FLOODING TOLERANCE AND SURVIVAL IN HIGHER PLANT STORAGE TISSUE

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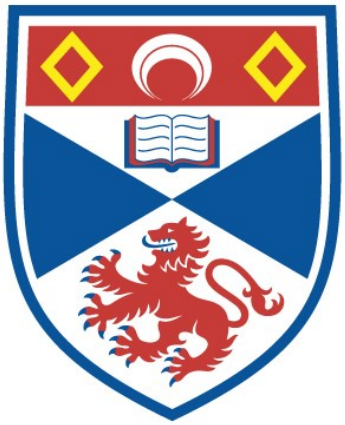
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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CERTIFICATE

I hereby declare that Mrs. Nashriyah Mat has been engaged upon research from September 1980 onwards to prepare the accompanying thesis for the degree of Doctor of Philosophy.

Signed :

St. Andrews,
August 1984.

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INTRODUCTION

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Chapter 1

INTRODUCTION

Flooding can occur continuously or seasonally and can affect a great area of otherwise an economically and ecologically important aerobic soil, including woodland (Gill, 1975) and pastures (Rhoades, 1967) . Some crop plants such as rice (Raalte, 1940; Plucknett et al., 1970; Plucknett and de la Pena , 1971) , edible aroids (Plucknett, 1978) especially taro (Greenwell, 1947; Barrau, 1953; Plucknett et al., 1970; Plucknett and de la Pena, 1971) , jute (Khanna and Chacravarti, 1949) and certain varieties of sugar-cane (Rege and Mascarenhas, 1956) , can flourish under flooding whereas for the majority of crop plants flooding can lead to death (Rhoades, 1967; Drew, 1979) or at least reducing grain yield (Ali, 1976) .

1.1 THE OBJECTIVE OF STUDY

In the less developed countries in the tropics or sub-tropics where the problem of the food shortages is the most acute, food crops which can give good yields while growing under difficult conditions have not received adequate scientific attention. Usually it is the ill-suited but cash-producing crops which are forced into cultivation in unfit areas resulting in failure of agricultural projects. There are however many

alternative more tolerant crops available; namely cassava (Manihot esculenta Crantz.) , edible aroids (Alocasia, Colocasia, Cryptosperma and Xanthosoma) , sweet potato (Ipomoea batatas (L.) Lam) , sago palm (Metroxylon sp.) and Plantain (Musa X paradisiaca L.) . Comments on their strengths or tolerance to sub-optimal land conditions are based more on observation rather than experimental results, hence the need for more scientific attention on this tolerance is recognised (Plucknett, 1978) .

In general, the objective of this study was to observe statistically the effect of prolonged shallow flooding and/or burial on growth and survival of plants with underground storage organ. Tubers, cormous and rhizomatous plants were collected from sites in Scotland and also from Malaysia. These plants were chosen because most of them are growing in wetland habitats and thus can tolerate the hypoxic conditions. Experimental results also showed that they can tolerate anoxia as well (see Barclay and Crawford, 1982). It is hoped that a better understanding on wetland plant's response to variation in soil aeration or flooding could be gained, which in the future will hopefully enable us to put into use in tropical agriculture.

1.2 SOIL ENVIRONMENT

In a well drained soil, individual soil particles and aggregates are surrounded by pore spaces. These spaces are filled with gas and interconnected with the atmosphere thus allowing the gaseous exchange which is necessary for the aeration of plant roots. Excess water from temporary flooding will rapidly drain through these spaces until only a thin oxidised moisture film of one third bar tension or less remains. This is moist enough to allow the uptake of water by plants (Gambrell and Patrick, 1978) . Upon flooding , soil becomes saturated with water (waterlogged) and this will cause the displacement of air from pore spaces (Grable, 1966; Drew, 1979) . As the replacement of soil air is largely by diffusion which is directly proportional to the amount of the air spaces (Bannister, 1976) , as well as the low solubility of gas such as oxygen in water (Drew, 1979) , flooding can cause restriction of oxygen transport into soil. Prolonged flooding and continued oxygen demand by respiration of plant roots and micro-organisms may eventually result in the depletion of the oxygen content of the soil solution within several hours to a few days (Turner and Patrick Jr. , 1968; Ponnamperuma, 1972; Jackson and Campbell, 1976; Drew and Sisworo, 1979) . Furthermore Howeler and Bouldin (1971) showed that oxygen is consumed not only through biological respiration but also through oxidation of chemical reductants such as ferrous iron. Such conditions happen beneath the surface of continuously flooded soil in swamp and marshland, ocean and lake sediments, as well as in agricultural soils (Mortimer,

1941; Ponnamperuma, 1965) .

This study is particularly concerned with shallow flooding which is a characteristic of some agricultural flooding (White, 1972) as well as wetland soils (Weaver and Himmel, 1930; Lieffers and Shay, 1981) . Under a shallow flooding of surface water , there usually exists a thin oxidised soil or sediment zone which ranges from a few millimetres to a few centimetres deep. In this zone, the photosynthesis of certain algae such as Ulothrix and Mougeotia (Weaver and Himmel, 1930) , together with surface water mixing and the oxygen transport across atmosphere-surface water interface, contribute to a significant oxygen input. The presence of only a small population of oxygen consuming organisms keeps consumption low and thus maintains a positive dissolved oxygen content of several μgm. $ml.^{-1}$ of the zone. Under this oxidised surface layer of uniform thickness, lies a deep anaerobic reduced subsurface horizon (Gambrell and Patrick, 1978) . When these reducing conditions occur in the soil, there will be an accumulation of soil produced toxins such as volatile fatty acids (Cho and Ponnamperuma, 1971, Chandrasekaran and Yoshida, 1973) , aromatic compounds (Wang et al., 1967; Patrick, 1971) and hydrogen sulphide (Allam and Hollis, 1972; Culbert and Ford, 1972) which has been proved to be closely associated with the damage of field crops. Large concentrations of iron (Jones and Etherington, 1970; Green and Etherington, 1977) and manganese (Graven et al., 1965) which can be found in the leaves of plant growing in waterlogged soil are also associated with plant injury and death.

When plants die in the aerobic soil, the primary end-products of organic matter degradation by the aerobic micro-organisms are carbon dioxide, water, nitrate and sulphate, plus some residual humic material. On the other hand, in the anaerobic soil, the anaerobic respiration of anaerobic micro-organisms results in the production of hydrogen, carbon dioxide, methane, ammonia, amines, , mercaptans, hydrogen sulphide, additional residual humic materials (Ponnamperuma, 1972) , low-molecular-weight organic acids such as acetic, propionic and butyric acids (Lynch et al., 1980) and ethylene (Smith and Russel, 1969) ; most of which are toxic to plant growth. Thus waterlogged soil does not only possess low or practically no oxygen (Weaver and Himmel, 1930) and a high amount of soil and root produced toxins (Drew, 1979) , but also typically has increased dissolved carbon dioxide concentration (Gambrell and Patrick, 1978) as well as toxic gases (Ponnamperuma, 1972) . Stagnant water also favours the growth of plant pathogens (Plucknett and de la Pena, 1971) .

1.3 FLOODING DAMAGE

Although plant injury and damage by flooding cannot be attributed to any particular factor and the cause is still unclear, oxygen deficiency is clearly the trigger (Drew, 1979) or the centre of the problem of excessive moisture (Rhoades, 1967) . As roots require oxygen for respiration (when

pyruvate, a glycolytic end-product is oxidised to carbon dioxide and water via the tricarboxylic acid cycle (TCA)) and other metabolic activities , plants will have to undergo anaerobic respiration when oxygen is insufficient. In anaerobic respiration, pyruvate is either reduced to lactate or decarboxylated to acetaldehyde than reduced to ethanol with the production of only two moles of ATP from one mole of glucose glycolated. On the other hand, in aerobic respiration, the breakdown of one mole of glucose will produce 36 moles of ATP (Beevers, 1961) . Thus carbohydrate fermentation will produce less energy than aerobic respiration, which can probably lead to a reduction or inhibition of the synthetic aspect of metabolism for example the protein synthesis (Bertani and Brambilla, 1982) . If prolonged, this will cause root cell to die and decay (Yu et al., 1969) . Thus rice plants cultivated in an oxygen free medium were shown to be affected by Brusone, a root rot (Brizi 1905 as reported by Raalte 1940) , and also the so-called "Omo Mentek" disease which consists of a severe root rot accompanied by a typical discoloration of the leaves (Elst, 1912 from Raalte, 1940) . On the other hand, Cannon (reported by Conway, 1937) has shown that at 23^{o}C, rice roots still continue to grow in an atmosphere containing less than 0.5% of oxygen. Recently, other experiments in solution culture showed effects such as inhibition of root and shoot growth (Russel, 1952) , depressed absorption of water (Mees and Weatherly, 1957) and nutrients (Hopkins et al., 1950:Hammond et al., 1955) and altering root/shoot hormone relations (Burrows and Carrs, 1969; Hiron and Wright, 1973) .

Soil oxygen supply may have little direct influence on root metabolism (Ingram, 1967) . He suggested that the accumulation and dispersal of carbon dioxide may be similarly important. Dubinina (1961) reported that the partial carbon dioxide pressure is of great significance in the creation of an anaerobic region in root environment. Anaerobiosis created by the use of gaseous mixture of 10% oxygen plus 30% carbon dioxide plus 69% nitrogen acts more powerfully than pure nitrogen gas on the change in roots' amino acids composition. The high carbon dioxide content also causes some deviation from typical anaerobiosis as caused by pure nitrogen; that is an excess of carbon dioxide reduces malic acid formation and enhances citric acid formation. Hence the role of aerenchyma as the remover of respiratory carbon dioxide from root was also suggested (Teal and Kanwisher, 1966) .

1.4 TOLERANCE TO WET HABITAT

In the wild, there are many species of grasses, sedges and dicotyledonous plants which thrive in the wet habitat (Billings and Godfrey, 1967) . These plants can also grow under a wide range of conditions, for example Typha latifolia naturally grows in waterlogged soil as well as a well aerated soil (Weaver and Himmel, 1930) and rhizomes of Nuphar advenum dug from under the ice of a frozen lake in mid-winter also possessed terminal clusters of coiled young leaves the same as those commonly seen

in early spring (Laing, 1940) . There are species which have selected races for flooded and unflooded conditions. For example, Veronica peregrina L. consists of ecologically distinct populations ; in and around vernal ponds (Linhart and Baker, 1973) and also the lowland and upland varieties of rice (Chang et al., 1972) , taro (Plucknett and de la Pena, 1971) and sugar-cane (Rege and Mascarenhas, 1956) .

1.4.1 MORPHOLOGICAL ADAPTATION

The basis of flooding tolerance may often lie more with the development of certain morphological attributes (Jackson et al, 1982) . For example, these morphological adaptations are the ability to form internal gas-filled channels (aerenchyma) that favour internal root aeration (Armstrong, 1979; Drew et al. , 1979) , the production of new adventitious roots (Gomes and Kozlowski, 1980) , rapid shoot elongation (Konings and Jackson, 1979) and to modify shoot behaviour to compensate reduced efficiency of anaerobic roots. The first two major adaptations were further discussed.

1.4.1.1 Numerous air channel and air spaces

It is well known that most wetland plants are characterised by numerous air-channels and intercellular air spaces within the roots (Raalte, 1940; Yu et al., 1969). Over the years there have been many microscopic studies of root aerenchyma (Yu et al., 1969; Jefferies, 1916). Barthelemy (1874) as reported by Raalte (1940) suggested that air could enter this plant through the stomata and passed through petioles and rhizomes to another leaves under suction. Furthermore, Conway (1937) proved that the air spaces in the plant was linked- up to a continuous system , where gas can pass down rather easily to stock and roots by the way of full -grown leaves. The meristematic region at the base of young leaves offered a great resistance to the passage of air whilst the already dead leaves of Cladium mariscus did not. The oxygen content was higher in the base of rice root than in the root tip, that is 14% compared to 8.1% oxygen in variety Brondol Puteh (Raalte, 1940) . This gradient was probably caused by external oxygen received by the base of root from stem and shoot. Chashchukhin (1979) found this same condition in the basal portion of the rhizome of common reed (Phragmites communis Trin.) when the growing point was under water. However with the emergence of the growing point above the water line, this pattern changes in the opposite direction resulting in the higher oxygen content in the apical growing part of rhizome. The apical metameres of this plant (with growing point above water) contain about the same concentration of oxygen (about 14%) in the rhizome spaces as from the plant growing under terrestrial

condition where the growing point is in moist soil. Both these results suggest that air channels and air spaces did provide the increases of oxygen content through the stems or the growing point, when exposed to air. However, lately doubt about the necessity of plant air spaces for the maintenance of root aerobic respiration under flooding was enhanced when attention was drawn to the low development of aerenchyma found in the roots of Phalaris arundinacea and Filipendula ulmaria; both wetland plants (Crawford, 1982 a) .

1.4.1.2 Production of adventitious roots

A majority of investigators suggest that the ability of species to survive flooding is wholly or partly due to production of adventitious roots in response to flooding (Jackson, 1955; Hosner and Boyce, 1962; Armstrong, 1968; Sartoris and Belcher, 1949) . Flood tolerant plants that do not show this response are rare. Furthermore, adventitious roots can also developed in certain flood intolerant plants such as maize, tomato, sunflower and tobacco as a consequence of experimental flooding. Finally, plants which produced adventitious roots most rapidly sustained less injury from flooding followed by a greater degree of recovery (Kramer, 1951) .

The adventitious roots of rice and maize which originated after flooding and then formed the larger part of root system have been shown to be more porous than ordinary roots (Luxmore and Stolzy, 1969). Yu et al. (1969) reported that experimental flooding also affected the root porosity of other crop seedlings (Inia variety of wheat, sunflower and corn) where a significant increase was observed under full flooding where water was kept one centimetre above the soil surface. A higher root porosity would also allow a greater internal oxygen supply, and under full flooding roots with increased porosity such as corn and sunflower appeared to survive better than tomato and barley which had not. On the other hand, plant dry weights were lower except for barley. Thus Yu et al. (1969) suggested that internal oxygen supply might only be adequate for minimum plant responses and metabolic activities but still inadequate for maximum growth requirements of the plant studied.

1.4.2 PHYSIOLOGICAL ADAPTATION

When whole plants were totally submerged under flooded water , no external oxygen could enter the roots either by ways of air spaces/channels or adventitious roots and under these conditions all plants are likely to experience totally anoxic conditions. These conditions so adversely affect the sugar-cane plant that they died out completely (Sartoris and Belcher, 1949) , the same happens to rice plants during monsoon (Crawford, pers .com

) . Both these species are well known as flood tolerant plants. On the other hand, the coleoptile of rice and the mesocotyl of barnyard grass seedlings (a common weed in rice fields) were observed to grow under totally anaerobic conditions (Pradet and Bomsel, 1978 ; Rumpho and Kennedy, 1981) . Several rhizomatous wetland plants also behaved similarly (Barclay and Crawford, 1982) . Hence, in plants where the function of morphological adaptations were largely restricted due to strict anoxia, the role of physiological adaptations became clearly recognised.

1.4.2.1 The abundance of food reserve

The rhizomes or tubers of either wetland or non-wetland plants contain abundant food reserves in the form of starch (Laing, 1940; Edelman, 1963) or fructosan found in several plants for example in Jerusalem artichoke, a non-wetland plant (Edelman, 1963) and in Iris pseudacorus, a wetland plant (Archbold, 1940) . In two species of Typha growing in wet habitats, more than 25% of rhizome biomass was found to be a total non-structural carbohydrates (Fiala, 1978; Grace and Wetzel, 1982) . Initial leaf growth is largely the result of these stored carbohydrates (Fiala, 1978) which are re-utilized(Jefford and Edelman, 1961) . Hence, the occurrence of bud-break in Rubus chamaemorus rhizomes would probably be the cause of a further reduction in carbohydrate content (Marks, 1978) . On the other hand, a high metabolic activity (especially respiration) that occurs endogenously within the tubers is also

thought to be the principle cause of loss during a storage of yam (Coursey, 1967) and potatoes (Burton, 1966) . Thus growth may be reduced due to excessive utilization of food in anaerobic respiration (Laing, 1940) .

Works on the tolerance of wetland plants storage organ to anoxia was advanced recently by Barclay and Crawford (1982) . From their results they listed and categorized some wetland plants into three categories based on shoot extension during seven days total anoxic incubation of intact plants together with the ability to grow in air afterwards. The plants which have the highest ability to tolerate this environment showed sustained shoot extension during the incubation period and continued to grow normally on being replaced in air. In this category are Shoenoplectus lacustris (L) Palla, Shoenoplectus tabernaemontani (C. C. Gmell.) Palla, Scirpus maritimus L. , Typha angustifolia L. and Potamogeton filiformis Pers. The second type of plant could actually survive the anoxic period and continue to grow normally on being replaced in air but did not show any shoot extension during the anoxic incubation. Among this category are Iris pseudacorus L. , Filipendula ulmaria (L.) Maxim and Spartina anglica C. E. Hubbard. The third type of plant was killed by anoxia, among this are Oryza sativa L. var Oeiras, Juncus effusus L. and Glyceria maxima (Hartm.) Holmberg. These results indicate that several of the rhizomatous species show a much greater ability to grow under anoxia than that of rice and barnyard grass seedlings because they can send shoots up and suffer no deleterious after-effects (Barclay and

Crawford, 1982) . In the wild the possession of much larger rhizome in Typha angustifolia is one of the factors that permit it to grow in deeper water than T. latifolia (Grace and Wetzel, 1982) .

Short periods of anoxia had little effect on the carbohydrate levels in Scirpus maritimus which can actually grow under anoxia. However in Glyceria maxima, the wetland species that dies under anoxia, the rapid depletion of sucrose, raffinose and storage carbohydrate was reported (Barclay and Crawford, 1983) . In roots of rice seedlings incubated completely in an oxygen-free medium, anaerobiosis immediately caused the reducing sugars and sucrose to decrease. However, the sucrose level increased afterwards and returned to initial values 96 hours later. Starch concentration did not change significantly (Bertani et al., 1981) . The roots of whole rice seedlings with attached seeds under anoxia were also reported to undergo changes in mitochondrial ultrastructure. Parallel cristae appeared one day after anoxia and developed into orderly arranged cristae within two days. However after three days of anoxia mitochondria begin to degrade (Vartapetian, 1978) . Moreover, these destructive changes can be postponed in excised roots and coleoptiles which contain little food reserve by adding glucose into the incubation medium (Vartapetian et al., 1978) . Thus his hypothesis (Vartapetian et al.., 1977) that carbon starvation of the cells under conditions of long term anaerobiosis that soon brings about the degradation of the cell ultrastructures which leads to plant death was proven. Nevertheless, application of

10% (v/v) of ethanol under anoxia in the growing medium also showed extensive mitochondrial membrane destruction after only 12 hours anoxia (Crawford and Vartapetian, unpublished data) . Furthermore, Barclay and Crawford (1983) suggested that the provision of large reserves of carbohydrate was not enough to ensure survival for wetland plant underground storage organs, in addition they need a metabolic conservation of these reserves.

1.4.2.2 The ability to tolerate by-products of fermentation.

Other than the importance of abundance food reserves, the ability to tolerate the by-products of fermentation could also be important in the endurance of wetland plants to their anaerobic habitats (Laing, 1940) . Tyler and Crawford (1969) suggested that the accumulation of the non-toxic malate and shikimate as the end-products of anaerobic respiration contributes to the flooding tolerance of five wetland species. Malate can be further metabolized on the return of aerobic conditions whilst shikimate can be further used in aromatic amino acids and lignin biosynthesis. Thus under flooding, wetland plants may deviate from normal metabolic route for the production of energy and yield 'tolerated' end-products of anaerobic respiration (Mazelis and Vennesland, 1957; McManmon and Crawford, 1971) .

Under anaerobic conditions, the majority of wetland plants produced ethanol (James, 1953) and/or lactate (Sherwin and Simon, 1969) which some workers (Fulton and Erickson, 1964; Andrews, 1977; Chirkova, 1978; Pradet and Bomsel, 1978) consider harmful to the plants. Lactic acid was first produced in potatoes placed under nitrogen storage at 10°C, followed only several days later by alcohol formation (Barker and el Saifi, 1953) . Under this anaerobic condition, lactate production from glucose could result in a fall in pH and subsequently to the stimulation of ethanol production (Davies et al., 1974) . However, in carrot tissue respiring under nitrogen, 97% of the carbon in the sugar consumed was recovered in alcohol and carbon dioxide, the relative amounts of both products agreed very closely with Gay-Lussac equation:

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2.$$

(James and Ritchie, 1955) .

Ethanol appeared to be the 'more favoured' end product of glycolysis than lactate in a study of rhizomes of wet and dryland species (Monk et al., 1984) , also in the apical zone of the roots of several species of flood-tolerant plants as well as root of Pisum sativum, a very intolerant plant (Smith and ap Rees, 1979) . About 68 to 88% of the carbohydrate consumed in anaerobic metabolism in Iris pseudacorus rhizome is fermented to ethanol (Boulter et al., 1963) .

A possible metabolic theory of flooding tolerance was proposed by McManmon and Crawford in 1971, based particularly on ethanol accumulation and its contribution to the poisoning of metabolism. They wrote:

> "In 'intolerant' roots, on flooding, normal respiration is blocked, and glycolysis proceeds to the production of acetaldehyde and ethanol. Acetaldehyde induces alcohol dehydrogenase activity which, together with a reduction in apparent Km value, accelerates glycolysis. Malate present is decarboxylated by 'malic' enzyme to pyruvate and thence to acetaldehyde, contributing further to ethanol production. Oxaloacetate and hence malate may be formed by carboxylation of phosphoenolpyruvate, but the malate will not accumulate. Ethanol and acetaldehyde do accumulate, and contribute to poisoning of metabolism.
>
> In 'tolerant' roots on flooding, normal respiration is at least partially blocked, and glycolysis may proceed to the production of acetaldehyde and ethanol, but the former failed to induce the ADH activity, the apparent Km value remains unchanged, and no acceleration of glycolysis ensues. Malate present is not

decarboxylated, because malic enzyme is absent. Oxaloacetate and hence malate are produced by the carboxylation of phosphoenol pyruvate, and malate accumulates. This is non-toxic, and may remain without harm to the plant until aerobic conditions are restored ".

The marked increase in glycolysis in flood-intolerant plants (see also Kennedy et al., 1983) is due to the operation of the Pasteur effect combined with inductive increase in glycolytic rate when hypoxic condition is prolonged. Consequently, tissue-ethanol content increases considerably followed by membrane leakage and organelle damage, microbial infection which subsequently leads to death (Crawford, 1982 a) . In other words, the accumulation of ethanol over long periods may damage tissues by causing membrane malfunction in organelles such as mitochondria (Nandini-Kishore et al., 1979; Crawford, 1977; Kiyosawa, 1975). This theory was criticised from many angles; the major one concerns the role of ethanol in the poisoning of metabolism. These objections gain ground when the externally applied ethanol was shown to cause no symptom of flooding damage when applied to the growing medium even in a much higher concentration than found internally in plant as well as normally found in anaerobic soil (Jackson et al., 1982) . However, in their experiment plants' shoots were exposed to air whilst Crawford (in press) reported that externally applied ethanol did cause symptom of flooding damage when plants' shoots were exposed to oxygen free gaseous media. Moreover, the internally

produced ethanol was considered more toxic than the externally applied ethanol (Nagodawithana and Steinkrauss, 1976) . The internal ethanol concentration of more than 60 mM (about 60 μmoles $gm.^{-1}$ fresh weight) (Barclay, pers comm.) has a deleterious effect on pea seedlings emergence (Barclay and Crawford, 1981).

Ethanol production was shown to be highest not only in the flood intolerant plants such as yam tubers (Ugochukwu and Anosike, 1979) but also in the flood tolerant plant such as rice seedlings (Bertani et al., 1980) and barnyard grass seedlings (Rumpho and Kennedy, 1981) when subjected to anaerobiosis. However in rice seedlings, 98% of ethanol produced in the tissue can be eliminated into the medium (Bertani et al., 1980) and 85% for barnyard grass seedlings (Rumpho and Kennedy, 1981) . In the rhizome tissue, Monk et al. (1984) measured 26 μmoles $gm.^{-1}$ fresh weight of ethanol in Iris pseudacorus -a flood tolerant plant- after 16 days incubation under nitrogen stream. According to Altenburger (1981) , about 95 μmoles $gm.^{-1}$ fresh weight of ethanol accumulated after 175 hours of storage under 'stagnant' nitrogen. In Iris germanica -a flood intolerant species- Monk et al. measured 71 μmoles $gm.^{-1}$ fresh weight of ethanol which was only a little lower than measured by Altenburger (87 μmoles $gm.^{-1}$ fresh weight) . Thus the rhizome tissue of flood tolerant plants is also capable of removing internal accumulated ethanol due to morphological adaptation favouring outward diffusion as in adventitious root system or porous rhizomes (Armstrong, 1979; Crawford, 1982 a) . Provided

a sufficient supply of carbohydrate for prolonged fermentation exists, a low metabolic rate under oxygen deficiency (hence accompanied by low ADH activity and low ethanol concentration in the tissue) plays an important role in the flooding tolerance only of races or species which do not have the ability to release ethanol produced into the surrounding gaseous or aqueous phase (Crawford, 1982 b, Monk et al., 1984) .

The malic acid content of helophytes increased under flooding whilst that from the non-helophytes decreased (Crawford and Taylor, 1969) . The experimental flooding of Veronica peregrina populations taken from the centre of vernal pools was also accompanied by an increase in malic acid content whilst the population taken from the periphery showed no regularity of behaviour (Linhart and Baker, 1973) , thus supporting the above theory. In chick peas, during anoxic period before the puncture of seed coat by the radicle malate was also accumulated (Aldasoro and Nicolas, 1980) . On the other hand, Smith and ap Rees (1979) found no detectable accumulation of malate in unaerated roots of three species of flood- tolerant plants. Malate was also found to accumulate in roots of flood-tolerance and also of flood-susceptible species (Dubinina, 1961). Even though Davies et al. (1975) found no absence of malic enzyme in marsh plants studied (a second defect of Crawford's flooding tolerance theory), Chirkova (1978) reported that it is inhibited by flooding in flood tolerant species, thus producing overall similar effect as suggested by McManmon and Crawford (1971) .

Another interesting idea of physiological adaptation was put forward by Davies. In (1973) he postulated the formulation (Figure 1.1) to explain the appearance of ethanol, lactate and malate during anaerobiosis. At the start of anaerobiosis, lactic and malic acids produced will lower the pH hence 'switching on' pyruvate decarboxylase which is responsible in decarboxylation of pyruvate to acetaldehyde. Under aerobic condition, this enzyme does not function for reason of unsuitable pH; hence the appearance of ethanol under anaerobic condition according to Davies is due to the increase acidity or low pH. In resting yam tubers, ethanol was produced only after seven days anoxic incubation under nitrogen gas whereas substantial amounts of lactate and malate were already accumulated from day one. Hence Ugochukwu and Anosike (1979) claimed that their results have confirmed the above formulation. In experiment with mature potato tubers, Barker and el Saifi (1953) results also support the postulation. However, at least during anoxic period of germination in chick peas (Cicer arietinum) , the accumulation of ethanol occurred simultaneously with that of malate and lactate (Aldasoro and Nicolas, 1980). Hence the idea that it was the lowering of pH by malate and lactate which 'switch on' the production of ethanol, is also liable to be criticised.

High levels of shikimate were also found to accumulate in wetland plants. Crawford and Taylor (1969) measured shikimate in the underground storage organs of Iris pseudacorus and Nuphar lutea (L) Sm. , taken from flooded sites. Nevertheless,

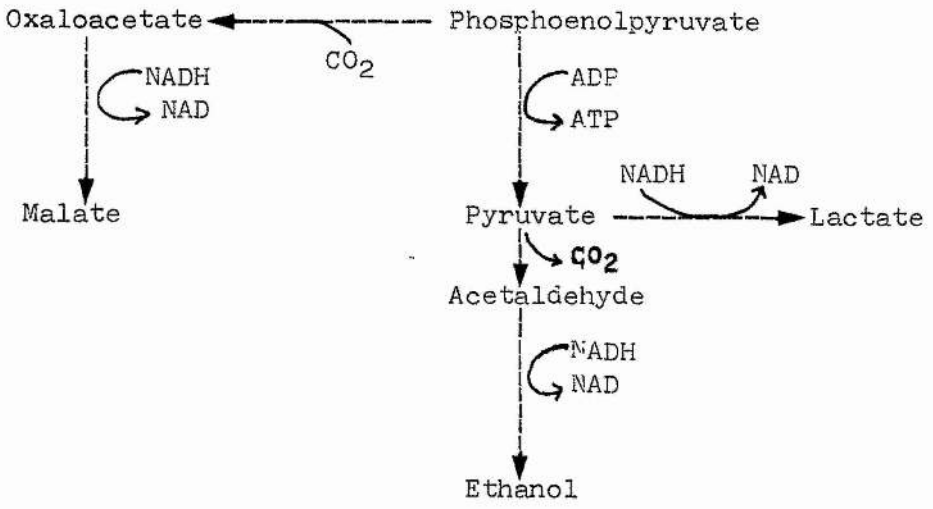


FIGURE 1.1 :

Metabolic formulation postulated by Davies (1973) to explain the appearance of ethanol, lactate and malate during anaerobiosis. From Ugochukwu and Anosike (1979).

shikimic acid was not detected in the non-rhizomatous species such as Juncus effusus. Moreover, the shikimic acid content varied according to season. In winter when the site is waterlogged the shikimate content of the root was high whereas in summer when the water-table has dropped to well below ground surface, the content was lower. The shikimate content was always higher in the rhizomes than the roots. Shikimate was also found when the plant was put under anaerobic treatment (Boulter et al., 1963) . The production of shikimic acid may follow from carbohydrate breakdown as shown in the metabolic pathway (Figure 1.2) postulated by Tyler and Crawford (1970) . Even though the Pentose phosphate pathway cannot function in the absence of oxygen (Forward, 1965 as reported by Ugochukwu and Anosike, 1979) , the step in which 5-Dehydroshikimate is reduced to shikimate would allow the referred pathway (Figure 1.2) to function anaerobically; the NADP produced can be re-used in oxidising 6-Phosphogluconate to Ribulose-5-phosphate in the absence of molecular oxygen.

Other than ethanol, lactate, malate and shikimate; some plants accumulate non-toxic Gamma-aminobutyrate and succinate (Streeter and Thompson, 1972 a, b; Dubinina, 1961) , alanine (Smith and ap Rees, 1979) and glycerol (Crawford , 1972) . Thus the possible pathway of anaerobic metabolism and accumulating metabolites according to Crawford (1982 a), Altenburger (1981), Zemlianukhin and Invanov (1978), Davies et al. (1974) and Streeter and Thompson (1972) were shown (

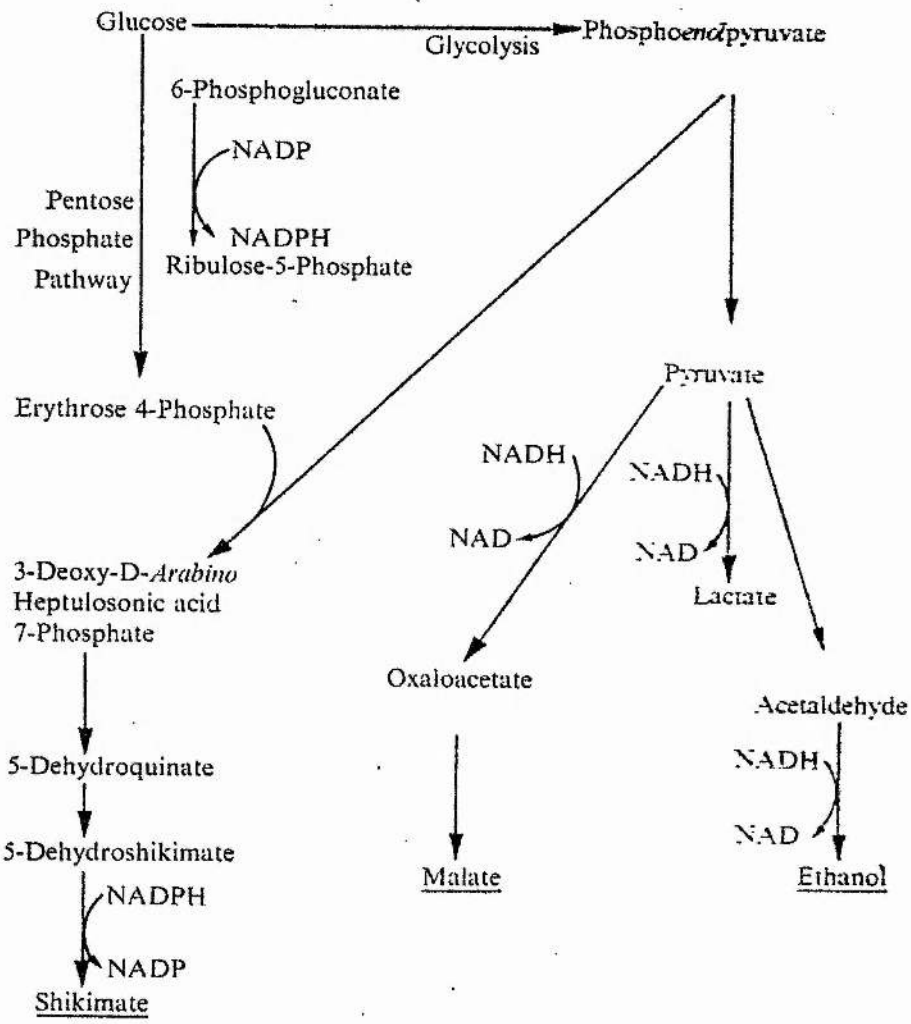


FIGURE 1.2 :

Metabolic pathways postulated by Tyler and Crawford (1970). Substances underlined have been shown to accumulate in anaerobic conditions depending upon species; Lactate in potato (Solanum tuberosum)and Equisetum sp.(James, 1953), Shikimate in Nuphar lutea and Iris pseudacorus (Crawford and Tyler, 1969), and malate in flooded roots of marsh plants (Crawford and Tyler, 1969; McManmon and Crawford, 1971), chick peas before the puncture of seed coat by the radicle (Aldasoro and Nicolas, 1980) and also in rhizomes of Iris pseudacorus stored under different aeration regimes (Altenburger, 1981).

FIGURE 1.3 :

The possible pathway of anaerobic metabolism and accumulating metabolites according to Crawford (1982a), Altenburger (1981), Zemlianukhin and Invanov (1978), Davies et al. (1974) and Streeter and Thompson (1972). Possible accumulating metabolites are lined. Details are explained in general introduction (page 22).

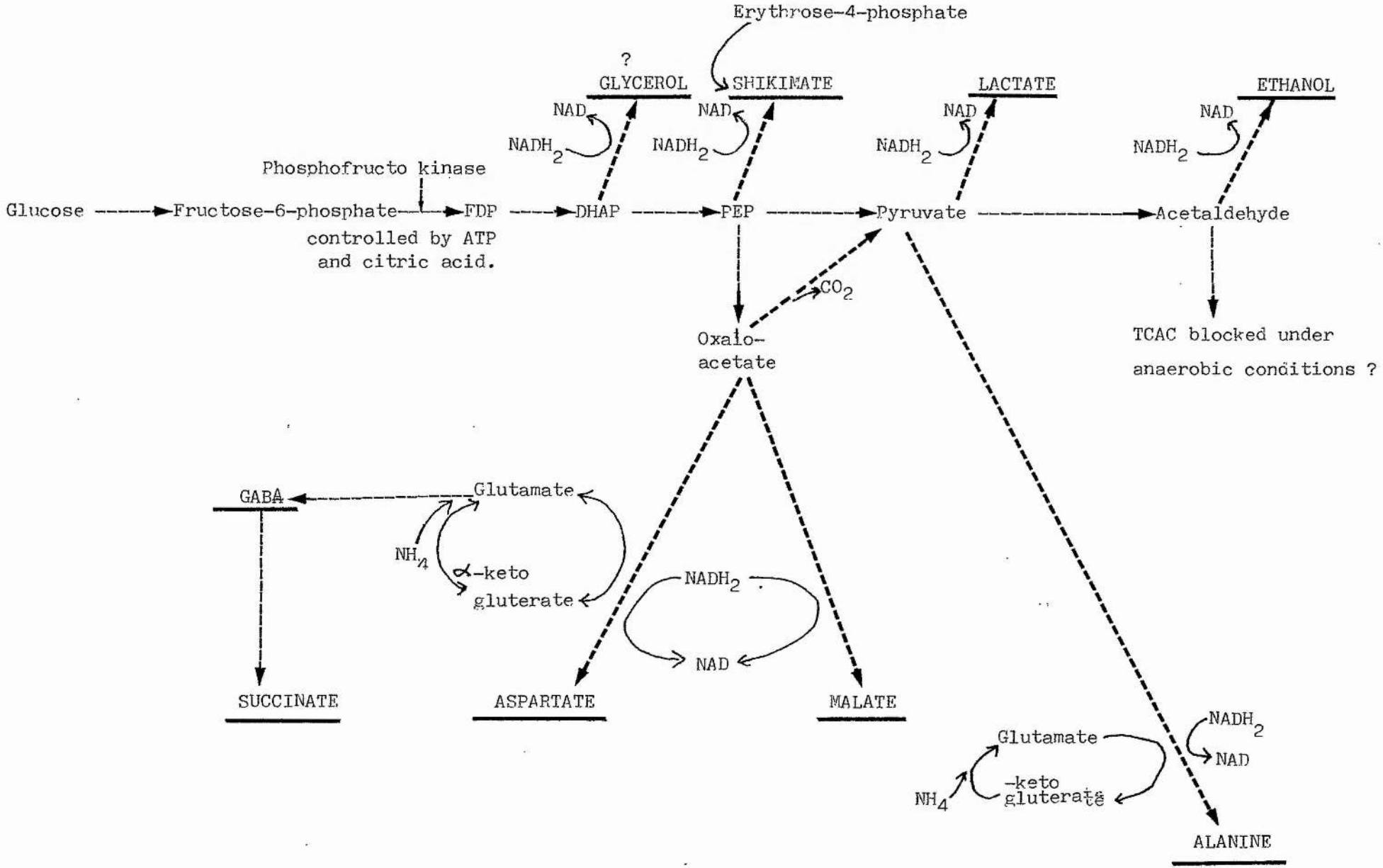

Erythrose-4-phosphate
?
GLYCEROL
SHIKIMATE
LACTATE
ETHANOL
NAD
NADH2
Phosphofructo kinase
Glucose
Fructose-6-phosphate
FDP
DHAP
PEP
Pyruvate
Acetaldehyde
controlled by ATP
and citric acid.
CO2
Oxalo-
acetate
TCAC blocked under
anaerobic conditions ?
GABA
Glutamate
NH4
α-keto
gluterate
NADH2
NAD
SUCCINATE
ASPARTATE
MALATE
Glutamate
-keto
gluterate
ALANINE

Figure 1.3). There is however a question mark on glycerol as the accumulating metabolite of the anaerobic respiration which is due to other possibility such as the product of lipid breakdown (Greulach and Adams, 1963; Davies, 1980) . As for malate, no significant quantities of malate were found in unaerated roots of flood tolerant plants (Smith and ap Rees, 1979) even though Crawford and Taylor (1969) and McManmon and Crawford (1971) have shown that it was formed under flooding conditions in the roots of marsh plants and also in chick peas before the puncture of seed coat by the radicle (Aldasoro and Nicolas, 1980). At this point, carbon balance sheet for anaerobic metabolism which can ascertain the significance of malate production in tolerant plant (for example a quantitative evidence of a conversion of carbohydrate to malate) should be produced (Davies, 1980). Until then the significance of malate production (and also of other accumulating metabolites) as compared to conventional ethanol accumulation cannot be justifiably argued.

CHAPTER 2 :
MATERIALS AND METHODS.

CONTENTS

Chapter 2

MATERIALS AND METHODS.

2.1 THE PROLONGED BURIAL AND/OR FLOODING TREATMENTS.

The rhizomes, corms and tubers were established for one month in sand under non-flooded condition and exposed to 16 hours day light regime in the heated glass house. About 24 to 40 plant materials from different species (Appendix I) were used. Prior to treatment, plants were washed free from sand. The senescend end of the rhizomes and corms were removed. The shoots were trimmed back leaving a two centimetres leaf bases. The longest root length was measured and the whole plants were weighed.

Four treatments (Figure 2.1) were performed:

A -------Not Buried and Flooded.

B -------Not Buried and Not Flooded.

C -------Buried and Flooded.

D -------Buried and Not Flooded.

In the BURIAL treatments (C and D) , the top of leaf bases or shoots were buried two centimetres below the sand surface which was eleven centimetres from the mouth of the 28 centimetres diameter pot. In the NON-BURIAL treatments (A and B) , shoots were exposed to air, seven centimetres from the lip of the pot. Nutrients in the form of Hoagland's solution (Appendix II) of one fifth strength was supplied twice a week to the NON-FLOODED pot (B and D) where it drained freely. For FLOODING treatments (A and C) , these nutrients were allowed to remain stagnant by putting each pot into a rubber bucket. The solution was then maintained not less than 1.5 centimetres above the surface of the sand, but never submerging the shoot in treatment A (Not Buried and Flooded) . After about seven weeks or more (Appendix I) , plants were harvested , washed free of sand with tap water and weighed. Roots were immediately excised followed by sectioning the rhizomes, corms or tubers. The sections were chosen at random for ethanol measurement (section 2.3) , dry weight measuring and sugar content determination (section 2.4).The exised roots were later kept in the solution of Formalin-glacial acetic acid-70% ethyl alcohol (5:5:90) at $4^{o}C$ for diameter measurement (section 3.2.) .

2.2 MEASUREMENT OF AEROBIC AND ANAEROBIC RESPIRATION .

Plant materials were kept in a sealed flask at 20°C and this temperature was maintained by a water bath. The flask was covered by black cloth and it was connected with plastic tubings to Infra-red gas analyser (Analytical Development Co. Ltd. , England). The environment of the whole system (Figure 2.2) was maintained in moist condition by lining the flask with wet filter paper. The atmosphere of this system could be changed from aerobic where air was circulated , to anaerobic, by gassing in pure nitrogen for about a quarter of an hour after which the air or nitrogen gas stream was kept at about 0.6 l. $min.^{-1}$ flow rate. The rate of respiration was then measured directly in closed system by evolution of carbon dioxide. The results were reported by a recorder connected continuously throughout the experiment with the gas analyser. Thus the rate of respiration could be calculated from the gradient of the curve and expressed as a percentage of carbon dioxide of the system evoluted (ppm) per gramma fresh weight per hour. By measuring the volume of the flask and adding it to the known volume of tubing in the gas analyser and the tubing which was connecting the flask and the analyser; the volume of air or nitrogen gas circulating in the system then could be calculated. From this total volume, the volume of the rhizomes was subtracted to give the net gas volume which enable the respiration rate to be expressed in μmoles per gramma fresh weight per hour of carbon dioxide evoluted.

2.3 ETHANOL MEASURING

The ethanol content of plant storage organ was measured by two methods;the enzymatic determination (Bergmeyer, 1963) and gas liquid chromatography (Ridgeon, 1971) .

2.3.1 Enzymatic determination of ethanol.

2.3.1.1 The principle of enzymatic ethanol analysis.

The principle of this analysis was based on the oxidation of ethanol in the presence of alcohol dehydrogenase enzyme (ADH) by nicotinamide adenine dinucleotide (NAD) to acetaldehyde:

$$\text{Ethanol} + NAD^{+} \xrightarrow{ADH} \text{acetaldehyde} + NADH + H^{+}.$$

The equilibrium of the above reaction lies to the left side, however in the presence of excess NAD, alkaline conditions and by trapping the acetaldehyde with semicarbazide, it can be completely displaced to the right. The amount of ethanol is stoichiometric with the amount of reduced nicotinamide adenine dinucleotide (NADH) . The amount of NADH produced was then determined by means of its absorption at 340 nm using UV Spectrophotometer (Unicam SP 1800 with programme controller) . The amount of ethenol present then can be calculated from the increase absorption values

obtained by adding ADH.

2.3.1.2 Preparation of extract.

The known weight plant materials were immediately killed in liquid nitrogen. The tissues were then fixed in cool 6% perchloric acid in plastic containers and were placed in deep freezer until required. On removal from the freezer, plant samples were partially thawed before being homogenized by ultra-turrax (Kika werk, Janke and Kunkel) . The extract obtained was then centrifuged (MSE, High Speed 18) at 15,000 rpm or ca 25,500 g for 30 minutes at $4^{o}C$. The volume of clear supernatant was measured. The excess of perchloric acid was removed by neutralizing the extract with 5 M K_2CO_3. A few drops of methyl orange were added followed by solution of 5 M K_2CO_3 until colour change from orange to yellow (or black/red due to phenolic compounds present in certain plant species) , and carbon dioxide bubbles have stopped coming out from the extract. The clear neutralized supernatant was decanted after half an hour standing and the volume was measured. This supernatant was further centrifuged for about 30 seconds in a microfuge (type B, Beckman, United States) at 8,730 g , before measuring the ethanol content by using the enzyme alcohol dehydrogenase (see Appendix IV and also section 6.2.3.1.) .

2.3.2 Ethanol measuring with GLC .

2.3.2.1 The principle of GLC ethanol measuring.

Ethanol GLC measurement was performed by external standard method. A new standard curve (Appendix III) was prepared for every run. Regression of the linear portion of this curve was calculated and used for estimation of samples' ethanol concentrations.

2.3.2.2 Preparation of extract.

It was performed with a similar procedure as for enzymatic analysis (section 2.3.1.2.) .

2.3.2.3 Preparation of column.

About 15 gm. of Chromosorb W High performance (BDH Chemicals Ltd. , product number 15175) as a support and 0.375 gm. of polyethylene glycol 20 M (Pye Unicam Ltd. , Catalogue number 12735) as a stationary phase was required to pack 1.52 metres long and 4 millimetres internal diameter coiled glass column, so that 2.5% stationary phase was coated on support. Only a small amount of solvent (chloroform) was needed to dissolve the

polyethylene glycol. The Chromosorb W was placed in the flask of a rotary evaporimeter. The dissolved polyethylene glycol 20 M was then added to the flask and more chloroform was also added to cover the support. The flask was slowly rotated throughout to ensure an even cover of stationary phase on the support. Care was taken to avoid "bumping" which could result in fracture of the support particles, forming finer particles which could impair column performance. The solvent was evaporated off at constant temperature of 20°C, maintained by water bath and the remaining material was placed on filter paper in a dry place to further remove the solvent. The dry coated support was then packed into column by using suction and gentle tapping. Prior to use, column was heated for about 24 hours at 25°C above its intended operating temperature (65°C) under a stream of nitrogen gas with 40 ml. $min.^{-1}$ flow rate whilst not connected to the detector, to mature it.

2.3.2.4 Gas liquid Chromatography of ethanol.

The column was fitted into a gas chromatograph (Pye Unicam Series 104) connected with a flame ionisation detector (FID) . Oxygen free nitrogen was used as a carrier gas with 40 ml. $min.^{-1}$ flow rate.

The heated column was run isothermally at 65°C. One µl of supernatant was then injected just below the surface of column packing using a one µl syringe (Scientific glass engineering pty. Ltd. , Australia) . The results of the analysis then were reported as a peak area by an integrator (Hewlett-Packard Co. , USA, model number 3390 A) which was connected to the chromatograph. A standard curve for ethanol (Appendix III) was prepared for each run with ethanol standard solution (0.08% , w/v, Sigma Chemical Co.) . This solution was diluted between 0.04% to 0.0025% so that their concentrations fell within the linear portion of the curve. Regression of this line was calculated so that ethanol concentrations in the known volume of samples can be estimated.

2.4 SUGAR EXTRACTION AND DETERMINATION BY GLC.

Soluble carbohydrates were determined by method developed by Sweeley, Bentley, Makita and Wells (1963) and Ellis (1969) .

2.4.1 The principle of GLC sugar analysis.

The principle of GLC preparation for soluble carbohydrate was based on the trimethyl silanization of sugars to the trimethylsilal (TMS) derivatives with a silating agent hexamethyldisilazine (HMDS) . Thus sugars were analysed in the form of their derivatives.

2.4.2 Preparation of column.

The method for column preparation for sugar measuring was the same as used for ethanol determination (section 2.3.2.3.) . However for sugar column , the support used was Diatomite CQ 60 - 70 mesh (Pye Unicam Ltd. , England, B/N 122 A) and the stationary phase was methyl phenyl silicone gum (E 52 , W. G. Pye and Co. Ltd. , Catalogue number:12731) . For 1.52 metres long and 4 millimetres internal diametre glass coiled column used, about 15 gm. of Diatomite CQ and 0.15 gm. of E 52 silicone gum were ample to give 1% stationary phase on support. Only a small amount of chloroform was needed to dissolve the gum.

2.4.3 Sugar measuring.

One µl of sugar derivatives from each sample was injected into the heated column. The temperature programme of the column was 100°C for 2 minutes, thereafter rising by 6°C per minute to 260°C and stayed at that temperature for 15 minutes. Within this programme, mono-, di- and tri-saccharide sugars peak could be detected. These peaks were identified by comparing them with known standard peaks and also with published retention time. The amount of each component was determined by comparing the areas under the peak, estimated by height x width at half height , with internal standard and sugar standard calibration mixture. By using an integrator, the peak area could be precisely estimated and reported directly through its recorder. The concentration of component i for internal standard method used thus could be calculated by using the formula:

$$C_i = A_i \times W_s \times 10^3 \times (A_s \times R_{f(i)} \times W_m)^{-1}.$$

(mg. gm.$^{-1}$ dry weight.)

where:

W_s = added grames of internal standard (10^{-4} gm.) .

W_m = added grames of sample (10^{-2} gm.)

A_i = peak area for component i.

A_s = peak area for internal standard.

$R_{f(i)}$ = relative response factor for component i calculated from the formula:

$$R_{f(i)} = A_i \times W_s \times (A_s \times W_i)^{-1}.$$

where:

W_s = mass of internal standard weighed into the calibration mixture .

W_i = mass of component i weighed into the calibration mixture.

A_s = peak area of internal standard.

A_i = peak area of component i.

Sorbitol or arabinose were used as the internal standard. The concentration of all the standard solutions used were 1 mg. sugar per millilitre DMSO.

2.5 STATISTICAL ANALYSIS OF RESULTS .

The results obtained were submitted to statistical examination by using the single factor analysis of variance (ANOVA 1) . In all cases the significant of the t-distribution is given by the conventional system in which *, ** and *** denote significance at the 5, 1 and 0.1 per cent levels respectively. The analysis was performed by a computer and one example is shown in Appendix V (a and b) . In Appendix VI both tests are explained further.

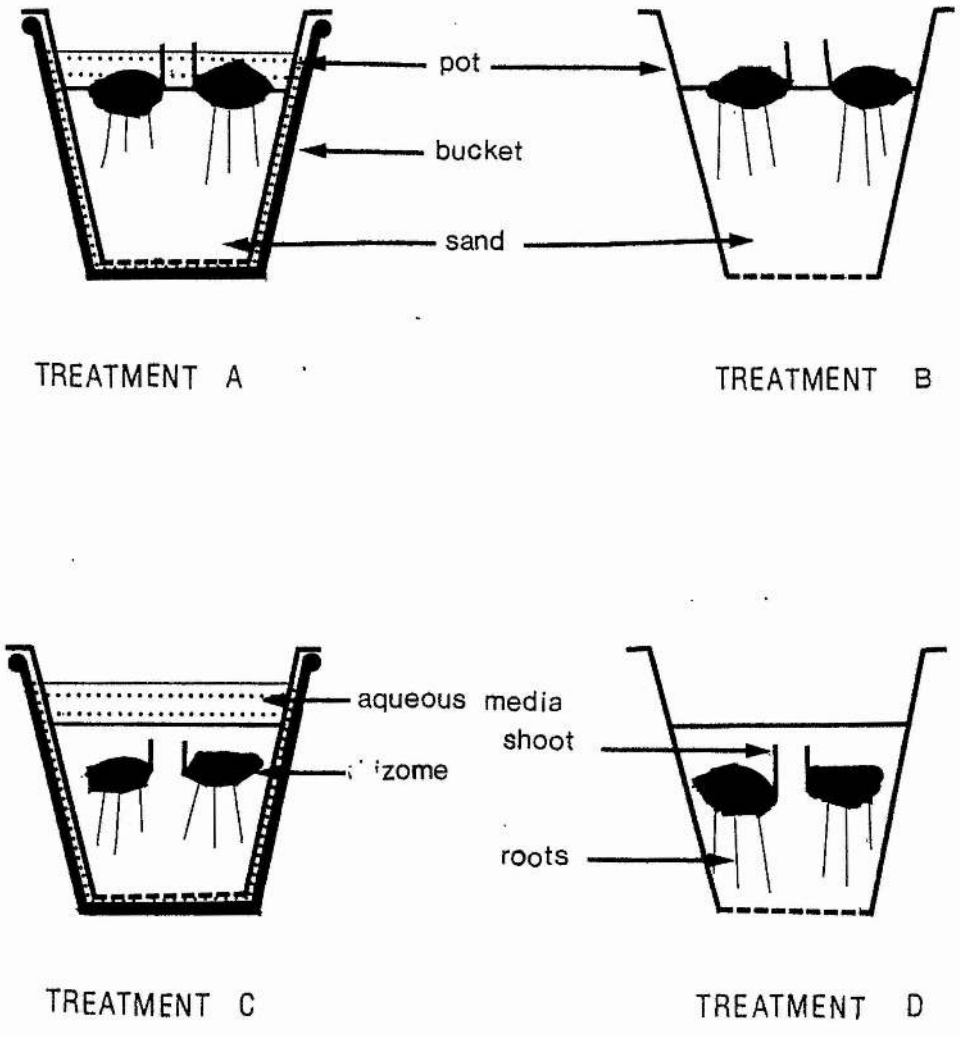


FIGURE 2.1 :

The four burial and/or flooding treatments for the underground storage organs ;A : Not buried-flooded, B: Not buried-not flooded, C: Buried-flooded and D: Buried-not flooded. The aqueous media is a one fifth strength Hoagland's solution (Appendix II).Plant materials used in this study are shown in Appendix I.

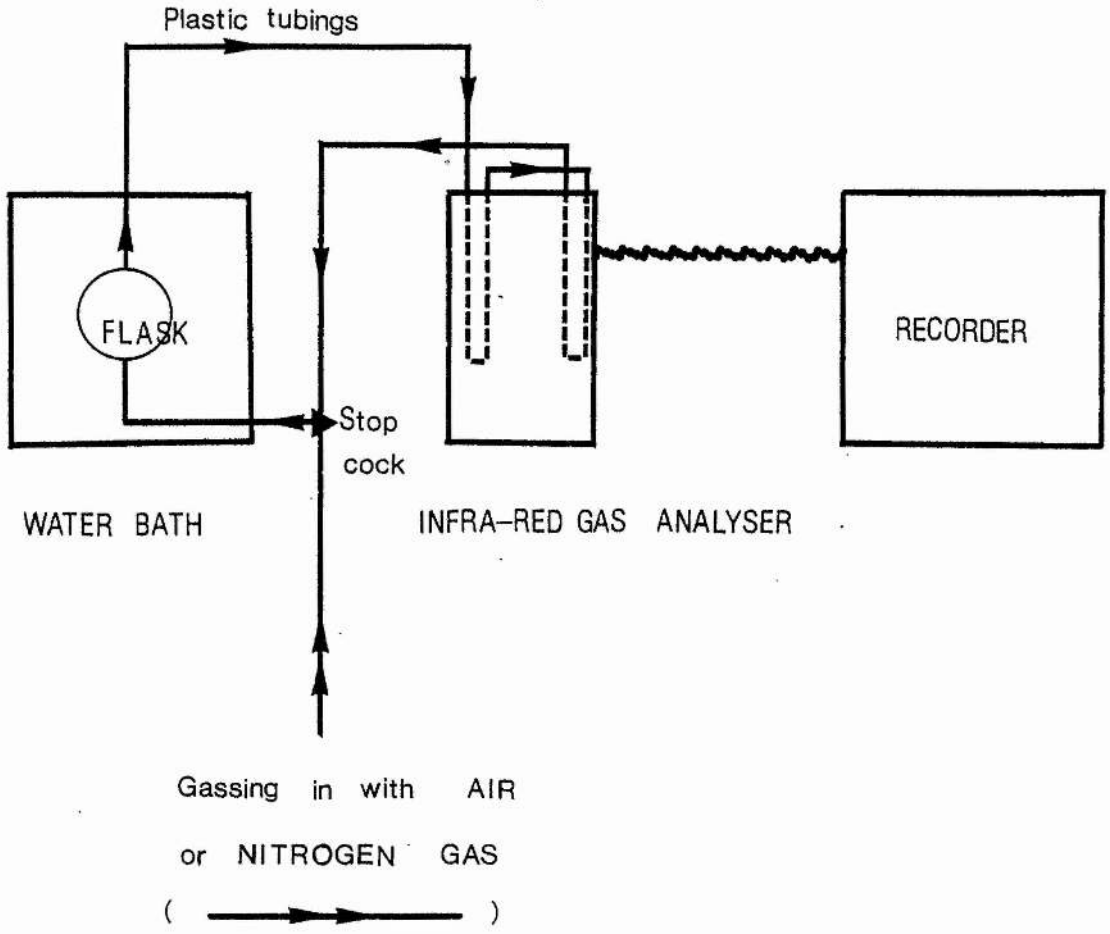


FIGURE 2.2 :

The closed system for measuring respiration rates (Carbon dioxide evolution rates). The stop cock was used to close the system while it was operating. Temperature was maintained at $20^{o}C$. The rhizome was kept in a sealed flask which was than covered by a dark cloth.

CHAPTER 3 :

CONTENTS

Chapter 3

A FACTORIAL EXPERIMENT ON THE EFFECTS OF BURIAL AND/OR FLOODING IN RELATION TO UNDERGROUND STEM MORPHOLOGY AND SURVIVAL.

3.1 INTRODUCTION.

Modifications in form and structure of the underground stems from the usual cylindrical above ground shape are found in many plant species. However, they still produce leaves and buds at the nodes externally , in conjunction with the internal tissues and vascular system typical of stems (Dittmer, 1972). The functions of these stems are vegetative reproduction and the storage of food reserves.

Rhizomes, tubers and corms were examined in this study. Rhizomes are more or less elongated stems growing horizontally underground and can be found in many species of plants; among them are certain species of iris (for example in Iris pseudacorus and Iris germanica), Filipendula ulmaria and Hedychium sp. as well as in many of the dominant grasses (Greulach and Adams, 1963). A mass of root and rhizomatous material formed by a grass sward on the top and in the upper few inches of the soil (Troughton, 1957) is known as the `mat' form if the material is mainly above ground (Davies, 1939;Bates, 1948) or a `sod-bound'

sward form if the material is mainly below ground (Myers and Anderson, 1942). The tuber is the thickened, fleshy end of rhizome (Greulach and Adams, 1963) or a very short rhizome with enlarged diameter (Dittmer, 1972), found in several plants such as the potato (Solanum tuberosum), the Jerusalem artichoke (Helianthus tuberosus) and `ubi kemili' (Coleus tuberosus), a tropical food plant. On the other hand, taro (Colocasia esculenta), `keladi kemahang' (Colocasia sp.) and `keladi telur' or yautia (Xanthosoma sp.); tropical food crops, and the Cuckoo-pint (Arum maculatum), have modified stems which fall in the corm category. A corm is a short, fleshy vertical underground stem (Greulach and Adams, 1963) or an abbreviated and stout rhizome (Fritsch and Salisbury, 1946). The rhizomes and corms persist for some time (Figure 3.1) but sooner or later the older parts decay thus detaching the sympodial or monopodial lateral branches (in rhizomes) or the cluster of daughter-corms or cormels around the remnant of the old corm. On the other hand, the `rhizomes' of tuber bearing plants (or stolon, Brook, 1965) together with the aerial shoots and also roots usually die out at the end of growing season in the autumn, leaving the isolated tubers more or less horizontally in the soil (Figure 3.1). The isolated tubers may each give rise to a new individual in the following spring, thus representing a lateral branch of the previous season's plant. Hence, tubers differ from rhizomes and corms in that the growth of previous year dies away rapidly.

Higher plants with underground storage organs offer very interesting material for study from the standpoint of the waterlogged or flooded environment. The essential feature of plants with these organs is that even though the old parts may die away, the new parts have been produced which may give rise to several new plants by means of vegetative propagation in addition to seed production, thus giving two modes of reproduction. By propagating vegetatively, they enable plants to form vigorous colonies which in turn enable them to hold their own against the attacks of a competitive species of plant especially under sub-optimal conditions. Some of the best examples of higher plant endurance to anoxia can be found among the rhizomatous (and also cormous) species:the rhizomes and corms being the best supplied with carbohydrates reserve of all buried plant organs (Barclay and Crawford, 1982). Their results however point to the fact that tolerance of anoxia varied widely even among these plants. Laing (1940) moreover argued that the ability of semi-submerged water plants to endure the condition of low aeration which persists at the muddy bottom of ponds is not due to any particular structural feature as rhizomes and corms of very diverse structures are found imbedded in the submerged mud. Nevertheless the relationship between morphology (in the context of this study included rhizome, corm and tuber forms together with structural features of plants) and survival under flooding (whether buried or not) and under burial (whether flooded or not) in plants with storage capacity should not be overlooked. This is due in part to the importance of some of these plants in

agriculture and horticulture and in part to the significance of examining their limit of tolerance in an ecological setting of sub-optimal conditions such as natural flooding.

3.2 MATERIALS AND METHODS.

Plant materials (from Flooding and/or Burial treatments; section 2.1) were photographed after each harvest, for all the treatments. Roots were randomly selected and sectioned. They were than stored in FAA solution (section 2.1) followed by measuring the diameter with vernier calliper.

3.3 RESULTS

3.3.1 RHIZOMES.

Among the four rhizomatous species studied, variations in the growth of shoot and root when buried and flooded for seven weeks (treatment C) were the most distinct. In *Iris pseudacorus* (Plate 3.1), a temperate wetland plant, growth commenced from both the terminal and axil buds. In *Hedychium* sp. (Plate 3.2), a tropical wild Zingiberaceae, growth commenced only from the axil bud. For *Filipendula ulmaria* (Plate 3.3), a meadow and marsh-plant, as well as for *Iris germanica* (Plate 3.4), a garden

plant, no growth was visible. Root growth was detected only in I. pseudacorus and Hedychium sp. however, when buried and flooded, Iris pseudacorus produced less root yet of relatively bigger diameter. When closely examined, all parts of the rhizome of Iris pseudacorus were found to be healthy (firm). However, some samples from treatment C (buried and flooded) of Hedychium sp. and F. ulmaria showed signs of softness in the proximal part of rhizome (just behind the shoot and including leaf base). In Iris germanica, only the distal part of rhizomes was healthy (firm) in this treatment.

The burial of these rhizomes when not flooded (treatment D) gave no visible effect on plant growth. However, the two centimetres leaf base which was buried underground was colourless, devoid of chlorophyll. Flooding of the rhizomes when planted on the sand surface (treatment A) also gave no negative effects except in Iris germanica where root and shoot growth was less vigour. In contrast, in Filipendula ulmaria this treatment produced vigorous shoot growth and in Hedychium sp. a more prominence root growth as compared to other treatments.

3.3.2 CORMS

The effect of burial and flooding (treatment C) was conspicuous. In Arum maculatum plants (plate 3.5) which can be found growing in woodland as well as marshland, daughter corms persist, and in one of the individual corms, shoot growth commenced from one of them (arrowed, Plate 3.5). However, in other plant species, no growth was visible even though taro (Colocasia esculenta) and `keladi kemahang' (Colocasia sp.) naturally grow in waterlogged or flooded environments. When closely examined, in Arum, only the distal parts of a few corms were healthy under burial and flooding treatment (treatment C); the reverse from other treatments where it was the proximal end that was firm. In `keladi kemahang' where all parts of the corms were healthy under the other experimental conditions, when flooded and buried only the distal part was found to be firm. Furthermore, after the ten weeks harvest half of the samples had almost rotted away. A similar effect was also observed in taro , yet no samples were rotting away after only seven weeks treatment.

The burial of these corms while not flooded (treatment D) gave no visible effect on shoot growth of taro (Plate 3.6), `keladi kemahang' (Plate 3.7) and `keladi telur' (Plate 3.8). However some of the roots produced were geotropically negative (arrowed) even though of the same diameter as those of other treatments (Figure 3.2). In Arum maculatum, burial increases the chances of survival because when they were not buried some of

the `mother corms' dried up. Root growth was also positively affected by burial in this species. Flooding without burial (treatment A) gave a positive effect on root and shoot growth in all the species studied.

3.3.3 TUBERS

The effect of burial and flooding (treatment C) was disastrous. Tubers became soft and putrid. In potato tubers, after seven weeks half of the samples had rotted away. Even in the remaining half only the remote part of tubers was firm. In this species even without burial, flooding could cause the lower part of tubers (the furthest from shoot) to become soft. In Jerusalem artichoke (Helianthus tuberosus), under flooding and burial it was the centre part of tubers that became putrid. However, in some samples the outside part was also affected. Only a few of the `kemili' (Coleus tuberosus) tubers were firm and healthy in their distal parts as under this condition most of the tubers of this species rotted badly.

The burial of all these tubers while not flooded (treatment D) gave a more vigorous shoot and root growth. More new tubers were produced in potato (unpublished data). A longer root was also visible from this treatment, together with the colourless `stem' bases of primary shoots, prominently shown by Coleus tuberosus (Plate 3.9). Along this aerial part, new roots were produced. Flooding without burial (treatment A) gave no visible

effect on shoot growth of Helianthus tuberosus (Plate 3.10). However, more profuse root growth commenced. In Coleus, flooding killed the aerial shoot, nevertheless, in some of the plants it was followed by renewed growth of buds from the base of the original shoot resulting in the formation of bush-form aerial shoots. New roots grew from the base of these shoots. Moreover, in Jerusalem artichoke as well as in potato, flooding enhanced the formation of hyperthrophied lenticels on the tubers planted above ground (arrowed), also no new tubers were found on the flooded plants. Roots of smaller diameter were formed in potato plants when flooded (Figure 3.2).

In all the plants studied (rhizomes, corms and tubers), flooding without burial resulted in the production of new roots which were often shallowly spread and nearer to the surface of a nutrient solution supplied.

3.4 DISCUSSION.

Four treatments employed in this study (A: not buried-flooded, B: not buried-not flooded, C: buried-flooded, D: buried-not flooded) could simulate partial inundation, above ground-no waterlogging condition, total inundation and below ground-no waterlogging condition, respectively. The aeration availability was lower in total inundation than in partial inundation due to complete submergence (Anderson, 1974). Meanwhile, water moisture was less in above ground-no

waterlogging than in below ground-no waterlogging condition prevailed. When not buried (treatment A and B), more light could be absorbed by plant especially during pre-emergence period.

When exposed to total inundation (buried and flooded -- treatment C), two species of rhizomatous plant and one corm species managed to grow and survive after seven weeks. Even in the rhizomatous species that could not grow, storage tissue remained relatively firm. Most part of corms from the same category also remained healthy. Yet no tuber species studied survived. In the wild, rhizomatous (creeping, woody, tuberous or monoliform types) water plants are more numerous than cormous water plants (Cook et al, 1974; Aston, 1973). On the other hand, only a few water plants arise directly from tubers (Ordinea purpurea den Hertog., Aponogeton bullosus van Bruggen.). Moreover, only one plant seem to produce abundant tubers (Triglochin procera R. Br., Family: Juncaginaceae), nevertheless its storage organs still consists of thick rhizomes which bear coarse roots each ending in one small tuber (Aston, 1973). Even though above results and arguments point to a certain superiority of rhizomes (followed by corms) over tubers in surviving under total inundation, this study can not confirm these until tubers from wetland plants are included in the test. Therefore, due to an understandable lack of available suitable plant material , the above confirmation could not be made.

3.4.1 Differential tissue survival.

Upon close examination, it was observed that differential tissue survival was shown within the rhizomes, corms and tubers studied. In susceptible rhizomatous species (tolerant Iris pseudacorus showed no injury) and corm species, the cut primary shoot and meristematic tissues behind it were more susceptible to prolonged total inundation (treatment C) then the remaining tissues. In 1983 Hetherington had already observed the same injurious effect caused by anoxic incubation of Iris germanica rhizomes. Accordingly, In Iris pseudacorus this response was not observed. In tubers however, the trend was not rhythmic. The injurious part was either the cut primary shoot and tissues nearest to shoot, the centre or outer part of tubers or the tissues furthest from shoot in Coleus tuberosus, Helianthus tuberosus and Solanum tuberosum, respectively.

It is possible that high metabolic activity found in the meristematic areas (Opik, 1980) of susceptible rhizomes and corms under anoxia may contribute to the injury. This effect could come from either carbon depletion or the accumulation of toxic product of fermentation, or both. In the tolerant plant (Iris pseudacorus), the ability of the rhizomes to withstand prolonged total inundation presents an important adaptation especially whenever O_2 supply from shoot to root is interrupted (due to flooding, silting or winter dieback), in that no immediate fatality occurred. This species hardiness has been proven when 2 months total anoxic incubation can still be

tolerated (Hetherington, 1983). In this study, growth was observed even after 7 weeks total inundation. It should be noted here however that constant application of solution media in the experiment employed and the present of photosynthesizing algae in the surface water (Weaver and Himmel, 1930), could render the flooding treatment as not totally anaerobic . In tuber species, the non-rhythmatical trend probably resulted from a lack of biochemical adaptation or from higher susceptibility to soil toxins, or both.

3.4.2 Stem modification.

When stems are flooded, hypertrophied lenticels are formed on certain swamp trees (Crawford, 1982 a; Hook and Scholtens, 1978; Chirkova and Gutman, 1972). This stem modification was also observed on the surface of tubers of potato and Jerusalem artichoke under non-burial and flooding condition. Hence, some subterranean storage stems (tubers) behaved similarly in response to partial submergence as their above ground counterpart. Lenticels could play a dual role in plant aeration; diffusion sites for atmospheric oxygen as shown by potato tubers (Wigginton, 1973) and also excretion sites for internal ethanol, acetaldehyde and ethylene. However, the second role was only observed in more flooding resistance plant species (Chirkova and Gutman, 1972).

3.4.3 Root modification.

In most flood tolerant species, morphological changes of roots were very prominent. This modification enables the plant (for example rice) to grow under low levels of soil aeration and they also include a development of a more porous roots and a shallow and more branching root system (Meek and Stolzy, 1978). Furthermore, de Wit (1978) classified these modifications into two types; increased branching of the roots and formation of adventitious roots.

The production of adventitious roots in response to flooding is considered as an asset to flooding survival (Drew, 1979; de Wit, 1978; Sartoris and Belcher, 1969; Armstrong, 1968; Kramer, 1951). In all the species studied, a vigorous growth of shoot under partial inundation (flooded without burial) could be partly attributed to the above factor as it was observed that adventitious roots were formed on all of the growing plants. This fact was further proven when the non-rooted tubers of potato and `ubi kemili' (Coleus tuberosus) showed suppressed growth or no growth at all, respectively as compared to the rooted tubers. However, in Hedychium sp. the abscence of adventitious roots in this treatment (treatment A) did not suppress bud growth thus stressing the partial contribution of the root in the hardy species.

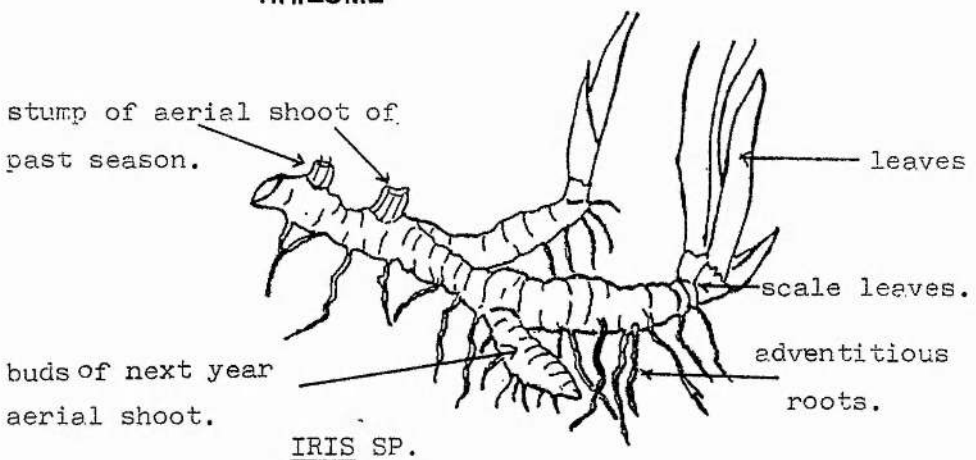
Under deficient aeration, the shallower root system of wetland plants could be differentiated into two distinct parts; a surface root system and a deeper part (Weaver and Himmel, 1930). In the upper few centimetres of best aerated surface layer, the root system consisted of a network of long, fine, profusely branched roots. These roots were found when Typha latifolia, Scirpus validus, Phragmites communis and Spartina michauxiana -- all wetland plants -- were grown in standing water and also in flooded flood-tolerant varieties of sugarcane (Eavis, 1972) and older rice plants (Alberda, 1953). Both the fine surface and also the coarser deeper root systems in the four wetland rhizomatous plants studied (Weaver and Himmel, 1930), consisted of roots of not more than 2 mm. in diameter. In the other conditions (drained, moist and dry treatments), no large differences in diameter were observed. The identical result was also exhibited by wetland rhizomatous plant (Iris pseudacorus and Filipendula ulmaria) in this study. Since a finer root system and an increase in branching will enhance the absorbing surface of roots for dissolved oxygen , as shown by rice plants (Alberda, 1953), wetland plants that inherit such roots did not exhibit any further modification under partial inundation (treatment A). Under total inundation (treatment C), few finer roots were produced in Iris pseudacorus which resulted in an increase in mean root diameter readings. The answer to why only a few roots were produced probably lies in the decreasing level of aeration . In taro (Colocasia esculenta), a corm-bearing wetland plant, roots of greater diameter (more than 2 mm.)

were measured. Under partial inundation, smaller roots were produced in taro whereas in Yautia (Xanthosoma sp.) also a corm-bearing but non-wetland plant, no modification was observed. A smaller root was also produced in potato under partial inundation even though it is a non-wetland plant , whereas in Iris germanica (a rhizomatous non -wetland plant) an increase in root diameter was observed. From the results obtained from non-wetland and wetland corm plants, it is obvious that no specific rules were followed under partial flooding concerning thickness of root. Therefore, there could be other factors involved which dictate the increase in root diameter in some of these plants such as root thickening found in the majority of plants which could increase root porosity and also aeration (Crawford, 1982 a). However in the wetland rhizomatous plant which had smaller roots it is most likely that by having finer roots there is no need for any size modification, at least under partial inundation. It is interesting to note that during 49 days flooding duration employed here, they have sufficient time to modify their root diameter (Meek and Stolzy, 1978) if they need to.

3.4.4 `Stem' (Aerial shoot) modification.

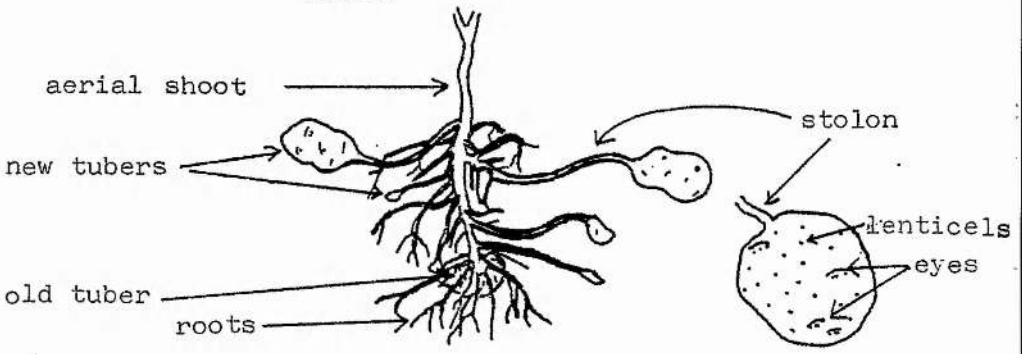
Apart from shoot growth under total inundation in rhizomatous Iris pseudacorus and Hedychium sp. and in one plant of Arum maculatum - a corm species -, no shoot growth that survived was observed . Under partial inundation , all plants grew except some of Coleus tuberosus. However in this species, new `stem' ( aerial shoot ) in the form of `bush' regrew on the survived tubers. This phenomena was also reported earlier by Crawford (1982 a) on a woody above ground stem of wetland alder.

RHIZOME



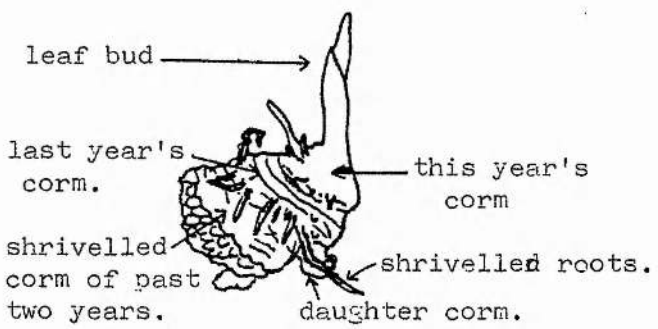
IRIS SP.

TUBER

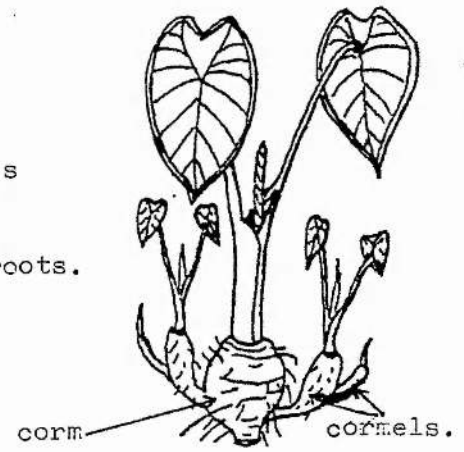


POTATO

CORM



ARUM MACULATUM



TARO

FIGURE 3.1 :

Rhizome, tuber and corm : the underground stem which functions as the storage organ. Potato and taro are important food plants.

PLATE 3.1 :

The effect of flooding and/or burial on rhizomatous plant of Iris pseudacorus.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

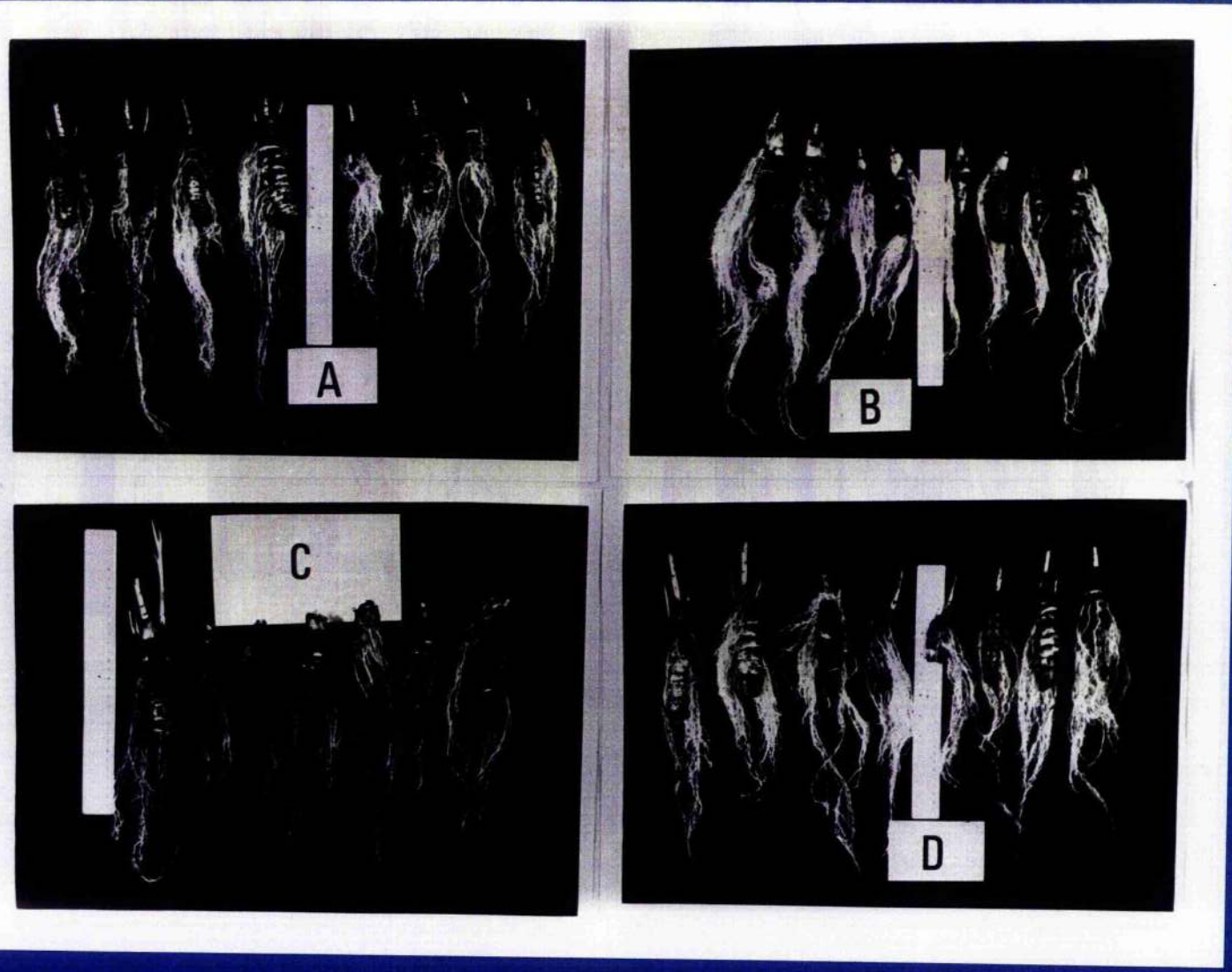

A
B
C
D

PLATE 3.2 :

The effect of flooding and/or burial on rhizomatous plant of Hedychium sp. (bud - Experiment II)
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

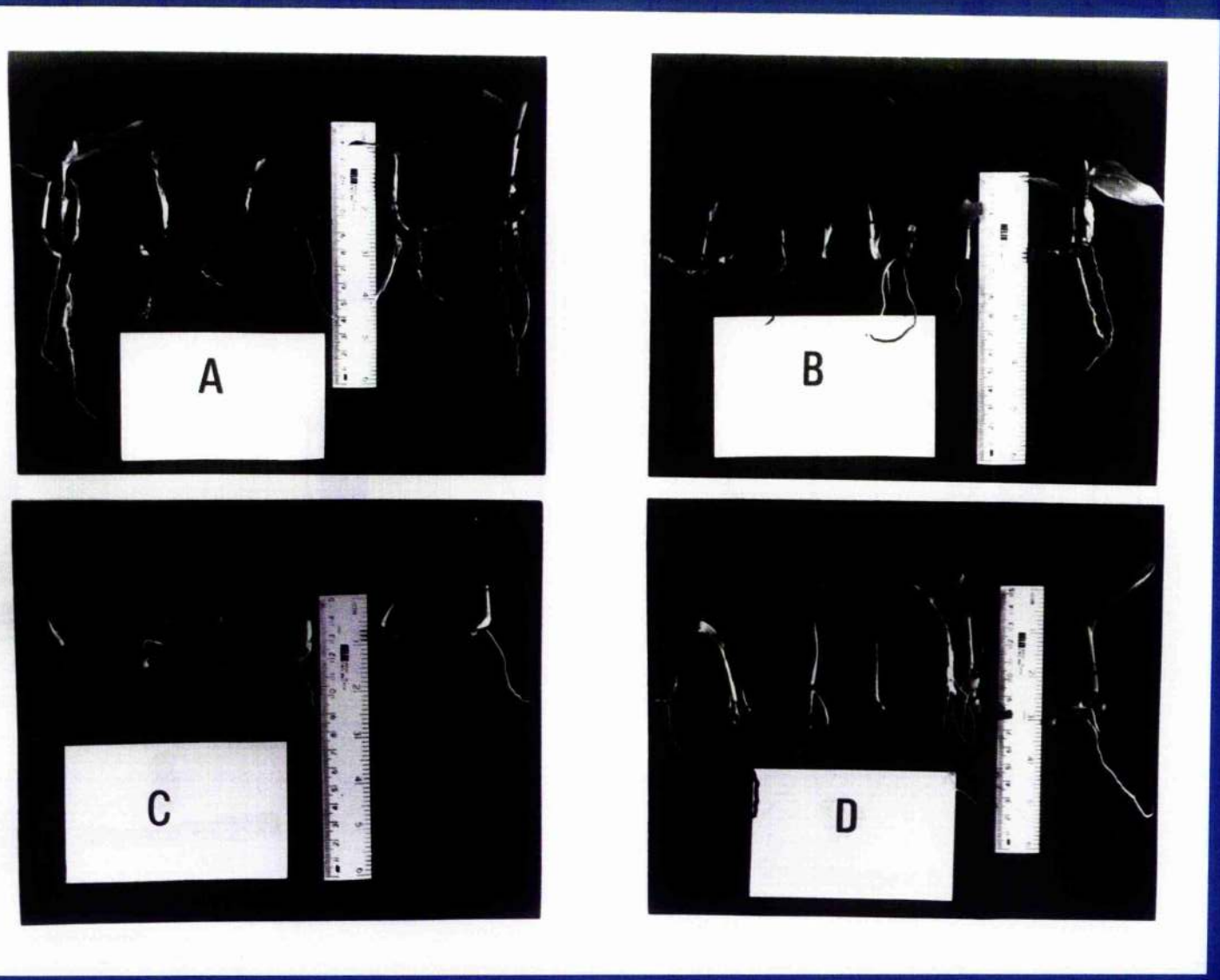
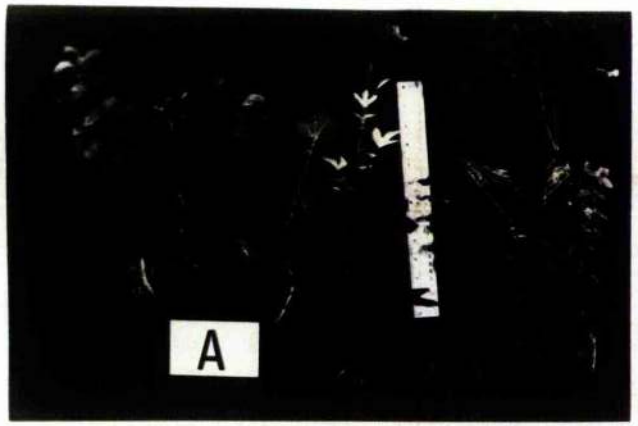
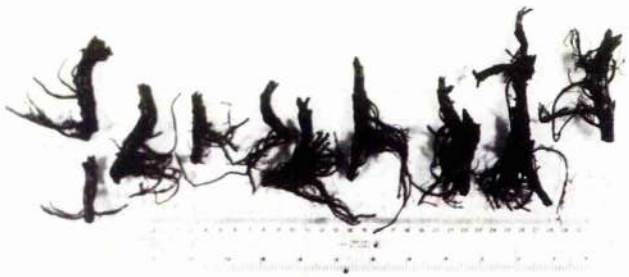

A
B
C
D

PLATE 3.3 :

The effect of flooding and/or burial on rhizomatous plant of Filipendula ulmaria.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.



C



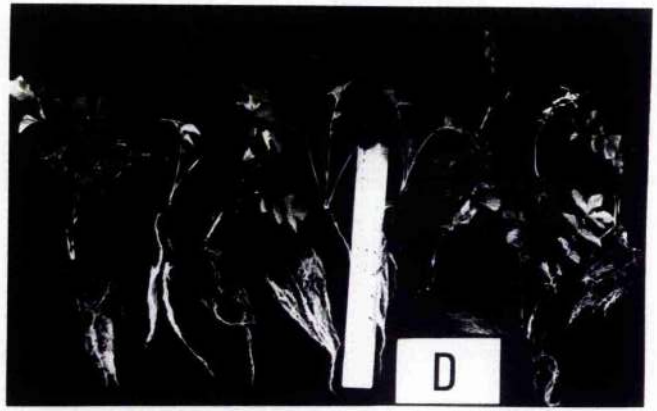


PLATE 3.4 :

The effect of flooding and/or burial on rhizomatous plant of Iris germanica.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

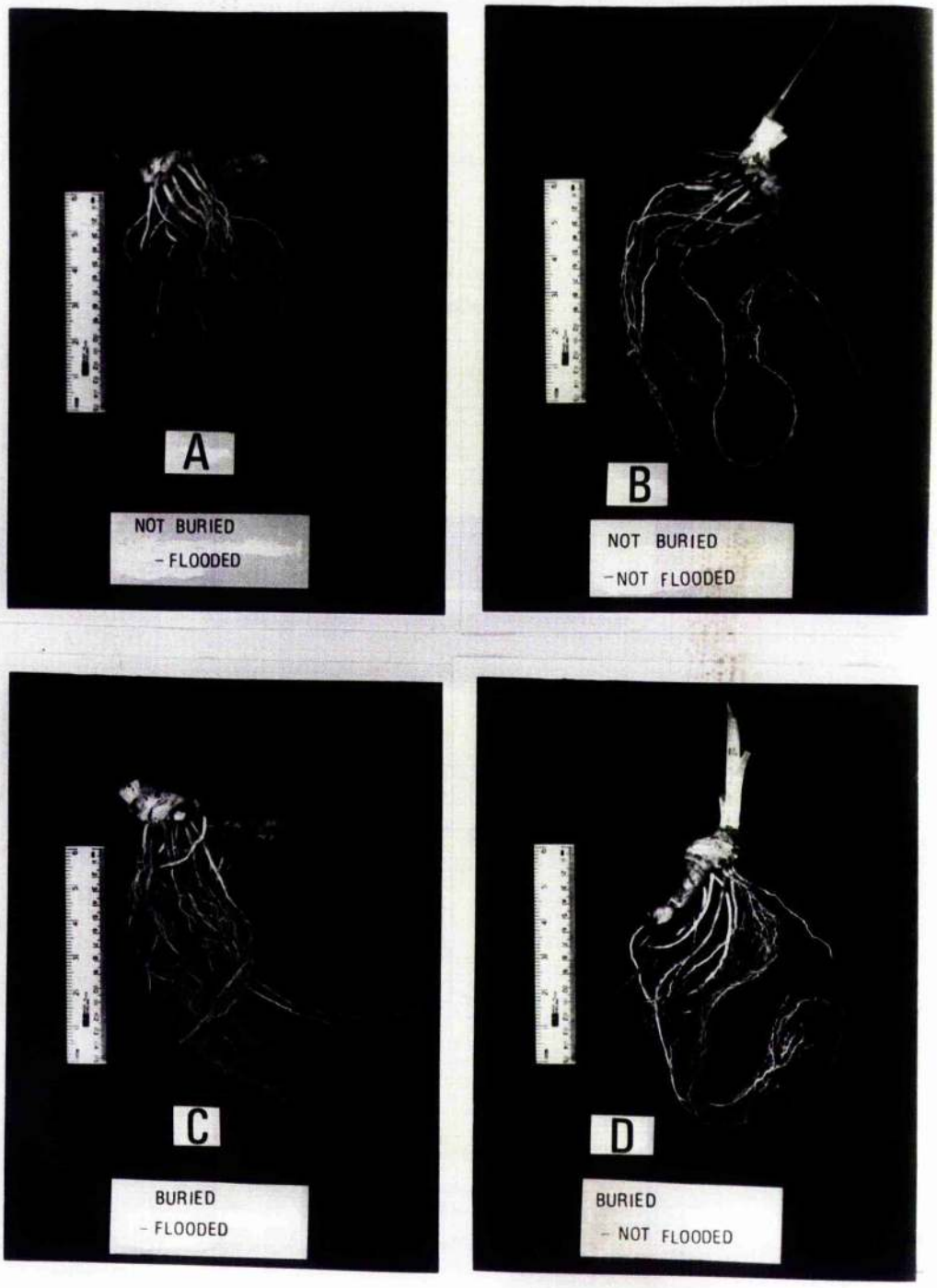
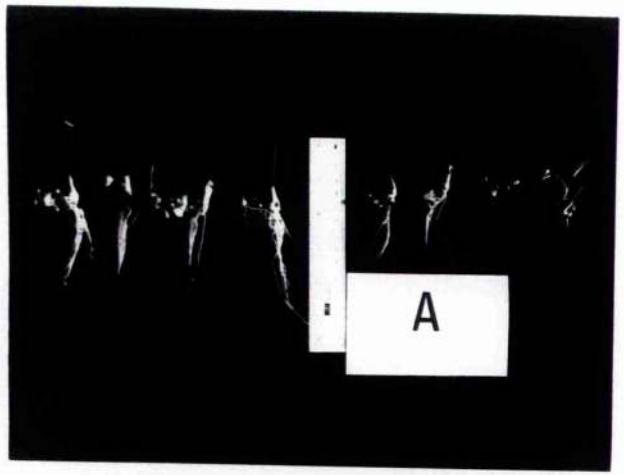
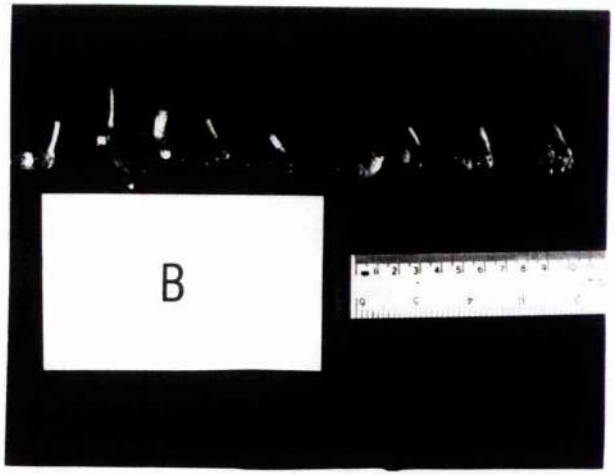
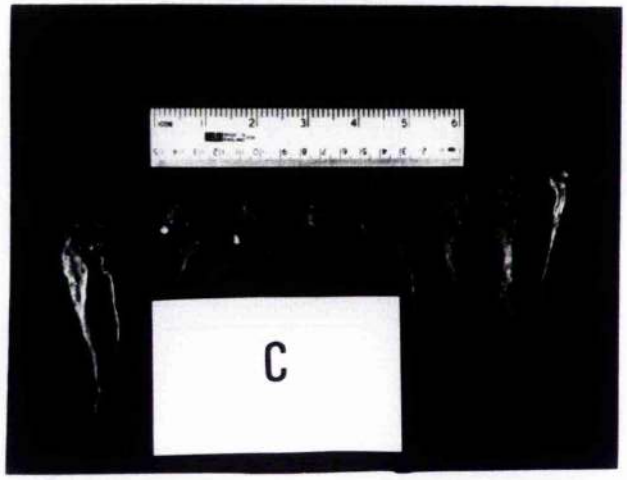

A
NOT BURIED
- FLOODED
B
NOT BURIED
-NOT FLOODED
C
BURIED
- FLOODED
D
BURIED
- NOT FLOODED

PLATE 3.5 :

The effect of flooding and/or burial on corm species of Arum maculatum (Experiment II).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.
Arrowed is a healthy growing shoot of one plant whilst the other plants succumbed to treatment C.


A


B


C

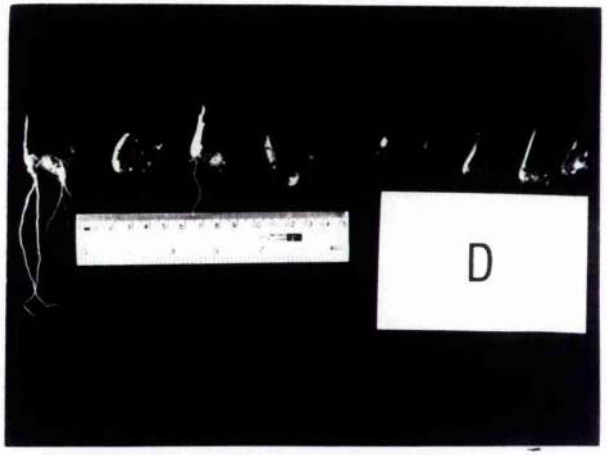

D

PLATE 3.6 :

The effect of flooding and/or burial on corm species of Colocasia esculenta (Taro).
Details of experiment:
A = Not buried and flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

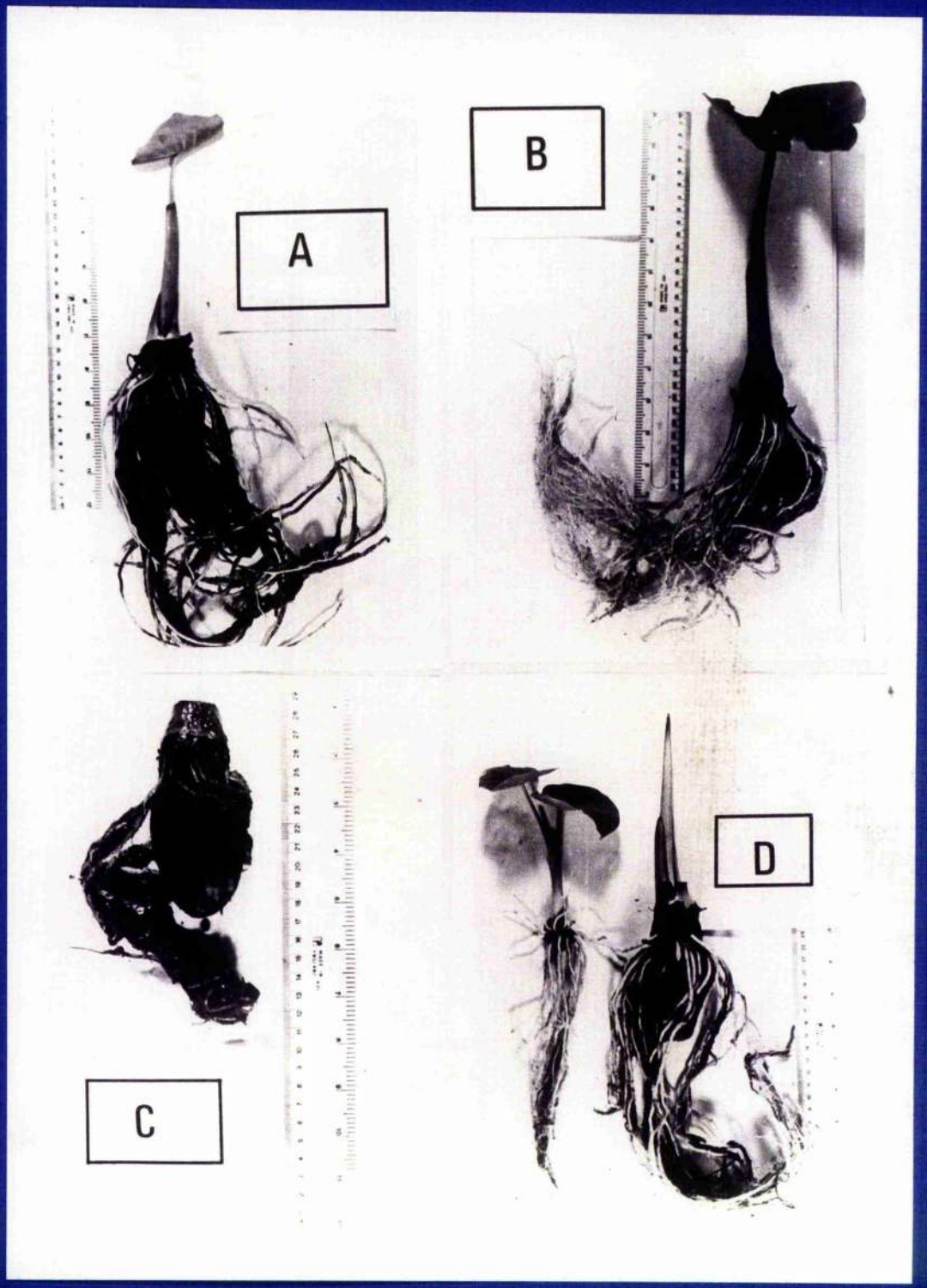
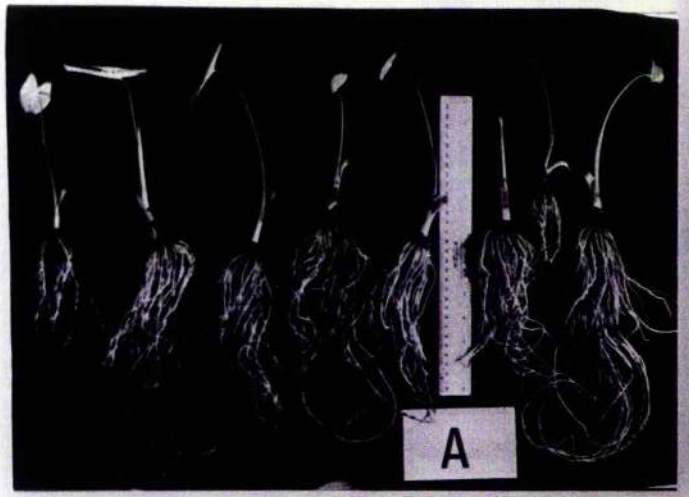
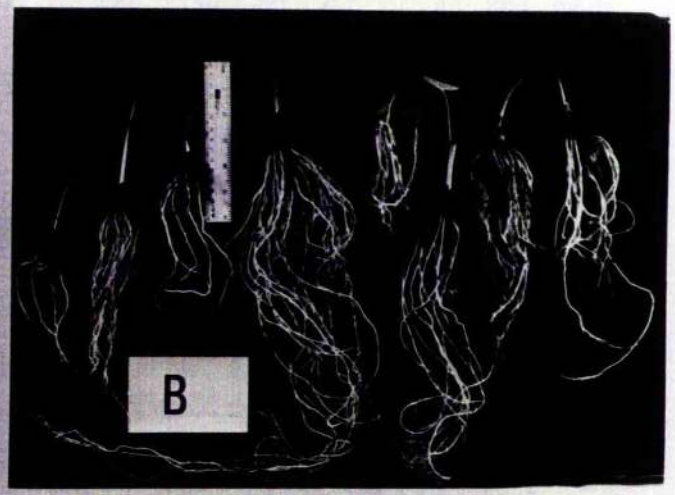
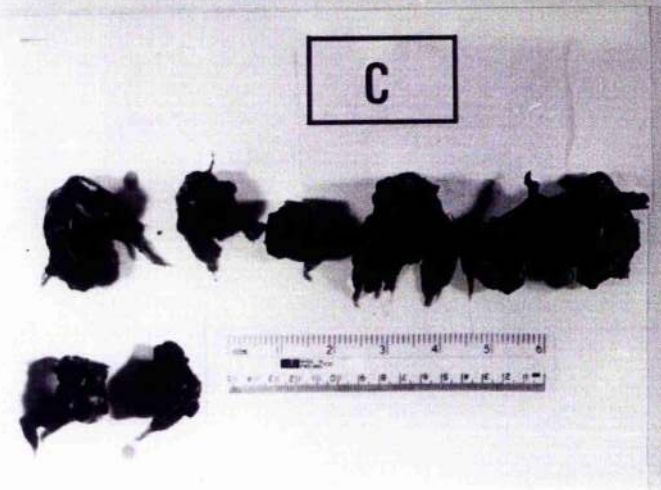

A
B
C
D

PLATE 3.7 :

The effect of flooding and/or burial on corm species of Colocasia sp. (Keladi Kemahang).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.


A


B


C

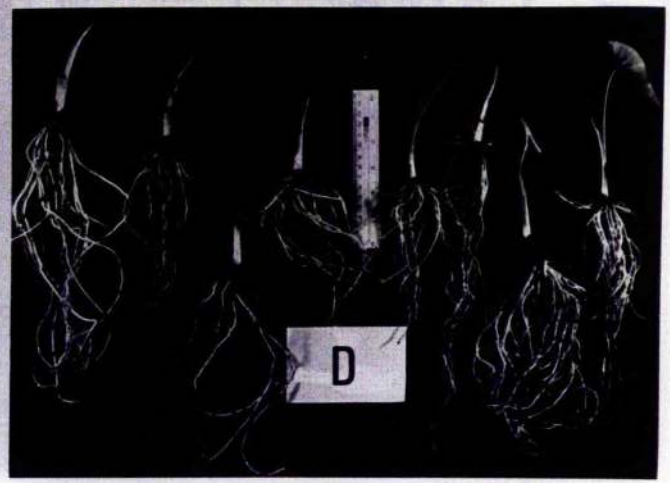
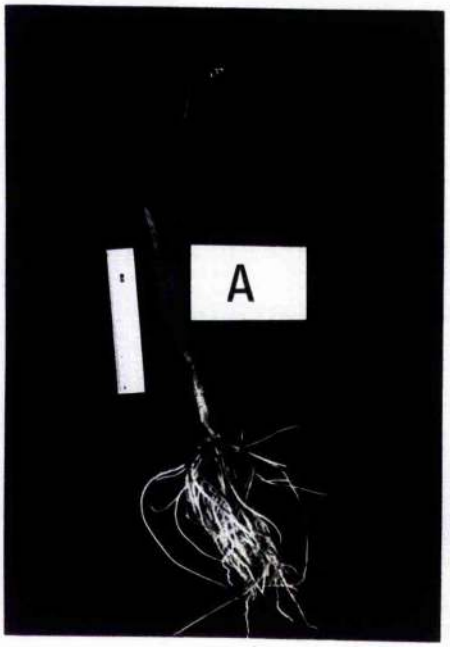
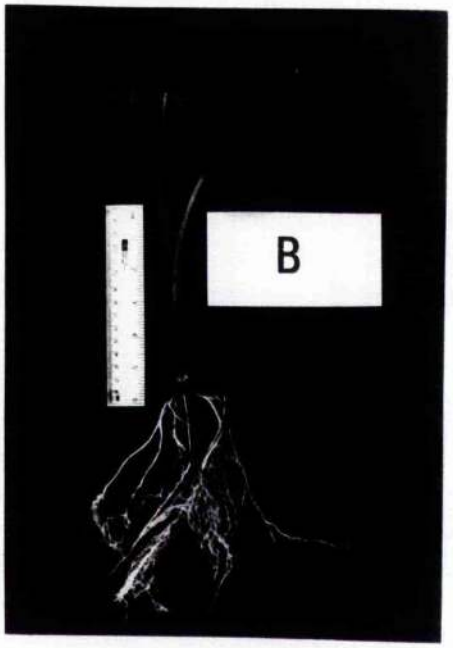

D

PLATE 3.8 :

The effect of flooding and/or burial on corm species of Xanthosoma sp. (Keladi Telur).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.





C

PLATE 3.9 :

The effect of flooding and/or burial on Coleus tuberosus (Ubi Kemili), a tuber species.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

A
B
C
D

PLATE 3.10 :

The effect of flooding and/or burial on Helianthus tuberosus (Jerusalem artichoke), a tuber species.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

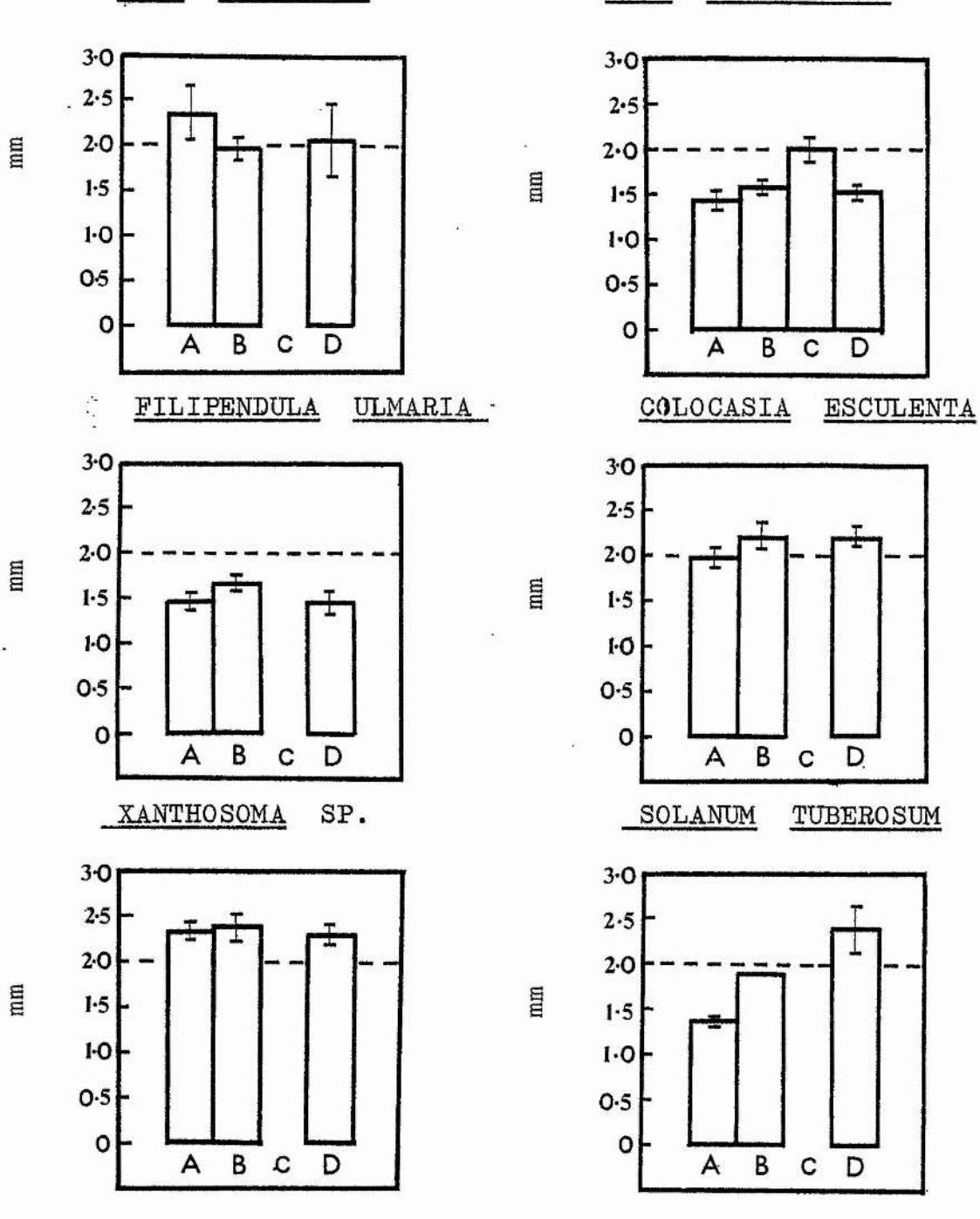


FIGURE 3.2 :

The effect of flooding and/or burial treatments on root diameter (mm). Details of treatment :
A = Not buried and flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Iris germanica, Iris pseudacorus and Filipendula ulmaria are rhizomatous plants, Colocasia esculenta and Xanthosoma sp. are corm producing plants whereas Solanum tuberosum is a tuber producing plant.

CHAPTER 4 :

CONTENTS

Chapter 4

A FACTORIAL ANALYSIS OF PLANT GROWTH UNDER FLOODING AND/OR BURIAL.

4.1 INTRODUCTION

A more precise idea of plant -- environment interaction can be gained through plant growth analysis. Thus the relationship of plant performance with the environment can be scanned even over a limited, short-term view of events (Hunt, 1978).

In this investigation four criteria of growth were employed:

(1) Increase in plant height.

(2) Mean relative growth rate ($\bar{R}$).

(3) Increase in root length.

(4) The rapidity of emergence under burial.

In general, plant yields are highly significantly correlated with plant height (Plucknett et al, 1970) whereas relative growth rate ($\bar{R}$) informs the ecologist about the integration of the combined plant performances, thus directly relating growth to the biomass (Hunt, 1978). Meanwhile, injury or death of the root systems will no doubt result in reduction of shoot growth and eventual death of plants (Rhoades, 1967) but plants which rapidly produced adventitious roots under flooding suffer least and shoot growth recovers rapidly (Sartoris and Belcher, 1949;Kramer, 1951). Thus shoot and root growth are mutually dependent (de Wit, 1978). However, as a measure of flooding treatment response, the number and length of roots were found to be a more sensitive parameter than stolon and leaf growth in strawberry clover varieties (Bendixen and Peterson, 1962).

The habitat in which plant is growing can influence the growth of shoot and root. In the submerged and the semi-submerged or emergent water plants there is the inherited ability to survive submerged in mud while growing progressively through it (Laing, 1940). There is no doubt that these plants can succeed in sending shoots through the anaerobic substrate to the surface. Yet, the ability to tolerate anoxia varied even among wetland plants that can tolerate flooding. For example, in rice -- a flood tolerant crop -- both shoot and root growth increased significantly under flooding (Saha et al, 1974) but were totally suppressed under complete anaerobiosis (Opik, 1973). On the other hand, in Scirpus maritimus and some wetland

plants, shoot growth also takes place (from unextended lateral buds) during eight weeks of total anoxic incubation (Barclay and Crawford, 1982) and in barnyard grass seedlings during seven days period under nitrogen gas (Rumpho and Kennedy, 1981). Among the flood-susceptible crop plants, severe reduction in both growth and crop yield of sunflower occurred under conditions of prolonged flooding (de Wit, 1978). Under short term flooding, however, sunflower plants are less injured and showed the development of good adventitious roots (Kramer, 1951). Root elongation of intact mustard seedlings (Sinapis alba) stopped when oxygen transport from the leaves was prevented while growing in solutions of low oxygen content (Greenwood and Goodman, 1971). In maize and bean seedlings, root growth was reduced in plant grown in non-aerated solution. Shoot growth was not affected in maize but was reduced in bean seedlings (de Wit, 1978). However, plants flooded in solution culture cannot be expected to behave in a similar manner when flooded in soil or sand. Plants potted in soil were injured more than those potted in sand or when they were simply submerged in water (Kramer, 1951).

Total inundation or 100% submergence severely affected plant growth. For example, Taxodium distichum a woody species noted for its flooding tolerance is killed by complete inundation (Demaree, 1932). The tall cultivars of Cenchrus ciliaris L. (a grass species) survive better than the short ones because when flooded with a constant depth of water, only 75% of the leaves in the tall cultivars became covered whilst in the short ones it was

totally submerged (Anderson, 1974). Total submergence of sugar cane --a crop plant which can withstand flooding -- will cause plant damage. So long as the growing point and the topmost leaves are above the level of flood water, growth will not be affected (Rege and Mascarenhas, 1956). Hence, the exposed leaves may served as a supplier of oxygen from air to the roots (Cannon, 1925; Greenwood, 1967) or as a means of transpiration of toxic substances such as ethanol (Kenefick, 1962). In the wild, in two rhizomatous Typha species growing in a small pond, those plants growing in deeper water had taller leaves and a greater allocation to leaves whilst allocation to sexual and vegetative reproduction decreased (Grace and Wetzel, 1982). A similar trend was also found in Scirpus maritimus, except that more seeds were produced under flooding (Lieffers and Shay, 1981); In taro --a tropical crop plant -- the relative proportion of top, corms and roots varied under flooded habitats; in wetland taro, a smaller proportion of corm is produced than top, whilst in upland taro more corm is produced (Hawai Agricultural Experiment Station, 1929). Whether a taller shoot system with an increase in biomass allocation to leaves is the means of adaptation of growth under flooding merits further investigation.

The seasonal cycle of environment also controls the growth of both shoot and root (Troughton, 1957). In a monsoon climate, the dry season causes a limitation in growth, just as the winter season does in temperate countries. Thus the underground storage organs are found in plants which die back to soil level in preparation for the winter or a dry season, leaving a dormant and

over-wintering or drought-avoiding organs underground (Villiers, 1975). In other words, only when plants are actively growing does the severity of injury depend upon the time of year when flooded. Generally, grass plants can withstand long periods of anoxia when they are dormant, semidormant or in early stages of growth while water and air are relatively cool (Rhoades, 1967).

The underground organs are not so deeply buried in the soil as the roots even though normally they are studied in conjunction with the roots (Troughton, 1957). Some even grow above ground. Yet, for crop plants, depth of planting is quite important because a shallow planted corm may develop new corms above the soil surface, thus exposing them to injury by insects, rodents and birds (Plucknett et al, 1970) or severe frosts and snow (Prime, 1960). Shallow rooting may also result from shallow planting thereby causing the increased possibility of moisture stress in upland plants (Plucknett et al, 1970) or a water loss from tubers exposed to air (Wiersum, 1966). Moreover, increasing the amount of buried stem from which the tubers grew will encourage the production of new tubers of potato and Jerusalem artichoke (Wood, 1979) and hence the yield.

4.2 MATERIALS AND METHODS

4.2.1 Shoot extension as a measure of plant growth.

During the experiment, shoot extension was measured every three to four days and used to plot plant growth curves.

4.2.2 Plant growth rate.

The fresh weight of the whole plant was also measured before planting and after harvesting. The changes in fresh weight were then determined and the mean relative growth rate, $\overline{R}$, was calculated by using the following formula:

$$\overline{R}_{1-2} = \frac{\log W_2 - \log W_1}{T_2 - T_1} \text{ gm. /gm. day}$$

where:

W_2 = final weight (gm.)

W_1 = initial weight (gm.)

$T_2 - T_1$ = duration of experiment (days).

4.2.3 The increase of maximum root length.

The longest root length was measured before planting the plant out and also after harvesting them. Thus the average increase in the maximum root length could be calculated.

4.2.4 Shoot emergence.

Shoot emergence in burial treatment (treatment C and D) was checked on every other day.

4.3 RESULTS.

4.3.1 GROWTH CURVE.

4.3.1.1 Rhizome species (Figure 4.1).

The growth of plant , measured as plant height, continued throughout the experimental period (see Appendix I) except for treatment C (buried and flooded) where only Iris pseudacorus showed shoot and bud growth and Hedychium sp. showed only bud growth (see Chapter 3). When planted on the sand surface, only Iris germanica -- which grows above ground in natural habitat -- showed a better shoot growth as compared to being grown buried. Meanwhile, other species (all with rhizomes which grow below soil level in wild habitats) favoured either being buried but not flooded (I. pseudacorus and Hedychium sp.) or flooded but not buried (Filipendula ulmaria). The growth rate in the latter species was rapid during the first two weeks as compared to other species, in all the treatments.

4.3.1.2 Corm species (Figure 4.2).

Shoot growth was greatest under the flooded but not buried condition for all the three species investigated. In Arum, growth rate was slowest and not all the plants in treatment D (buried and not flooded) emerged, thus plant height in this treatment could not be measured. Growth in taro (Colocasia esculenta) was slow during the first two weeks, however in the following weeks a very fast growth rate was recorded. In yautia (Xanthosoma sp.), a slow initial phase of growth was recorded. Nevertheless after the fifth week a rapid shoot elongation took place.

4.3.1.3 Tuber species (Figure 4.3).

Potato (Solanum tuberosum) and `ubi kemili' (Coleus tuberosus) favoured the burial but non-flooded condition for maximum shoot growth. Moreover, in Coleus flooding even under the non- buried condition caused shoot damage. Nevertheless, after 40 days new shoot in the form of `bush' appeared in several of the plants. Potato behaved irregularly -- in the presence of roots, flooding without burial gave rise to a taller plant but whenever non-rooted tuber was flooded as above , a very short plant was produced. Hence this plant is unique in this respect. In Jerusalem artichoke (Helianthus tuberosus), rapid growth was recorded in treatments A, B and D. For all the species studied, burial and flooding caused no shoot extension (except in H.

tuberosus where one shoot managed to emerge but died off very rapidly).

4.3.2 PLANT HEIGHT.

4.3.2.1 Rhizome species (Figure 4.4).

Plant height in all the species studied followed a similar trend in relation to the experimental condition; treatment D (buried and not flooded) produced the greatest growth followed by treatment A (not buried and flooded) and treatment B (not buried and not flooded). There were only a few exceptions to this pattern. In Iris pseudacorus shoot growth was also recorded under buried and flooded (treatment C) which did not take place in the other species. Other differences were observed in Filipendula ulmaria where the not buried and flooded treatment gave the biggest increment whilst in Iris germanica this was achieved with the not buried and not flooded regime. Flooding while not buried suppressed plant height in the latter species.

Statistically (Table 4.1 a, b), a significant increase in plant height ($p < 0.001$) was observed I. pseudacorus, Hedychium sp. and F. ulmaria under the non-buried condition due to the flooding effect and under the non-flooded condition due to the burial effect. In I. germanica, a significant decrease ($p <$

0.001) was observed under the former condition however under the latter condition no significant change was recorded. In the buried condition, flooding could significantly decrease ($p < 0.001$) plant height in all the species studied. The same result was also obtained whenever the burial factor was imposed on the flooded habitat. When interaction of flooding and burial factors were examined (Table 4.1 c), both factors showed an interaction in the species studied except in Iris germanica. In this species the flooding factor gave the greatest effect on reducing plant height when both factors were imposed together.

4.3.2.2 Corm species (Figure 4.5).

Yautia (Xanthosoma sp.) and taro (Colocasia esculenta) showed a similar trend of plant height increment; the tallest plants growing under treatment A, followed by treatment B and D. In treatment C no shoot growth was shown except from one Arum maculatum plant harvested after 7 weeks. In this species, plant height in treatment A, B and D was about the same when harvested after 14 weeks.

Statistically (Table 4.1 a, b, c), flooding will significantly ($p < 0.001$) enhance plant height in non-buried corms of taro and yautia. Yet in Arum, no significant effect was observed. Burial caused significant reduction in plant height ($p < 0.001$) in yautia but no significant effect was observed in taro and Arum. Burial together with flooding will significantly

suppressed plant height in all the species studied. When these factors occurred together , burial gives the greatest effect in yautia and possibly also in taro. Nevertheless, in Arum both these factors contributed to the severe damage.

4.3.2.3 Tuber species (Figure 4.6)

The trend of plant height in three tuberous plants studied (Appendix I) were ;the biggest increment in treatment D, followed by treatment A, B and no shoot growth in treatment C, where it decayed. Nevertheless, there was a great variability of results in treatment A. Jerusalem artichoke (Helianthus tuberosus) showed the biggest height, followed by potato (Solanum tuberosum) and finally in `kemili' (Coleus tuberosus) this treatment produced the smallest height increment of new morphologically different shoot (bush form). Thus in the latter species treatment B produced a higher plant than treatment A ; a deviation from the above generalized trend.

Statistically (Table 4.1 a, b, c), flooding when not buried will significantly ($p < 0.001$) increase plant height in Helianthus and decrease it in Coleus. In potato no significant effect due to flooding was observed. On the other hand, burial when not flooded significantly ($p < 0.001$) enhanced plant height in all the species studied. Flooding of buried tubers caused a significant decrease in height. Hence both these factors interacted in causing a severe plant height reduction, but in

Coleus the flooding factor gave the greatest effect.

4.3.3 MEAN RELATIVE GROWTH RATE.

4.3.3.1 Rhizome species (Figure 4.7, 4.8 and Table 4.2 a,b,c).

Flooding without burial significantly ($p > 0.001$) enhanced relative growth rate in Iris pseudacorus, the rhizomes and buds of Hedychium sp. and Filipendula ulmaria rhizomes (Table 4.2 a, b, c). In Iris germanica there was no significant decrease in growth rate. Burial when not flooded caused a significant increase ($p < 0.001$) in Hedychium sp. (bud and rhizome) and Iris germanica. In Filipendula ulmaria and Iris pseudacorus, the increase and decrease of growth rate , respectively, under this treatment was not significant ($p < 0.05$). When buried, flooding significantly ($p < 0.001$) enhanced growth rate only in Iris pseudacorus whilst in Hedychium sp. rhizome the increase was not significant. In Hedychium there was a significant decrease in bud growth rate in this treatment ($p < 0.05$). The same was also found in the rhizomes of F. ulmaria and I. germanica ($p < 0.001$).

Under flooded condition, the effect of burial was to increase significantly ($p < 0.05$) plant growth in Iris pseudacorus, and suppress it ($p < 0.001$) in Hedychium buds and also the rhizomes of Filipendula ulmaria and Iris germanica. In Hedychium sp. rhizomes the increase was not significant. Hence in most of the species studied, burial and flooding complimented each other in giving a severe reduction of growth when they occurred together. However, in Iris pseudacorus growth was enhanced due only to flooding effect in the buried and flooded treatment (treatment C) or in other words no interaction between flooding and burial occurred in this species.

4.3.3.2 Corm species (Figure 4.9 and Table 4.2 a, b, c).

Flooding when not buried significantly ($p < 0.001$) enhanced plant growth rate in yautia (Xanthosoma sp.) and Arum maculatum whilst in taro (Colocasia esculenta) no significant increase was observed. Burial when not flooded significantly ($p < 0.001$) enhanced plant growth rate in Arum but in taro and yautia there was no significant increase.Upon flooding and burial, a significant reduction in plant growth was observed in all the species. Thus the flooding and burial factors together gave the severe effect on plant growth when acting together.

4.3.3.3 Tuber species (Figure 4.10 and Table 4.2 a, b, c).

Flooding will significantly ($p < 0.05$) enhance plant growth under non-buried condition in Helianthus tuberosus. Yet in Solanum tuberosum and Coleus tuberosus there is a non-significant increase and decrease, respectively. Burial when not flooded caused a significant increase in rhizome growth ($p < 0001$) in C. tuberosus whilst in H. tuberosus and S. tuberosum the increases were not significant. With buried and flooding, growth rate was significantly suppressed ($p < 0.001$). Thus both these factors interacted when employed together, giving the severest effect.

4.3.4 ROOT GROWTH. (Figure 4.11 and Table 4.3 a, b, c).

4.3.4.1 Rhizome species.

Flooding under non-buried condition will significantly ($p< 0.001$) enhanced root elongation in I. pseudacorus but reduced it in I. germanica ($p < 0.001$). In Hedychium sp., a non-significant increase was recorded ($p > 0.05$). On the other hand, burial under non-flooded condition significantly ($p < 0.001$) enhanced root growth in I. pseudacorus and Hedychium sp. whereas in I. germanica a non- significant increase was recorded.

Flooding upon burial caused a severe decrease in root length. Similar result was obtained when burial factor was imposed on flooding, however in Hedychium sp. the latter treatment showed a non-significant decrease probably due to a small number of root present in this species hence a smaller sample size, bigger standard error and a non-significant result. Nevertheless, in all the species studied interaction between flooding and burial occurred.

4.3.4.2 Corm species.

Only one corm species was examined. After 7 or 14 weeks, a significant increase in root length was observed upon flooding the non-buried corms of Arum maculatum. The same result was obtained when the burial factor was imposed on a non-flooded corm. When burial and flooding factors acted together, a significant decrease in root length was observed only at the 14 weeks harvest, whilst at 7 weeks it was not significant. Hence both factors gave a severe effect.

4.3.4.3 Tuber species.

Flooding of non-buried Helianthus tuberosus tubers caused a non -significant increase in root elongation. Burial on flooded tubers also resulted in a non-significant increase. However flooding of buried tubers could significantly ($p < 0.001$) reduce root length. There is therefore the possibility that a burial factor gave the most damaging effect.

4.3.5 SHOOT EMERGENCE UNDER BURIAL (Figure 4.12, 4.13 and 4.14)

In all the species studied (rhizome, corm and tuber) only one plant of Iris pseudacorus managed to surface and grow in treatment C (buried and flooded). In this plant, the buds elongated faster and emerged first, followed later by the terminal shoot (Plate 4.1). In H. tuberosus, one plant also managed to surface, however this aerial shoot died off after a few days. Nevertheless in treatment D (buried and not flooded) all the species showed shoot emergence and growth also continued afterwards. In the latter treatment , there was 100% emergence before the seventh week harvest in all the species studied except in Arum maculatum.

4.3.5.1 Rhizome species.

Filipendula ulmaria and Hedychium sp. emerged mostly in the first week. Yet in the latter species 15% of the sample emerged only after the fifth due to partial shoot rot. In I. pseudacorus and I. germanica, 50% and 60% of the plants emerged in weeks two and weeks three, respectively.

4.3.5.2 Corm species.

Most of the taro plant (Colocasia esculenta) emerged in weeks three. In yautia (Xanthosoma) it was a week or two later whilst in Arum maculatum 50% emerged in weeks six.

4.3.5.3 Tuber species.

In all the tubers studied, plants started to emerge even during the first week. However, only in H. tuberosus did 80% of the plant surface in week one whilst in C. tuberosus ('ubi kemili') and S. tuberosum (potato) the majority of the plants emerged in weeks two.

4.4 DISCUSSION.

In plants with storage organs, patterns of biomass allocation through time generally showed the same trends; initially biomass stored in the rhizomes (or corms or tubers) is diverted to leaves (Fiala, 1978), then flowering increased which is finally followed by increase in lateral ramet production (daughter plant which consists of rhizome and its associated leaves, roots and flowering structures). The increase in growth of lateral ramets could be measured by weight (Grace and Wetzel, 1982). Except in Filipendula ulmaria and Solanum tuberosum where the flowering stage was achieved under flooding and non-burial, the other species has not yet shown the tendency to flower. Hence the results from this study could indicate that data was only collected during the initial stage of growth where biomass is mainly allocated from the storage organs to leaves and aerial shoot production.

It was apparent that flooding or burial increased height growth in most of the species studied regardless of its natural habitat or form. The significant reduction of height growth due to burial was observed only in Yautia (Xanthosoma sp.) a cormous species cultivated on dry land in the tropics. On the other hand, height growth was significantly less because of flooding in Iris germanica, a rhizomatous garden plant, and Coleus tuberosus, a tuber crop of moist tropical environments. Slower leaf elongation in wheat grown in anoxic culture solution was attributed to lack of oxygen (Trought and Drew, 1980) and

it could also be the case here. Burial could envelope the tuber in moist soil, thus reducing moisture stress (Wiersum, 1966). It was then seen in this study that burial could significantly result in increase height growth of tubers, hence the usual practise of burying sprouted tubers in agriculture is clearly beneficial.

In the flooded environment, the increase in plant height to above the water level will undoubtedly enable the plant to expose part of its organs to air. Leaves exposed to air could supply oxygen to the submerged part (Cannon, 1925; Raalte, 1940; Greenwood, 1967) or remove the respired CO_2 (Boulter et al., 1963; Chashchukhin, 1979) and even volatile toxic substances (Kenefick, 1962) from the underwater parts. Moreover, the taller leaves were shown to be produced on flooded wetland rhizomatous species (Grace and Wetzel, 1982; Lieffers and Shay, 1981). Growth height was also increased with increasing water depth (Lieffers and Shay, 1981). In the saturated soil, the maximum height of Typha latifolia, Phragmites communis and Spartina michauxiana, all plants of wetland habitat, was also enhanced as compared to drier better aerated controls (Weaver and Himmel, 1930). Hence it is possible that adapted plants of flooded conditions will increase height growth under flooding as a positive tolerance response.

The value of mean relative growth rate ($\bar{R}$) was very small when compared to achievement in plant height growth. It could be attributed to the measurement which was based on a daily basis (Hunt, 1978) or more importantly on the smaller change in plant total weight. As growth was observed during the initial phase (Vegetative stage) only, buds, cormels and daughter tubers were in the early stage of development when samples were harvested. At this stage, the small increase in biomass in this new growth was at the expense of the parent rhizome (Grace and Wetzel, 1982). This factor could influence the mean relative growth rate ($\bar{R}$), which was measured on the basis of change in whole plant weight before and after the transfer of the biomass. Hence the value of $\bar{R}$ in the plant storage organs studied (after 49 days treatment) was as expected, smaller than in other plants reported elsewhere (Hunt, 1978).

Even though the value of $\bar{R}$ is small, flooding or burial showed a significant effect in enhancing it. Flooding will significantly increase $\bar{R}$ in Iris pseudacorus, Hedychium sp., Filipendula ulmaria, Xanthosoma sp., Arum maculatum and Helianthus tuberosus , whereas burial will significantly increase the value of $\bar{R}$ in Hedychium sp., Iris germanica, Xanthosoma sp., Arum maculatum and Coleus tuberosus. When buried and flooded (total submergence), only Iris pseudacorus exhibited a significant increase in $\bar{R}$ indicating its ability to tolerate this severe condition. Flooding rather than burial was the cause of this positive behaviour. It should be noted here that plant

height was not significantly increased in comparison with $\bar{R}$, however shoot growth was observed and at the same time bud development was maintained indicating short and long term viability. Hence it is likely that this plant is in a waiting stage before water level subside. Moreover, during total anoxic incubation this plant did not produce shoot growth (Barclay and Crawford, 1982), nevertheless it is better adapted because if it is compared with Iris germanica (which showed no shoot growth under buried-flooded), two weeks total anoxia caused only 20% of its rhizomes to perish (Hetherington, 1983). In Hedychium sp., another well adapted plant, the value of $\bar{R}$ increased when no shoots are produced but not when shoots are produced. It is most likely that during initial shoot burst plant weight decreases due to greater biomass allocation to leaves. Starch stored in the rhizomes was catabolised to sugar which was then translocated for cell division, differentiation and elongation in the shoot. During the period when shoot production was at its peak, plant weight (mainly concentrated in the storage organ) decrease resulted in smaller value of $\bar{R}$.

In this study, root length increment was studied as a measure of survival ability. It was known that root growth was much more inhibited than shoot growth by differences in aeration level (Trought and Drew, 1980) where the longest root are usually obtained in loose, well aerated and moist soil (Troughton, 1957). Nevertheless, other workers (Weaver and Himmel, 1930), have also shown that saturated culture of wetland plants would produce longer roots as compared to dry culture. In

this study, flooding induces root growth in the basis of maximum length in Iris pseudacorus and Arum maculatum. In Iris germanica, it was significantly inhibited. Burial also induces root growth of Iris pseudacorus and Hedychium sp. However the drastic effect was found under burial and flooding except in Iris pseudacorus where a small non-significant increase was shown instead. It is possible that shading of shoot in this treatment lowered root production and elongation as observed elsewhere (Langer,1979). In Hedychium rhizomes, shoot growth in the form of axial buds, was exhibited even though there was a non-significant root growth. Hence there is the possibility that damaged roots present on this plant could still provide nutrient for its growth (Trought and Drew, 1980).

It seems that rapid emergence under burial (not flooded) has a correlation with increase in shoot length in tuber species. Hence in corm species, no identical increase in growth maybe due partly to a slow rate of emergence. However in the rhizomatous species no correlation was shown, suggesting the non-significant effect of emergence on their growth. In corm producing plants, species which showed total emergence also showed increase in shoot length under flooding. As a rapid, luxuriant growth during the early crop period usually gave a higher yield (Plucknett and de la Pena, 1971), and early emergence produced greater shoot growth, the normal practice of planting setts or hulis of taro (Colocasia esculenta) and yautia (Xanthosoma sp.) with leaf base above the soil or water surface (Plucknett and de la Pena, 1971) is clearly a

profitable act to the farmer.

As a summary, plants well adapted to waterlogging can grow even under total submergence (buried and flooded -- treatment C). Under this condition, axial bud growth is more rapid than shoot growth (terminal bud). Once axial buds have emerged on top of the water surface, shoot growth soon followed. Growth rate in terms of biomass ($\bar{R}$) is rather slow but significantly increased. It was shoot and root lengths which were more affected.Less adapted plants grow under partial flooding (not buried and flooded -- treatment A) but succumb under total submergence. Plant growth rates under partial flooding are usually rapid, however root length can sometimes be affected. On the other hand, susceptible plants are affected by even partial flooding; it reduces growth in terms of plant height, root length and even $\bar{R}$ when statistically compared with the non-flooded treatments.

Except for plants that naturally grow above ground, most rhizomatous plants grow better when buried. Burial of tubers also increased its shoot growth. However cormous plants seem to thrive better when shoot is exposed above ground (not buried) probably due to slower rate of emergence when buried.

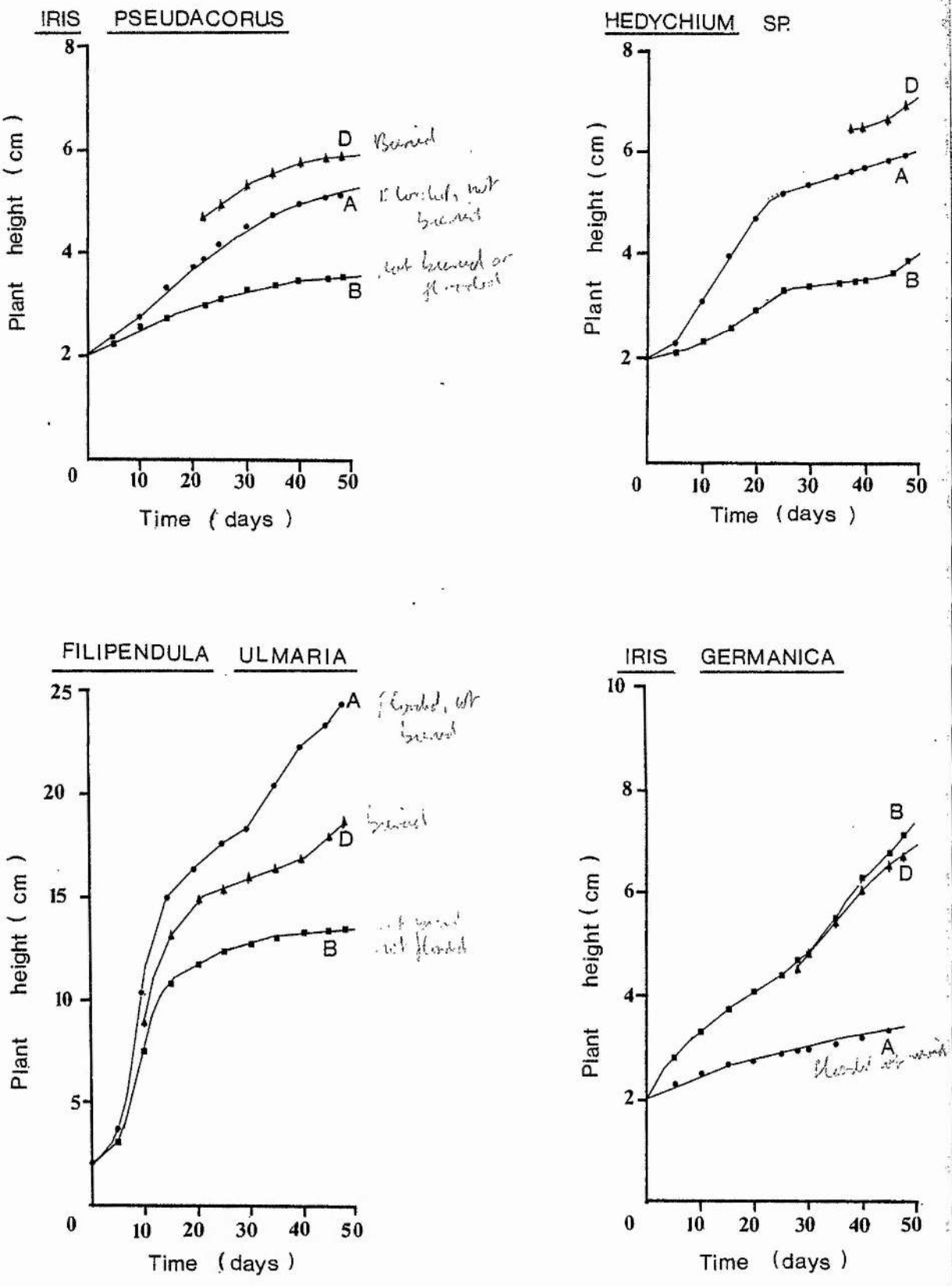
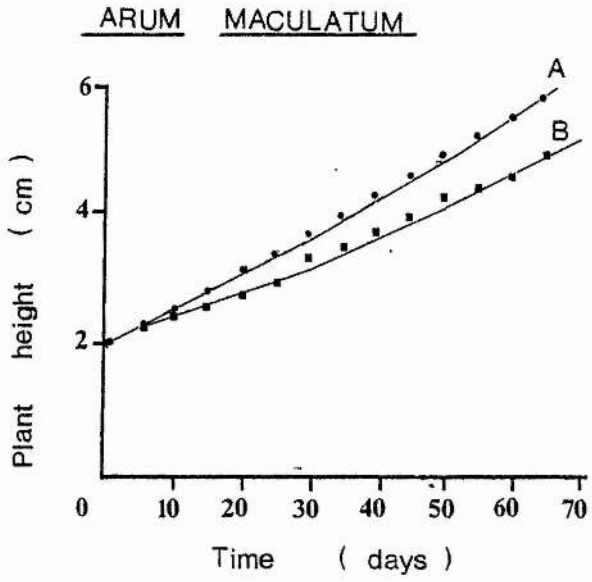
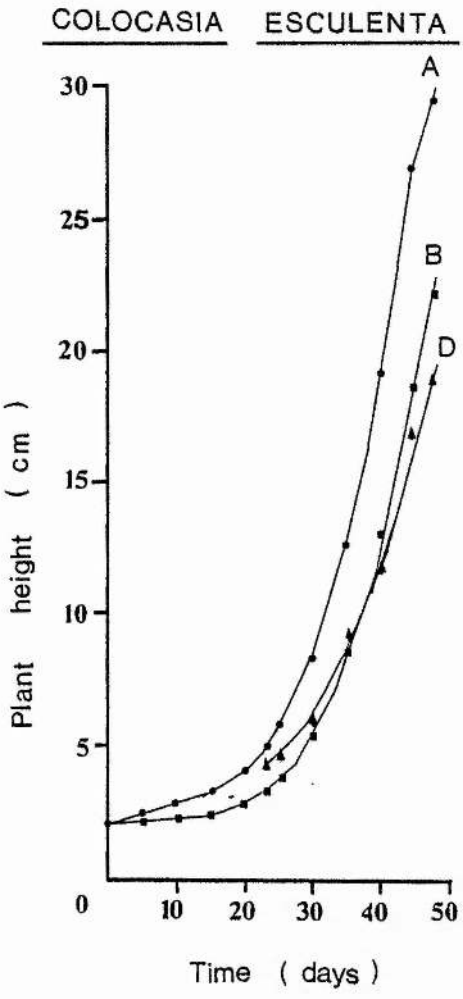


FIGURE 4.1 :

The growth curve of rhizomatous species under flooding and/or burial treatments. Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.





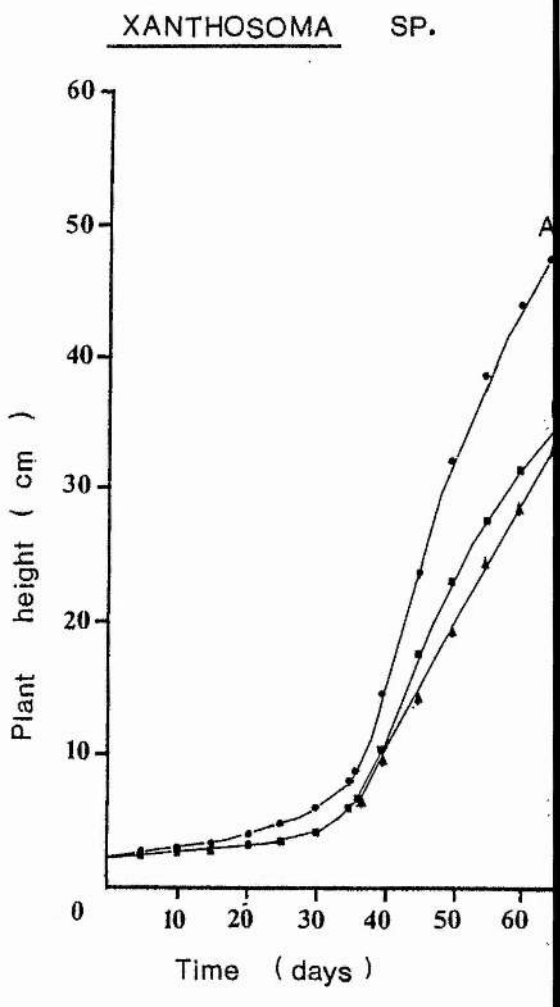
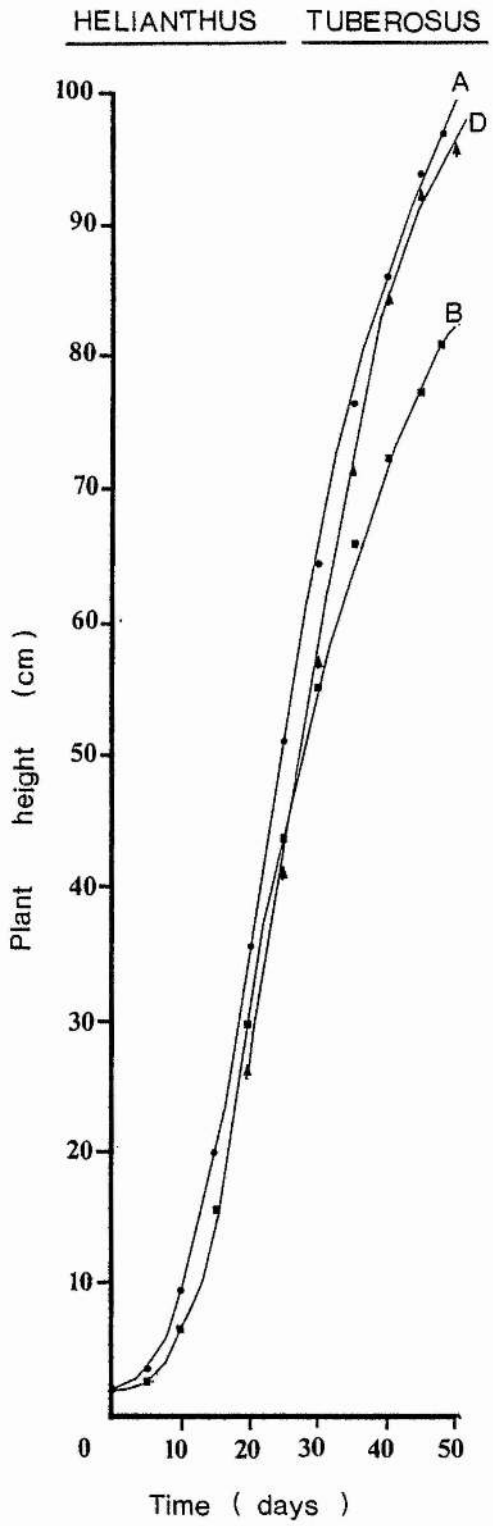
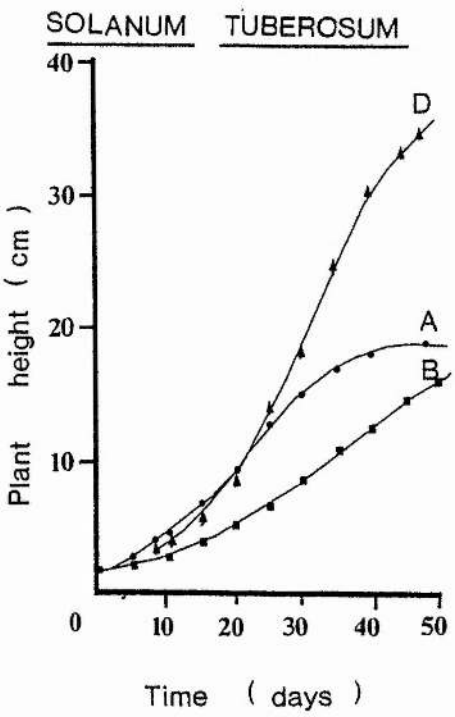


FIGURE 4.2 :

The growth curve of corm species under flooding and/or burial treatments. Details of experiment :
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.





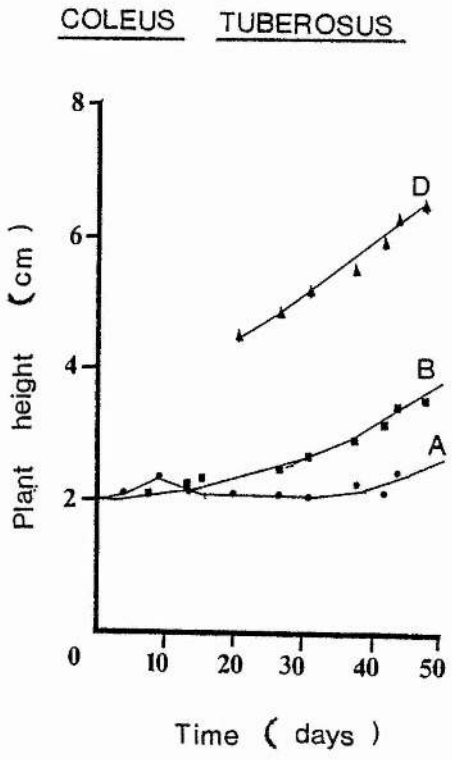


FIGURE 4.3 :

The growth curve of tuber producing plants under flooding and/or burial treatments.Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

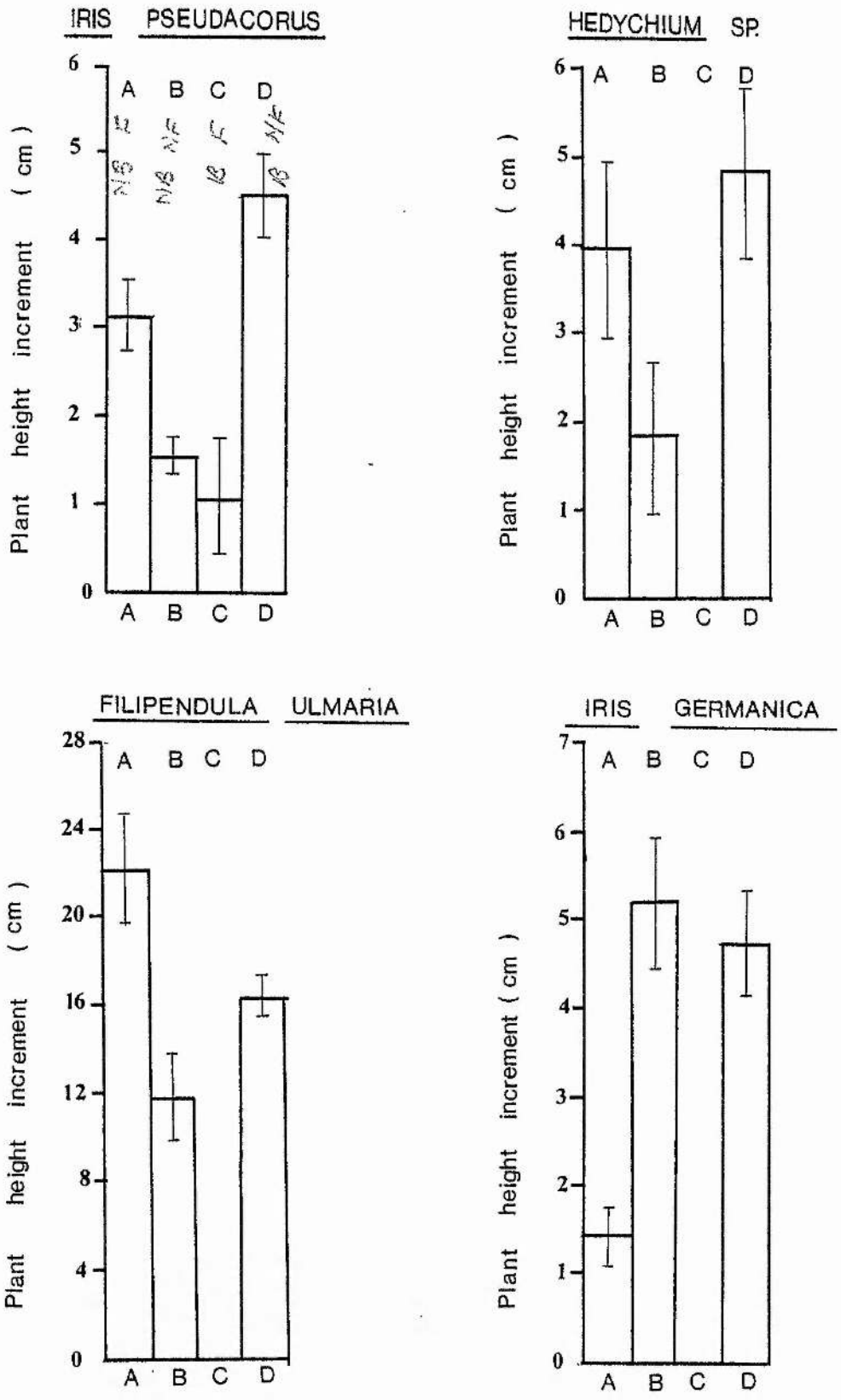


FIGURE 4.4 :

The increase in maximum plant height after flooding and/or burial treatments (see Appendix I for duration of experiments) of rhizomatous plants. Detail of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

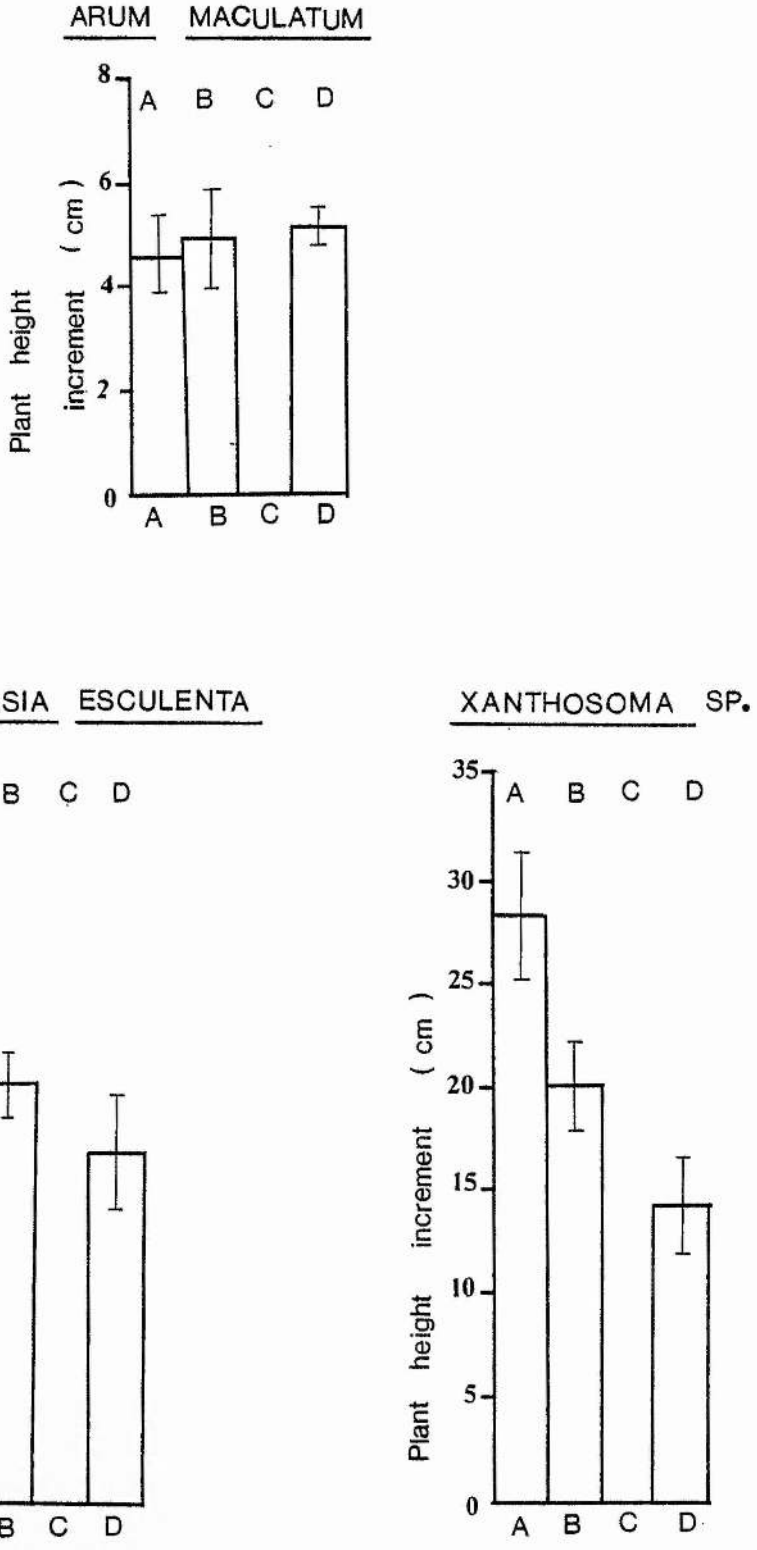


FIGURE 4.5 :

The increase in maximum plant height after flooding and/or burial treatments of corm species (see Appendix I for duration of experiments).
Details of experiment:
A = Not buried and flooded.
B = Not buried and not flooded.
C = Buried and flooded.
D = Buried and not flooded.

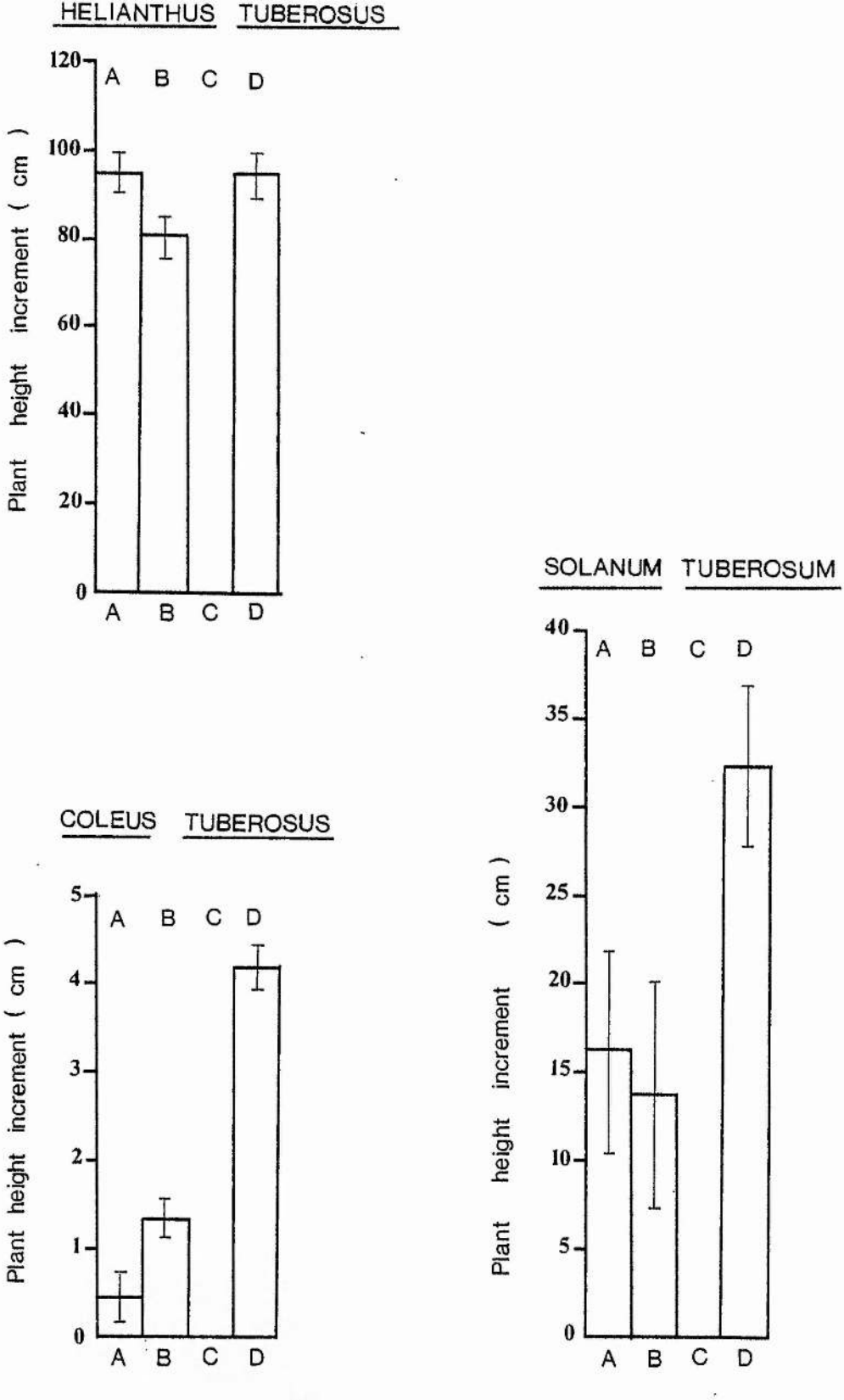


FIGURE 4.6 :

The increase in maximum plant height after flooding and/or burial treatments of tuber producing plants (see Appendix I for duration of experiments).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 4.1a : Statistical analysis of plant height in relation to flooding and/or burial of rhizomes, corms and tubers.

I. The effect of flooding.

	Not buried	Buried
i. Rhizome species.		
Iris pseudacorus	+ (***)	- (***)
Hedychium sp.	+ (***)	- (***)
Filipendula ulmaria	+ (***)	- (***)
Iris germanica	- (***)	- (***)
ii. Corm species.		
Colocasia esculenta	+ (***)	- (***)
Xanthosoma sp.	+ (***)	- (***)
Arum maculatum(I)	N.S.	- (***)
iii. Tuber species.		
Helianthus tuberosus	+ (***)	- (***)
Solanum tuberosum	N.S.	- (***)
Coleus tuberosus	- (***)	- (***)

Keynote;

+ = increase
- = decrease
N.S. = no significance changes occured.
*** = significance at 0.1% level .
** = significance at 1% level.
* = significance at 5% level.

Table 4.1b : Statistical analysis of plant height in relation to flooding and/or burial of rhizomes, corms and tubers.

II. The effect of burial.

	Not flooded	Flooded
i. Rhizome species.		
Iris pseudacorus	+ (***)	- (***)
Hedychium sp.	+ (***)	- (***)
Filipendula ulmaria	+ (***)	- (***)
Iris germanica	N.S.	- (***)
ii. Corm species.		
Colocasia esculenta	N.S.	- (***)
Xanthosoma sp.	- (***)	- (***)
Arum maculatum (I)	N.S.	- (***)
iii. Tuber species.		
Helianthus tuberosus	+ (***)	- (***)
Solanum tuberosum	+ (***)	- (***)
Coleus tuberosus	+ (***)	- (***)

Keynote;

See legend under Table 4.1a.

Table 4.1c : Interaction of flooding with burial factor on plant height of rhizomes, corms and tubers. The interaction is studied from both flooding (not buried versus buried) and burial (not flooded versus flooded) factors.

	FLOODING FACTOR	BURIAL FACTOR
i. Rhizome species.		
Iris pseudacorus	✓	✓
Hedychium sp.	✓	✓
Filipendula ulmaria	✓	✓
Iris germanica	X	✓ (N.S.P.)
ii. Corm species.		
Colocasia esculenta	✓	X (N.S.P.)
Xanthosoma sp.	✓	X
Arum maculatum (I)	✓ (N.S.P.)	✓ (N.S.P.)
iii. Tuber species.		
Helianthus tuberosus	✓	✓
Solanum tuberosum	✓ (N.S.P.)	✓
Coleus tuberosus	X	✓

Keynote;

✓ ; interaction between burial and flooding occured.

X ; no interaction , the factor concerned gave the most effect when both factors occured together (treatment C -- buried and flooded).

(N.S.P.) ; the interaction or no interaction cannot be statistically proved because of a non-significant effect of one side (or both sides) of the tests. (Buried versus not buried -- Table 4.1a and Flooded versus not flooded -- Table 4.1b).

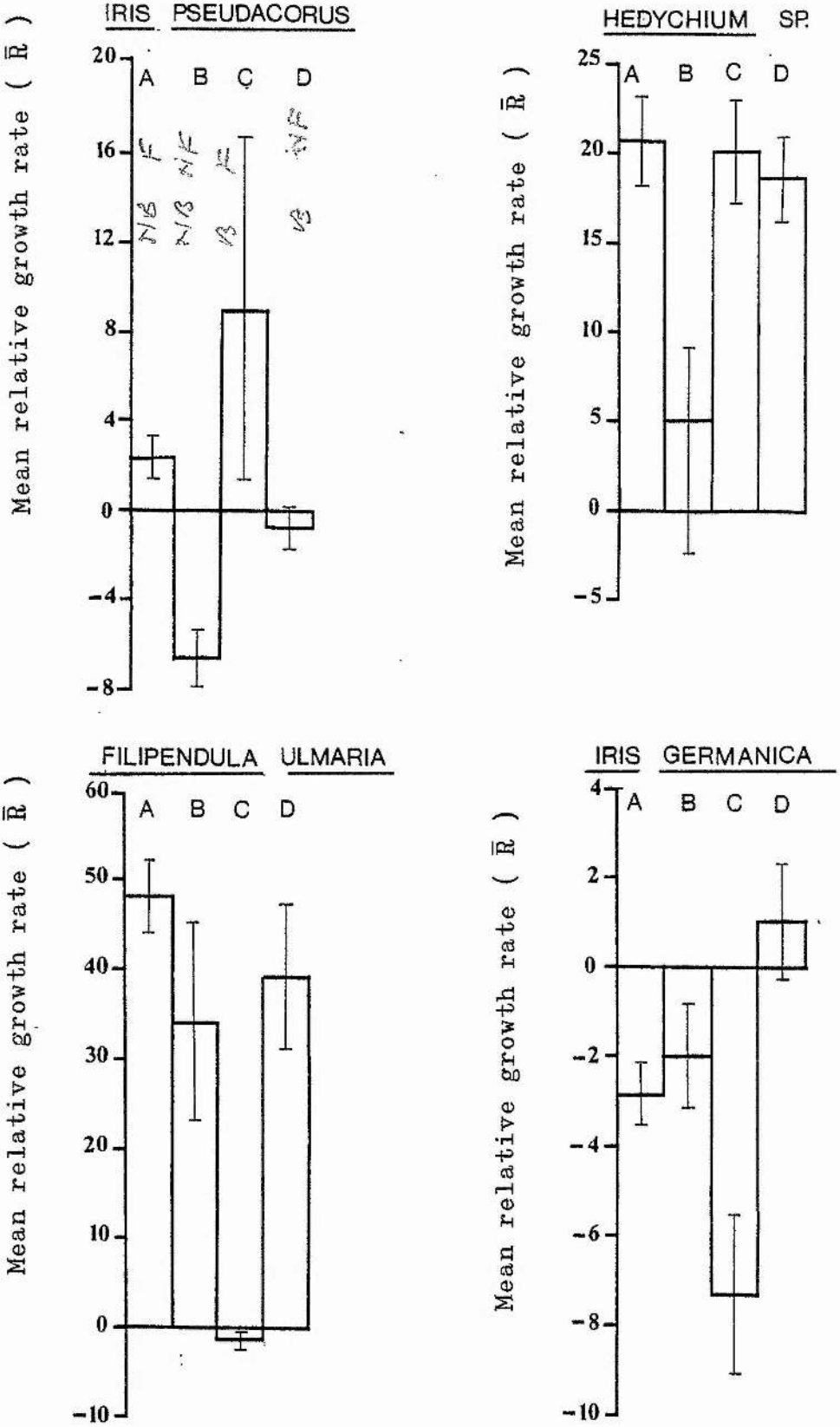
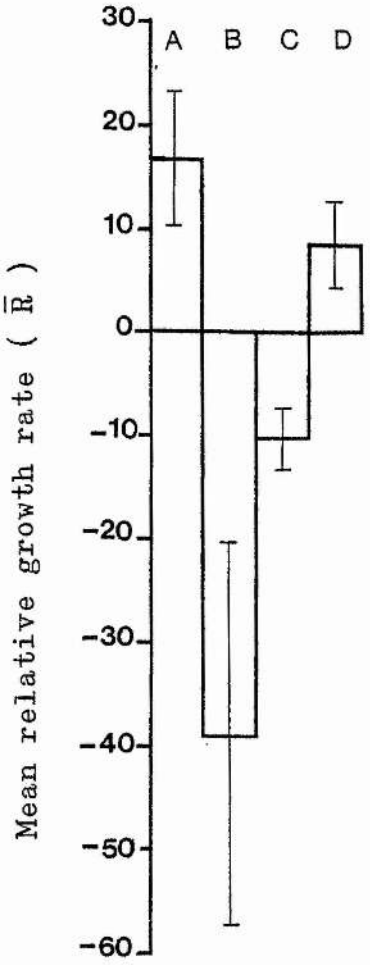


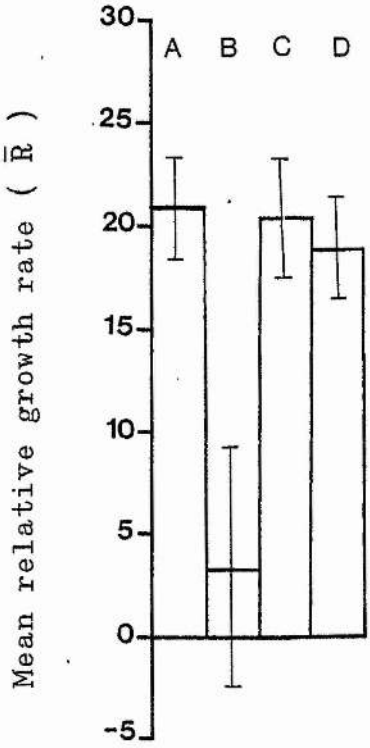
FIGURE 4.7 :

The mean relative growth rate ($\bar{R}$) of rhizomatous plants. Results are expressed as day^{-1} ($\times 10^{-4}$ day^{-1}). Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.



a) With shoot growth (bud) Experiment II(Appendix I).



b) With no shoot growth (rhizome). (Experiment I in Appendix I).

FIGURE 4.8 :

The mean relative growth rate ($\bar{R}$) of Hedychium sp. (x 10^{-4} day^{-1}) ; a rhizomatous species. In(a) young plants (hence called Bud) are studied whereas in (b) mature rhizomes are studied (hence called rhizomes). In (b), growth was observed in the form of healthy axial buds.
Details of experiment:
A = Not buried and flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

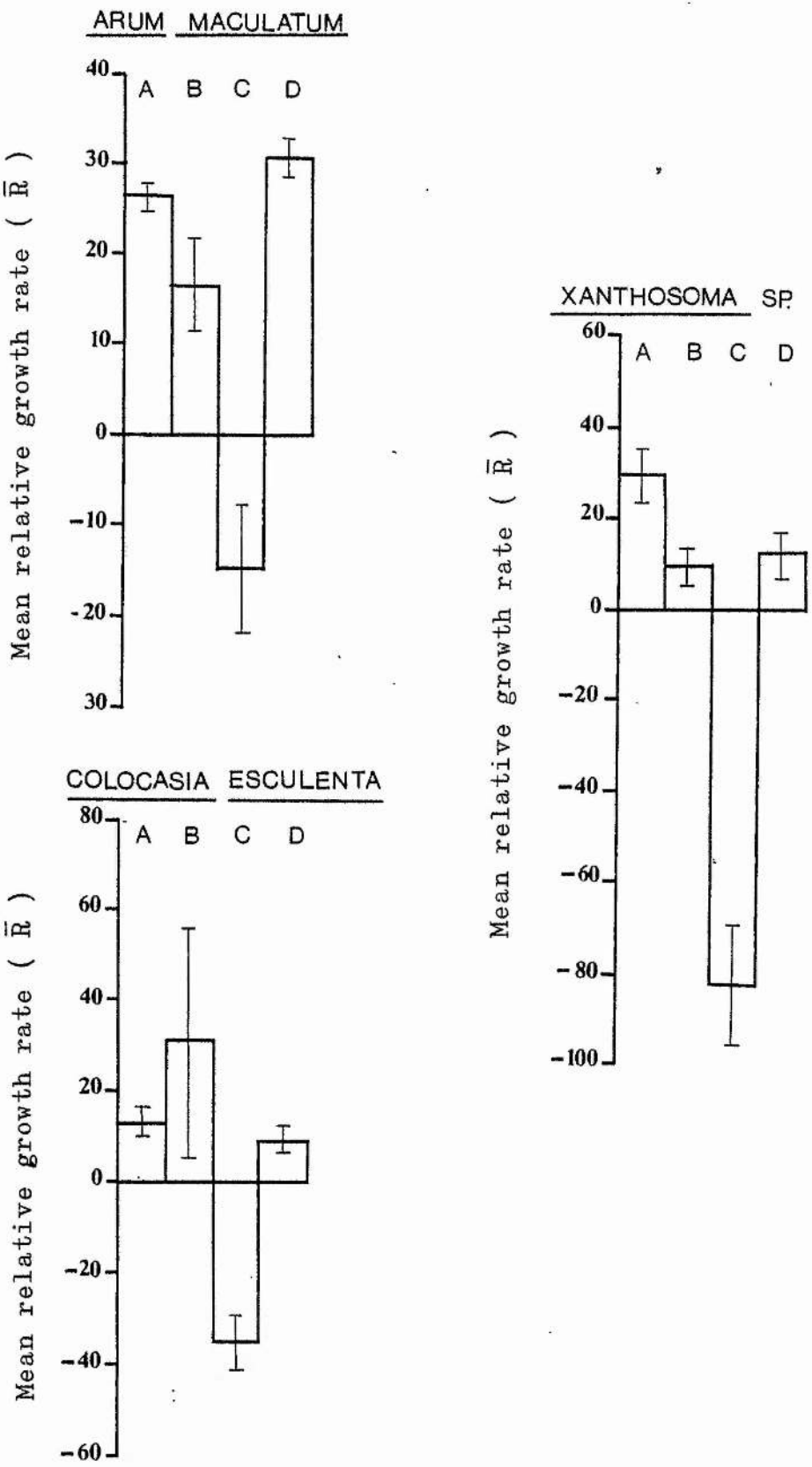
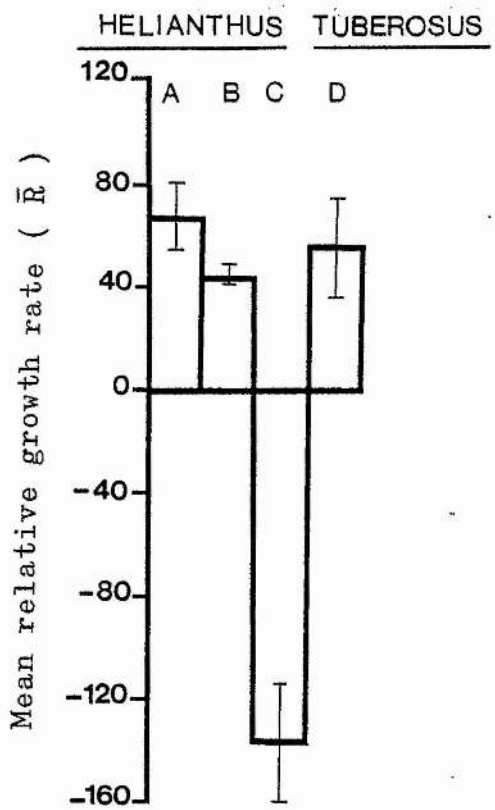
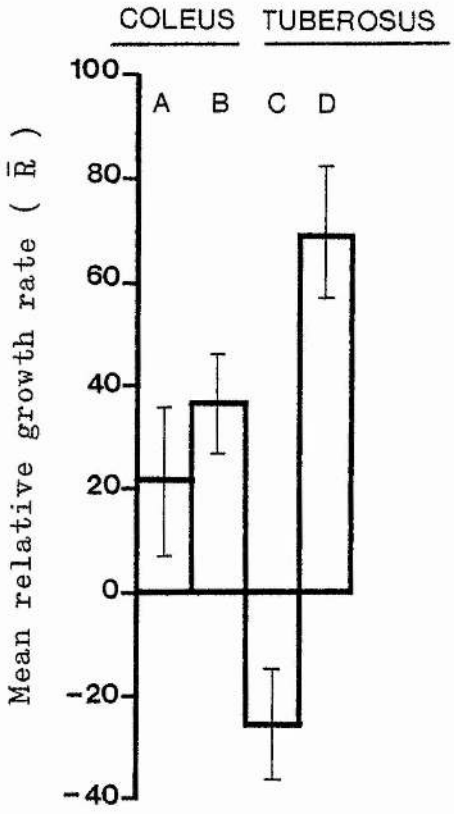


FIGURE 4.9 :

The mean relative growth rate ($\bar{R}$) of corm species (x 10^{-4} day^{-1}). Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.





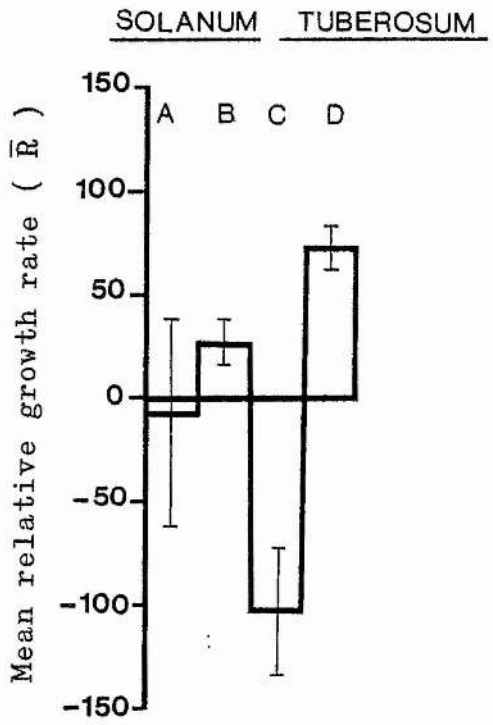


FIGURE 4.10 :

The mean relative growth rate ($\bar{R}$) of tuber species (x 10^{-4} day^{-1}). Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and flooded.
D = Buried and Not flooded.

Table 4.2a : Statistical analysis of mean relative growth rate per day ($\bar{R}$) in relation to flooding and/or burial of rhizomes, corms and tubers.

I. The effect of flooding.

	Not buried	Buried
i. Rhizome species.		
Iris pseudacorus	+ (***)	+ (***)
Hedychium sp.		
a) rhizomes (I)	+ (***)	N.S.
b) buds (II)	+ (***)	- (*)
Filipendula ulmaria	+ (***)	- (***)
Iris germanica	N.S.	- (***)
ii. Corm species.		
Colocasia esculenta	N.S.	- (*)
Xanthosoma sp.	+ (***)	- (***)
Arum maculatum (I)	+ (***)	- (***)
iii. Tuber species.		
Helianthus tuberosus	+ (*)	- (***)
Solanum tuberosum	N.S.	- (***)
Coleus tuberosus	N.S.	- (***)

Keynote;

See legend under Table 4.1a.

(I) = Experiment I

(II) = Experiment II , see Appendix I.

Table 4.2b : Statistical analysis of mean relative growth rate per day ($\bar{R}$) in relation to flooding and/or burial oof rhizomes, corms and tubers.

II. The effect of burial.

	Not flooded	Flooded
i. Rhizome species.		
Iris pseudacorus	N.S.	+ (*)
Hedychium sp.		
a) rhizomes (I)	+ (***)	N.S.
b) buds (II)	+ (***)	- (***)
Filipendula ulmaria	N.S.	- (***)
Iris germanica	+ (***)	- (***)
ii. Corm species.		
Colocasia esculenta	N.S.	- (***)
Xanthosoma sp.	N.S.	- (***)
Arum maculatum (I)	+ (***)	- (***)
iii. Tuber species.		
Helianthus tuberosus	N.S.	- (***)
Solanum tuberosum	N.S.	- (***)
Coleus tuberosus	+ (***)	- (***)

Keynote;

See legend under Table 4.1a.

(I) = Experiment I

(II) = Experiment II , see Appendix I.

Table 4.2c : Interaction of flooding with burial factor on mean relative growth rate per day ($\bar{R}$) of rhizomes, corms and tubers. The interaction is studied from both flooding (not buried versus buried) and burial (not flooded versus flooded) factors.

	FLOODING FACTOR	BURIAL FACTOR
i. Rhizome species.		
Iris pseudacorus	X	✓ (N.S.P.)
Hedychium sp.		
a) rhizomes (I)	✓ (N.S.P.)	✓ (N.S.P.)
b) buds (II)	✓	✓
Filipendula ulmaria	✓	✓ (N.S.P.)
Iris germanica	✓ (N.S.P.)	✓
ii. Corm species.		
Colocasia esculenta	✓ (N.S.P.)	✓ (N.S.P.)
Xanthosoma sp.	✓	✓ (N.S.P.)
Arum maculatum (I)	✓	✓
iii. Tuber species.		
Helianthus tuberosus	✓	✓ (N.S.P.)
Solanum tuberosum	✓ (N.S.P.)	✓ (N.S.P.)
Coleus tuberosus	✓ (N.S.P.)	✓

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See legend under Table 4.1c.

(I) = Experiment I

(II) = Experiment II , see Appendix I.

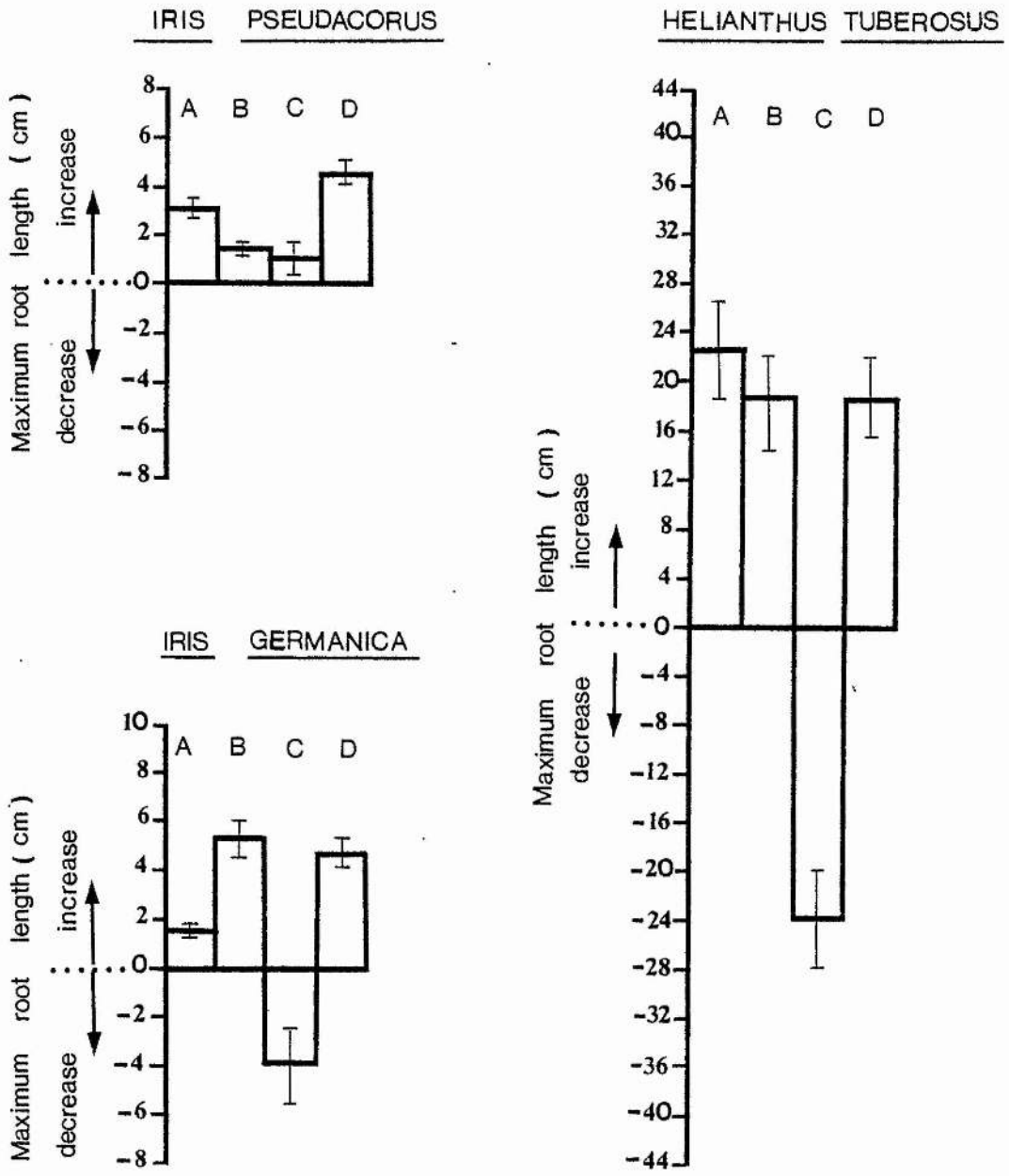

FIGURE 4.11 :

The increase or decrease in maximum root length (cm.) after flooding and/or burial treatments (see Appendix I for duration of experiment). Iris germanica and Iris pseudacorus are rhizomatous plants whilst Helianthus tuberosus is a tuber producing plant. Details of experiment are shown in legend under Figure 4.10.

Table 4.3a : Statistical analysis of root growth in relation to flooding and/or burial of rhizomes, corms and tubers.

I. The effect of flooding.

	Not buried	Buried.
i. Rhizome species.		
Iris pseudacorus	+ (***)	- (***)
Hedychium sp.	N.S.	- (*)
Iris germanica	- (***)	- (***)
ii. Corm species.		
Arum maculatum		
a) 7 weeks treatment (II)	+ (***)	N.S.
b) 14 weeks treatment (I)	+ (*)	- (***)
iii. Tuber species.		
Helianthus tuberosus	N.S.	- (***)

Keynote;

See legend under Table 4.1a.

Table 4.3b : Statistical analysis of root growth in relation to flooding and/or burial of rhizomes, corms and tubers.

II. The effect of burial.

	Not flooded	Flooded
i. Rhizome species.		
Iris pseudacorus	+ (***)	- (***)
Hedychium sp.	+ (***)	N.S.
Iris germanica	N.S.	- (***)
ii. Corm species		
Arum maculatum		
a) 7 weeks treatment (II)	+ (***)	N.S.
b) 14 weeks treatment (I)	+ (***)	- (***)
iii. Tuber species.		
Helianthus tuberosus	N.S.	N.S.

Keynote;

See legend under Table 4.1a.

Table 4.3c : Interaction of flooding with burial factor on root extension of rhizomes, corms and tubers. The interaction is studied from both flooding (not buried versus buried) and burial (flooded versus not flooded) factors.

	FLOODING-FACTOR	BURIAL FACTOR
i. Rhizome species.		
Iris pseudacorus	✓	✓
Hedychium sp.	✓ (N.S.P.)	✓ (N.S.P.)
Iris germanica	✓	✓ (N.S.P.)
ii. Corm species.		
Arum maculatum		
a) 7 weeks treatment.	✓	✓
b) 14 weeks treatment.	✓ (N.S.P.)	✓ (N.S.P.)
iii. Tuber species.		
Helianthus tuberosus	✓ (N.S.P.)	X (N.S.P.)

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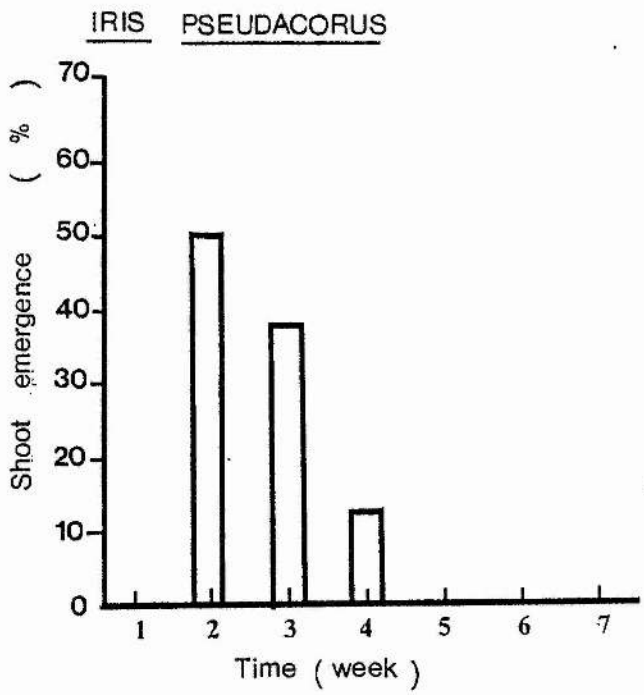
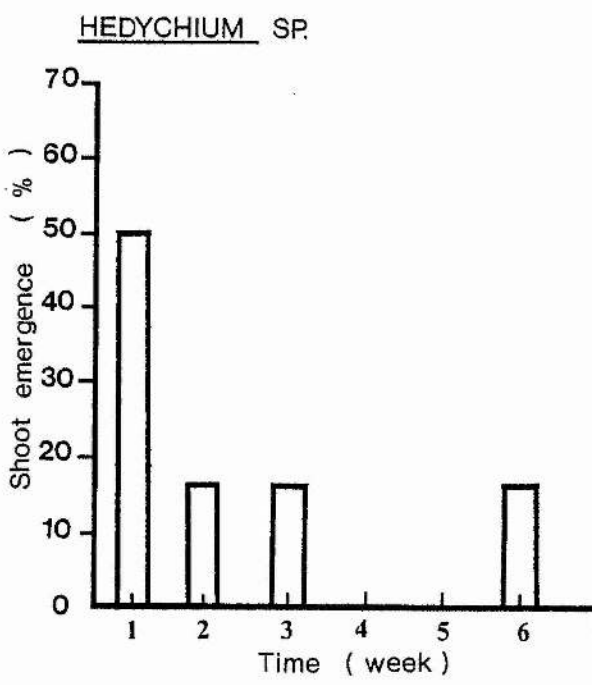
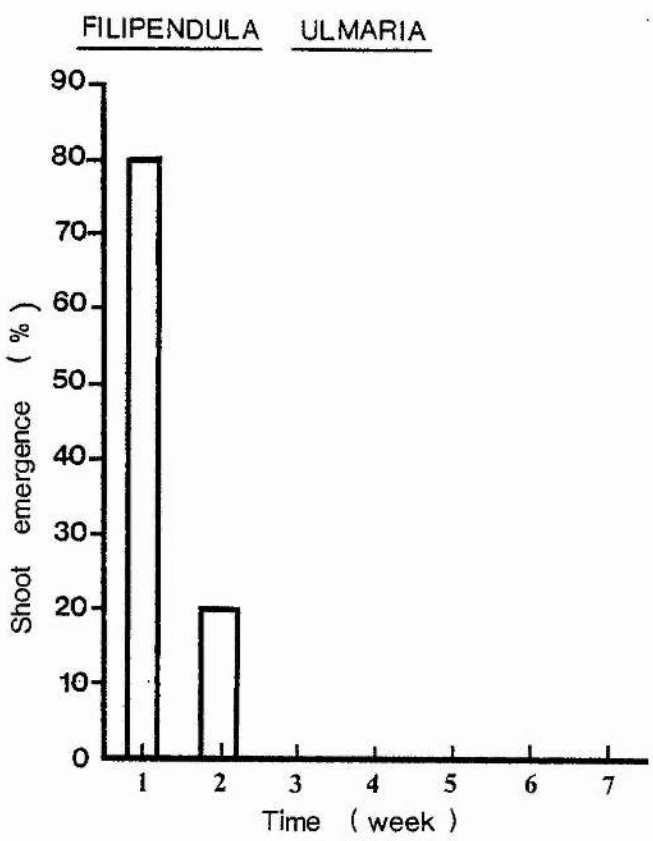
See legend under Table 4.1c.







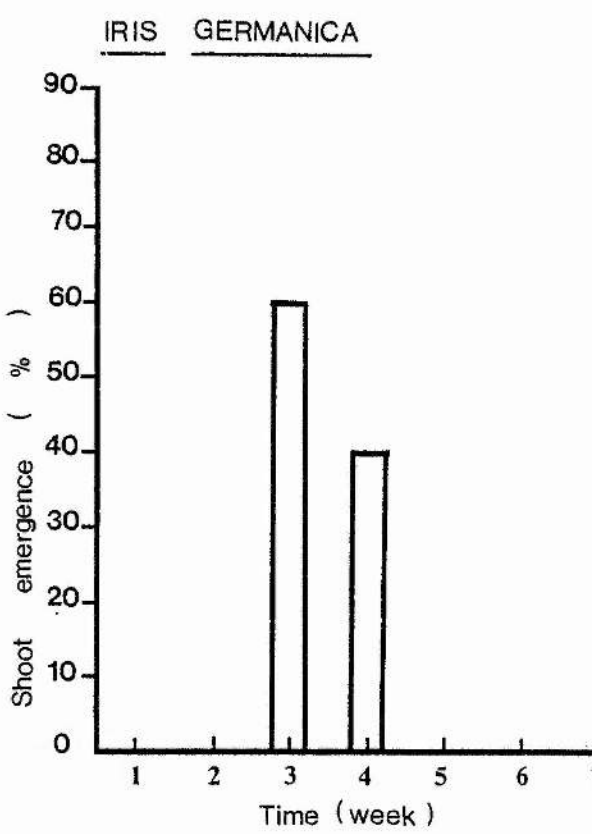
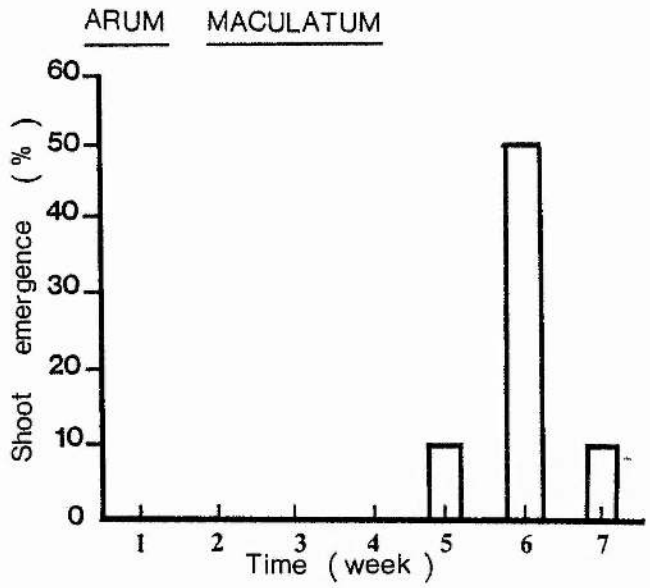
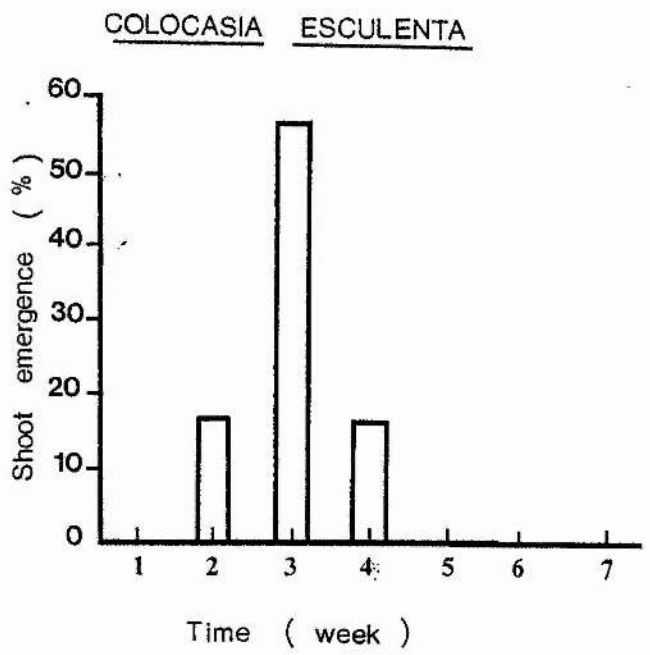


FIGURE 4.12 :

Emergence of shoot in treatment D (Buried and Not flooded) of rhizomatous plants studied. All the plants (100%) emerged during 7 weeks treatment.





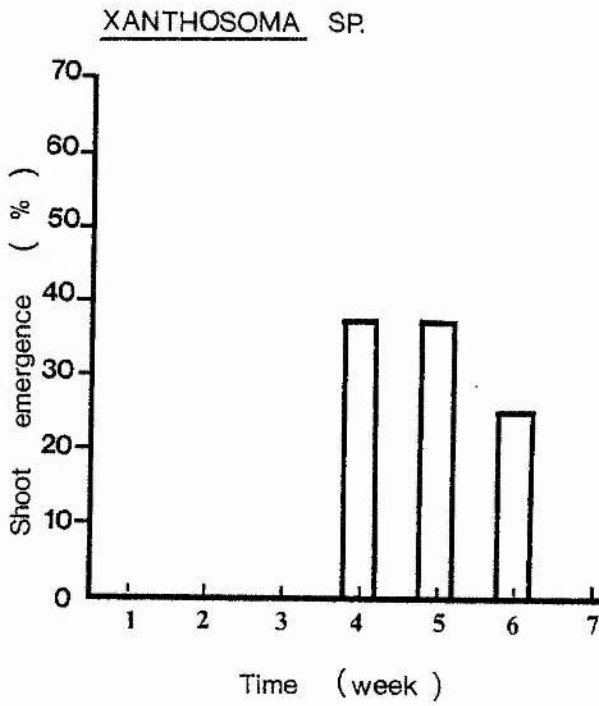
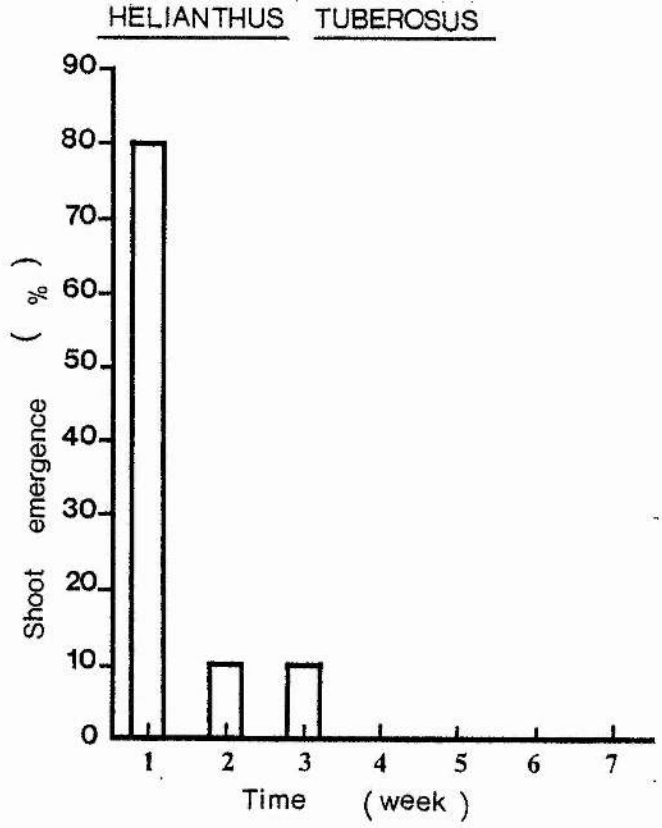
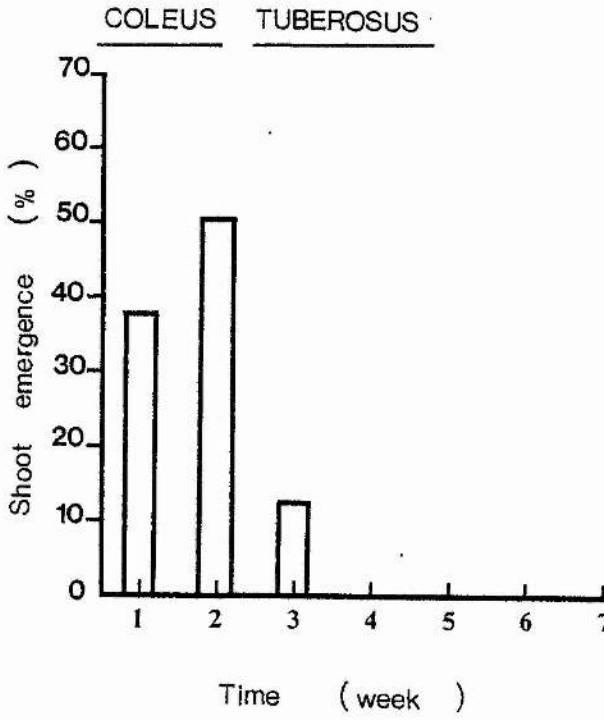


FIGURE 4.13 :

Emergence of shoot in treatment D (Buried and Not flooded) of corm species studied. Except in Arum maculatum, others showed a total emergence during seven weeks duration.





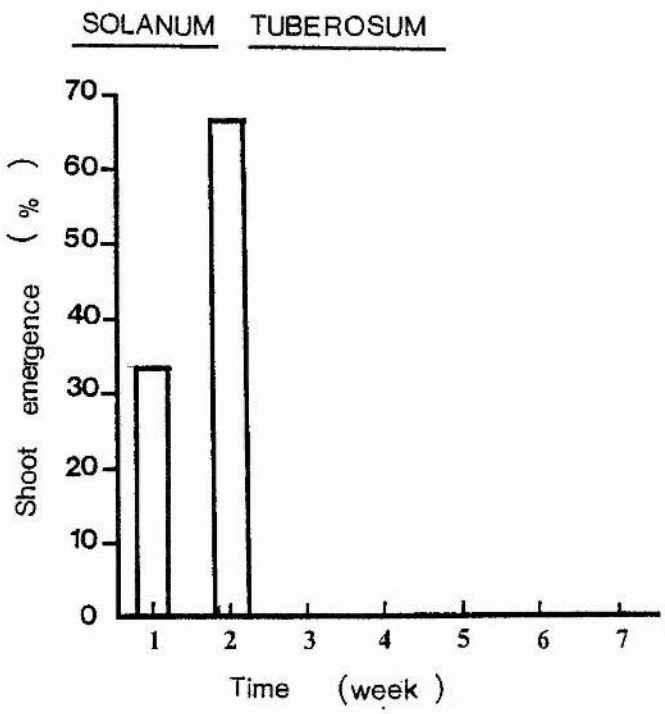


FIGURE 4.14 :

Emergence of shoot in treatment D (Buried and Not flooded) of tuber species studied. Total emergence was shown in all species during 7 weeks treatment.

PLATE 4.1 :

The emergence of Iris pseudacorus above the sand and water levels in treatment C (Buried and Flooded or total submergence). Arrowed is a terminal shoot. Note a vigorous growth of bud on the right.

CHAPTER 5 :

CONTENTS

Chapter 5

SUGAR LEVELS AND SURVIVAL OF PLANT STORAGE TISSUES AS AFFECTED BY FLOODING AND BURIAL.

5.1 INTRODUCTION.

As wetland habitats are characterised by flooded, waterlogged and anaerobic soils, they are therefore a difficult land for crop cultivation. However,apart from some of the starch- producing crop plants (Plucknett, 1978), a large number of wild species which also possess underground storage organs also exist in this environment (Laing, 1940). It was recognized that these wild plants with rhizomatous (and cormous, author) organs were amongst the most tolerant plants of anaerobiosis (Barclay and Crawford, 1982; Monk et al., 1984).

Vartapetian et al. (1977) advanced a hypothesis that carbon starvation rather than oxygen starvation is the immediate factor responsible for the injury of mesophytic root cells during anaerobiosis. Their results indicated that tolerance to anaerobiosis can be significantly elevated in both mesophytic (pumpkin) and hydrophytic (rice) roots by the application of exogenous glucose fed to these roots. Hence their findings demonstrated that carbon compounds played an important role in the resistance of root cells during oxygen-deficit periods.

Anaerobiosis did not cause any significant effects on the carbohydrate levels in Scirpus maritimus, an extremely tolerant rhizomatous wetland species which can grow under strict anoxia. In Phalaris arundinacea which can resume healthy aerobic growth after seven days anoxia, no alteration of glucose and sucrose concentrations was observed in response to anaerobic treatment. However, a large decrease in raffinose concentration was observed, balanced by an equally large increase in fructose levels. On the other hand, in Glyceria maxima, the rapid depletion of carbohydrate was the most striking effect of anaerobiosis on this least tolerant plant species. After only four days anaerobiosis under an atmosphere of 85% nitrogen , 10% hydrogen and 5% carbon dioxide at 22^{o}C ; sucrose, raffinose and total non-structural carbohydrates concentrations of this species were significantly ($p < 0.001$) reduced (Barclay and Crawford, 1983). Fifteen days anoxia in a stream of oxygen-free- nitrogen did not bring about any significant differences in the amounts of reducing sugars (fructose,glucose and total reducing sugars) in the five wetland species studied (Acorus calamus, Iris pseudacorus, Phragmites australis, Typha latifolia and Shoenoplectus lacustris). With the exception of A. calamus, no significant reductions in sucrose content were observed. In this species, a slight accumulation of sucrose was found. Nevertheless, in Iris germanica -- a non-wetland species -- the concentrations of reducing sugars and sucrose were found not to be affected by anaerobiosis (Monk et al.,1984). In potato, a crop plant susceptible to flooding, incubation under a nitrogen

gas stream caused sucrose content to fall followed by its eventual complete disappearance. In contrast, the hexose sugars showed either little change or even increased in amount (Barker and el Saifi, 1953). A large increase in invert sugars (Khanna and Chacravarti, 1949) and a continuous fall in the sucrose content in sugar-cane juice, together with the drop in percentage of sugar in cane was among the adverse effects of prolonged flooding on this crop plants (Rege and Mascarenhas, 1956).

The flooding and/or burial treatments in the present study were carried out after plants were subjected to partial defoliation. Following defoliation, water-soluble carbohydrates (glucose, fructose, sucrose and fructosan) of roots and stubble decreased rapidly. Plants placed under shade also showed a decrease in carbohydrate reserves in the rhizomes as the carbohydrate produced by the plant was utilised in the production of top growth (Watkins, 1940). In the normal light (not shaded), sufficient photosynthetic tissue was produced to enable carbohydrates to be manufactured in excess of those required by current growth. Hence, following defoliation plants placed in darkness showed the same initial changes as those in the light. However, in the dark (such as in the continuous burial) the concentrations of soluble carbohydrates continued to decline almost to the point of exhaustion (Sullivan and Sprague, 1943; Sprague and Sullivan, 1950).

Different anoxic treatments were shown to give different results. On the other hand , exogenous glucose feeding to root cannot match internal controlled supply from reserved carbohydrates as seen in plant storage organs. With that in mind, flooding treatments were applied directly to assess flooding tolerance in the rhizomatous plants.

5.2 MATERIALS AND METHODS.

5.2.1 Sugar Analysis.

The sugar analysis was performed on pieces of rhizomes from prolonged flooding-burial treatments (Section 2.1).

5.2.1.1 Plant extraction.

Plant materials used were first killed in liquid nitrogen and finely ground by pestle and mortar before freeze drying to 0.05 torr in the freeze drier (Edwards High Vacuum Ltd.). About 100 mg. of these tissues were extracted three times in 80% ethanol at 20^{o}C for five minutes each. This was followed by another three extractions in 60% ethanol at the same temperature, maintained by a water bath.

5.2.1.2 Evaporation of plant extracts.

The combined extracts were evaporated to dryness *in vacuo* in the Vortex evaporimeter (Searle Analytic Inc., New Jersey, USA) at 50°C. It was dried further in the freeze drier and finally stored in the desiccator over $CaCl_2$ at -20°C until required.

5.2.1.3 Gas-liquid chromatography preparation.

Each dried sugar extract was redissolved in one ml. dimethyl sulphoxide, DMSO (Sigma Chemical Co.) by shaking vigorously on a mechanical shaker. Exactly 0.2 ml. of this aliquot were mixed in a cherry flask with the same amount of hexamethyldisilazine, HMDS (BDH Chemical Ltd., Poole, England), 0.1 ml. of internal standard and 0.1 ml. Trimethyl chlorosilane, TMS (BDH Chemicals Ltd., England). It was then stoppered against water vapour contamination and shaken vigorously for 90 seconds. The flask was left standing between three hours and overnight. Two phases appeared, DMSO in the lower layer and sugar derivatives in the upper phase, which was analysed. Sugars standard mixture were treated in the same way. One µl. of sugar or standard derivatives were injected into the heated column (see section 2.4.2.).

5.2.2 Test of carbohydrate reserves .

5.2.2.1 Starch.

Fresh longitudinal sections of rhizomes were cut free-hand and stained with an iodine solution (0.3 gm. iodine + 1.5 gm. IKI in 100 cc water, Johansen,1940) to ascertain the presence of starch grains. In the presence of starch dark blue coloration was observed.

5.2.2.2 Fructosan.

The rhizome tissues were macerated with water followed by precipitating the aqueous liquid with alcohol. Fructosans are soluble in cold water but not in alcohol (Archbold, 1940).

5.3 RESULTS.

In these experiments only the rhizomatous species were studied for example: Iris pseudacorus, Hedychium sp., Filipendula ulmaria and Iris germanica (see Appendix I). This was because the above rhizomes were the only underground storage organs that had been found to be firm (except part of Iris germanica rhizomes) after seven weeks burial and flooding (treatment C).

This treatment was the most damaging of all. Among these four species, there was shoot growth in all the treatments except in Iris germanica and F. ulmaria where no shoot growth was observed after seven weeks burial and flooding (treatment C).

5.3.1 STORED CARBOHYDRATES (Table 5.1).

Fructosan and starch were found to accumulate in the rhizomes of F. ulmaria and I. germanica. In I. pseudacorus only fructosan was stored whilst starch was the only non-structural carbohydrate stored in the rhizomes of Hedychium sp., a tropical plant from the Zingiberaceae family, and in Iris germanica, a temperate garden plant.

5.3.2 SUGAR LEVELS.

(Table 5.2 and Figure 5.1 ,5.2, 5.3 and 5.4).

The total sugar levels were the highest in Iris germanica (more than 100 mg. $gm.^{-1}$ dry weight) in treatment B (not buried and not flooded) and treatment D (buried and not flooded). It was followed by Filipendula ulmaria in treatment A (more than 70 mg. $gm.^{-1}$ dry weight, excluding sugar Z, Table 5.2). In Iris pseudacorus, nearly 60 mg. $gm.^{-1}$ dry weight of total sugar (excluding sugar Z) was observed in treatment A (not buried and flooded) and B (not buried and not flooded). Finally, Hedychium

sp. which has the thinnest rhizomes contains the least amount of sugar; nearly 50 mg. $gm.^{-1}$ dry weight in treatment A and D. Sugar Z was an unidentified sugar, of four carbon structure, most likely to be erythritol (Retention time 0.43,relative to sorbitol).

The total of three major sugars (fructose, glucose and sucrose) was also the highest in Iris germanica (more than 70 mg. $gm.^{-1}$ dry weight in treatment B and D, Table 5.3), followed by Iris pseudacorus and Filipendula ulmaria (more than 65 mg. $gm.^{-1}$ dry weight) in treatment A. In Hedychium sp., about 45 mg. $gm.^{-1}$ dry weight of fructose plus glucose plus sucrose were accumulated in treatment A and D.

The largest proportion of sugars accumulated was in the form of fructose in Iris pseudacorus. In Hedychium sp. and F. ulmaria it was sucrose whereas in Iris germanica more raffinose was detected than sucrose.

More than 95% of total sugars was accumulated (Table 5.4) in the form of fructose plus glucose plus sucrose in all the treatments in Iris pseudacorus. In Hedychium sp., nearly all the sugars were accumulated in the form of these three main sugars except in treatment C. In this treatment it was only 90% of total sugars. About 80% of total sugars found in F. ulmaria were fructose, glucose and sucrose in treatment A, B and D. However, in treatment C it was only 30%. In Iris germanica, treatment C has the highest proportion of these three major sugars; that is

more than 90% whereas it was only between 65 to 75% in treatment A, B and D, the reverse from all the other species.

5.3.3 THE FLOODING AND/OR BURIAL EFFECT ON SUGAR CONTENT.

5.3.3.1 Fructose content (Figure 5.5).

The flooding factor has no significant effect on fructose content of *Iris pseudacorus*, *Hedychium* sp. and *Filipendula ulmaria* rhizomes when not buried (Table 5.5a). In *Iris germanica* the above condition will cause a significant ($p < 0.001$) decrease. When buried, except in *I. pseudacorus*, flooding can significantly reduce fructose content in all the species studied.

The burial factor did not significantly alter fructose content in *Iris pseudacorus*, *Hedychium* sp. and *Iris germanica* whether under flooding or not. However, a significant ($p < 0.001$) rise and fall while not flooded and flooded, respectively, was seen in *Filipendula ulmaria*.

When both factors occurred together (for example in treatment C), both burial and flooding factors contributed to the decrease in fructose content in *Filipendula ulmaria*. In *Iris germanica* it was the flooding factor which gave the most effect

(Table 5.5b).

5.3.3.2 Glucose content (Figure 5.6).

The flooding factor has no significant effect when not buried on glucose content of Iris pseudacorus and Hedychium sp. (Table 5.6a). In F. ulmaria there was a significant ($p < 0.001$) increase whilst in Iris germanica a significant decrease. When buried, flooding caused a significant ($p < 0.001$) decrease in glucose content of Iris pseudacorus, F. ulmaria and Iris germanica rhizomes, yet no significance effect ($p > 0.05$) was seen on Hedychium sp.

Burial when not flooded caused a significant ($P < 0.05$) reduction in glucose content of I. pseudacorus, a significant ($p < 0.001$) increase in F. ulmaria but no significance changes in Hedychium sp. and I. germanica. When flooded, the burial factor can significantly ($p < 0.001$) reduce glucose content of Iris pseudacorus and F. ulmaria. However, no significant change was observed in Hedychium sp. and I. germanica.

Whenever burial and flooding factors occurred together it was the burial factor which gave the greatest effect in reducing glucose content in Iris pseudacorus, whereas in I. germanica it was the flooding factor. Both these factors interacted in F. ulmaria in reducing the glucose content (Table 5.6b).

5.3.3.3 Sucrose content (Figure 5.7).

Flooding when not buried gave no significant effect on sucrose content of Iris pseudacorus and Iris germanica (Table 5.7a). In Hedychium sp. and F. ulmaria however, this factor caused sucrose content to rise significantly ($p < 0.05$ and $p < 0.001$, respectively). When buried, a significant rise ($p < 0.01$) was observed in I. pseudacorus, but in the other three species there was a significant reduction of sucrose content.

The burial factor gave no significant effect on sucrose content in I. pseudacorus and Hedychium sp. when not flooded. There was a significant ($p < 0.001$) drop in F. ulmaria whereas in I. germanica a significant rise ($p < 0.01$) was observed. When flooded, all the species showed a decrease ($P < 0.001$) except in I. pseudacorus where a rise of sucrose content was observed ($p < 0.001$).

Both burial and flooding factors when operating together (for example in treatment C) interacted in giving the suppressed effect in Hedychium sp. and I. germanica. In F. ulmaria however, it was most likely that burial factor gave the most effect in depleting sucrose content.

5.3.3.4 Melibiose content (Figure 5.8).

Melibiose was not found in Iris germanica rhizomes. In Hedychium sp., flooding whether accompanied with burial or not caused no significance ($p > 0.05$) change (Table 5.8a). In I. pseudacorus and F. ulmaria, flooding when not buried caused melibiose level to increase ($p < 0.05$ and $p < 0.001$, respectively). When buried, no significant alteration was observed due to flooding factor.

Burial when not flooded gave no significant effect except in F. ulmaria. In this species, burial of flooded rhizomes can significantly reduce the melibiose content. When both factors occurred together (Table 5.8b), no significant interaction was observed.

5.3.3.5 Maltose content (Figure 5.9).

Maltose sugar was present only in the rhizome of Iris germanica. In this species, less than 2 mg. $gm.^{-1}$ dry weight of maltose was detected and it could be increased by flooding when the rhizomes were buried ($p < 0.001$).

5.3.3.6 Raffinose content (Figure 5.10).

In Iris germanica, there was a high amount of raffinose (more than 25 mg. gm.$^{-1}$ dry weight) in treatment A, B and D; as compared to other species which accumulated less than 2 mg. gm.$^{-1}$ dry weight. Raffinose was not detected in treatment B and D of Hedychium sp., but it was detected in treatment C (buried and flooded) and A (not buried and flooded). Higher raffinose content was observed in the former treatment than in the latter.

Flooding when not buried significantly ($p < 0.001$) increased raffinose content in Iris pseudacorus. In F. ulmaria and I. germanica, the increase was not significant. When buried, flooding caused a drop in raffinose content ($p < 0.001$).

Burial when not flooded significantly ($p < 0.001$) suppressed raffinose level of Iris pseudacorus whilst an increase ($p < 0.001$) was observed in F. ulmaria. In I. germanica the effect was a non-significant decrease. When flooded, raffinose content was found to be significantly depleted ($P < 0.001$) in the three species studied.

Whenever both factors (flooding and burial) were overlapping, it was most likely that burial factor gave the most effect in Iris pseudacorus. However interaction between flooding and burial could also possibly gave the severe effect observed (see comment in Table 5.10b). In F. ulmaria, both flooding and burial factors interacted in causing raffinose levels to decrease

and in I. germanica it was possible that the condition was identical. Hence in the latter species, the large variation in readings give the big standard errors and thus a non-significant statistical test (see also comment in Table 5.10 b).

5.3.3.7 Sorbitol content (Figure 5.11).

Sorbitol was not present in the rhizomes of E. ulmaria. In I. pseudacorus and I. germanica it was detected only in treatment C (buried and flooded) in a small quantity (about 1 mg $g.^{-1}$ dry weight). In Hedychium sp., more sorbitol was detected in the growing buds (in treatment B) than in the matured rhizomes. The Treatment B (not buried and not flooded) gave the highest level of sorbitol.

Flooding factor when not buried caused a significant decrease ($p < 0.05$) of sorbitol. In other conditions (Table 5.11 a,b) ,no significance effect was observed in Hedychium sp.

5.3.4 STORAGE FOOD CONTENT (Figure 5.12).

The `storage food content' was crudely measured as the percentage of dry weight over fresh weight of tissue chosen at random from each sample .

Flooding when not buried caused it to decrease significantly ($p < 0.001$) in Iris pseudacorus and to increase in Iris germanica. In Hedychium sp. and Filipendula ulmaria, no significant change was observed (Table 5.12a). When buried, flooding caused it to decrease ($p < 0.01$ and $p < 0.001$, respectively) in Hedychium sp. and F. ulmaria. However, in I. pseudacorus and I. germanica there was no significant change.

Burial when not flooded caused the 'storage food content' to decrease significantly ($p < 0.001$) in I. pseudacorus, whereas in the other species it was not significant. The effect of burial of flooded rhizomes of all the species studied was to decrease significantly the storage food content.

When both factors operating together, it was the burial factor which gave the greatest effect in reducing the storage food content of Iris pseudacorus. In the other species, it was possible (but not statistically proved significant) that both factors contributed to its depletion in Hedychium sp. and F. ulmaria (Table 5.12 b).

5.3.5 THE OCCURRENCE OF SUGAR Z (Figure 5.13).

Substance Z was the unknown sugar of four carbon structure possibly erythritol (Retention time 0.43, relative to sorbitol). It was found only in Filipendula ulmaria and Iris pseudacorus, two plants which normally grow in wet habitats. In both species, the largest quantity of sugar Z (up to 15% of total peak area) was found in treatment A (flooded and not buried) whilst the lowest quantity was found in treatment C (flooded and buried). In the buried/not buried and not flooded treatment (D and B), the treatment D (buried) gave a smaller value than treatment B (not buried). In F. ulmaria where vigorous shoot growth was observed in treatment A, sugar Z was accumulated in a much higher quantity than in I. pseudacorus where shoot growth was much slower.

5.3.6 MAJOR SUGARS OF RHIZOMES VERSUS BUDS IN HEDYCHIUM SP.

a) Fructose content.

In young rhizomes with growing shoots (buds), fructose content (Figure 5.14) was found to be higher than in mature rhizomes in treatment A and D. However in treatment B and C the trend was reversed.

b) Glucose content.

The trend was the same as for fructose.

c) Sucrose content.

Higher amounts of accumulated sucrose were observed in buds in treatment A only, as compared to matured rhizomes. In other treatments more sucrose was detected in the matured rhizomes except in treatment D where both types of rhizomes accumulated about the same amount of sucrose.

5.4 DISCUSSION

The four species; *Iris pseudacorus*, *Hedychium* sp., *Filipendula ulmaria* and *Iris germanica* differ in their ability to grow and also show differences in sugar response to flooding and/or burial treatment. The flooding factor alone will not cause any sugar reduction except for glucose and fructose contents of *Iris germanica* rhizome and sorbitol in *Hedychium* sp. On the other hand, a rise of sucrose content was exhibited in *Hedychium* sp. and *F. ulmaria*, glucose and melibiose in *F. ulmaria* and melibiose and raffinose in *Iris pseudacorus*. This result differs from previous studies (Barclay and Crawford, 1984; Monk, Brandle and Crawford, 1984; Rege and Mascarenhas, 1956; Barker and el Saifi, 1953) , except that the increase in sucrose

had also been observed in Acorus calamus but after 15 days anoxia (Monk et al., 1984). Burial separately caused a drop in glucose and raffinose content of Iris pseudacorus and sucrose content in F. ulmaria. Nevertheless the levels of fructose, glucose and raffinose rise in F. ulmaria and sucrose content also increase in I. germanica. The rapid depletion of sugar is observed whenever flooding with burial treatment (treatment C) is employed. However, when examined through both tests (The effect of flooding on buried rhizome and The effect of burial on flooded rhizome), some of the significant effect exhibited by one factor are not shown by the other as significance. It is probably because the effect of flooding on burial is obtained by comparing treatment D to treatment C (both are buried but whereas C is flooded, D is not), whilst the effect of burial on flooding is obtained by comparing treatment A to treatment C (both are flooded but whereas C is also buried, A is not). This non-regularity of statistical result appeared in one third of the sugar readings. However, from the growth results (Chapter 3) it only appeared in a few readings. As the experiment was not repeated this could be caused by biological error which can be eliminated by increasing the sample number i.e. repeating the experiment. It was first thought that by using a large number of samples in one experiment, it is no need to repeat the experiment (the long duration of experiment makes it inconvenient to repeat it). Nevertheless, to avoid more complication, the significance shown by only one test is considered valid even though it is not shown by the other test (for more detailed explanation see Appendix VI).

Flooding combined with burial caused the most dramatic effect. Except in Iris pseudacorus, the fructose and sucrose content of all the other rhizomes studied is reduced. The levels of glucose and raffinose also drop in all the species except in Hedychium sp. In this species only traces of raffinose are found under flooding conditions (alone or combined with burial) which is not detected under non-flooded conditions. Melibiose level also fell in F. ulmaria and maltose in Iris germanica. The only significant increase of sugar (sucrose) is observed in Iris pseudacorus. Under this severe condition however this species is similar to all the other species in that the storage food (% DW/FW) levels also decrease. Since only sucrose in Iris pseudacorus increased under flooding and burial treatment, as reported elsewhere in Acorus calamus after 15 days anoxia (Monk et al., 1984), this flooding and burial treatment can be considered as severe enough as far as flooding is concerned.

Fructose, sucrose, glucose and raffinose are the four sugars most negatively affected. When the level of fructose plus sucrose plus glucose is compared to total sugar level (excluding sugar Z in Iris pseudacorus and Filipendula ulmaria), the plants which showed growth in this treatment i.e. Iris pseudacorus and Hedychium sp. exhibited only small changes when compared to other less severe treatments. Filipendula ulmaria exhibited drastic reduction but in Iris germanica , the level has risen. It is most likely that in F. ulmaria these three sugars are utilised in anaerobic respiration and become exhausted as its

source (the rhizome) is small and the shoot cannot photosynthesized in darkness (Sullivan and Sprague, 1943; Sprague and Sullivan, 1950). This could eventually lead to its death. As for the other two endurant species (Iris pseudacorus and Hedychium sp.), these three major sugars are not severely affected which is probably due to a lower rate of anaerobic respiration or other sugars are utilised instead; raffinose content drop considerably in I. pseudacorus. This drop in raffinose has also been shown in F. ulmaria and Iris germanica and has been observed by other workers elsewhere (Barclay and Crawford, 1983). The hydrolyzation of raffinose by mild acid or by invertase produced melibiose and fructose (Pigman, 1948). On the other hand, it has been suggested that Iris pseudacorus lacks a Pasteur effect (Bown et al., 1968) which means that its anaerobic rate of respiration is slower than most of other plants. Even though less ATP is formed as the result of lowering its anaerobic respiration rate under anaerobiosis (Grineva, 1964; Rumpho and Kennedy, 1981), by doing this it can conserve its storage carbon i.e. carbohydrates hence avoiding carbon starvation which could lead to cell death (Vartapetian et al., 1976, 1977, 1978). Hence, in this study only glucose is affected whereas other sugars are not. Whether it is their ability to conserve carbon that eventually leads to the survival and growth in I. pseudacorus and Hedychium sp., it cannot be confirmed due to lack of knowledge of their Total Nonstructural Carbohydrate (TNC) content. However Barclay and Crawford (1983) have shown that in a plant that tolerates anoxia, sugar and TNC levels are unaffected whereas in plants that cannot tolerate anoxia, they

were reduced. By using % DW/FW (Table 5.12) as a crude measure of storage food content the results obtained are found to be in contrast to the above published work, hence this measurement cannot represent the true value of storage food content. It is probable that other factors i.e. water content (Baker and Moorby, 1969) or the changes in the properties of rhizome tissues have been involved in the % DW/FW values measured. In Iris germanica, the rise of the percentage of fructose plus glucose plus sucrose under burial and flooding treatment when compared to total sugar from other treatments, is most likely due to rhizome decomposition. Flooding and burial (treatment C) caused its rhizome (especially part behind leaf base) to become soft and clearly no growth is possible from the meristem. As during decomposition, starch is breakdown to smaller unit, it is possible that fructose, glucose and sucrose are also among the product. However, as the levels of all sugars measured are reduced it is possible that this plant is only in the early stage of decomposition, after its sugars have become exhausted during accelerated anaerobic respiration. The large rhizome can become a source of sugar, however there is no sign that its sugar debt is paid by this way. Hence it is most likely that death is quite swift, that is before any adaptation can take place which is normally observed in plants of well-drained habitats (Crawford, 1982 a). The other possibility is that other sugars (such as raffinose) are metabolised instead of these three sugars. Raffinose is present in a large amount and significantly depleted under burial and flooding in this species.

Sorbitol is found in water animals during diving and linked with their ability to stay under water (Crawford, 1978). In plants, it is detected in Iris pseudacorus and Iris germanica under flooding and burial treatment. In Hedychium sp., sorbitol is detected in quite a substantial amount but flooding (alone) reduced its concentration. Hedychium sp. and Iris pseudacorus are plants which can endure flooding and burial treatment. However Iris germanica is very intolerant , yet also show traces of sorbitol under this condition. Hence the role of sorbitol in flooding tolerance of plant is not yet clear. Other than sorbitol, maltose is detected in Iris germanica only and its level is drastically reduced under flooding and burial. Since its occurrence is rare, this study cannot ascertain any significant role to this sugar. In the two temperate wetland plants studied (Iris pseudacorus and Filipendula ulmaria), an unknown sugar (sugar Z) is produced in quite a substantial amount (up to 15% of total peak area). When compared with the standard it is most likely that this unknown sugar is erythritol. Treatment A (flooded and not buried) produced the largest amount whereas treatment C (buried and flooded) the lowest. Therefore, it is most likely that under flooding and burial, this sugar is consumed in metabolism. Nevertheless, under anoxia no such sugar is reported (Barclay and Crawford, 1983).

The experiments on buds (with shoot growing) and rhizomes (no shoot growing, growth is shown by healthy development of axillary bud) of Hedychium sp. has been performed to provide the opportunity of looking at the effect of shoot on sugar content of rhizome. This species was chosen because it has small rhizomes which were thought to be easily depleted of their sugar content whenever there is no supply of sugar from the shoots. As expected, flooding or burial (separately) caused the glucose and fructose contents of buds to rise above the rhizome level. Flooding alone gave a similar result in the accumulation of sucrose whereas burial alone showed no varied effect. On the other hand, in buried and flooded and also in not buried and not flooded treatments glucose and fructose levels are higher in rhizomes than in buds. Hence it is not possible to clearly see the effect of shoot here, probably because this effect is dampened by the effect of carbon conservation exhibited by the rhizome. Also flooding versus burial factor interacted with each other i.e. in reducing the sucrose content. Better results may be obtained when other species are studied instead.

As a summary, it is of advantage to point out that in Iris pseudacorus it is the burial factor which was statistically proved to cause a reduction of glucose and also raffinose content, but for glucose the interaction of burial and flooding is also significant. In the rhizome of Hedychium sp. where sugar is most proficiently conserved, the interaction of burial and flooding only caused sucrose levels to decline. F. ulmaria,

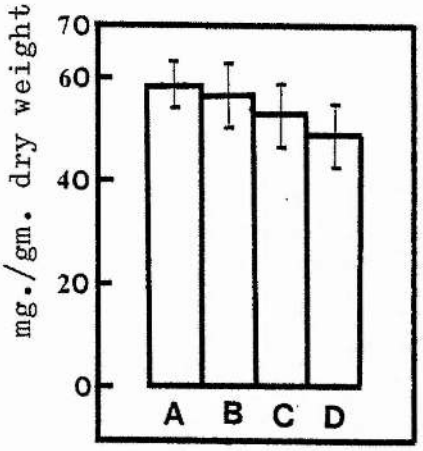
a plant of wetland-edged habitats, was severely affected by burial combined with flooding, the interaction of flooding and burial lowered its fructose, glucose, raffinose and sucrose levels. The burial factor separately can also reduce the sucrose level in this species. Finally, in Iris germanica, a garden plant severely damaged by flooding and burial, it is the flooding factor which caused a fall in its fructose and glucose levels whereas the interaction of flooding with burial resulted in a fall in sucrose concentration. Hence it is not only flooding that effect the sugar level of rhizome but also whether it is flooded on the soil/sand surface (Not buried) or below (Buried) also have the effect. Further research should also consider the aspect of burial whenever flooding aspect is discussed.

Table 5.1. Types of stored carbohydrate in the four rhizomatous plants studied.

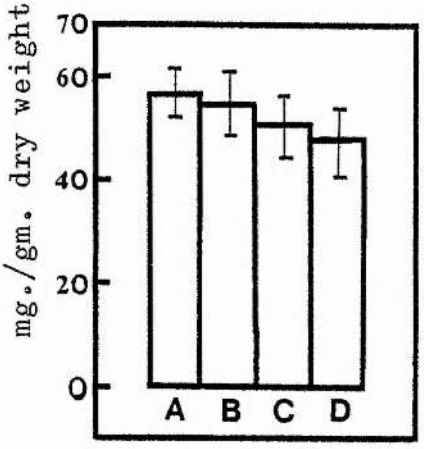
Plant species	Types of stored carbohydrate presents.
1. *Iris pseudacorus*	fructosan (Irisin).
2. *Hedychium* sp.	starch.
3. *Filipendula ulmaria*	fructosan and starch.
4. *Iris germanica*	starch.

a) TOTAL SUGAR

(excluding sugar Z).



b) FRUCTOSE + GLUCOSE + SUCROSE



c) FRUCTOSE + GLUCOSE + SUCROSE AS A PERCENTAGE OF TOTAL SUGAR.

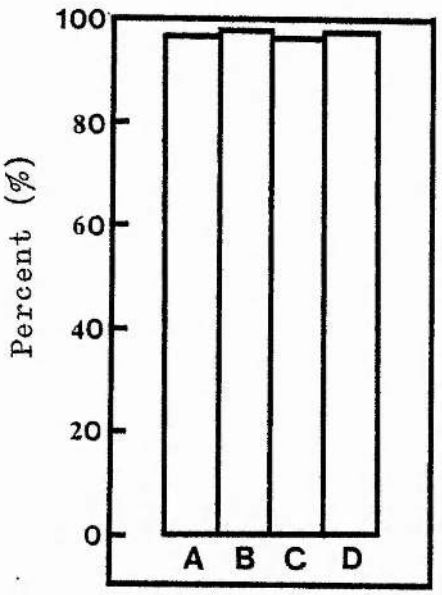
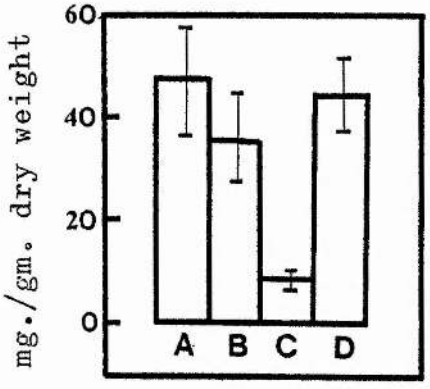


FIGURE 5.1 :

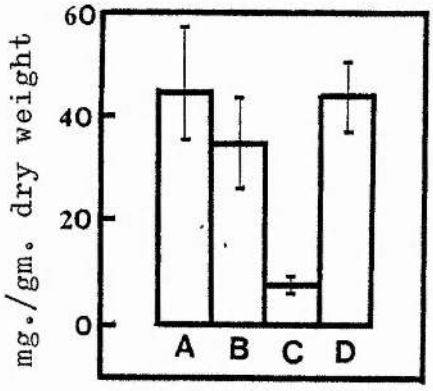
The relative changes of fructose + glucose + sucrose as compared to total sugar content (excluding substance Z) in the rhizome of Iris pseudacorus. Details of treatment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

a) TOTAL SUGAR



b) FRUCTOSE + GLUCOSE + SUCROSE



c) FRUCTOSE + GLUCOSE + SUCROSE AS A PERCENTAGE OF TOTAL SUGAR.

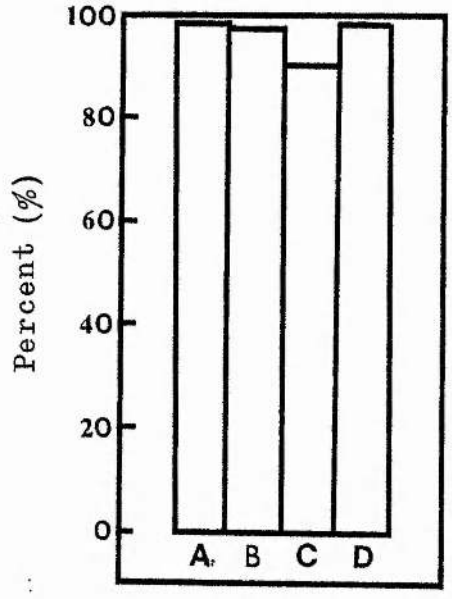


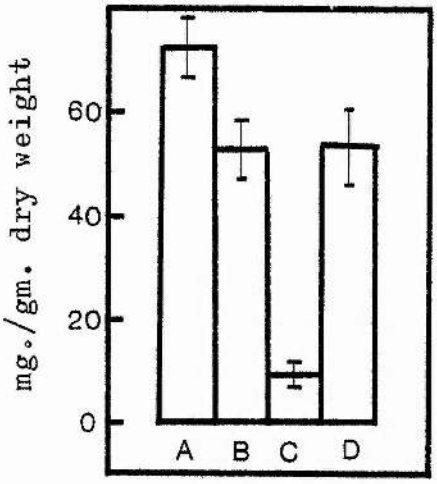
FIGURE 5.2 :

The relative changes of fructose + glucose + sucrose as compared to total sugar content in the rhizome of Hedychium sp. Details of experiment:

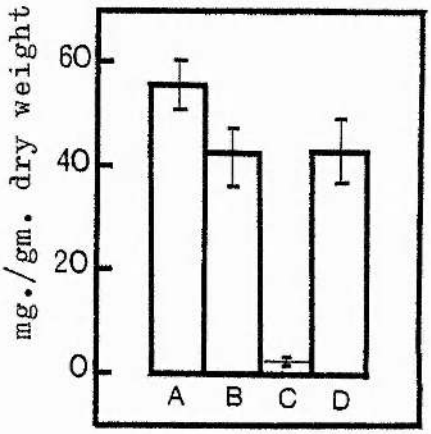
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

a) TOTAL SUGAR

(excluding sugar Z).



b) FRUCTOSE + GLUCOSE + SUCROSE.



c) FRUCTOSE + GLUCOSE + SUCROSE AS A PERCENTAGE OF TOTAL SUGAR.

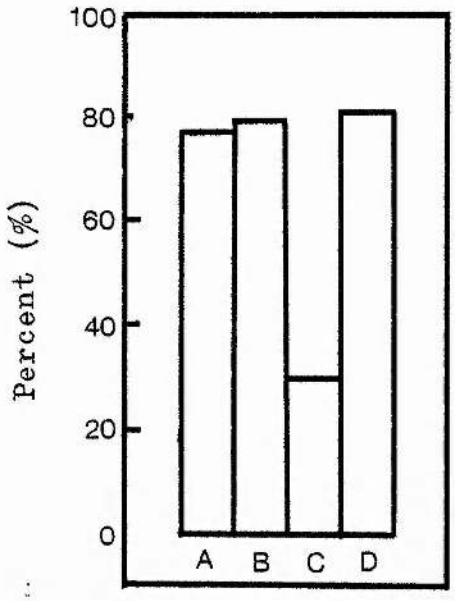
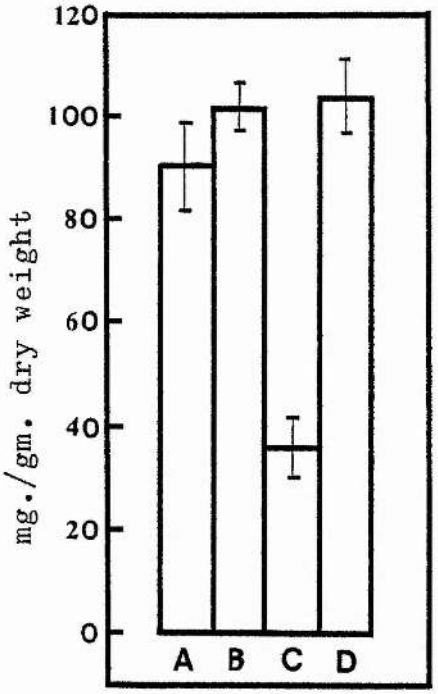


FIGURE 5.3 :

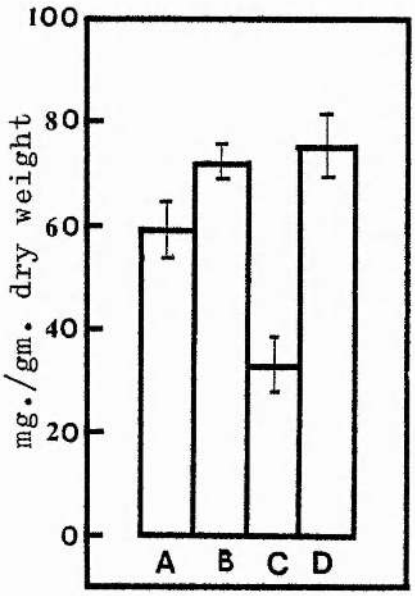
The relative changes of fructose + glucose + sucrose as compared to total sugar content (excluding sugar Z) in the rhizome of Filipendula ulmaria.Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

a) TOTAL SUGAR



b) FRUCTOSE + GLUCOSE + SUCROSE



c) FRUCTOSE + GLUCOSE + SUCROSE AS A PERCENTAGE OF TOTAL SUGAR.

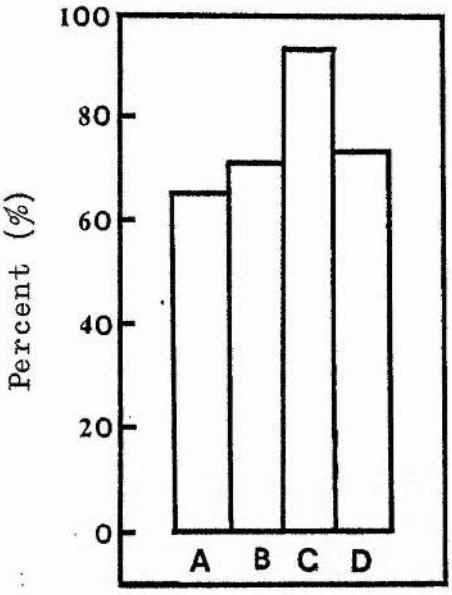


FIGURE 5.4 :

The relative changes of fructose + glucose + sucrose as compared to total sugar content in the rhizome of Iris germanica. Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.2 : The total sugar level (mg. per gm. dry weight) ± S.E. in the rhizomes of Iris pseudacorus (excluding sugar Z), Hedychium sp., Filipendula ulmaria (excluding sugar Z) and Iris germanica. Sugar Z is the unknown sugar of four carbon structures most likely to be erythritol (Retention time 0.43, relative to sorbitol standard).

Species	Treatments			
	A	B	C	D
1. Iris pseudacorus	58.75 ± 4.46	56.415 ±6.27	52.41 ±6.3	48.655 ±6.28
2. Hedychium sp.	47.05 ±10.66	35.84 ±8.55	8.50 ±1.70	44.79 ±7.15
3. Filipendula ulmaria	72.67 ±5.69	52.92 ±5.69	9.47 ±2.19	53.35 ±7.02
4. Iris germanica	90.18 ± 8.39	101.77 ± 4.62	35.95 ±5.83	103.7 ±7.07

Table 5.3 : The level of three major sugars (fructose + glucose + sucrose) ± S.E. in the rhizomes of four rhizomatous plants studied. The value is expressed as mg per gm. dry weight of rhizome tissues.

Species	Treatments			
	A	B	C	D
1. Iris pseudacorus	56.66 ±4.45	54.72 ±6.27	50.24 ±6.09	47.18 ±6.26
2. Hedychium sp.	46.51 ±10.65	35.08 ±8.66	7.81 ±1.77	44.27 ±7.07
3. Filipendula ulmaria	55.96 ±4.79	42.18 ±5.4	2.83 ±0.69	48.62 ±6.23
4. Iris germanica	59.33 ±5.255	72.71 ±3.05	33.45 ±5.34	75.84 ±6.14

Table 5.4 : Ratio of three major sugars (sucrose + glucose + fructose) over Total sugar content presented as a percentage. Note that in Iris pseudacorus and F. ulmaria, the Total sugar content is not inclusive of sugar Z (unknown sugar). However only in treatment A in F. ulmaria that this sugar is present in large amount (up to 15% of total peak area measured, see also Figure 5.13), so the actual percentage could be much lower than actually shown in this table for this treatment.

Species	Treatments			
	A	B	C	D
1. Iris pseudacorus	96.44%	97.0%	95.86%	96.97%
2. Hedychium sp.	98.85%	97.88%	91.88%	98.8%
3. Filipendula ulmaria	77.0%	79.7%	29.88%	81.76%
4. Iris germanica	65.79%	71.45%	93.05%	73.1%

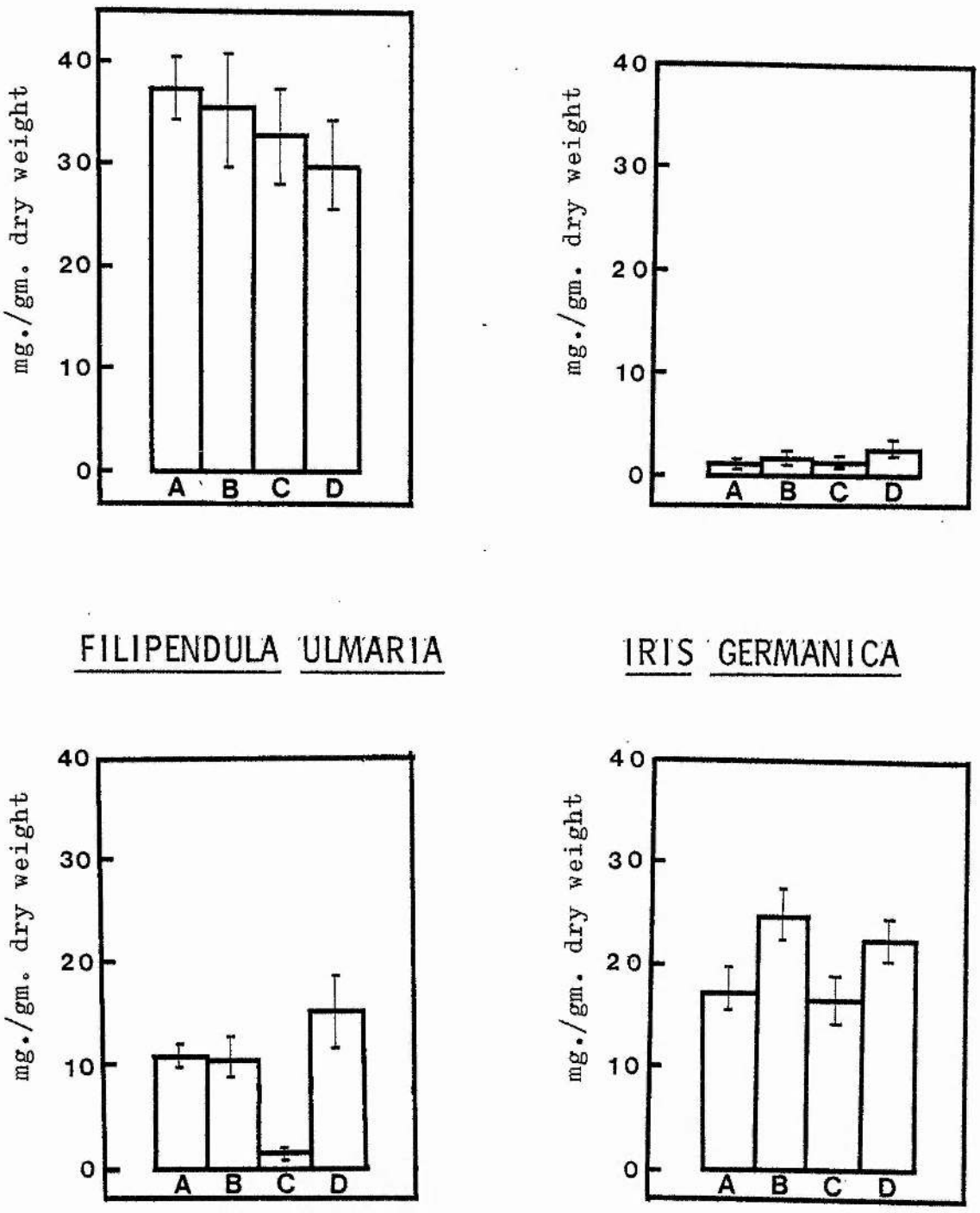


FIGURE 5.5 :

The effect of flooding and/or burial treatment on fructose content of rhizome (mg. per gm. dry weight).
Details of treatment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.5a : The effect of flooding or burial on rhizome fructose content.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris pseudacorus	N.S.	N.S.
2. Hedychium sp.	N.S.	- (***)
3. Filipendula ulmaria	N.S.	- (***)
4. Iris germanica	- (***)	- (***)

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	N.S.	N.S.
2. Hedychium sp.	N.S.	N.S.
3. Filipendula ulmaria	+ (***)	- (***)
4. Iris germanica	N.S.	N.S.

Footnote;

+ = increase

- = decrease

N.S. = no significance changes occured.

*** = significance at 0.1% level.

** = significance at 1% level.

* = significance at 5% level.

Table 5.5b : The interaction of flooding and burial factors on changes in rhizome fructose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	(N.S.P.)	(N.S.P.)
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	(N.S.P.)	✓
4. *Iris germanica*	X	(N.S.P.)

Footnote;

✓ = interaction between burial and flooding occurred.

X = no interaction, the factor concerned gave the most effect when both factors occured together (treatment C -- buried and flooded).

(N.S.P.) = the occurence of interaction or not cannot be statistically proved because of a non-significant effect of one side (or both sides) of the test (Buried versus not buried and Flooded versus not flooded -- Table 5.5a).

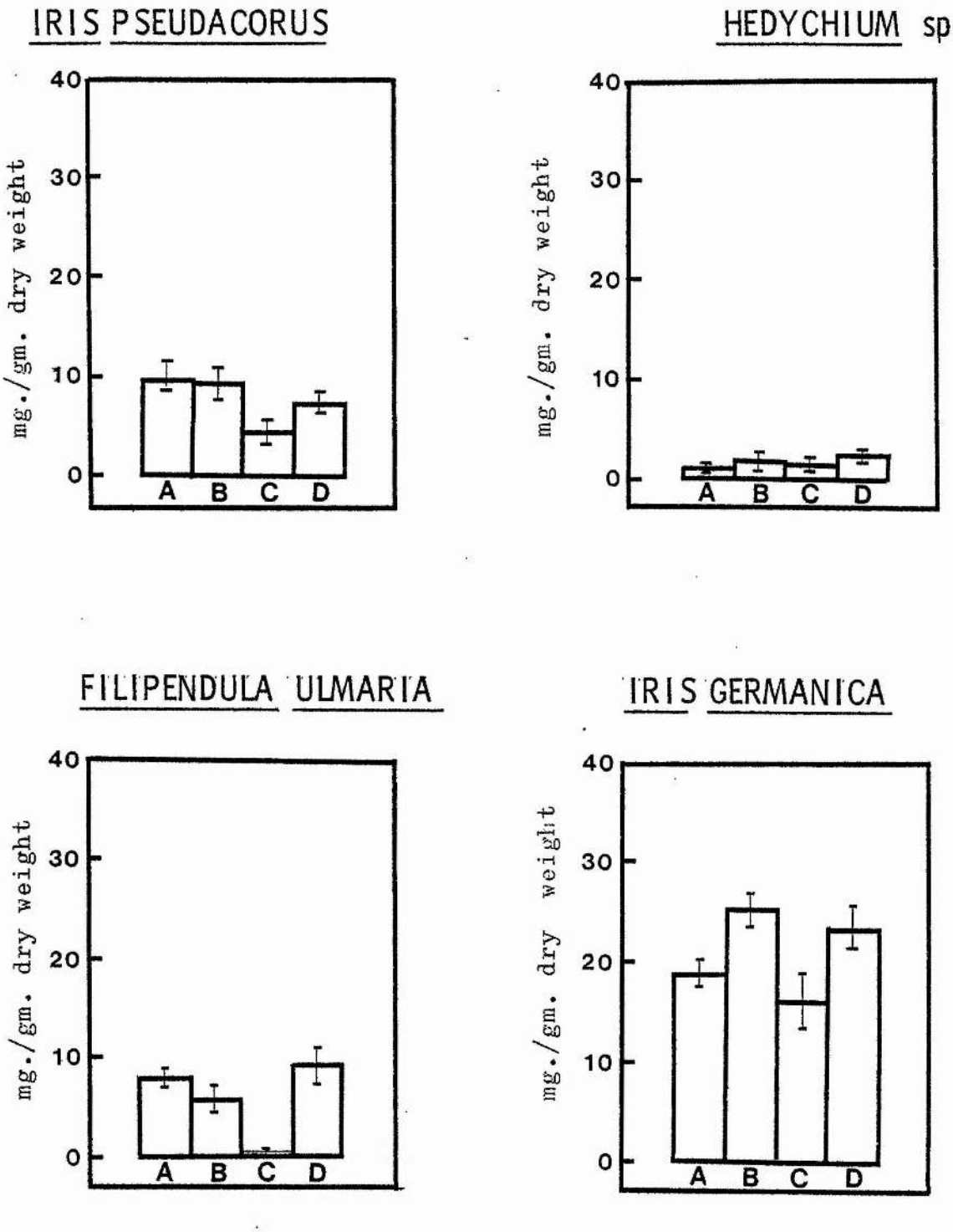


FIGURE 5.6 :

The effect of flooding and/or burial treatment on glucose content of rhizome (mg. per gm. dry weight).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.6a : The effect of flooding or burial on rhizome glucose content.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris pseudacorus	N.S.	- (***)
2. Hedychium sp.	N.S.	N.S.
3. Filipendula ulmaria	+ (***)	- (***)
4. Iris germanica	- (***)	- (***)

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	- (*)	- (***)
2. Hedychium sp.	N.S.	N.S.
3. Filipendula ulmaria	+ (***)	- (***)
4. Iris germanica	N.S.	N.S.

Footnote;

See legend under Table 5.5a.

Table 5.6b : The interaction of flooding and burial factors on changes in rhizome glucose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	(N.S.P.)	X
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	✓	✓
4. *Iris germanica*	X	(N.S.P)

Footnote;

See legend under Table 5.5b.

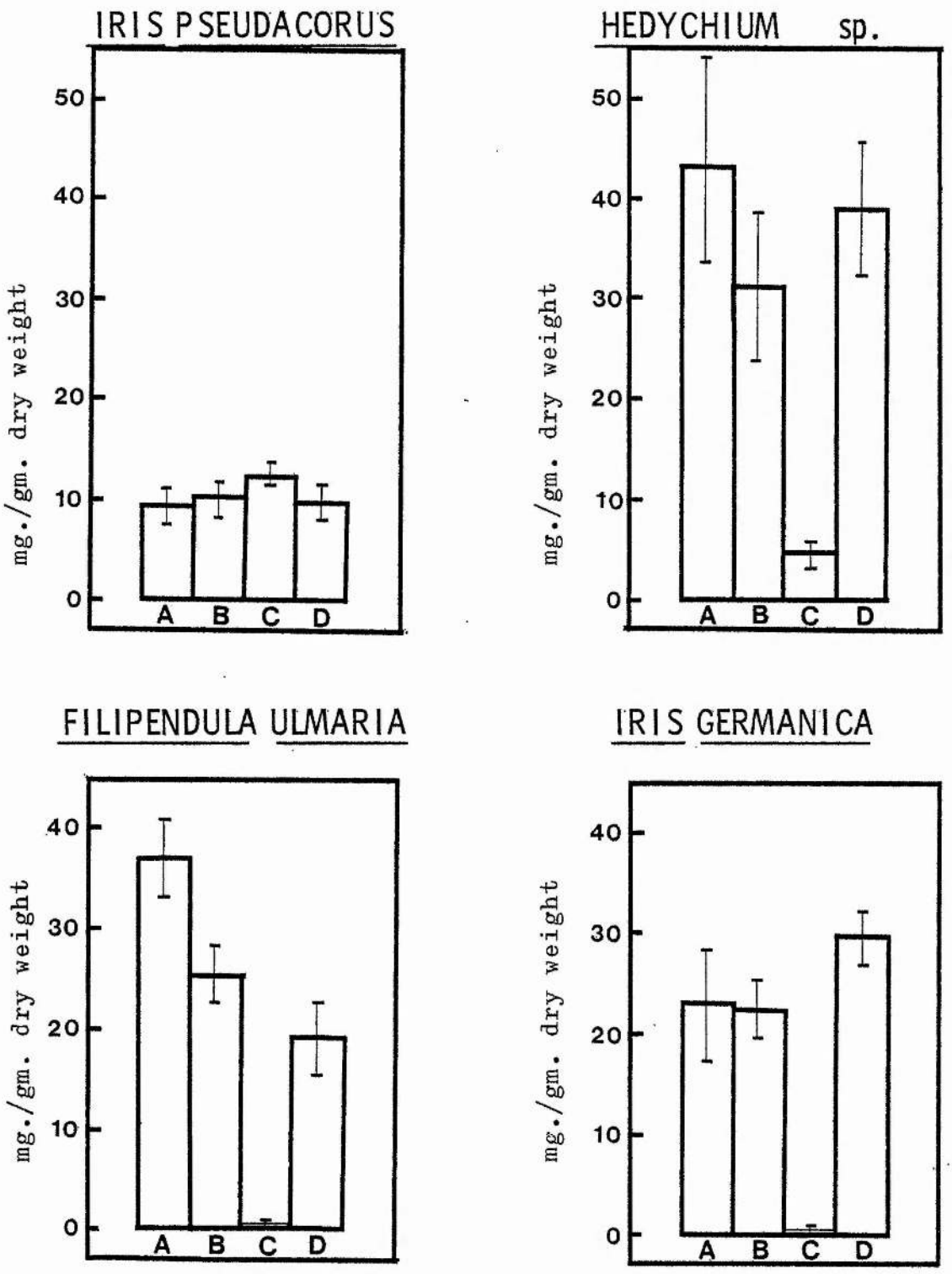


FIGURE 5.7 :

The effect of flooding and/or burial treatment on sucrose content of rhizome (mg. per gm. dry weight).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.7a : The effect of flooding or burial on rhizome sucrose content.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris pseudacorus	N.S.	+ (**)
2. Hedychium sp.	+ (*)	- (***)
3. Filipendula ulmaria	+ (***)	- (***)
4. Iris germanica	N.S.	- (***)

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	N.S.	+ (***)
2. Hedychium sp.	N.S.	- (***)
3. Filipendula ulmaria	- (***)	- (***)
4. Iris germanica	+ (**)	- (***)

Footnote;

See legend under Table 5.5a.

Table 5.7b ; The interaction of flooding and burial factors on changes in rhizome sucrose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	(N.S.P.)	(N.S.P.)
2. *Hedychium* sp.	✓	(N.S.P.)
3. *Filipendula ulmaria*	✓	X
4. *Iris germanica*	(N.S.P.)	✓

Footnote;

See legend under Table 5.5b.

Comment;

Although there is a significant interaction , the effect of burial is also highly significant in *Filipendula ulmaria*.

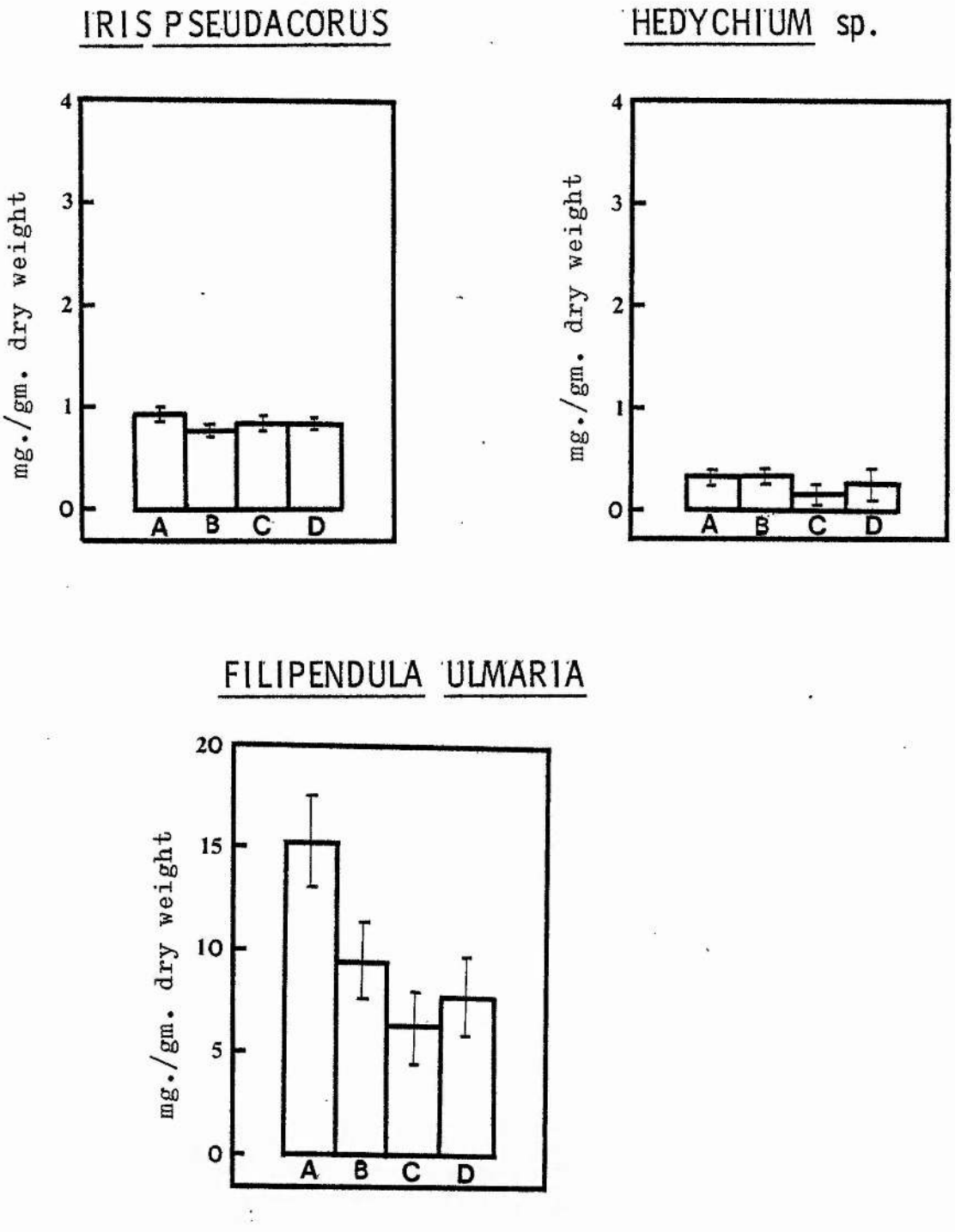


FIGURE 5.8 :

The effect of flooding and/or burial treatment on melibiose content of rhizome (mg. per gm. dry weight). Melibiose was not detected in Iris germanica rhizome.
Details of experiment:
A = Not buried and flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.8a : The effect of flooding or burial on rhizome melibiose content.

I. The effect of flooding.

	NOT-BURIED	BURIED
1. Iris pseudacorus	+ (*)	N.S.
2. Hedychium sp.	N.S.	N.S.
3. Filipendula ulmaria	+ (***)	N.S.
4. Iris germanica	N.D.	N.D.

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	N.S.	N.S.
2. Hedychium sp.	N.S.	N.S.
3. Filipendula ulmaria	N.S.	- (***)
4. Iris germanica	N.D.	N.D.

Footnote;

See legend under Table 5.5a.

N.D. = not detected in the species concerned.

Table 5.8b : The interaction of flooding and burial factors on changes in rhizome melibiose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	(N.S.P.)	(N.S.P.)
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	(N.S.P.)	(N.S.P.)
4. *Iris germanica*	N.D.	N.D.

Footnote;

See legend under Table 5.5b.

IRIS GERMANICA

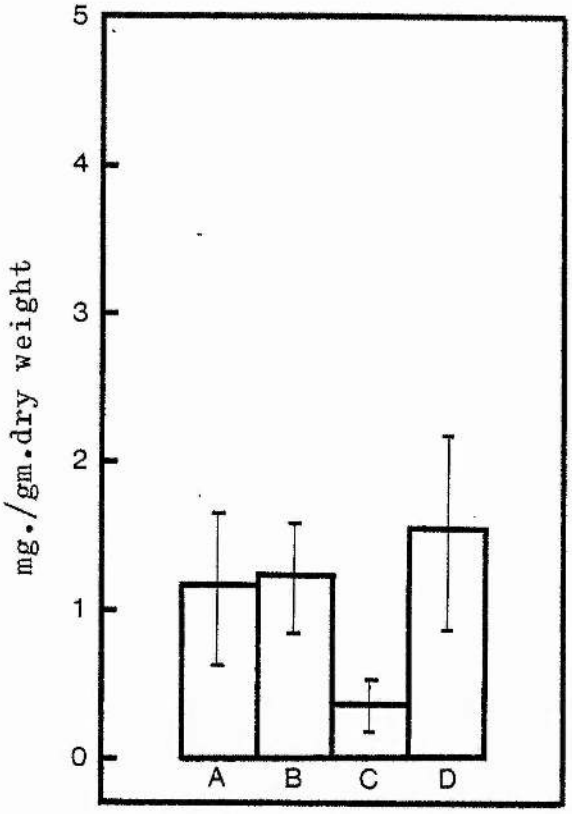


FIGURE 5.9 :

The effect of flooding and/or burial treatment on maltose content of Iris germanica rhizome (mg. per gm. dry weight). Maltose was absent from the other species.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.9a : The effect of flooding or burial on rhizome maltose content.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris germanica	N .S.	- (***)

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris germanica	N.S.	N.S.

Footnote;

See legend under Table 5.5a.

Table 5.9b : The interaction of flooding and burial on changes in rhizome maltose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. Iris germanica	(N.S.P.)	(N.S.P.)

Footnote;

See legend under Table 5.5b.

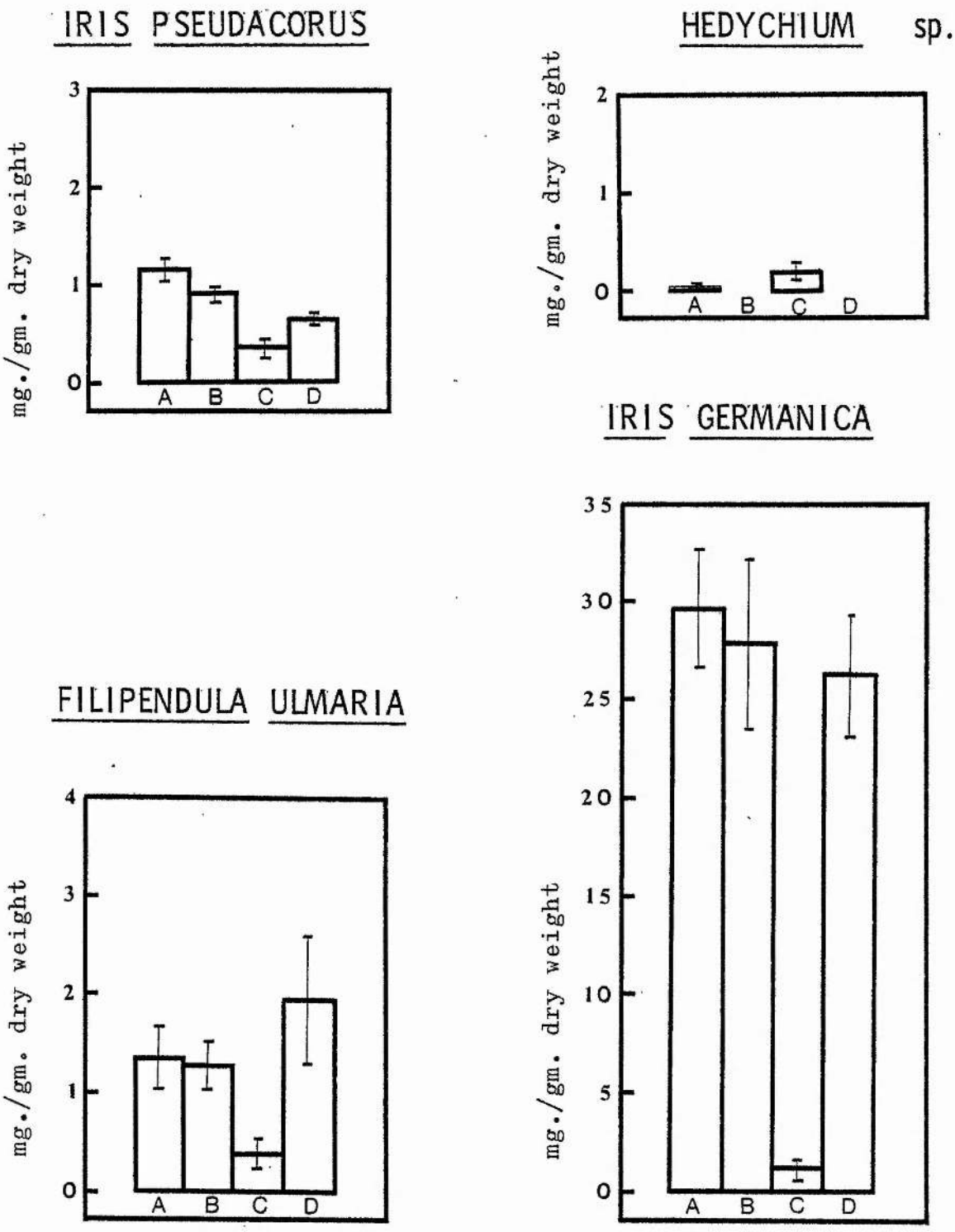


FIGURE 5.10 :

The effect of flooding and/or burial treatment on raffinose content of rhizome (mg. per gm. dry weight).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.10a : The effect of flooding or burial on rhizome raffinose content.

I. The effect of flooding.

	NOT BURIED	BURIED.
1. Iris pseudacorus	+ (***)	- (***)
2. Hedychium sp.	See comment	See comment
3. Filipendula ulmaria	N.S.	- (***)
4. Iris germanica	N.S.	- (***)

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	- (***)	- (***)
2. Hedychium sp.	See comment	See comment
3. Filipendula ulmaria	+ (***)	- (***)
4. Iris germanica	N.S.	- (***)

Footnote; See legend under Table 5.5a.

Comment; Raffinose was detected only under flooded treatments, whether not buried (A) or buried (C) in the rhizome of Hedychium sp.

Table 5.10b: The interaction of flooding and burial factors on changes in rhizome raffinose levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	✓	X
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	(N.S.P.)	✓
4. *Iris germanica*	✓ (N.S.P.)	✓ (N.S.P.)

Footnote;

See legend under Table 5.5b.

Comment;

a) In *Iris pseudacorus*, there is a significant interaction between flooding and burial. However, the effect of burial separately is also highly significant.

b) The content of raffinose was significantly lowered (p less than 0.001) under flooding and burial (treatment C) but the interaction/or no interaction cannot be statistically proven because under non burial flooding does not significantly effect this sugar level and under non flooded burial does not significantly effect its level as well (Table 5.10a).

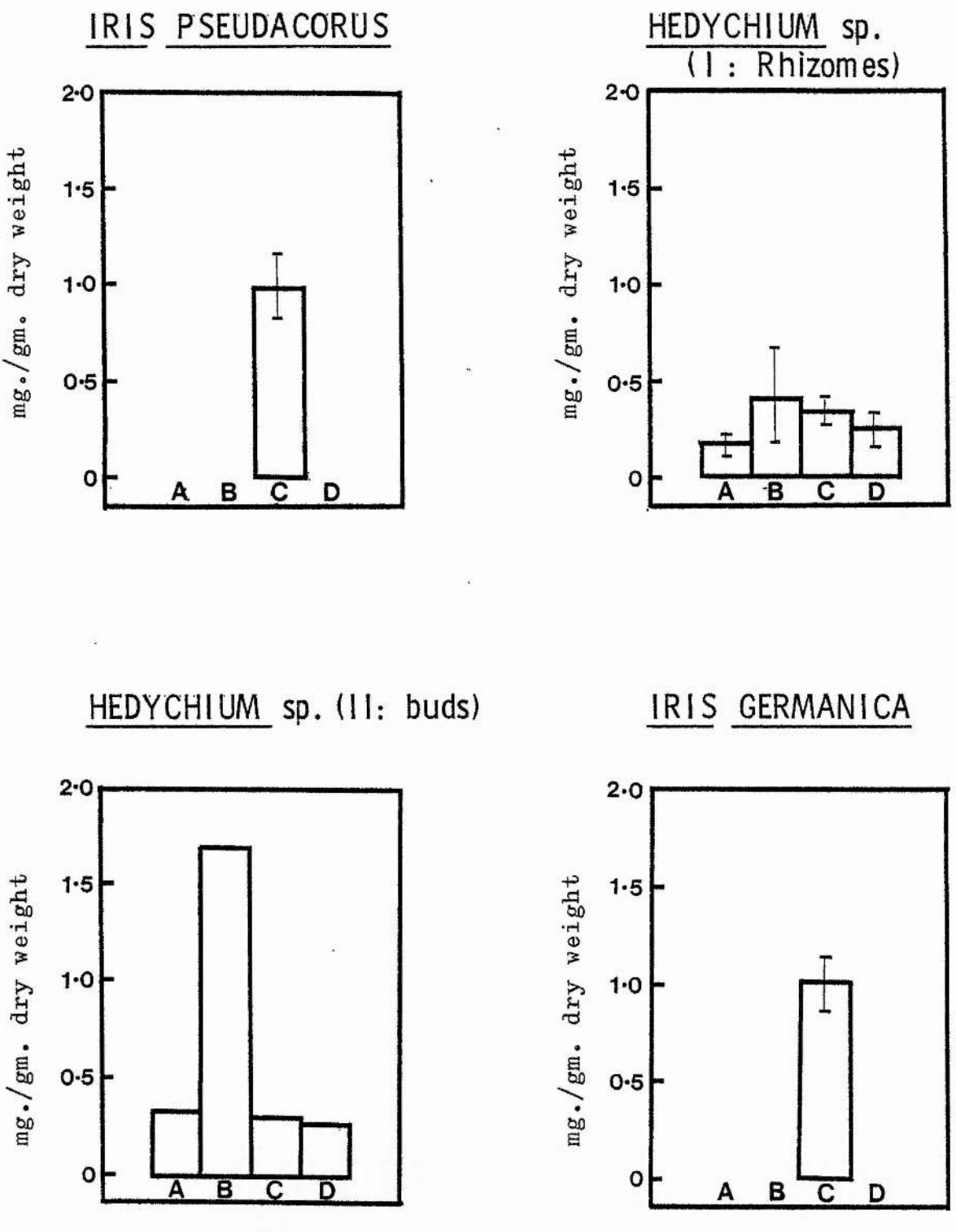


FIGURE 5.11 :

The effect of flooding and/or burial treatment on sorbitol content of rhizome (mg. per gm. dry weight). Sorbitol was not present in Filipendula ulmaria. In Hedychium sp. (Experiment II : buds), only one sugar analysis was performed due to a very little sample available. Details of experiment see legend under Figure 5.10 or 5.12.

Table 5.11a: The effect of flooding or burial on rhizome sorbitol content.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris pseudacorus	See comment	See comment
2. Hedychium sp. (rhizome)	- (*)	N.S.
3. Filipendula ulmaria	N.D.	N.D.
4. Iris germanica	See comment	See comment

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	See comment	See comment
2. Hedychium sp.(rhizome)	N.S.	N.S.
3. Filipendula ulmaria	N.D.	N.D.
4. Iris germanica	See comment	See comment

Footnote;

See legend under Table 5.5a

N.D. = not detected in the species concerned.

Comment; Sorbitol was detected only under flooded and buried treatment (C) in Iris pseudacorus and Iris germanica.

Table 5.11b : The interaction of flooding and burial factors on changes in rhizome sorbitol levels.

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	See comment	See comment
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	N.D.	N.D.
4. *Iris germanica*	See comment	See comment

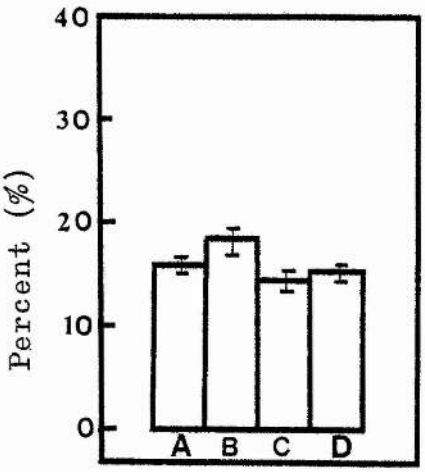
Footnote;

See legend under Table 5.5b.

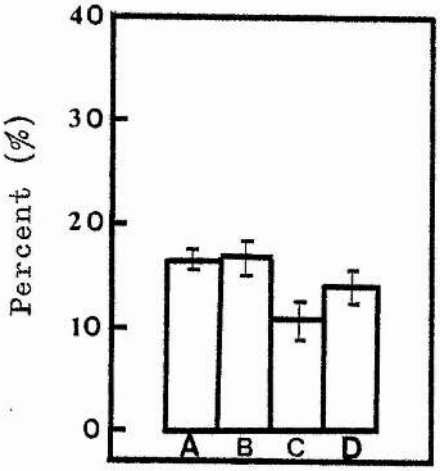
Comment;

Flooding with burial factors caused sorbitol to be accumulated in *Iris pseudacorus* and *I. germanica* rhizomes (Figure 5.11).

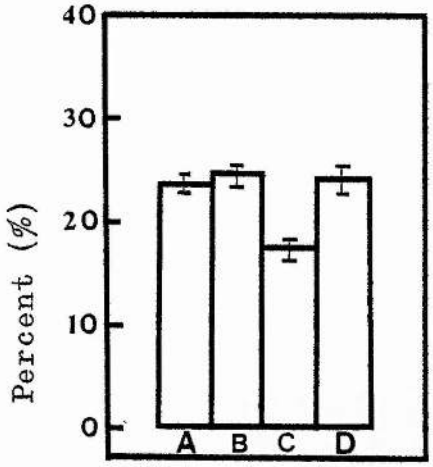
IRIS PSEUDACORUS



HEDYCHIUM sp.



FILIPENDULA ULMARIA



IRIS GERMANICA

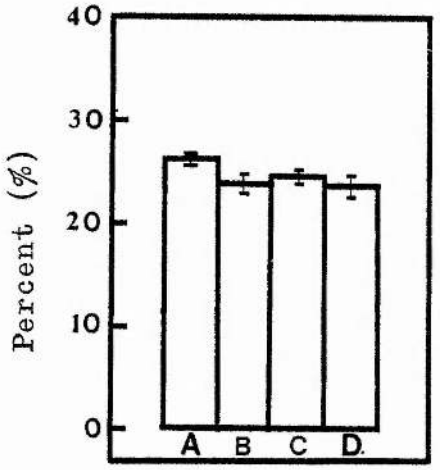


FIGURE 5.12 :

The effect of flooding and/or burial treatment on Storage food content (measured as % dry weight/fresh weight) of rhizome. Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

Table 5.12a : The effect of flooding or burial on rhizome storage food content (% DW/FW) of rhizome tissue.

I. The effect of flooding.

	NOT BURIED	BURIED
1. Iris pseudacorus	- (***)	N.S.
2. Hedychium sp.	N.S.	- (**)
3. Filipendula ulmaria	N.S.	- (***)
4. Iris germanica	+ (***)	N.S.

II. The effect of burial.

	NOT FLOODED	FLOODED
1. Iris pseudacorus	- (***)	- (*)
2. Hedychium sp.	N.S.	- (***)
3. Filipendula ulmaria	N.S.	- (***)
4. Iris germanica	N.S.	- (***)

Footnote;

See legend under Table 5.5a.

DW = dry weight.

FW = fresh weight.

Table 5.12b : The interaction of flooding and burial factors on changes in rhizome storage food levels (% DW/FW).

	FLOODING FACTOR	BURIAL FACTOR
1. *Iris pseudacorus*	(N.S.P.)	X
2. *Hedychium* sp.	(N.S.P.)	(N.S.P.)
3. *Filipendula ulmaria*	(N.S.P.)	(N.S.P.)
4. *Iris germanica*	(N.S.P.)	(N.S.P.)

Footnote;

See legend under Table 5.5b.

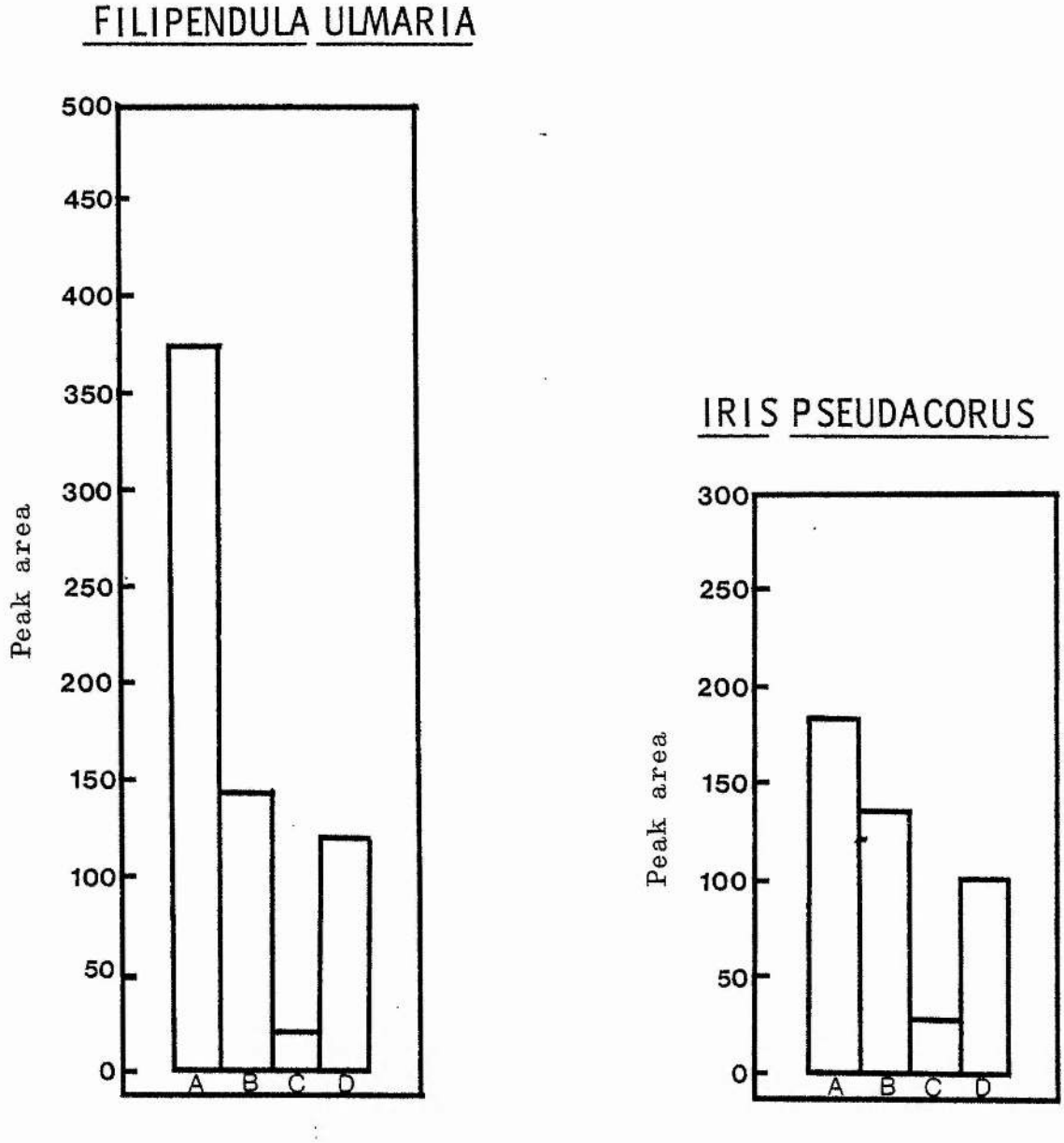


FIGURE 5.13 :

The effect of flooding and/or burial treatment on sugar Z content of Filipendula ulmaria and I. pseudacorus rhizomes (peak area). Sugar Z is most likely to be erythritol (4 carbon structure, Retention time 0.43 relative to sorbitol).
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

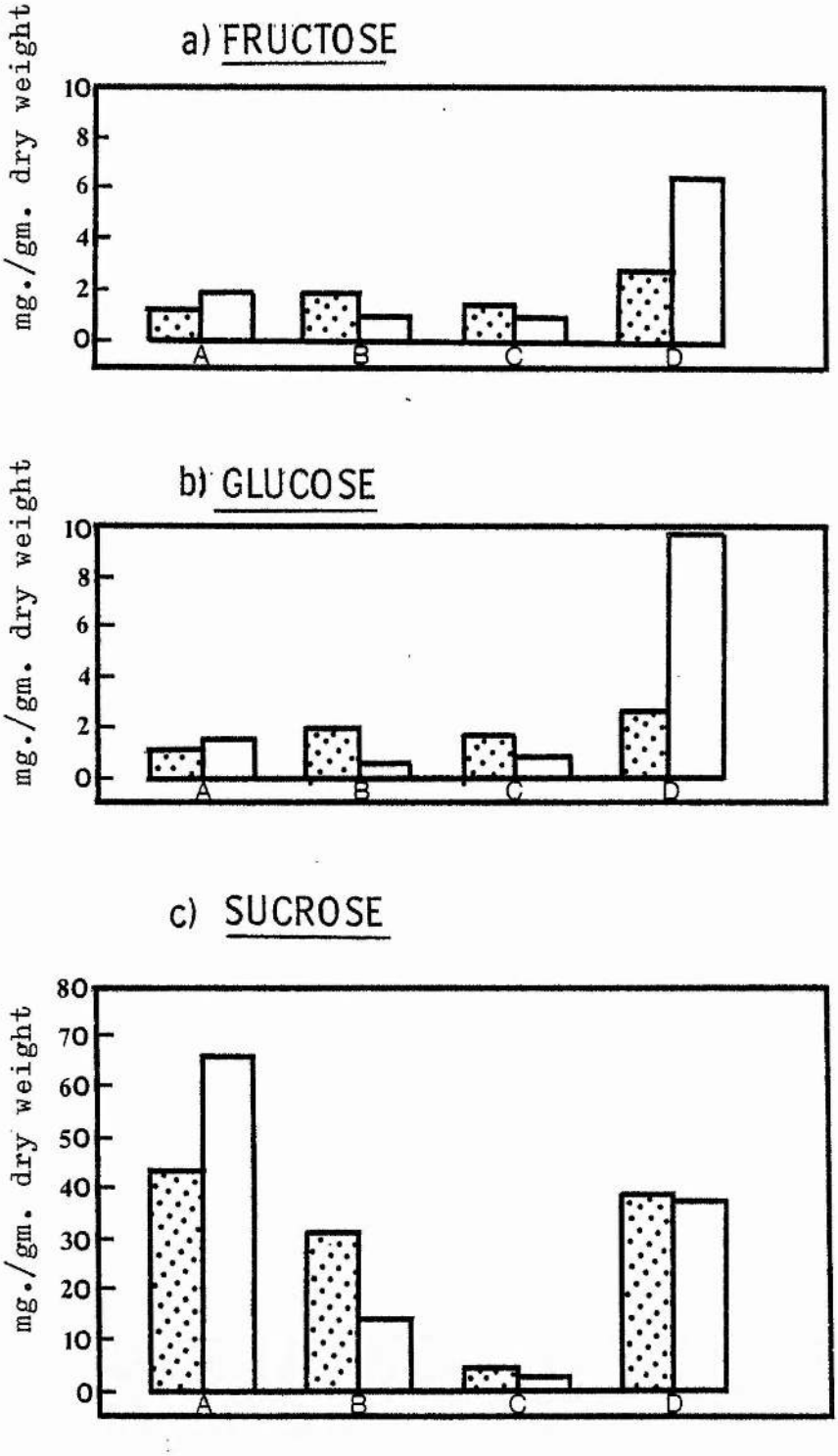


FIGURE 5.14 :

The effect of flooding and/or burial treatment on fructose, glucose and sucrose content of Hedychium sp. rhizome (Experiment I) and bud (Experiment II)

Details of experiment:

A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

CHAPTER 6 :

CONTENTS

Chapter 6

RESPIRATORY METABOLISM UNDER DIFFERENT AERATION REGIMES.

6.1 INTRODUCTION.

Conventional interpretations of anaerobic metabolism suggest that plant species intolerant to anaerobiosis (as caused by anoxia and flooding) have a Pasteur effect where under low O_2, carbohydrate is metabolized at a faster rate as compared to aerobic control (Effer and Ranson, 1967). The respiratory activity of flood-tolerant seedlings (oryzicola and rice) was greater in air than under anaerobic conditions where it was suppressed. In a flood-susceptible plant (pea) this reduction of respiration under anoxia was not observed (Kennedy et al, 1983). It was generally accepted that the enhancement of rate of respiration under anoxia in intolerant species (for example in yam tubers) if prolonged may cause a significant loss of carbohydrate and yield in term of weight (Passam et al, 1978). In root where a small amount of carbohydrate was stored, this factor could lead to carbon starvation which in turn could subsequently lead to cell death (Vartapetian et al, 1978). Hence, in tolerant species such as Iris pseudacorus, a common marsh plant, a low rate of respiration under anoxia could lead to carbon conservation and a limitation of glycolytic rate thus

associating it to tolerance of anaerobic conditions.

On the other hand, ethanol was also produced as a result of carbohydrate breakdown under anoxia and flooding. Ethanol was regarded by the majority of workers as toxic to plant root systems (Eisenmeger, 1930; Fulton and Erickson, 1964; McManmon and Crawford, 1971; Andrews, 1977; Pradet and Bomsel, 1978). Recently, debates on whether plant produced substantial amounts of ethanol enough to cause its death have developed worldwide as a follow-up of Crawford's flooding tolerance theory. A most recent work (Crawford and Zochowski, 1984) discussed problems faced by laboratory workers in this field and presented new data to suggest that ethanol accumulation may play a significant role in chickpea seedling death under anoxia. In this experiment, seedlings incubated in a closed anaerobic gas system (where ethanol was allowed to steadily accumulate) produced only a small percentage of emergence when later planted in glass house. Nevertheless, the majority of seedlings incubated in an open system where ethanol was removed by moving gaseous stream emerged even after 80 hours anaerobiosis. However, after 100 hours anoxia this figure dropped considerably.

Flooding by stagnant or flowing water could also simulate the close and open system of anoxic treatment. It was known that stagnant water is more injurious than flowing water (Sabau, 1967). Various factors could contribute to this phenomenon; the direct effect of anaerobiosis on roots and also the indirect effects for example from the accumulation of toxic products in

stagnant water (Gill, 1975). Among the first category, the severe effect is probably due to complete absence of oxygen (Robinson, 1930; Pearsall and Mortimer, 1939) or to high levels of nitrogen and carbon dioxide dissolved in waterlogged or flooded soils (Russel and Appleyard, 1915). Under flowing water, the less severe injury sustained is associated with richer oxygen status even though anaerobiosis may still be severe (Gill, 1975).

Under flooding conditions, carbon dioxide toxicity is often considered as an influencing factor such as restricting growth of the submerged but not the exposed stolons (Bendixen and Peterson, 1962). Greenwood (1967) suggested that evolution of carbon dioxide may influence the pH; by moving it to a more acid range (Small, 1954) which, in turn influences root growth. However, the extent of sensitivity depended on the species; radish and pea root-tips were severely affected. When storage roots of flood-tolerant and flood-susceptible sweet potato cultivars were held totally submerged underwater for 48 hours at 22°C, the internal gas atmosphere was replaced rapidly by CO_2 (Figure 6.1). Furthermore, roots held in 100% CO_2 gas in all the cultivars except in Caromex (a flood- susceptible cultivar) contained the highest concentration of ethanol, followed by complete submergence , 100% N_2 ,but a very low concentration in air control roots. In Caromex, complete submergence exhibited the highest level of ethanol, followed closely by 100% CO_2 (Chang et al, 1983). When chickpea seedlings were exposed to anaerobic incubation, 5-24% CO_2 accumulated in the closed system modified

the glycolytic activity by increasing the ethanol content (Crawford and Zochowski, 1984). Therefore, high concentration of CO_2 found in the internal atmosphere of the submerged storage roots and also in the gas atmosphere of flooded soils (Takai et al, 1963; Cho and Ponnamperuma, 1971) could be of considerable importance in flood damage, even though O_2 starvation was the main cause (Drew, 1979). In roots (for example rice roots), after 18 hours of complete submergence of whole plant, CO_2 content also increased as compared to partial submergence (Table 6.1). However, the O_2 content only indicated a small drop (Raalte, 1940) and was not completely depleted as with the sweet potato tubers (Chang et al, 1983). There is clearly a need for a separate study of roots and storage organs in relation to the effect of CO_2 on anaerobic metabolism.

This study was performed as an attempt to assess the physiological tolerance of rhizomatous species to anoxia and stagnant water. In gaseous anoxic treatment (under N_2 stream or N_2 incubation), the build up of micro-organisms-related compound toxic to root was avoided. Under waterlogged condition, partial and complete submergence (combine with burial) was employed. Respiration rate as a measure of metabolic tolerance was examined by both carbon dioxide evolution and ethanol accumulation (Crawford, 1969). The effect of CO_2 (low and high concentration) was also observed on rhizomes and also rhizome buds on Iris germanica, a flood-susceptible plant, and Iris pseudacorus, a flood-tolerant plant.

6.2 MATERIALS AND METHODS.

6.2.1 Plant Materials.

The rhizomes of *Iris pseudacorus* L. and *Iris germanica* var. *quechei* L. supplied by the University Botanic Garden were planted out for about one month in sand under 16 hours light in heated glass-house and were watered regularly. Following harvest, plants were washed carefully under the tap. The rhizome surface was gently rubbed to remove outer dead layers of tissue and reduce the microflora present there. They were than pre-treated homogenously by submerging them in distilled water leaving shoot and leaf base in the air. During this period, the bathing solution was continuously aerated. Plants were kept under this condition overnight in order to achieve a state of steady metabolism before the start of the experiment. After about 24 hours, the roots were immediately excised and leaves were cut leaving only about 2 centimetres from leaf base. The senescent end of rhizomes was removed and the wound was thinly lanolined. Fresh weight of the rhizomes was measured. Volume was also measured by the displacement of water, taking care not to submerge the top of shoot which has been cut previously.

6.2.2 The aerobic and anaerobic respiration rate measurements.

Rhizomes and rhizome buds were used for respiration rates (rate of CO_2 evoluted per hour per gram fresh weight) measurement with infra-red gas analyser (Section 2.2. ; 0 - 1% CO_2, 0 - 5% CO_2 or 0 - 12% CO_2 systems). Aerobic respiration was measured for 30 hours at 20°C after which plants were harvested for enzymatic ethanol measuring. However, in some rhizomes the aerobic treatment was followed by the anoxic treatment under N_2 stream in a close system (Figure 2.2). The aerobic respiration rates after this anoxic period were also recorded.

6.2.3 Ethanol measuring.

The ethanol concentration was measured by enzymatic analysis and also by Gas-liquid chromatography (GLC).

6.2.3.1 Enzymatic analysis.

The rhizomes were pre-treated as in section 6.2.1. Cores were than cut from the middle part of rhizomes by a cork borer with one centimetre internal diameter and surface sterilised with Streptomycin sulphate , 0.3 gm. in 100 ml. water (Dista products Limited, England). These plant materials were each put into flask covered by black plastic sheet. For the aerobic treatment or control, the flasks were left open to laboratory

air. For the anaerobic treatment, flasks were stoppered with rubber bungs, through each of which were inserted two glass tubes. A set of two flasks, one containing rhizome or core respectively of Iris germanica whilst the other flask of Iris pseudacorus, was linked together by one of the glass tube. Nitrogen gas was passed through both flasks for about half an hour and thereafter the flasks were sealed so that plant materials were left to incubate in anoxic atmosphere. Flasks for both aerobic and anaerobic treatments were kept at 20°C in a water bath. After 30 hours, plant materials were immediately harvested. Buds were separated and rhizomes were sectioned into 2 centimetres pieces. Fresh weights of buds, cores and rhizome pieces were measured followed by preparation of extract which was already described in section 2.3.1.1.

The ethanol content was measured spectrophotometrically by measuring the increase in absorption values of reduced nicotinamide adenine dinucleotide (NADH) in the extract, formed in the presence of alcohol dehydrogenase (ADH) enzyme. The reaction mixture contained phosphate buffer (Pyrophosphate, 75 mmol l^{-1}; semicarbazide, 75 mmol l^{-1}; glycine, 21 mmol l^{-1}; pH 8.7); nicotinamide adenine dinucleotide solution, NAD (Boehringer-Mannheim, GMBH, Catalogue number 127990, ca. 16 mmol l^{-1}), sample or standard respectively and distilled water. The ethanol standard used (0.08% w/v, Sigma chemical Company) was diluted fourteen times to give 0.0571 gm. l^{-1} concentration which was within the range (0.01 - 0.15 gm. l^{-1}) of measurement at 340 nm. The assay of the reaction mixture was shown in

Appendix IV. The reaction was started with the addition of ADH enzyme (Boehringer-Mannheim, GMBH, Catalogue number 127540). The increase in absorbance of NADH at 340 nm was followed in a Unicam SP 1800 Ultraviolet recording sprectrophotometer against a blank. After about 10 to 15 minutes, a plateau was achieved indicating that the reaction had ended. NADH concentration was then calculated from the increase of at least two dilutions of one extract. As the amount of NADH formed is stoichiometric with the amount of ethanol present, therefore this value could be expressed as μmoles of ethanol formed per gramma fresh weight. A molar extinction coefficient of 6.3 ($1 \times mmol^{-1} \times cm^{-1}$) was used.

6.2.3.2 Ethanol measuring with GLC.

The ethanol content in rhizomes of Iris pseudacorus, Filipendula ulmaria, Hedychium sp. and Jerusalem artichokes (Helianthus tuberosus) tubers from flooding and/or burial treatments (section 2.1) was measured with this rapid technique. However, ethanol contents in rhizomes of Iris germanica from the same treatments was measured spectrophotometrically with Enzymatic test kit (Boehringer-Mannheim, GMBH) at Hg 366 nm with Eppendorf photometer (Netheler and Hintz, GMBH, Hamburg). The extinction coefficient of NADH used was 3.4 ($1 \times mmol^{-1} \times cm^{-1}$). The preparation of extract and GLC measuring were described in Section 2.3.2. Total volumes of extract were estimated as 180 ml. for Iris pseudacorus, 65 ml. for Filipendula ulmaria and

Hedychium sp., and 80 ml. for Jerusalem artichokes, giving the amount of ethanol present as μmoles gm.$^{-1}$ fresh weight.

6.3 RESULTS.

6.3.1 RESPIRATION RATES.

Aerobic and anaerobic respiration rates were measured within the first day of incubation. During this period, CO_2 evolution rate of whole rhizomes or buds were scanned. During the course of study, it was found that instead of conventional rate versus time presentation, it was also possible to exhibit rate versus concentration of CO_2. Hence the effect of low (0 - 1% CO_2) or high (more than 1%) CO_2 on respiration was discussed.

6.3.1.1 Aerobic respiration.

The aerobic respiration of rhizomes of Iris pseudacorus (wetland plant) and Iris germanica (non-wetland plant) was low (less than 0.6 μmoles gm.$^{-1}$ hour^{-1}) as compared to buds (Figure 6.2). Bud respiration was higher in Iris pseudacorus than in Iris germanica. In I. pseudacorus, bud respiration rate dropped initially however increased after 4 hour period until 24 hour period where it gained maximum value of 4.56 μmoles gm.$^{-1}$ hour^{-1},

before dropping to a lower level. In Iris germanica, bud respiration rate also dropped initially, and continued to drop to a minimum level at 24 hours (2.49 μmoles $gm.^{-1}$ $hour^{-1}$), thereafter gaining a small rise.

During three hours aerobic incubation under high CO_2 concentration (up to 10 % CO_2), the CO_2 evolution rate was initially high for both rhizomes and buds. In rhizomes, both species showed a rapid drop of respiration rate followed by a level off at about 1% CO_2 to ca. 40% initial rate (Figure 6.3 a,b). This drop was also observed on buds, however I. germanica buds exhibited a dramatic drop to less than 50% initial aerobic rate as compared to I. pseudacorus (drop to more than 50%).

6.3.1.2 Anaerobic respiration.

Under low CO_2 (0 - 1% CO_2), the initial drop of respiration rate under air was 60% (I. pseudacorus) and ca. 30% (I. germanica). When N_2 was gassed over the rhizome (Figure 6.4), the anaerobic respiration rate of Iris germanica shoot up to a maximum of 280% of initial aerobic rate, however within 14 minutes a rapid drop was observed to 140%. Henceforth, anaerobic rate increased to ca. 170% and was maintained at a constant level until up to 1% CO_2. A small rise was observed when aerobic environment was once again employed, followed by a drop of about 30%. In I. pseudacorus, the initial rise under anoxia was small (20%), followed by a gradual drop to ca. 80% of the initial

aerobic rate at 1.0% CO_2. The aerobic regime which followed further reduced the rate to ca. 60% at ca. 0.3% CO_2 and thereafter was maintained at this level even until 1.0% CO_2.

Under high CO_2 (up to more than 4 % CO_2), a rapid drop from initial anaerobic rate was observed in I. pseudacorus (Figure 6.5) to 30% level. However, after 1% CO_2 the tendency to level off was exhibited which was further maintained between 25 - 30% even up to 10% CO_2. In Iris germanica (Figure 6.6), the drop was rather gradual and no tendency to level off was shown even after 4% CO_2. At this high CO_2 level, lower respiration rates (15% of initial anaerobic rate) were observed and graph (Figure 6.6) suggests a further drop when the experiment was continued for a second day of incubation which is out of the scope of this experiment.

6.3.2 ETHANOL ACCUMULATION.

6.3.2.1 Effect of anoxic incubation (close system).

In Iris pseudacorus, aerobic ethanol content was low (mean value 3.54 µmoles $gm.^{-1}$ fresh weight). When incubated in N_2 gas for 30 hours in a static and close system where CO_2 accumulated, 91.07 µmoles $gm.^{-1}$ fresh weight of ethanol were measured (Figure 6.7 a). Greater amounts of ethanol were found in buds for both

treatments. The ethanol content in the core was also high in air (10.42 µmoles gm.$^{-1}$ fresh weight) and in N_2 (165.44 µmoles gm.$^{-1}$ fresh weight) as compared to buds.

In Iris germanica, very minute quantities of ethanol were found (mean value = 0.087 µmoles gm.$^{-1}$ fresh weight) in air. Under anoxia, 58.87 µmoles gm.$^{-1}$ fresh weight was accumulated. In air, bud and core showed no presence of ethanol. Under N_2, core contained the highest amount of ethanol (144.71 µmoles gm.$^{-1}$ fresh weight), followed by front buds (101.78 µmoles gm.$^{-1}$ fresh weight), front section of rhizomes (90.4 µmoles gm.$^{-1}$ fresh weight), middle buds (61.51 µmoles gm.$^{-1}$ fresh weight), and other parts of rhizomes (Figure 6.7 b). Comparing the increase of ethanol in both species, Iris germanica exhibited a bigger increment (99.86% - rhizomes, 100% - core) than Iris pseudacorus (96.11% - rhizomes, 93.7% - core). In buds, Iris germanica also showed bigger increments (100%) as compared to I. pseudacorus (93.58%).

6.3.2.2 Effect of flooding and/or burial.

Five species of rhizomatous (Iris germanica, I. pseudacorus, Hedychium sp. and Filipendula ulmaria) and tuber producing (Helianthus tuberosus) plants were examined. Growth was shown in all the treatments except in treatment C (buried and flooded) for all the species. However, I. pseudacorus and Hedychium sp. could still grow under this severest environment. Details of

treatments were discussed in Chapter 2.

In Iris germanica, flooding caused the ethanol level to rise and when combined with burial (treatment C), ethanol content rose to a maximum level (37.56 μmoles $gm.^{-1}$ fresh weight). Burial also increased the accumulation of ethanol even when not flooded, however to a lesser extent than flooding (Figure 6.8). In I. pseudacorus, flooding with burial (total submergence) also enhanced ethanol accumulation, but flooding without burial (partial submergence) or burial without flooding (Figure 6.8) caused lower levels of ethanol accumulation. In this species, planting the plant on the surface of sand could enhance ethanol accumulation (treatment B). In Hedychium sp., burial with flooding also gave the highest level of ethanol but the lowest level was shown under burial without flooding (Figure 6.8). When planted on the sand surface (not buried), flooding did not affect ethanol accumulation. In Filipendula ulmaria, ethanol levels in treatment A, B and D were about similar, as burial and flooding separately did not affect ethanol production. However, when both factors occurred together (total submergence), ethanol content rose. In Helianthus tuberosus (a tuberous plant), flooding and burial (treatment C - total inundation) gave the highest level, followed by not flooded - not buried (treatment B), not flooded - buried (treatment D) and flooded - not buried (treatment A - partial submergence).

When treatment B (Not buried and not flooded) was assumed as a control and the percentage of increase or decrease was calculated from the other treatments, the increase in Iris germanica was the most prominent (Figure 6.10). In all the other species, an increase is shown by treatment C and a decrease in treatment D. In treatment A, Iris pseudacorus and Helianthus tuberosus exhibited a decrease whereas Hedychium sp. and Filipendula ulmaria an increase.

6.4 DISCUSSION

In their natural habitats, roots and rhizomes of marsh plants are often submerged in anoxic or hypoxic environments. However, these organs could still receive oxygen and release respiratory products such as CO_2 and ethanol vapour via the exposed leaves or even dead stumps. Nevertheless, whenever leaves, stems or stumps are wholly covered under water, these plants have to face a severe condition as far as normal respiration is concerned (Boulter et al., 1963). The results from this chapter are obtained from exposing rhizomatous plants of marshland (Iris pseudacorus) and dryland (Iris germanica) to complete anaerobiosis (anoxia) and also to different concentrations of CO_2. Low level of O_2 and raised CO_2 level are characteristic of flooded soil (Weaver and Himmel, 1930; Gambrell and Patrick, 1978). In this way an ecological approach is combined with an examination of a physiological response.

6.4.1 The rate of aerobic and anaerobic respiration.

As expected, the aerobic respiration of the non-dormant buds is higher than in rhizomes. Physiological studies have shown that this is due to the increase in the efficiency of the respiratory enzyme systems in the non-dormant bud tissue (Villiers, 1975). Under higher and increasing CO_2 levels (up to 10%), rhizomes of both wetland and dryland plants are similarly affected whereas buds of dryland plant (Iris germanica) exhibit a drop of respiration rate to well below 50% of the initial level when compared to Iris pseudacorus (above 50%). It seems that buds are more sensitive to high CO_2 than rhizomes i.e. above 1% CO_2 , the level of aerobic respiration of rhizomes becomes constant with no apparent effect of rising CO_2 level even up to 10% CO_2. In buds, high CO_2 has the effect of breaking dormancy (Villiers, 1975), hence the drop in respiratory rate is unlikely to be caused by imposing dormancy on this non-dormant organ. In the study on the effect of asulam (herbicide) on bracken rhizomes, Veerasekaran et al. (1977) also demonstrated the greater inhibition of bud respiration (O_2 uptake) than in storage tissue. Within three days of application, the RNA levels in meristematic tissue were reduced by 5 percent. The important point of their findings which could be relevant in this study is that RNA metabolism is more rapidly affected before any reduction in protein content and O_2 uptake takes place. The inhibition of RNA metabolism would undoubtedly inhibit meristematic activity of buds. This factor is of considerable importance because further new shoot growth from that bud will be prevented. The results

from Chapter 4 (growth chapter) showed that in Iris pseudacorus growth was observed from buds whereas no such growth was seen in Iris germanica after 49 days burial and flooding.

Anaerobic respiration was measured under a stream of N_2 gas circulating in a closed system. Under low levels of CO_2 (0 - 1%) an increased rate of respiration under anoxia was observed in Iris germanica when compared to air. In Iris pseudacorus, CO_2 evolution was reduced under anoxia, providing further evidence (see also Bown et al., 1968) that carbon conservation (the so-called Pasteur effect) is shown by this plant. The production of extra CO_2 has been ascribed to the oxidation of organic acids such as malic acid (Turner, 1960) but the balance sheet produced by Neal and Girton (1955) indicates that most of this extra CO_2 came from carbohydrate ; some of it may arise from carbohydrate in the hexose monophosphate shunt (Figure 6.10). Hence, the raised CO_2 level would increase the rate of diminution of this substance (Bown et al.,1968).

In Crawford's metabolic theory (McManmon and Crawford, 1971), the tolerance of flooding is associated with a lack of acceleration of glycolysis which was induced by the anaerobic condition. In other words, tolerant plants lack a Pasteur effect. In a more recent work, Rumpho and Kennedy (1981) have shown that seedlings of Echinochloa crus-galli var oryzicola (barnyard grass), common weeds of rice fields, also lack a Pasteur effect under anaerobic conditions. Under these conditions, the oxidative pentose phosphate pathway is

operating. Moreover, Pesis and Ng (1984 a,b), have exhibited the association between high vigour in muskmelon seeds with the absence of an apparent Pasteur effect. In the present study the result also suggested the association between the lack of Pasteur effect with growth e.g. Iris pseudacorus. This effect created by anoxia is irreversible when air is reintroduced. When exposed to increasing higher level of CO_2 concentration (up to 10%), a constant level of respiration is achieved whether under air or nitrogen at about 1% CO_2 level even up to 10% level in Iris pseudacorus. In Iris germanica, under N_2, the rate of CO_2 evolution continues to drop steadily indicating the absence of homeostatic property noted above. High internal concentrations of CO_2 in Iris pseudacorus (up to 13.8% v/v) are measured even during mid-summer when in full leaves (Boulter et al., 1963). During this time the air space system from leaf to rhizome could provide the rhizome with O_2 supply and CO_2 release. As an adaptation to aquatic environment requires greater tolerance to low oxygen and raised CO_2 levels, I. pseudacorus appears to develop the intrinsic adaptation to high CO_2 level as shown by its internal CO_2 level (Boulter et al., 1963) and also by the results from this study.

6.4.2 Ethanol accumulation.

The question on whether ethanol can be toxic or not has been answered in one case by Crawford and Zochowski (1984). In the present study (under anoxia) both Iris pseudacorus and Iris germanica show an increase in their ethanol content with buds as the major accumulator. Since buds are the active part of the rhizome (Villiers, 1975) and a major sink, ethanol could be produced in situ or transported to this sink. In rhizomes, higher amounts of ethanol were measured in Iris pseudacorus, a flood-tolerant plant, as compared to Iris germanica, a non-flood-tolerant plant, contrary to results from other workers (Monk et al., 1984). However, in the present study, rhizomes were held in static environment where ethanol vapour could not escape. In this static environment, Altenburger (1981) also measured a high similar amount of ethanol in Iris pseudacorus. The advantage of releasing ethanol to the medium around the roots has been shown in rice and barnyard grass seedlings ,both very tolerant to anoxia (Bertani et al., 1980; Rumpho and Kennedy, 1981) and by rhizomes of wetland plants (Monk et al., 1984) and chickpea seedlings (Crawford and Zochowski, 1984) ; growth response or better growth were observed. In this present study, ethanol is absent in Iris germanica core and bud under aerobic environment whereas a substantial amount of ethanol accumulated in the tolerant Iris pseudacorus core and bud in air. The ethanol content of Iris germanica rhizome in air was less than 0.1 μmoles $gm.^{-1}$ fresh weight as compared to Iris pseudacorus with more than 3 μmoles $gm.^{-1}$ fresh weight. The presence of

higher concentrations of ethanol in rhizomes and buds of a flood-tolerant plant suggests that the internal tissues are better buffered (Hook and Scholtens, 1978). In this bulky tissues where diffusion is limited, this property would be of significance. However, in the natural environment moving water and wind could produce a non-static environment which eventually facilitates gaseous and ethanol vapour diffusion.

Flooding or burial (separately) would inhibit ethanol production in Iris pseudacorus. In this species only flooding combined with burial (total inundation) would greatly raise the ethanol level. However, in the latter treatment growth is still observed, indicating its tolerance of high levels of accumulated ethanol. Nevertheless, under burial or flooding, shoot growth is more rapid. Flooding and burial gave the highest levels of accumulated ethanol in Iris germanica, Hedychium sp., Filipendula ulmaria and Helianthus tuberosus. However, the actual concentration in this less tolerant (Hedychium sp.) and non-tolerant (Filipendula ulmaria and Helianthus tuberosus) plants are less except in Iris germanica. In H. tuberosus it is most likely that ethanol is dissipated into the aqueous medium due to decay of tubers and the rupture of the epidermis. In other species (Filipendula ulmaria) where small part of rhizome became soft, the lower ethanol content could possibly mean that these somewhat woody species are not better buffered; several woody flood-tolerant plants can contain more ethanol than intolerant ones (Hook and Brown, 1973). In Iris germanica, a tremendous increase of ethanol content after burial and flooding may poison

the tissue as part of rhizomes becomes soft. There is also no evidence (Turner, 1960) that alcohol formed in the initial fermentation is oxidised anaerobically, producing extra CO_2.

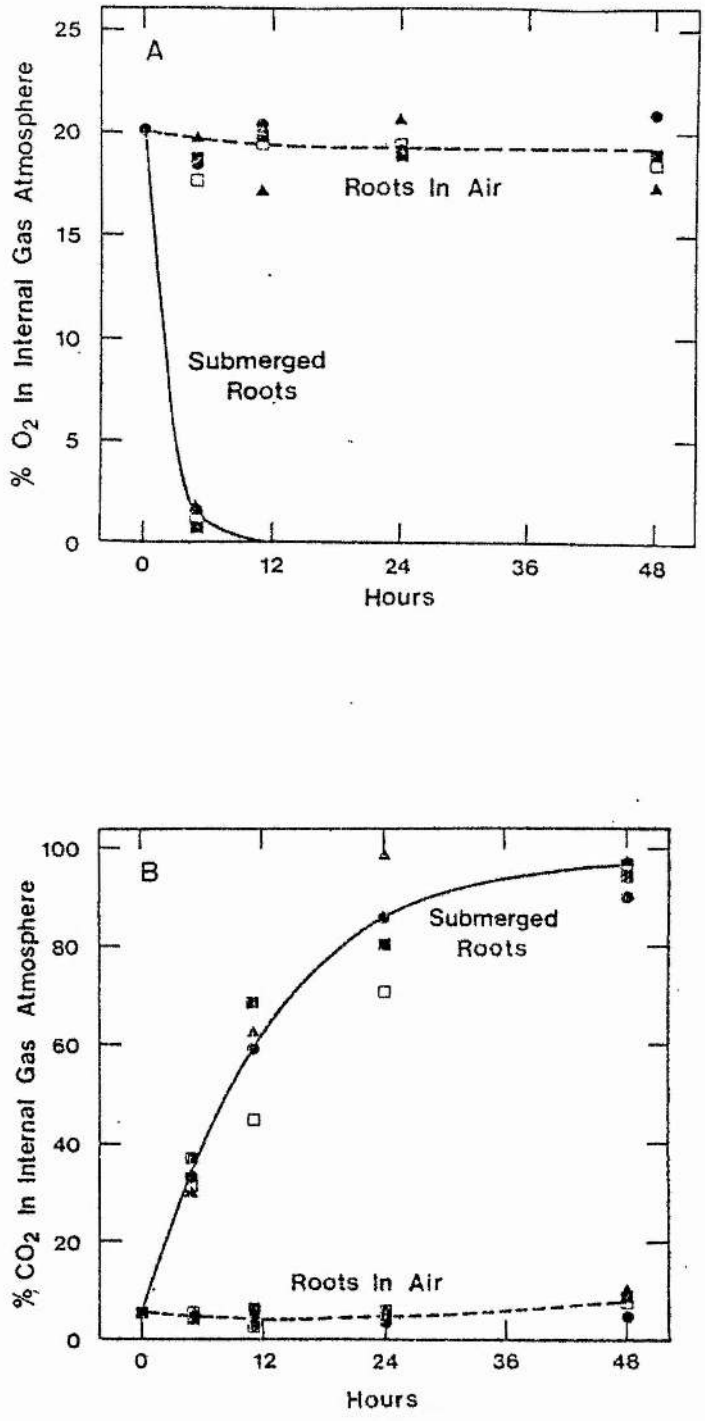


Figure 6.1 :

Changes in internal O_2 (A) and CO_2 (B) gas concentrations in storage roots of four sweet potato cultivars when held submerged under water for 48 hours at 22°C. The following cultivars were used: (▲), Caromex; (□), Jewel; (●), Centennial; (■), Jasper.
From L.A. Chang, L.K. Hammet and D.M. Pharr, (1983), Plant Physiology, 71 , 59 - 62.

Table 6.1 : Changes in internal O_2 (%) and CO_2(%) gas concentrations in roots (apical 5 cm. of the roots) of lowland rice when completely submerged as compared to other treatment in which 5 cm. of stem (basal) was surrounded by air. Analysis was done after 18 hours.

1. basal 5 cm of the roots in air.			2. roots completely submerged.		
roots No.	CO_2%	O_2%	root No.	CO_2%	O_2%
1	3.9	8.6	1	8.1	5.0
2	4.4	8.0	2	6.5	5.1
3	5.4	5.9	3	8.1	7.0
4	4.0	8.6	4	8.5	7.9
5	4.1	8.9	5	6.6	5.6
6	3.5	8.3			
mean	4.2 ± 0.06	8.0 ± 0.15	mean	7.6 ± 0.18	6.1 ± 0.7

From Van Raalte M.H. (1940), Annales du Jardin Botanique de Buitenzorg, 50, 99 - 114.

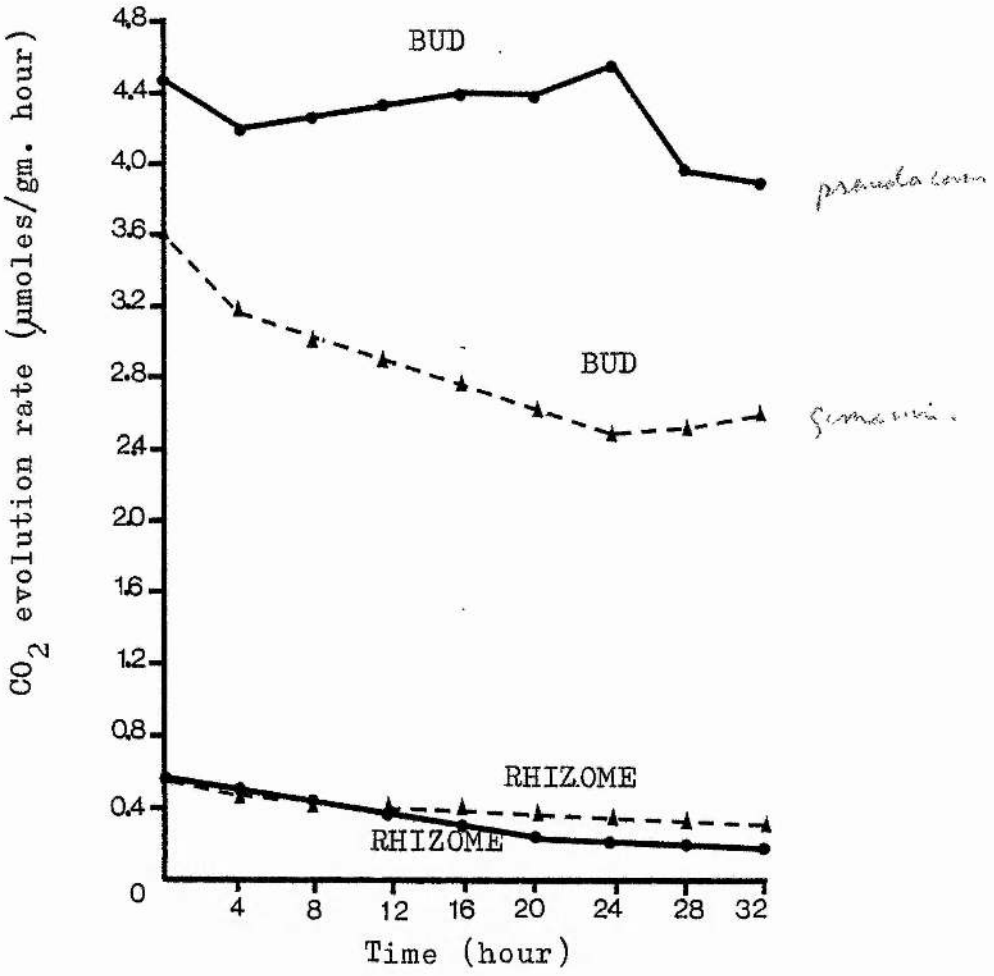
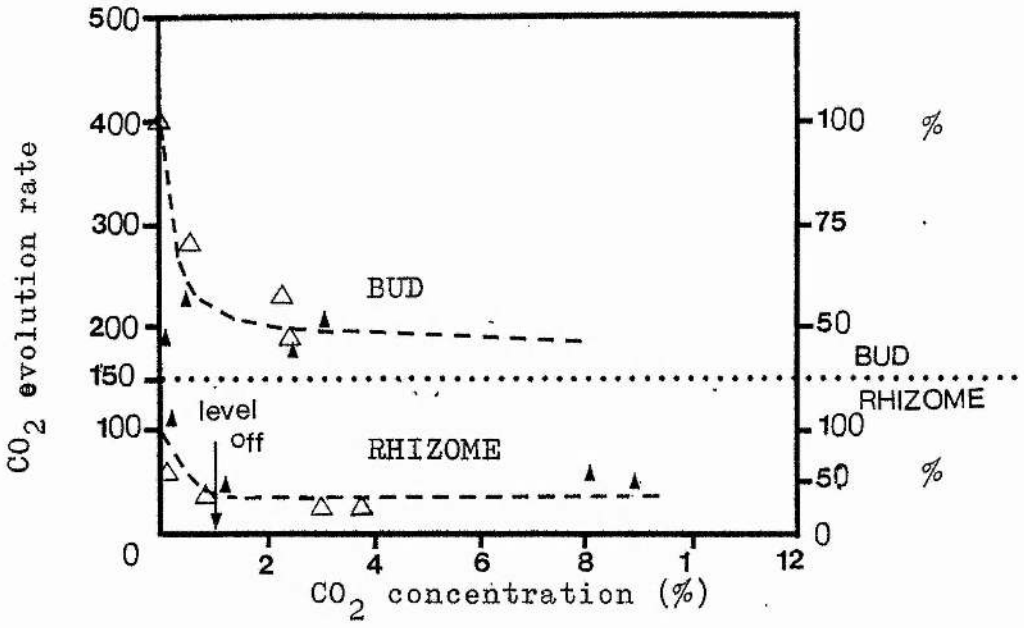


FIGURE 6.2 :

Aerobic respiration of Iris pseudacorus (●——●) and Iris germanica (▲- - - - - -▲) rhizomes and buds. CO_2 evolution rate was measured in a closed system where air is circulating.

a) IRIS GERMANICA



b) IRIS PSEUDACORUS

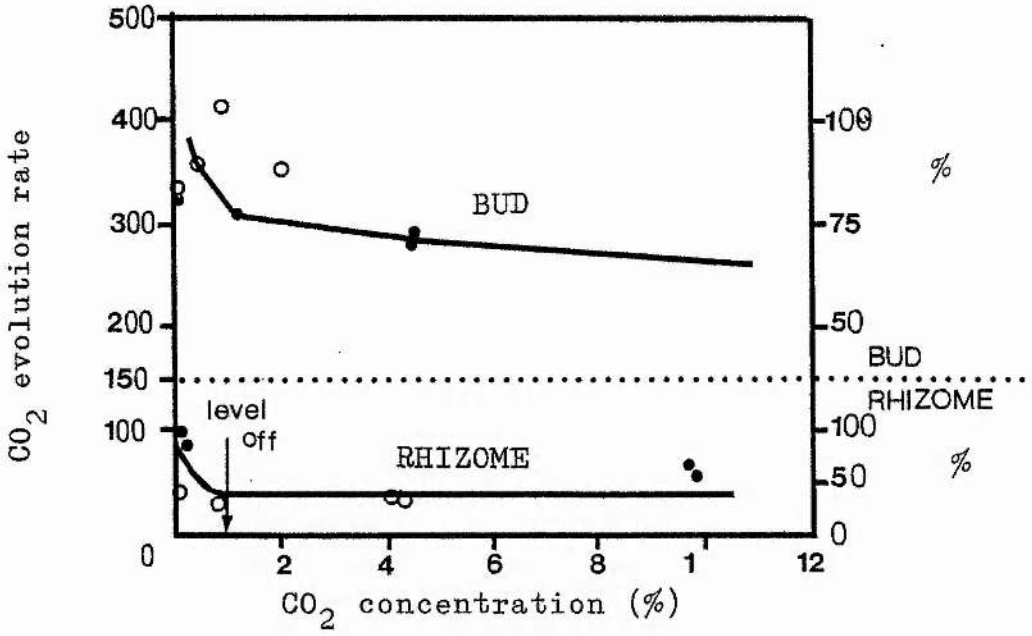


FIGURE 6.3 :

Aerobic respiration of Iris germanica (▲-△-▲) and Iris pseudacorus (●——○——●) rhizomes and buds under high and increasing CO_2 concentrations. Carbon dioxide evolution rate is presented as ppm CO_2 per gram fresh weight. The percentage of decrease of respiration rate with increasing CO_2 concentration of rhizome and bud are also shown.

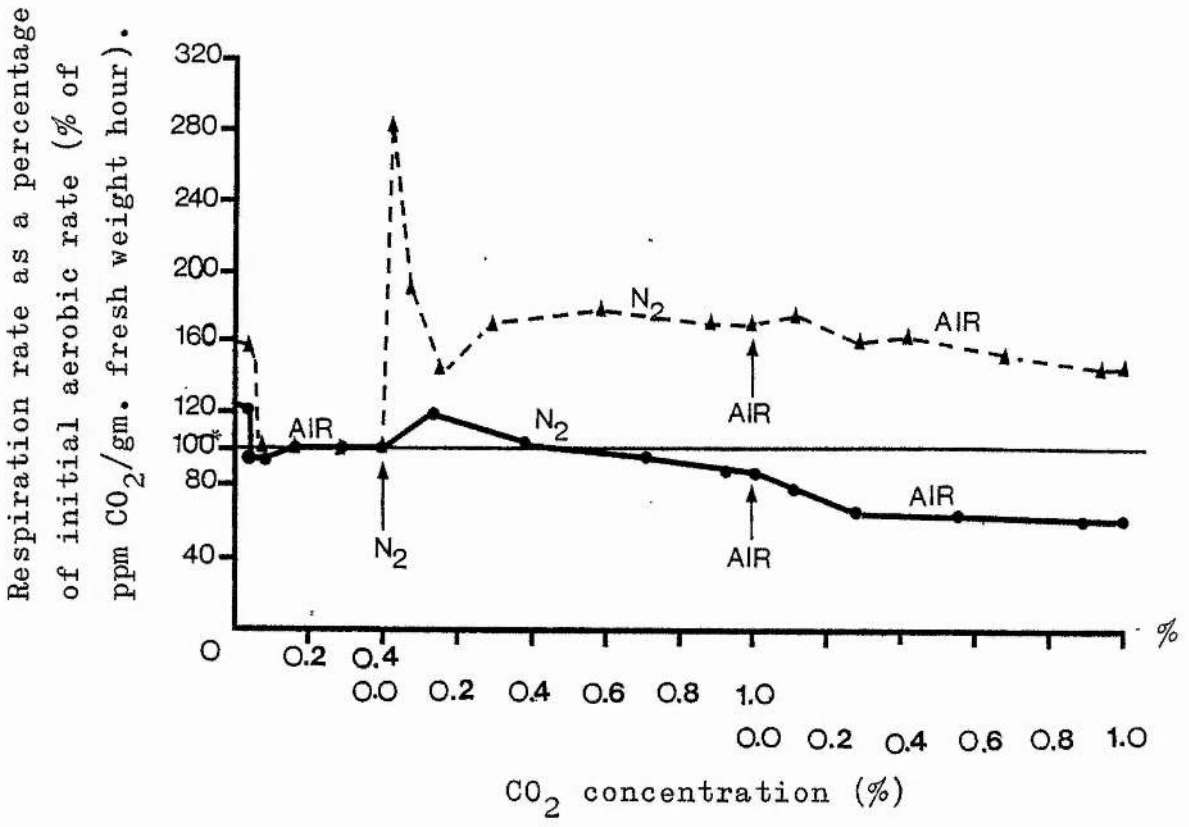


FIGURE 6.4 :

Respiration rate (Aerobic and Anaerobic) in Iris germanica (▲ - - - ▲) and Iris pseudacorus (●——●) rhizomes under low CO_2 concentrations (0 - 1 %).Respiration rate is presented as a percentage of initial aerobic rate*

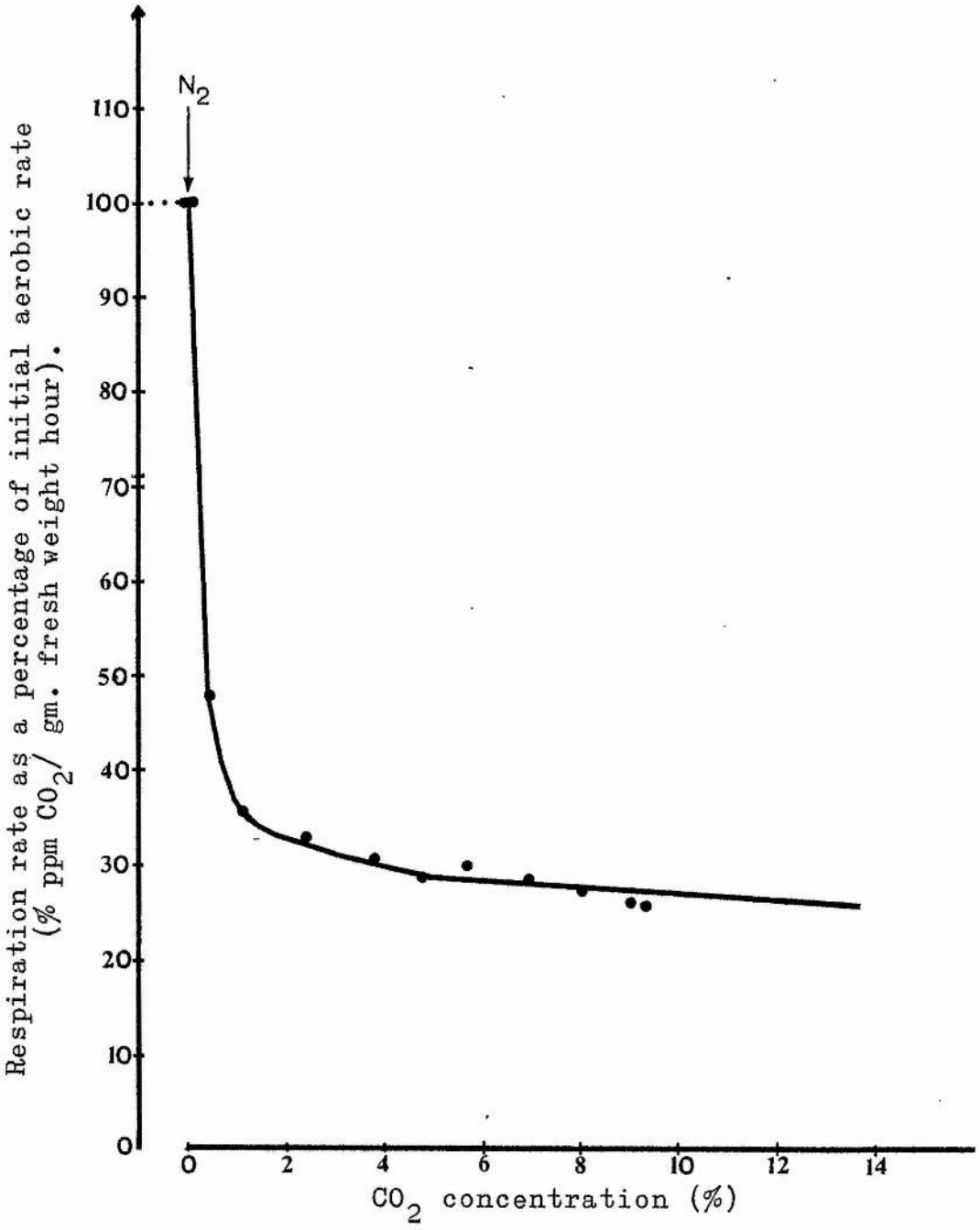


FIGURE 6.5 :

Anaerobic respiration rate as a percentage of initial rate under high increasing CO_2 concentrations in Iris pseudacorus rhizome.

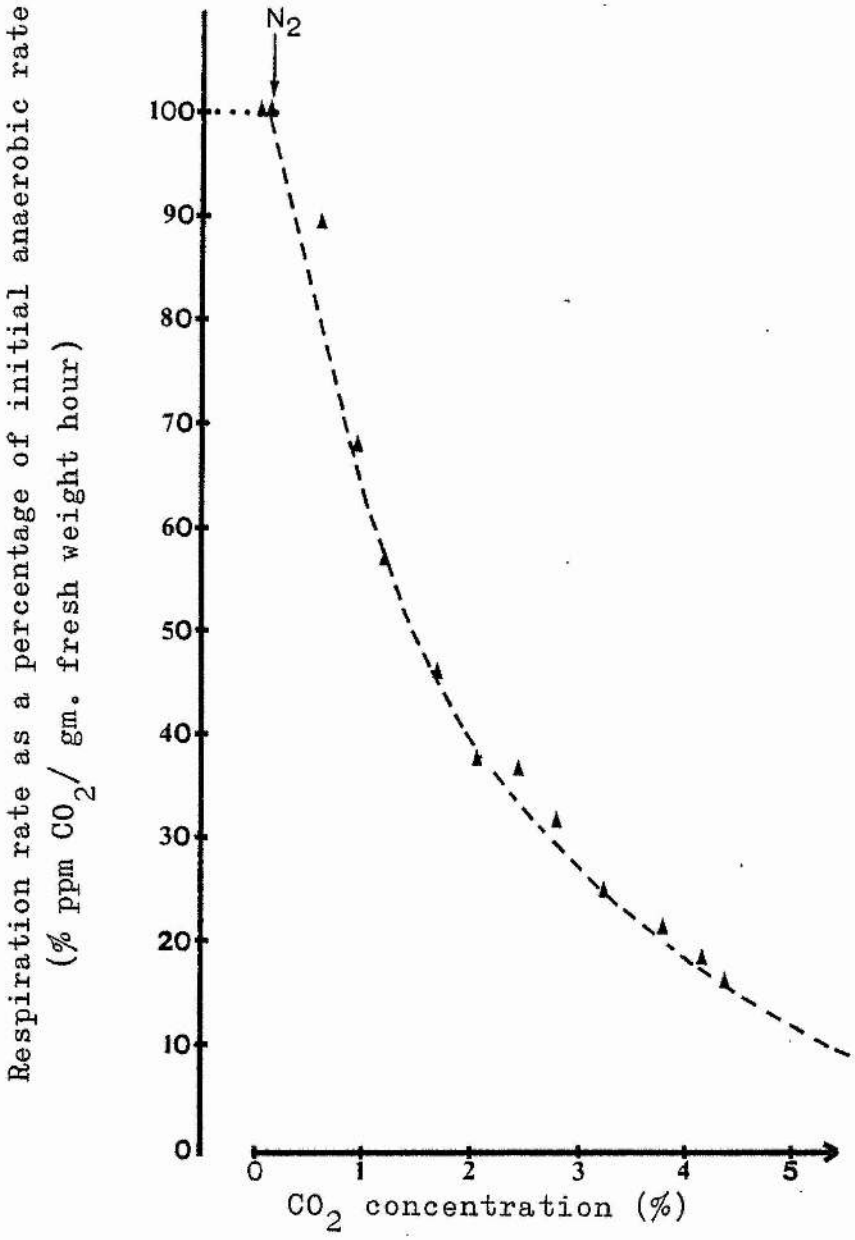
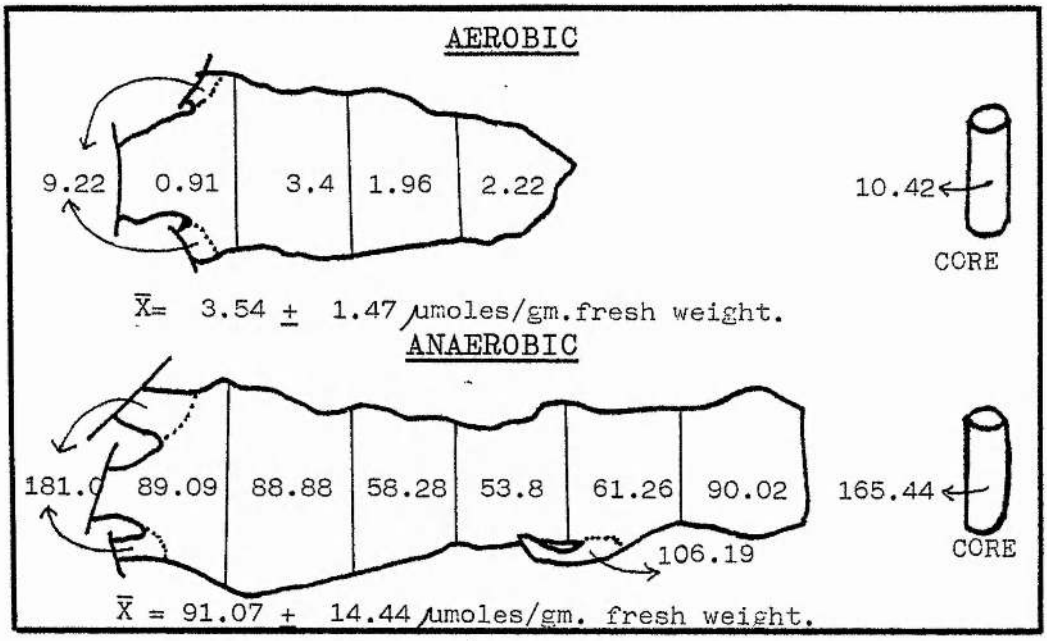


FIGURE 6.6 :

Anaerobic respiration of Iris germanica rhizome under increasing high CO_2 concentration, presented as a percentage of initial rate.

a) IRIS PSEUDACORUS



b) IRIS GERMANICA

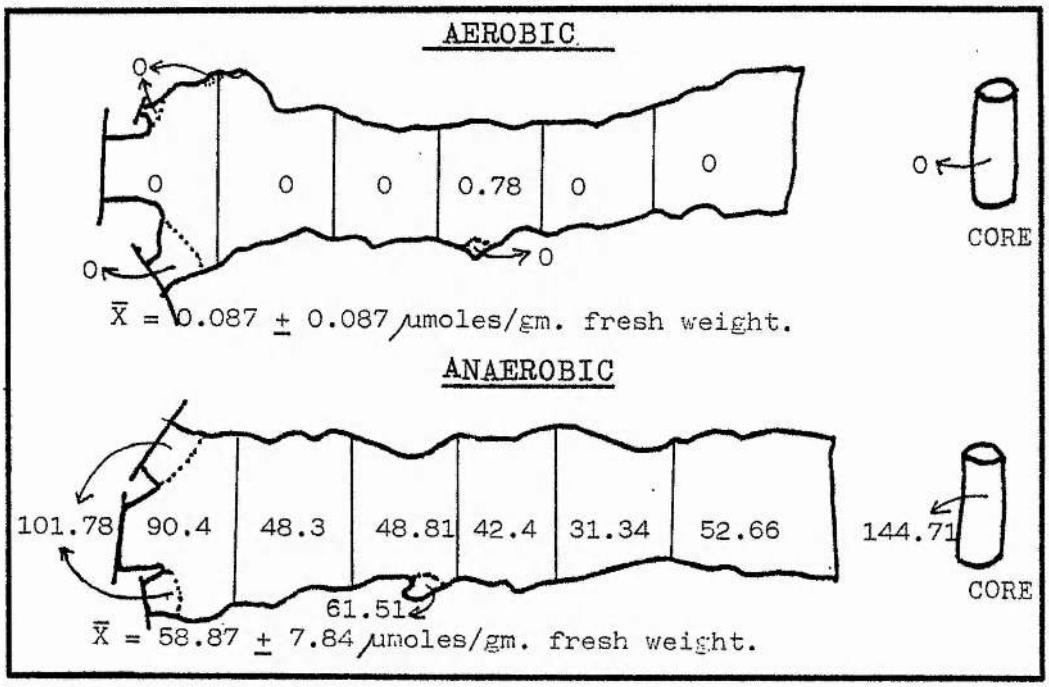
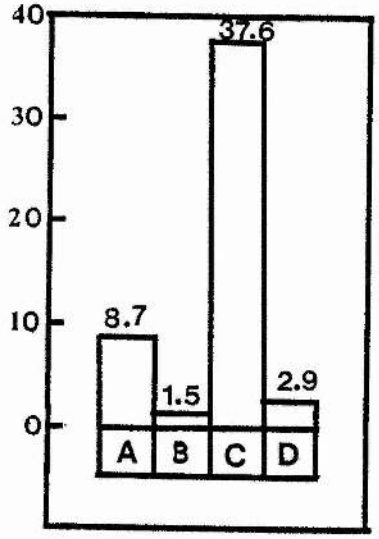


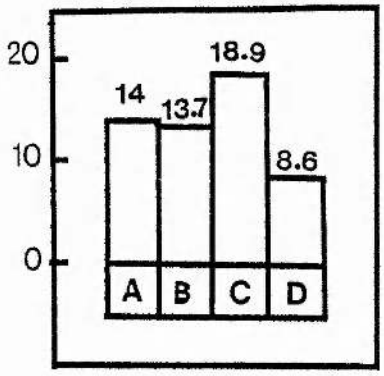
FIGURE 6.7 :

Ethanol content of Iris germanica and Iris pseudacor rhizomes, buds and cores under anoxic incubation (static) or air (static) for 30 hours. Results are expressed as µmoles per gram fresh weight (± S.E.).

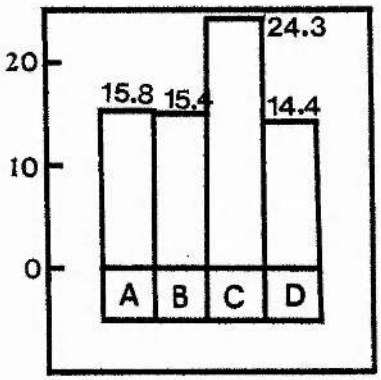
IRIS GERMANICA



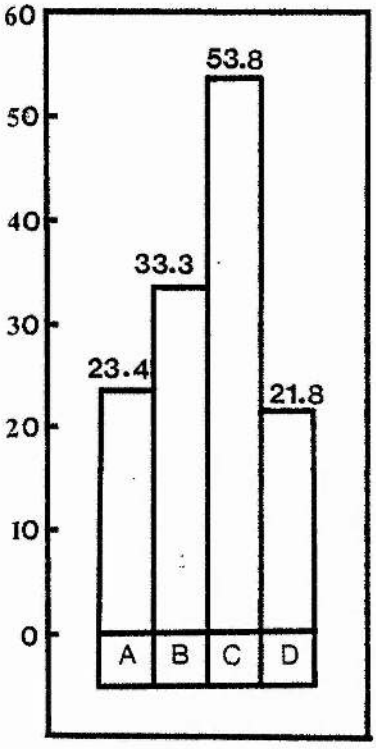
HEDYCHIUM SP.



FILIPENDULA ULMARIA



IRIS PSEUDACORUS



HELIANTHUS TUBEROSUS

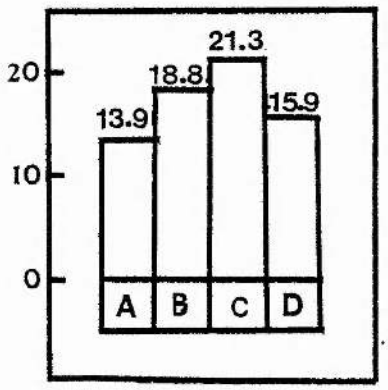
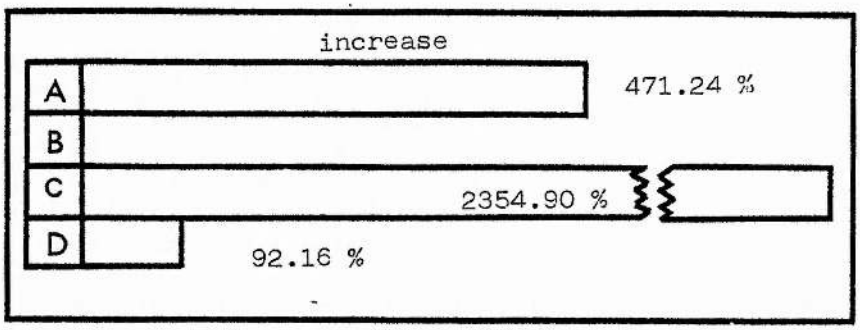


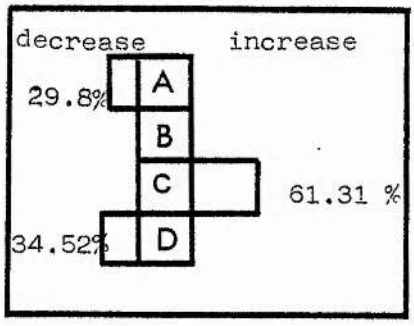
FIGURE 6.8 :

Ethanol accumulation (µmoles/ gm. fresh weight) as affected by flooding and/or burial treatments. Except Helianthus tuberosus (a tuber producing plant), all other species are rhizomatous plants. Details of experiment are explained in legend under Figure 6.9.

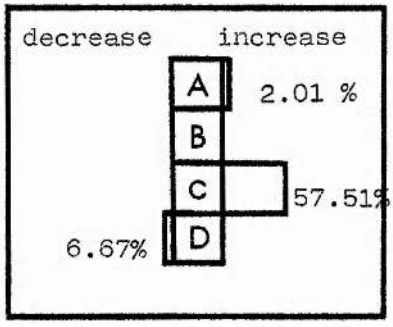
IRIS GERMANICA



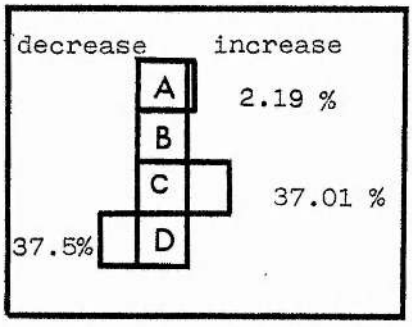
IRIS PSEUDACORUS



FILIPENDULA ULMARIA



HEDYCHIUM SP.



HELIANTHUS TUBEROSUS

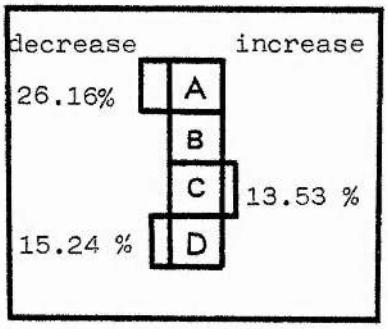


FIGURE 6.9 :

The percentage of increase or decrease of ethanol content as compared to treatment B (Not buried and Not flooded) which is assumed as a control.
Details of experiment:
A = Not buried and Flooded.
B = Not buried and Not flooded.
C = Buried and Flooded.
D = Buried and Not flooded.

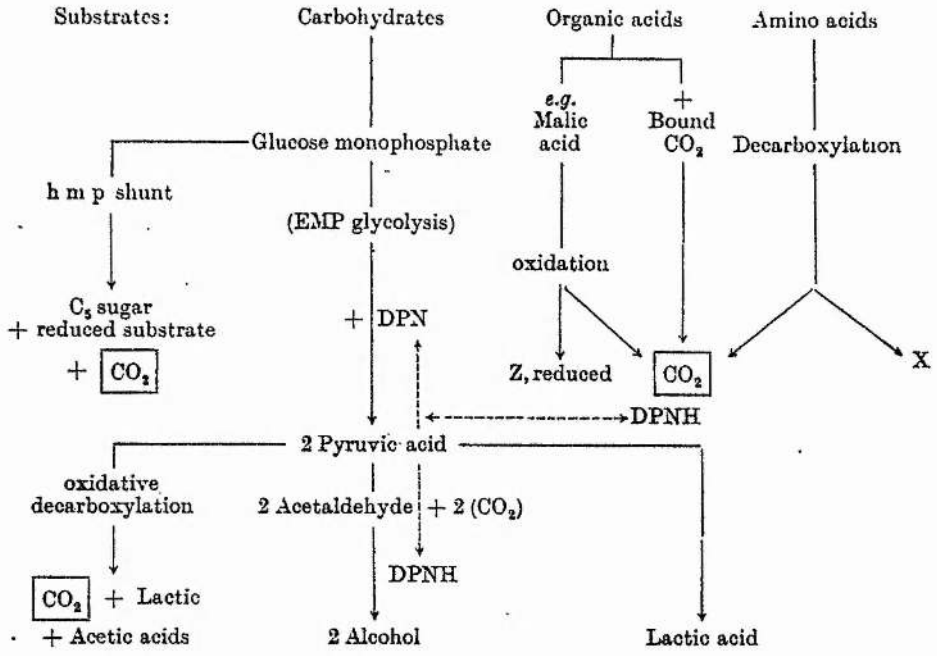


FIGURE 6.10:

Possible fermentation in higher plants according to Turner (1960). $\boxed{CO_2}$ represents the extra carbon dioxide measured in the presence of Pasteur effect. (CO_2) represents carbon dioxide of zymasis.

From:

Turner J. S. (1960), Fermentation in higher plants, its relation to respiration, The Pasteur effect, Encyclopaedia of Plant Physiology, 12, Part 2, 42-87.

CHAPTER 7 :

Chapter 7

GENERAL CONCLUSION

The objective of this study was to observe and record the effect of flooding and/or burial on various plants with storage organ. Underground stems (rhizomes, tubers and corms) were subjected to different aeration regimes under the four treatments employed:

A = Not buried and Flooded.

B = Not buried and Not flooded.

C = Buried and Flooded.

D = Buried and Not flooded.

Flooding whether combined with burial or not is widely acknowledged as one of the major hazard to plants especially crop plants. When combined with burial such as silting of river bank and deltas during monsoon periods, their devastating effects on crop plants are maximal. To know the limit of tolerance of higher plants to these severe conditions would be most helpful especially to agriculture. Since carbohydrate is the major

substance accumulated in the storage organs under investigation, its metabolism was further studied.

Growth is the most reliable indicator of plant viability. In this study (Chapter 3 and 4), rhizomes are better adapted to combined burial and flooding conditions than corms, whereas tubers were the worst affected. Wetland rhizomatous plants which normally grow near the water-edge also succumb whenever flooding occurred with burial but one non-wetland plant did not. Since in wetland habitats, shoots or even dead stumps whenever exposed to air can function as aeration channels to submerged rhizomes, under flooding and burial (total submergence) these facilities are severed, causing the severest effect. Hence these morphological attributes are not the ultimate adaptation to flooding.

The idea that physiological adaptation is a major causative factor for flooding tolerance has been championed by Crawford and co-workers. However, possibly due to different 'anaerobic' treatments (partly or totally submerged under water, under vacuum, under N_2 incubation, under N_2 stream systems or under gaseous mixtures of N_2, CO_2 and H_2) the results reported were sometimes contradictory i.e. see Chapter 5 (Sugar analysis). In this study, flooding tolerance was studied and the anaerobic respiration under anoxia (N_2 stream) was also measured together with their effect on ethanol content.

A definition of flooding tolerance was based on the growth response of rhizomatous or non-rhizomatous plants to one month flooding whilst planted on the sand surface (Not buried and Flooded - Treatment A in this study) (Crawford, 1966). In this study, a flood tolerant plant is defined as a plant which shows growth even under seven weeks total submergence (Buried and Flooded - Treatment C). In this category are rhizomatous Iris pseudacorus and Hedychium sp. Their sugar contents were also least affected by this severe condition especially the three major sugars; fructose, glucose and sucrose. Iris pseudacorus also exhibited a reduction in the rate of respiration under anoxia (N_2 stream) suggesting carbon economy. High external CO_2 levels and internal ethanol accumulation (Chapter 6) were also tolerated suggesting a good buffer system in the rhizome.

On the other hand, a non-flood tolerant plant is defined as a plant which cannot tolerate 7 weeks total submergence hence succumbed even though under partial submergence (Flooded and Not-buried, Treatment A) growth was vigorous in some of them. Among these plants were rhizomatous Filipendula ulmaria and Iris germanica , cormous Arum maculatum, taro and yautia and also tuber producing plants such as potato, Jerusalem artichoke and Coleus tuberosus (ubi kemili). Except in the rhizomatous species, most corms and tubers decayed. In the rhizomatous species, even though rhizomes tissues were quite firm after the treatment, their sugar content was greatly depleted suggesting carbon starvation. Nevertheless ethanol level was variable (very

high or low) possibly indicating that their tissues are not balancely buffered against high level of ethanol (Hook and Scholtens, 1978) or not tolerant of ethanol even at low concentrations (Crawford, pers.com.). These interpretations may seem contradictory to each other. However, it is important to note that here ethanol was measured after a period of stress where the tissues examined were already in many stages of decomposition. The possibility of ethanol leaking into the medium further complicated the interpretation. In Arum maculatum , a species which consist of many genetical varieties (Prime , 1960) , it is possible that a flooding tolerant variety could be found (See Chapter 3) in nature. Interestingly the occurrence of this species on marshy land is also sometimes observed.

Among crop plants, rhizome species are possibly the best choice for farming of lowland area prone to flooding. Whenever shoots are not buried (up to several weeks) under mud or flooded water, yautia and taro could also be grown. Tuber producing plants are the most affected by flooding, hence not advised for growing in flood-prone areas.

Comment.

This study was performed when plants were subjected to restricted (growing in pot and only for several weeks duration) and controlled conditions (under glass house environment) and well supported with nutrient solution. Competition among plants was also reduced. Hence the results obtained may be applicable

only to the above set of conditions.

Future Research.

In the interest of tropical agriculture, a longer term of flooding experiments especially on taro and yautia are clearly beneficial, especially its effect on yield (corm production). Field experiments such as those used to ascertain the advantage of crop rotation between rice and taro are also of considerable importance. In upland farming, the possible application of flooding or burial in increasing food production also should not be neglected. The search for better adapted varieties or tolerant mutants from irradiation treatments are also needed.

APPENDIX.

APPENDIX I : SPECIES USED IN FLOODING AND/OR BURIAL TREATMENTS.

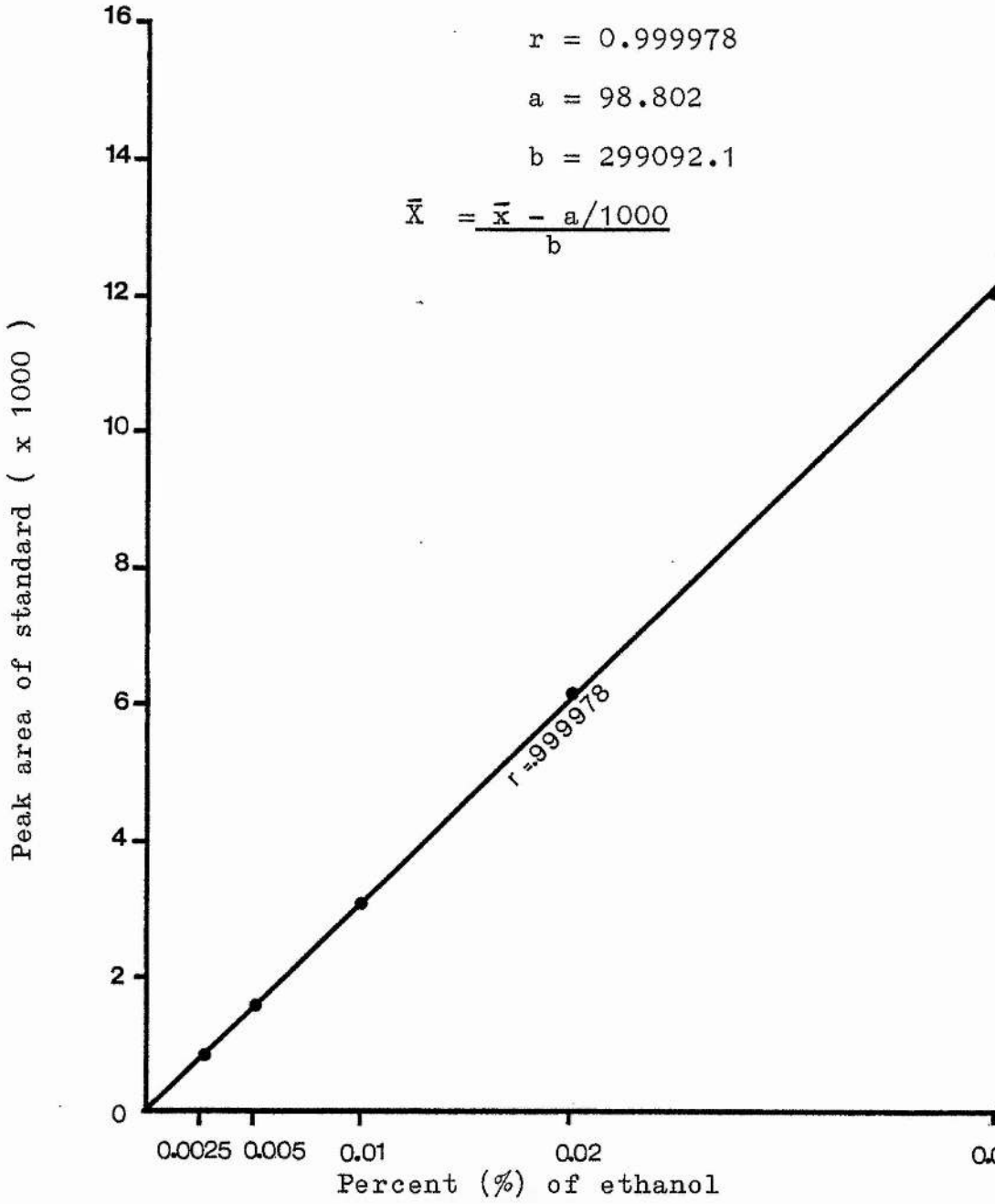
NO.	SPECIES	SITE OF COLLECTION	TIME OF EXPERIMENT	DURATION (weeks)
A.	RHIZOME SPECIES.			
1.	Iris pseudacorus L.	Botanic garden.	December - January.	7
2.	Hedychium sp.	"	I. January-March	7
			II. May-June	7
3.	Filipendula ulmaria (L.) Maxim	St. Andrews	March-April	7
4.	Iris germanica var. queechei L.	Botanic garden.	November - December	7
B.	CORM SPECIES			
1.	Arum maculatum	Dyers Brae, St. Andrews	I. Oct.-Jan.	14
			II. Feb.-April	7
2.	Colocasia esculenta (Taro)	shop	April-June	7
3.	Colocasia sp. (Keladi kemahang)	Malaysia	September - November	9
4.	Xanthosoma sp. (Keladi telur)	Malaysia	September - December	10
C.	TUBER SPECIES			
1.	Solanum tuberosum	shop	March-May	7
2.	Helianthus tuberosus	Botanic garden	Feb.-April	7
3.	Coleus tuberosus Benth. (Ubi kemili)	Malaysia	June-July	6.5

APPENDIX II : MODIFIED HOAGLAND'S SOLUTION (JOHNSON).

COMPOUND	g/litre in stock solution.
1. KNO_3	101.1 gm.
2. $Ca(NO_3)_2.4H_2O$	236.16 gm.
3. $NH_4H_2PO_4$	115.08 gm.
4. $MgSO_4.7H_2O$	246.49 gm.
5. Micronutrient solution	
KCl	3.728 gm.
H_2BO_3 (Boric acid)	1.546 gm.
$MnSO_4.H_2O$	0.338 gm.
$ZnSO_4.7H_2O$	0.575 gm.
$CuSO_4.5H_2O$	0.125 gm.
H_2MoO_4 (Molybdic acid)	0.018 gm.
$MnSO_4.4H_2O$	0.446 gm.
6. Fe-EDTA	
If (Molecular weight 346.08) ----------	6.922 gm.
If (Molecular weight 367.05) ----------	7.341 gm.

VOLUME OF STOCK/LITRE FINAL SOLUTION.

1. KNO_3	6.0 ml.
2. $Ca(NO_3)_2.4H_2O$	4.0 ml.
3. $NH_4H_2PO_4$	2.0 ml.
4. $MgSO_4.7H_2O$	1.0 ml.
5. Micronutrient solution	1.0 ml.
7. Fe-EDTA	1.0 ml.
TOTAL	15 ml.



APPENDIX III : Ethanol Standard Curve for GLC measuring (external standard method). The percentage of ethanol in the extract ($\bar{X}$) was estimated from the mean value of the areas of sample measured ($\bar{x}$).

APPENDIX IV : THE ASSAY OF THE REACTION MIXTURE OF ENZYMATIC ETHANOL MEASURING.

Cuvette number / Volume added (ml)	1 (Blank)	2 (Dilution factor 10X)	3 (Dilution factor 5X)
Buffer	2.0	2.5	2.5
Water	1.0	0.25	-
Sample (extract)	-	0.25	0.5
NAD	0.1	0.1	0.1
Total amount	3.1	3.1	3.1
Enzyme (ADH)	0.02	0.02	0.02

Footnote ;

NAD : nicotinamide adenine dinucleotide.

ADH : Alcohol dehydrogenase.

APPENDIX Va : DATA AND STATISTICAL TEST CARRIED OUT BY THE COMPUTER SHOWN HERE AS ONE EXAMPLE FOR ALL THE RESULTS SHOWN IN TABLES THROUGHOUT THE THESIS.

I. AMOUNT OF GLUCOSE IN F.ULMARIA +/- BURIAL AND FLOODING

mg/g dry weight

ROW #	NOT BURIED FLOODED	NOT BURIED NOT FLOODED	BURIED FLOODED	BURIED NOT FLOODED
1	8.26	3.05	.35	17.4
2	8.82	8.5	.45	8.6
3	7.06	1.55	.75	12.4
4	2.28	6.2	1.23	7.4
5	7.95	3.55	.4	6.45
6	11.3	8.9	.6	18.7
7	7.85	11.85	1.05	3.15
8	14.25	1.65	1.15	11.35
9	5.45	9.85	.55	2.8
10	6.85	4.35	.65	4.07
$\bar{X} \pm$ S.E.	8.008 $\pm$ 1.012	5.945 $\pm$ 1.154	0.718 $\pm$ 0.101	9.232 $\pm$ 1.787

II. The single factor Analysis of variance (ANOVA 1).

---> ANOVA1: GLUCOSE CONTENT IN FILIPENDULA ULMARIA RHIZOMES +/- BURIAL AND FLOODING.

	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE
TREATMENT	423.74	3	141.247
ERROR	500.31	36	13.8975
TOTAL	924.051		

F-TEST RATIO: 10.1635

APPENDIX Vb : STUDENT'S T-DISTRIBUTION TEST, DATA ARE FROM EXAMPLE (APPENDIX Va).

$$\hat{\sigma}^2 = \frac{\text{sum of squares (error)}}{\text{degrees of freedom (error)}}$$

$$\hat{\sigma}^2 = \text{mean square}$$

Estimated variance of difference between 2 means each of n observation is :

$$\sqrt{\text{mean square } (1/n + 1/n)}.$$

from Appendix Va, mean square = 13.8975

n = 10

degrees of freedom = 36.

Therefore,

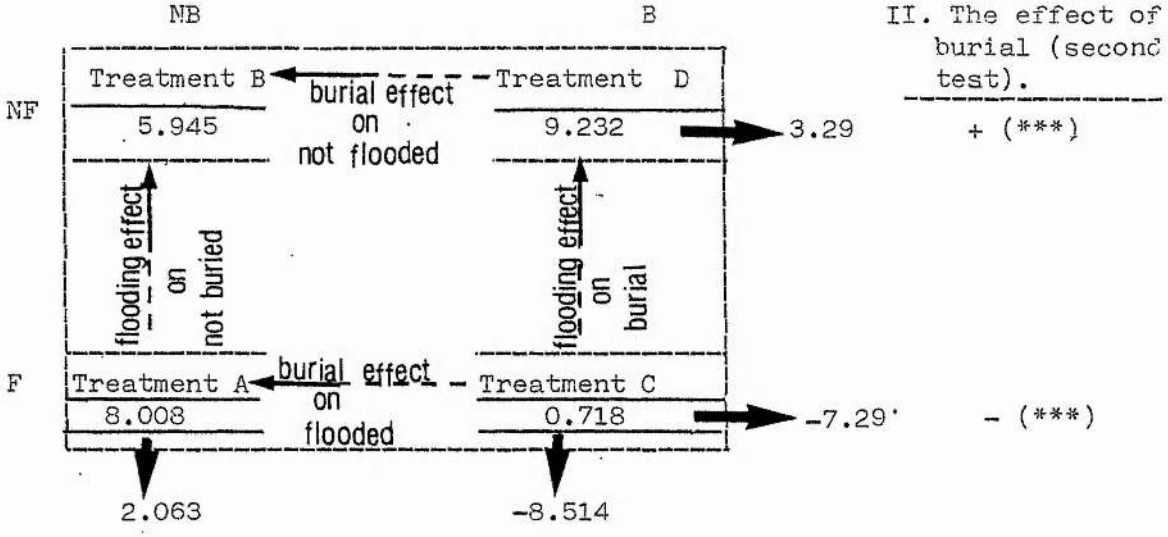
$$t_{36} = \sqrt{13.8975\ (2/10)} = 0.746$$

Two means differ at 5% if their difference is $>$ than $t_{36}^{0.05}$ 0.746

Two means differ at 1% if their difference is $>$ than $t_{36}^{0.01}$ 0.746

Two means differ at 0.1% if their difference is $>$ than $t_{36}^{0.001}$ 0.746

APPENDIX VI : DETAILED EXPLANATION OF STATISTICAL TEST OF BURIAL AND/OR FLOODING TREATMENTS.



I. The effect of flooding (first test):

+ (***) - (***)

(Data is from Appendix V)

The interaction of flooding and burial is exhibited whenever:

1). The increase is shown (+) under flooding effect on unburied rhizome (NB), but the decrease is found (-) instead under flooding effect on buried rhizome (B). For example in I above.

2). The increase is shown (+) under burial effect on non-flooded rhizome (NF), but the decrease is found (-) instead under burial effect on flooded rhizome (F). For example in II above.

3). The increase or decrease can be shown by either side of the test, however, these two should not be the same (+/-) before an interaction can be proven.

4). There are cases where the result of one or both sides from one or both tests (I and II) are not significant. In cases where one test show interaction (for example in I or II only), the significance of even from one test is already valid. However, whenever both tests show no significant increase or decrease, the interaction or no interaction cannot be statistically proved.

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