.f.)= 82%

The ED_{20} and ED_{50} values calculated from this equation was 4.8 p.p.m. and 25 p.p.m. respectively.

4.1.2.3 Shoot Death

Figure 4.3 shows the dose-response relationships for the effects of bromacil on shoot death 18 days after treatment. A steep slope reaching a maximum response around 16 p.p.m. was evident. The regression equation for this phase of the curve was:

$$Y = -5.65 + 83.8X$$

$$R^2 \text{ (adjusted for d.f.)} = 95.4\%$$

The ED_{20} and ED_{50} values computed from the regression equation was 2 p.p.m. and 4.6 p.p.m. respectively.

Table 4.2 shows the time-course data for the mean number of dead shoots 10,13 and 18 days after treatment.

FIGURE 4.4: DOSE RESPONSE CURVE,
BROMACIL EFFECTS ON ROOT FRESH WEIGHT
after 31 days (18 days after treatment)

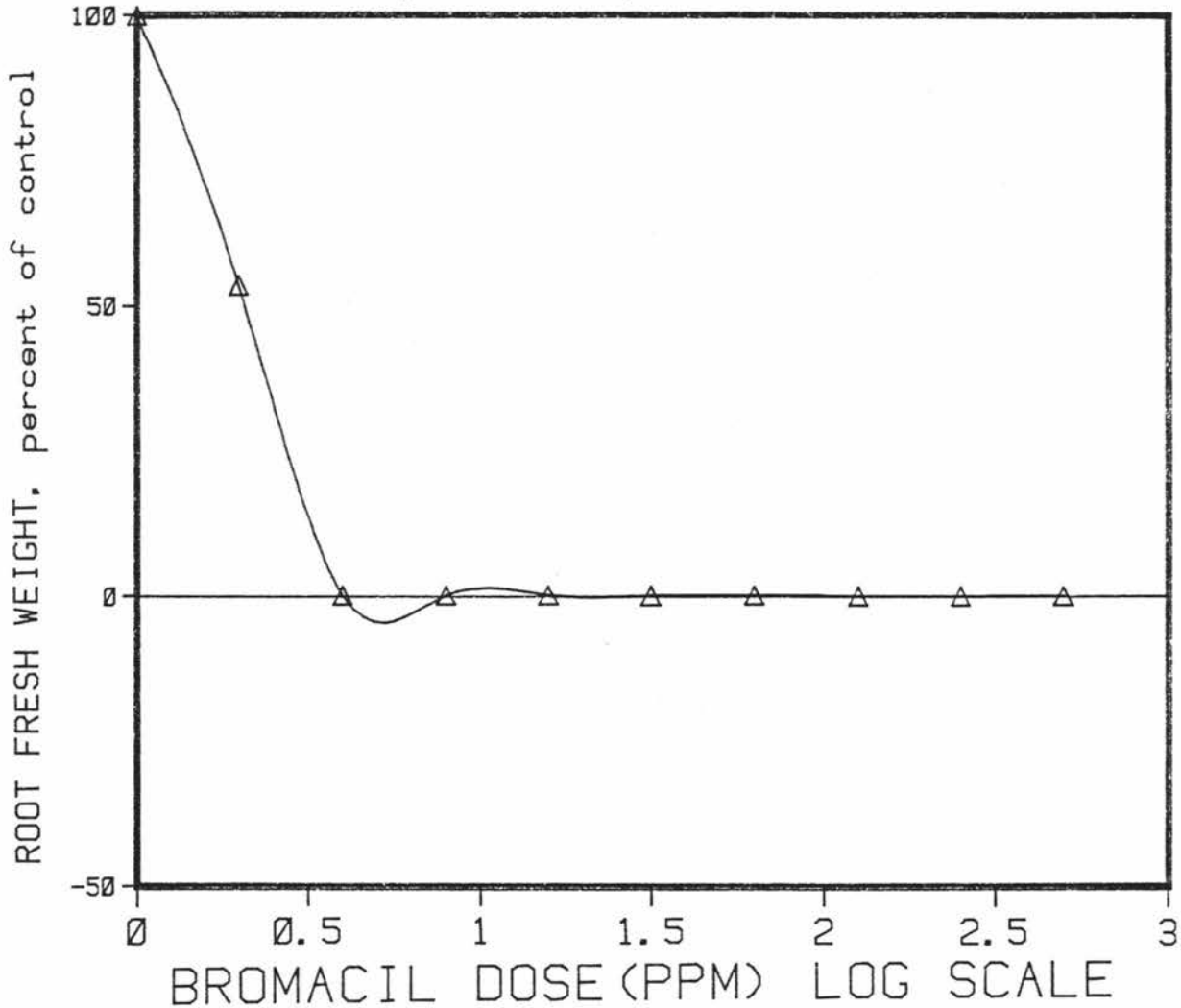


Table 4.2: Time-course data for dead shoots

HERBICIDE DOSE (p.p.m.)	MEAN NUMBER OF DEAD SHOOTS		
	DAYS AFTER TREATMENT		
	10	13	18
0	0	0	0
2	0	0.2	0.5
4	0.8	*1.4	*1.8
8	*1.4	*1.8	*1.9
16	*2.1	*3.1	*4.1
32	*1.7	*2	*3
64	*2	*2.3	*3.5
128	*2.8	*3	*3.8
256	*3.5	*4	*4.7
512	*2.2	*2.6	*3.2

* Significantly different from untreated control at p=0.05

These time-course data show that shoot death occurs in a progressive manner with a greater number of shoots dying with increasing time and dose. These data also support the findings reported earlier on the visually assessed damage score. Cladophylls at 2 p.p.m. showed signs of injury (chlorosis and bleaching) but these symptoms were generally not followed by shoot death indicating that the shoots were able to recover from the somewhat milder effects of the lower dose.

4.1.2.4 Root Fresh Weight

Figure 4.4 shows root fresh weight 31 days after topgrowth was removed and 18 days after application of bromacil. Again strong dose-response relationships were evident. The dose-response curve shows that bromacil at 2 p.p.m. caused an almost 50% reduction in root fresh weight and at 4 p.p.m., no increase or a slight reduction in root fresh weight, compared to the untreated control.The regression equation computed for the linear phase of the curve (0 to 4 p.p.m.) gave the following equation:

$$Y = 101 - 167X$$

$$R^2 \text{ (adjusted for d.f.)} = 99.7\%$$

The ED_{20} and ED_{50} values derived from the regression equation was 1.34 p.p.m. and 2.1 p.p.m. respectively.

The results of a single application of a wide range of bromacil doses to 18-month-old asparagus plants in sand-culture and growing in a non-draining system can be summarised as follows:

1. Good dose-response characteristics were evident for all the parameters measured. The steep slopes at the lower range of the bromacil doses indicate that the asparagus plants used were very sensitive to relatively small changes in the herbicide concentration.

2. Although the effect of bromacil on asparagus plants was directly related to the dose applied, the degree and rate of effect varied according to the parameter measured. The ED_{20} and ED_{50} values computed gave a convenient basis for comparing these differences.

3. The ED_{20} and ED_{50} values for the parameters measured were:

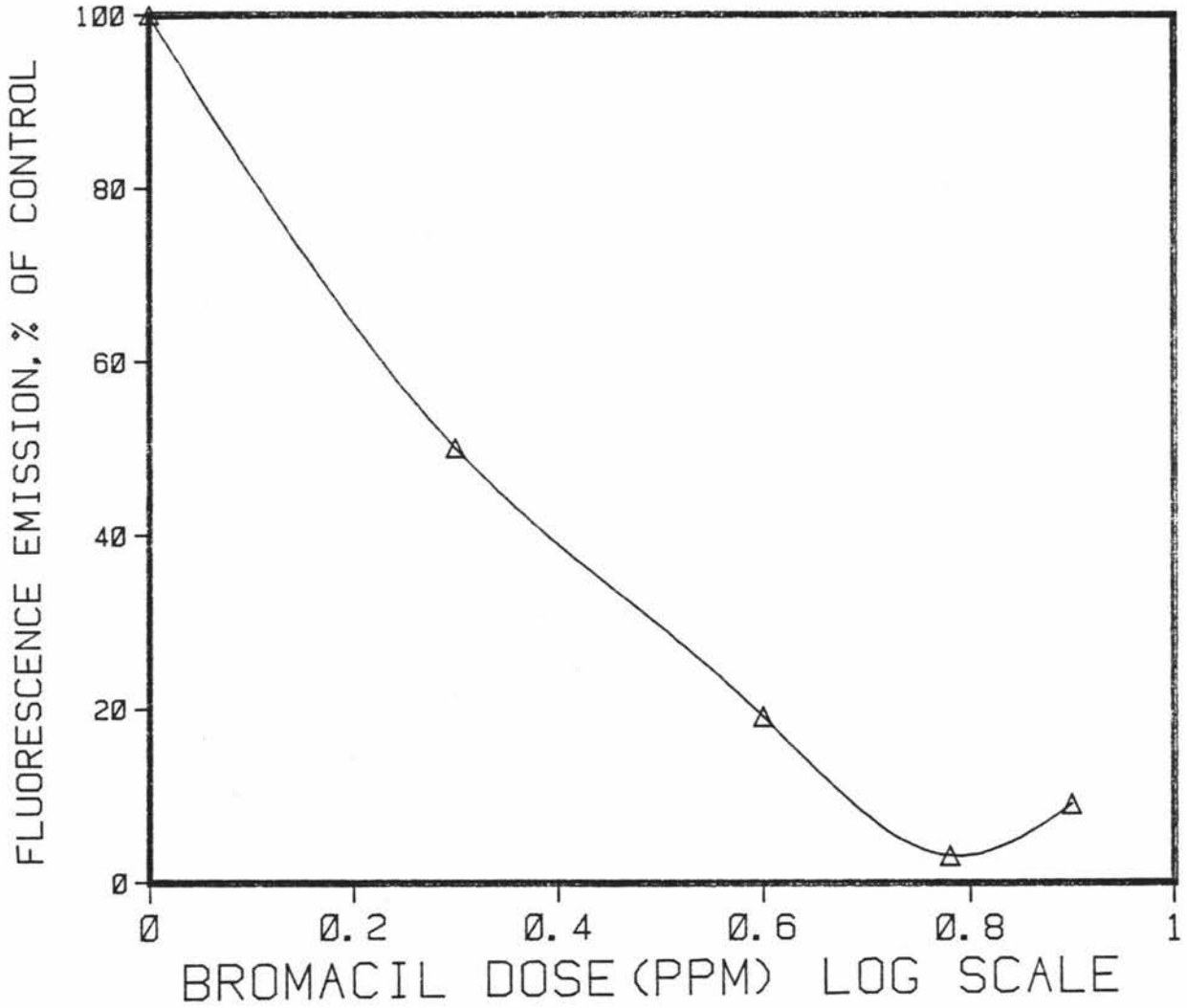
	ED_{20}	ED_{50}
V.A.D. Score	-	2.7 p.p.m.
Shoot Growth	4.8 p.p.m.	25 p.p.m.
Shoot Death	2 p.p.m.	4.6 p.p.m.
Root Fresh Weight	1.3 p.p.m.	2.1 p.p.m.

4. Shoot growth was affected more slowly than the other parameters; root fresh weight and the V.A.D.score were the most sensitive. For these parameters the ED_{50} values

derived from regression lines indicate that severe damage to the plants was caused at bromacil doses slightly over 2 p.p.m.

5. The time-course data for V.A.D.scores and mean number of dead shoots also indicated that the rate at which damage occurred was directly related to bromacil dose, the higher the dose the more severe the effects and the quicker the injury was manifested.

FIGURE 4.5: DOSE RESPONSE CURVE, BROMACIL EFFECTS ON FLUORESCENCE INDUCTION OF CLADOPHYLL TIPS 156 HOURS AFTER TREATMENT



4.2 PART 111: EQUAL INCREMENT DOSE-RESEPONSE EXPERIMENTS

4.2.1 FLUOROMETRIC MEASUREMENTS

4.2.1.1 Cladophyll tips from intact plants

Fluorescence emission measurements at wavelengths greater than 710nm. were made with the Kautsky apparatus. Plates 4.7 and 4.8 show the type of fluorescence induction curves obtained. These were taken for display purposes only. For analysis, all data were recorded on a chart recorder. Plate 4.7 shows a typical fluorescence induction curve for an untreated control sample. Plate 4.8 shows a decrease in induced rise in chlorophyll fluorescence due to bromacil damage to the photosynthetic apparatus.

The slope of the rising phase of the fluorescence induction curve was recorded on a chart recorder and analysed. Figure 4.5 shows the decrease in fluorescence yield in cladopohyll tips, expressed as a percent of control, 156 hours after bromacil application. Each point is the mean of 20 observations. At a dose of 2 p.p.m. an almost 50% reduction in fluorescence yield was evident, decreasing to less than 20% of the control value at 4 p.p.m. The regression equation for the linear phase of the dose-response curve (0 to 6 p.p.m.) was:

$$Y = 0.948 - 1.23X$$

$$R^2 \text{ (adjusted for d.f.)} = 97.0\%$$

The ED_{20} and ED_{50} values calculated from the regression equation was 1.3 p.p.m. and 2.3 p.p.m. bromacil dose.

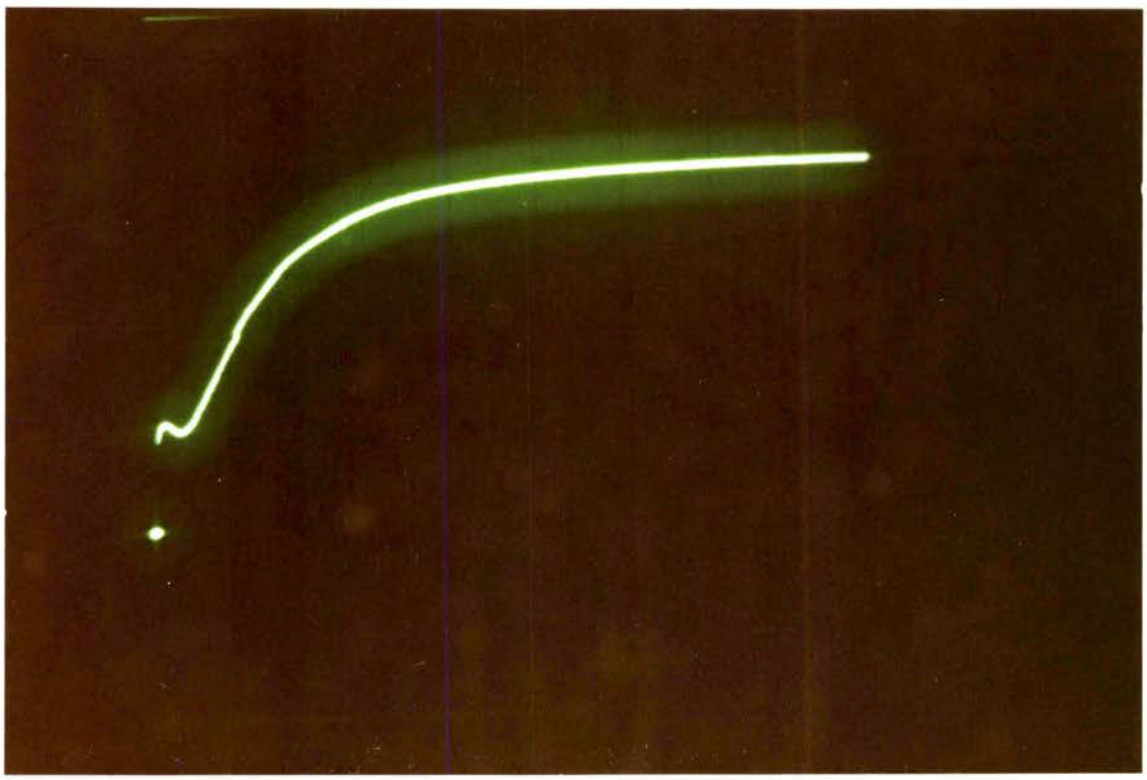
Time-course data on Table 4.3 show significant differences ($p=0.05$) in fluorescence emission measurements for the 6 p.p.m. and 8 p.p.m. doses after 108 hours. After 156 hours, measurements from all treatments were significantly different from the untreated control.

Plate 4.7

Fluorescence induction curve on storage oscilloscope screen of untreated control sample

Plate 4.8

Fluorescence induction curve showing a decrease in induced rise in chlorophyll fluorescence due to bromacil damage to the photosynthetic apparatus



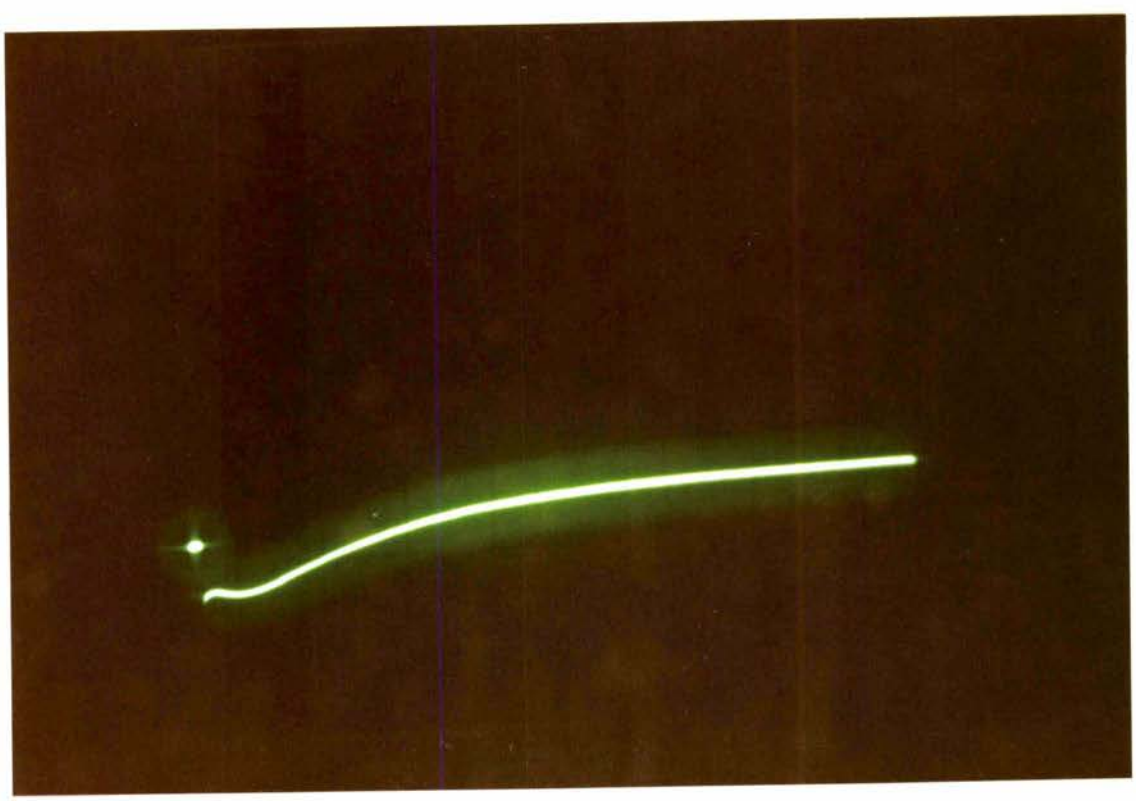


FIGURE 4.6: DOSE RESPONSE CURVE, BROMACIL EFFECTS ON FLUORESCENCE INDUCTION OF CLADOPHYLL TIPS FROM EXCISED SHOOTS 17 HOURS AFTER TREATMENT

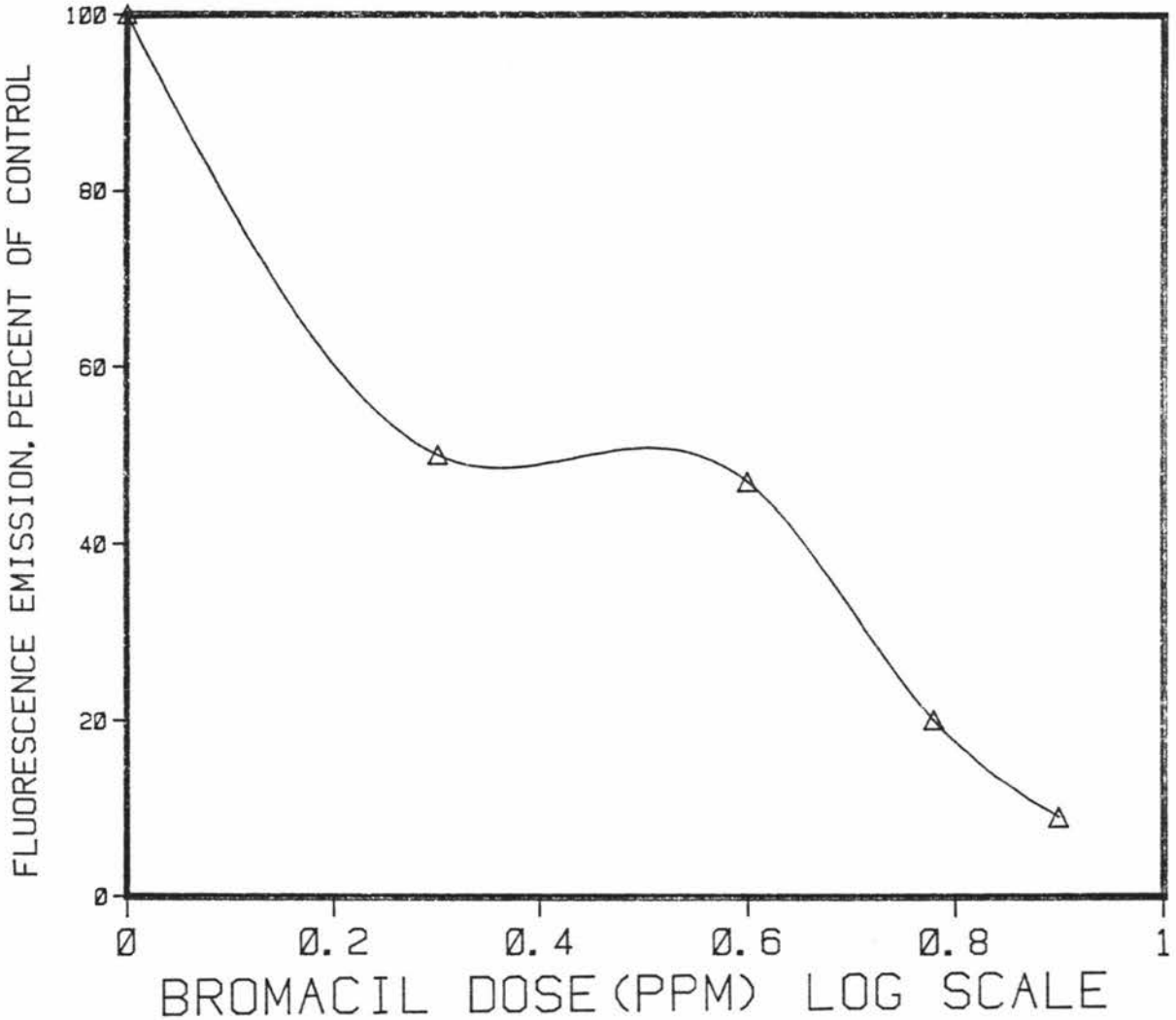


Table 4.3:Time-course data for fluorescence emission measurements

TIME AFTER HERBICIDE APPLICATION	FLUORESCENCE EMISSION MEASUREMENTS, PERCENT OF CONTROL				
	BROMACIL DOSE (p.p.m.)				
	0	2	4	6	8
60 hours	1.0	0.53	0.42	0.33	0.34
108 hours	1.0	0.79	0.40	*0.21	*0.13
156 hours	1.0	*0.5	*0.19	*0.03	*0.09

* Significantly different from untreated control at p=0.05

4.2.1.2 Cladophyll tips from excised shoots

Figure 4.6 shows the fluorescence emission measurements of cladophyll tips from excised shoots immersed in 0, 2, 4, 6 and 8 p.p.m. bromacil after 17 hours. Again a steep slope showing a relatively rapid and drastic decrease in fluorescence emission was apparent. The regression equation for the data was:

$$Y = 0.93 - 0.926X.$$

$$R^2 \text{ (adjusted for d.f.)} = 90.0\%$$

The ED_{20} and ED_{50} values derived from this equation were 1.38 p.p.m. and 2.9 p.p.m. respectively. Table 4.4 shows that after 96 hours the relative fluorescence emission of all treatments had decreased to almost nil, showing that severe and irreversible damage to the photosynthetic mechanisms had occurred.

Table 4.4:Time-course data for fluorescence emission of cladophyll tips from excised shoots

TIME FOR WHICH EXCISED SHOOTS WERE IMMERSED IN BROMACIL SOLUTION	FLUORESCENCE EMISSION, PERCENT OF CONTROL				
	BROMACIL DOSE (p.p.m.)				
	0	2	4	6	8
17 hours	1.0	*0.5	*0.47	*0.2	*0.09
96 hours	1.0	*0.02	* 0	* 0	* 0

* Significantly different from untreated control at p=0.05

4.3 CHLOROPHYLL ANALYSIS

Using the 80% acetone extraction method, chlorophyll a, chlorophyll b and total chlorophyll concentrations were determined for the samples used for fluorometric measurements [Section (1)]. Table 4.5 shows the relative chlorophyll concentrations, expressed as percent of control, for samples of cladophyll tips 156 hours after treatment.

Table 4.5: Relative chlorophyll concentration 156 hours after treatment

BROMACIL DOSE (p.p.m.)	RELATIVE CHLOROPHYLL CONCENTRATION, PERCENT OF CONTROL (Mean of 5 samples)		
	Chlorophyll a	Chlorophyll b	Total Chlorophyll
0	1.0	1.0	1.0
2	0.89	0.79	0.87
4	0.94	1.32	1.01
6	0.88	1.08	0.92
8	0.96	1.29	1.02

None of the treatment values were significantly different from the untreated control at p=0.05

The results of the experiments from Part 111 of the project can be summarised as follows:

1. The effects of bromacil on asparagus plants observed in Part 11 of the project have been reflected in Part 111 using Fluorometric methods.

2. Fluorescence emission measurements showed bromacil injury effects on the photosynthetic apparatus more rapidly than was observed in the parameters used in Part 11.

3 The data revealed that fluorescence yield at wavelengths greater than 710 nm. from the photosynthetic cladophyll tips deteriorated rapidly with increasing bromacil dose. Even at 2 p.p.m., a significant and dramatic decrease in fluorescence

yield was evident 156 hours after treatment.

4. Fluorescence emission data of cladophyll tips from excised shoots immersed in bromacil solution showed that damage to the photosythetic apparatus occurred within 17 hours. This indicates that without the roots, untake and translocation of bromacil was very rapid. The injury effects observed were severe and progressive, resulting in irreversible and total damage within 96 hours of treatment.

5. The ED_{20} and ED_{50} values obtained for the fluorescence yield were:

	ED_{20}	ED_{50}
Cladophyll from intact plants (156 hours after treatment)	1.3	2.3
Cladophyll from excised shoots (17 hours after treatment)	1.3	2.9

These values correspond fairly well with the ED_{20} and ED_{50} values obtained for Root Fresh Weight, V.A.D.score and Shoot Death in Part 11.

6. Analysis of photosynthetic pigments by acetone extraction revealed no significant decline in chlorophyll a, chlorophyll b and total chlorophyll concentration during the period in which a dramatic decline in fluorescence yield occurred.

7. Fluorescence emission measurements using a relatively simple and portable fluorometer provided sensitive and reliable data for determining bromacil damage to asparagus plants.

Chapter 5

5.1 DISCUSSION

In field conditions the kinetics of solution processes, particularly with materials with relatively low water solubility, make it extremely difficult to predict what the concentration of the herbicide in the soil will be at a certain rate of application (Hance, 1976). Further, research on a number of perennial crops has indicated that the effectiveness of xylem-transported herbicides can be affected by rainfall, light intensity, temperature, humidity, depth of plant root system, age of plant; and such soil factors as water-holding capacity, adsorption potential and organic matter content (see review by Ashton & Crafts, 1973). These variables impose limitations in interpreting data from field experiments on the effects of soil-acting herbicides like bromacil.

Various techniques have been used to overcome some of these limitations. Ivens (1964) and Holloway (1968) grew crops in pots of soil or compost into which the herbicide had been incorporated, thus ensuring that the roots were in contact with the herbicide. Tweedy and Ries (1966) planted crops into beds of treated soil. Orth (1967) dipped the roots of test plants in herbicide solution prior to planting in soil.Sand-culture techniques were used by Caseley (1964) (cited in Luckwill & Caseley, 1966) to study the effects of herbicides on fruit crops and to compare the tolerance of different cultivars of one crop. Clay and Davidson (1978) cited Julliard and Ancel (1969) as having used a similar method to study the effects of herbicides on vines. Leonard and Lider (1961) and van Oorschot and Haker (1964) used nutrient culture methods for comparing the tolerance of perennial crops to herbicide. Clay and Davidson (1978) reported that the sand-culture method using a single application of herbicide made to pots with a closed draining system, to prevent leaching, was the best treatment method for studying the tolerance of plants to residual soil-applied herbicides.

In the project reported here sand-culture methods were used to determine the dose-response characteristics of bromacil on 18-month-old asparagus plants under carefully controlled environmental conditions.

The ED_{20} and ED_{50} values for several parameters were determined by using regression analysis on the linear phase of the log dose-response curves. The ED values were a convenient method for determining the extent of injury and comparing the response on various parameters. Genetic variation in crop cultivar and effects of plant age were minimised by the use of clonal material already described. In Part 11 a wide bromacil dose-range increasing geometrically up to 512 p.p.m. was used to establish dose-response relationships over the total effective range from initiation of damage symptoms to shoot death. Time-course studies were conducted to follow the sequence of events that lead to irreversible damage.

The parameters measured indicated good dose-response characteristics suggesting strong cause-effect relationships.The phytotoxic effects observed indicated that bromacil was readily absorbed by asparagus roots and translocated acropetally to the shoot and cladophyll tips. The directly proportional relationship observed between phytotoxic effects and bromacil dose suggests that the herbicide tends to accumulate in the foliage and the rate of accumulation was determined by the rate of absorption, translocation and retention. These results also suggest that bromacil is not readily degraded or detoxified by asparagus plants. Indeed the steep slope in the early phase of the dose-response curves for most of the parameters measured indicates that relatively small increments in bromacil dose produced substantial injury. The low capacity of asparagus plants for detoxification and the high rates of uptake and transport in the plant would result in a low selectivity index (S) for bromacil; where selectivity can be described by the function:

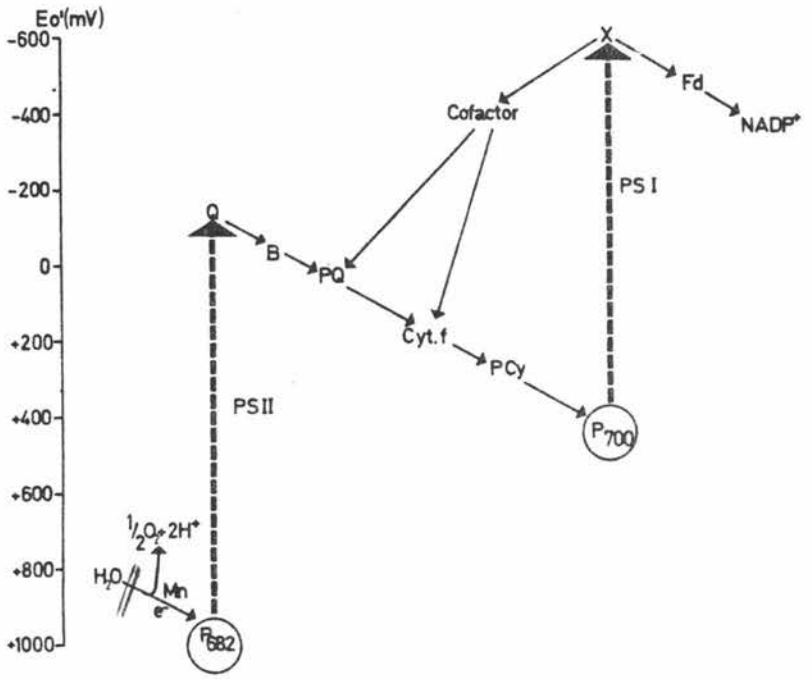
$$S = \frac{\text{detoxification}}{\text{uptake x transport}}$$ (Fedtke, 1982)

Bromacil was found to inhibit shoot growth and elongation, decrease visually assessed damage score, increase shoot death and reduce root fresh weight. The higher ED_{20} and ED_{50} values for shoot growth indicate a slower response suggesting that bromacil does not have an immediate effect on the meristematic regions and that inhibition of growth is a secondary effect. Similarly, observations of the roots

indicated no direct effects such as lesions or necrosis. However, a reduction in the number of feeder roots and the presence of hollow storage roots was apparent. This would account for the dramatic decline in root fresh weight as a result of a depletion of carbohydrate reserves due to the inhibition of photosynthesis. The appearance of phytotoxic symptoms such as chlorosis, bleaching and necrosis of the foliage supports the findings in the literature that bromacil is a photosynthetic inhibitor. Further, the results of the chlorophyll fluorescence studies also suggests that the basic primary site of action is associated with the photosynthetic process.

When a chlorophyll molecule, excited by light, returns from its singlet excited state to its ground state, there is light emission within a few nanoseconds. This emission is red in colour, and is the fluorescence of chlorophyll. Of the plant pigments in vivo, Chlorophyll a and the algal biliproteins (phycobilins) fluoresce weakly; other pigments of photosynthetic importance do not have have any measurable fluorescence. These, including chlorophyll a and phycobilins dissipate their electronic excitation energy also by other processes, of which the most important is the transfer of energy to other chlorophyll a molecules - ultimately to special chlorophyll a molecules (the reaction centres) where the energy is used for photosynthesis. The fluorescence characteristic of a molecule are governed both by its chemical nature and interaction with its environment. Thus fluorescence of chlorophyll a in vivo has been used as a powerful tool in the analysis of photosynthesis, particularly of the process of excitation energy transfer (see review by Govindgee and Papageorgiou, 1971).

The sequence of events in the photosynthetic electron transport chain, arranged according to redox potential, is presented in Figure 5.1. Energy that has travelled through the pigment complex to the "chlorophyll- trap" (P_{682} or P_{700}) may eventually be captured in an energised electron, leaving a cationic Chlorophyll^{+}. The electron is replaced from water by an enzyme complex containing manganese, and the splitting of water leads to the liberation of molecular oxygen. The energised electron derived from P_{682} may now reduce other compounds, depending on the redox potential. The native electron acceptor for electrons from photosystem II is the quencher Q, a compound whose molecular identity is not yet clear (Fedtke,1982). The two pigment systems (PS I and PS II) work in sequence in the generation of the



// site of action of bromacil

Figure 5.1: Schematic presentation of photoinduced electron transport system showing postulated site of action of bromacil (adapted from Fedtke, 1982).

Abbreviations: PS I, photosystem I; PS II, photosystem II; P_{682}, reaction centre chlorophyll of PS II; Q, primary electron acceptor for PS II; PQ, plastoquinones; cyt.f, cytochrome f; PCy, plastocyanin; P_{700}, reaction centre chlorophyll of PS I; X, unidentified electron acceptor; Fd, ferredoxin; NaDP, nicotinamide adenine dinucleotide phosphate; E_o, standard redox potential.

redox potential necessary for the reduction of ferredoxin. The exact molecular identity of X, a native electron acceptor of photosystem I is not known.

Since chlorophyll fluorescence and electron flow along the photosynthetic transport chain draw on the excited chlorophyll a population, any change in the photosynthetic rate is reflected as a change in the yield of fluorescence. These changes in fluorescence yield are known to be dependent on the oxidation-reduction state of the photochemical reaction centre, the rates of reactions associated with it, the intermediates of the electron transport chain and the physical state of the pigment system. Thus, fluorescence is a measure of the photochemistry at the reaction centres of Photosystem II.

Chlorophyll fluorescence yield is known to be greatest when the primary electron acceptors of Photosystem II are fully reduced and are unable to extract energy from the active centres of Photosystem II. Conversely, the yield is lowest when the primary electron acceptors are oxidised (Hetherington et al.,1983). Hence, an inhibition of photosynthetic electron transfer on the photo-reducing side of Photosystem II blocking reoxidation of the primary electron acceptors will increase the yield of fluorescence, while inhibition on the water splitting or photo-oxidising side of photosystem II will decrease the yield (Papageorgiou, 1975). The reduction of the fluorescence yield evident in this study support the findings in the literature that bromacil is a Hill Reaction inhibitor, that is, inhibition first develops in the water splitting process of photosystem II. The site of action of bromacil is shown in Figure 5.1.

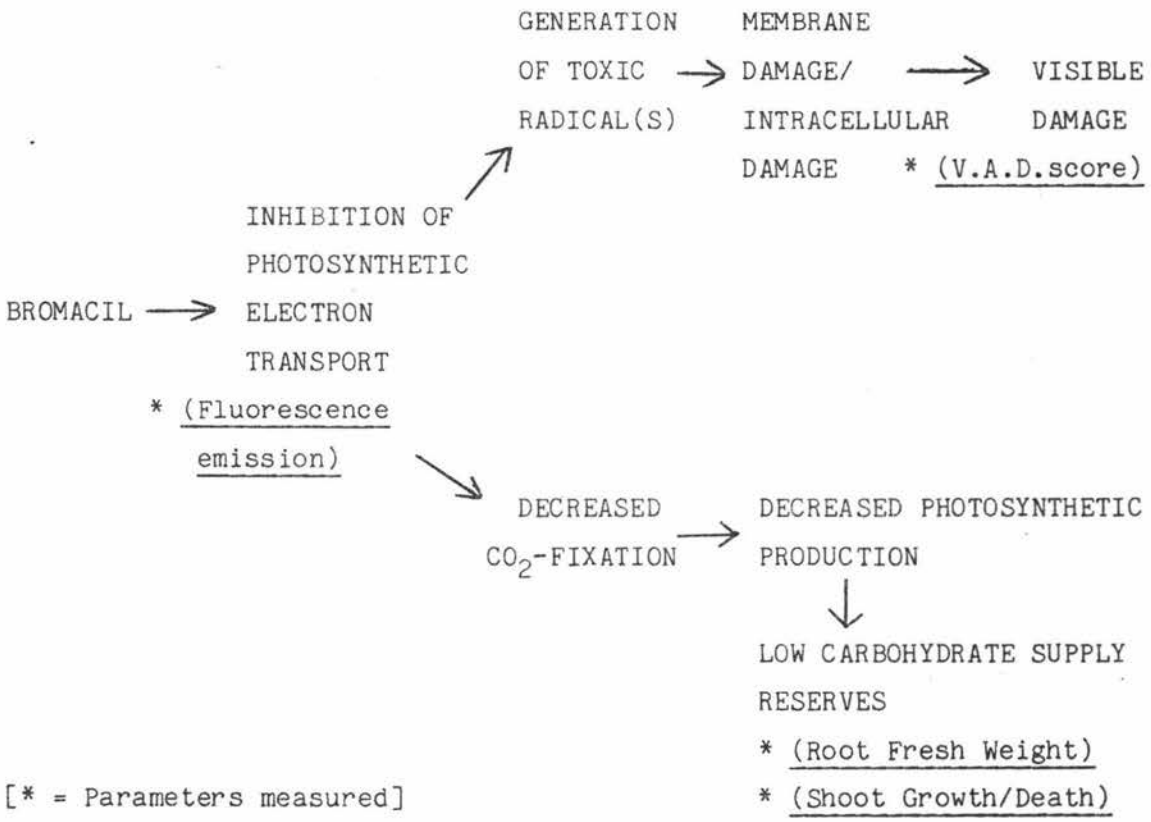
The principle of the fluorometer detection of inhibition of photosystem II electron transport herbicides is based on the different shapes of the fluorescence induction curves in the presence or absence of the inhibitor. In the presence of bromacil a decrease in fluorescence rise occurs due to damage to the photosynthetic apparatus. This enables a means of calculating the extent of damage by determining the slope of the initial fluorescence rise in the induction curve. The sensor was a light probe that illuminated the cladophylls for 2-4 seconds with red light centering around a wavelength of 670 nm. Then, with the aid of a red filter that blocked out light waves shorter than 710 nm, it measured the far-red fluorescence. Such measurement was relatively

rapid; 96 cladophyll samples could be processed within 20 minutes, with the data being recorded on a chart recorder.

The inhibition in fluorescence yield was manifested relatively rapidly, and reliably reflected bromacil damage as evident from parameters such as V.A.D scores, root fresh weight, shoot death and shoot growth. The very rapid inhibition in fluorescence yield observed on samples from excised shoots immersed in bromacil indicates more rapid uptake and translocation without the roots. This suggests that the passage of bromacil through the roots is relatively slow and lends support to the findings in the literature cited in Chapter 1 that bromacil is transported apoplastically and passage across the Casparian strip is a relatively slow process. However, the nature of this study does not give any clues to the mechanism by which bromacil crosses the Casparian strip in root cortical cells.

The lack of any significant differences in extractable chlorophyll a, chlorophyll b and total chlorophyll concentrations in samples which showed a marked decline in fluorescence emission suggests that bromacil does not have an immediate effect on chlorophyll synthesis. The visible toxic responses observed later were probably a result of the build up of a toxic radical.

The possible chain of events for bromacil action on asparagus can be outlined as follows:



The use of a simple portable fluorometer for measuring chlorophyll fluorescence to help rapidly and accurately identify tolerance to photosynthetic inhibitors is a recent development (Ali & Machado, 1981). Although the Kautsky apparatus has recently been used by some research groups to study chilling tolerance (e.g. Hetherington et al., 1983), the use of a portable plant productivity fluorometer such as Model SF-10 in herbicide research has not been widely reported. The results presented in this report do confirm the usefulness of this instrument as a basic tool in herbicide research. Fluorescence emission measurements appear to be an appropriate analytical tool for monitoring the inhibitory photosynthetic activity of herbicides such as bromacil not only because fluorescence yield changes are due to modified photosynthetic activities but also fluorescence emission is

easily detectable in intact leaves. Measurement of fluorescence induction in plant tissue *in situ* provides an elegant, relatively simple and rapid technique for assessing bromacil damage.

5.2 CONCLUSION

The results from this study have clearly established that the asparagus plants tested were susceptible to bromacil damage at very low concentrations. However, the methods used in this project, while overcoming many of the limitations of field studies, probably overestimate the risk of injury in the field situation because they are based on environmental and growth conditions in which herbicide activity is likely to be at its maximum. Further, there may be varietal differences in susceptibility between different cultivars of asparagus.

The results of this study also establish reproducible background information upon which to base interpretation of the phytotoxic action of bromacil on asparagus. Further controlled environment studies on varietal differences for bromacil tolerance, persistence and movement of bromacil in various soils and their availability to asparagus root systems would give a better basis for making reliable predictions on risk of herbicide damage in the field situation. For the present, the increasing-dose effect on several parameters showing the apparent lack of tolerance by asparagus plants to bromacil calls for caution in the repeated use of bromacil on asparagus plots without monitoring residue levels in the soil.

APPENDIX A

INORGANICS - BASAL MEDIUM FOR ASPARAGUS

1. MACRO ELEMENTS X 20 conc.

	mg/l
NH_4NO_3	1320
KNO_3	1520
$CaCl_2 . 2H_2O$	440
KH_2PO_4	170
$MgSO_4 . 7H_2O$	370

Dissolve each compound before adding the next.
Rate of use: 50 ml / litre medium

2. MICRO ELEMENTS X 200 conc.

	mg/l
H_3BO_3	6.2
$MnSO_4 . 4H_2O$	22.3
$ZnSO_4 . 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 . 2H_2O$	0.25
$CuSO_4 . 5H_2O$	0.025
$CoCl_2 . 6H_2O$	0.025

Dissolve each compound before adding the next.
Rate of use: 5 ml / litre medium

3. IRON X 200 conc.

	mg/l
NaFe EDTA	36.7

Dissolve and make up to volume.
Rate of use: 5 ml / litre medium

APPENDIX B

MODIFIED HOAGLAND'S SOLUTION - 1/2 STRENGTH

4 ml/l water

	gm/l concentrate
Stock solution A:	**100 l**
$Ca(NO_3)_2 \cdot 4H_2O$	14,759.5
10% DTPA NaFe (Sequestrene)	520.0
Stock solution B:	
KH_2PO_4	1,701.0
KNO_3	6,319.5
$MgSO_4 \cdot 7H_2O$	6,162.0
H_3BO_3	35.75
$MnCl_2 \cdot 4H_2O$	22.62
$ZnSO_4 \cdot 7H_2O$	2.75
$CuSO_4 \cdot 5H_2O$	1.00
$Na_2MoO_4 \cdot 2H_2O$	0.335
KCl	78.75

pH of final solution = 6.5 - 7.5

APPENDIX C

CONTROLLED ENVIRONMENT ROOM OR CLIMATE ROOM

The experiments were carried out in Climate Room Number 17 at the D.S.I.R. Climate Laboratory at the Plant Physiology Division in Palmerston North.

Conditions in the Cliamte Room were as follows:

Photosynthetic Photon Flux Density ($\mu E\ m^{-2}\ s^{-1}$)*		
	pre-expt	716
	post-expt	657
	mean	687
Photosynthetic irradiance ($W\ m^{-2}$)**		
	pre-expt	152
	post-expt	111
	mean	132
Daylength (h)		14
Temperature (plus or minus 0.5°C)		
	Day	23
	Night	23
Relative Humidity (plus or minus 5%)		
	Day	70
	Night	70

* Licor LI 185 Meter with LI 190S Quantum Sensor

** Licor LI 185 Meter with LI 190SE Flat Response Photosynthetic Irradiance Sensor

APPENDIX D

FORMULATION AND CALCULATION OF HERBICIDE

HYVAR X has 80% of the active ingredient bromacil

Solubility in water: 815 p.p.m.

Bromacil conc. p.p.m.	mg of Hyvar X/litre of water	mg of Hyvar X/50 ml i.e. mg/pot
0	0	0
2	2.5	0.125
4	5.0	0.25
8	10.0	0.5
16	20	1.0
32	40	2.0
64	80	4.0
128	160	8.0
256	320	16.0
512	640	32.0

Bromacil concentration p.p.m.	$\log 10^X$
2	0.3
4	0.6
8	0.9
16	1.2
32	1.5
64	1.8
128	2.1
256	2.4
512	2.7

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